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Edited by L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen

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Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture,
Department of Nuclear Sciences and Applications,
International Atomic Energy Agency, Vienna, Austria



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PREFACE

Global food production must increase by 70% to feed the projected growth in the world's population from about seven to nine billion people by 2050. This cannot be achieved without increasing land productivity and conserving soil and water resources in the face of the severe challenges posed, including climate change, soil erosion and salinization, and drought and flooding — all of which contribute to reducing the quantity and quality of soil and water resources. Global R&D efforts are accelerating to both develop and put into practice "win–win" systems of agricultural production, targeting a range of scales from field plots to farm and catchment levels, which are resilient against the negative consequences of these challenges, while at the same time enhance land productivity for sustainable food production and minimize the greenhouse gas (GHG) emissions which potentially contribute to climate change and variations.

This publication is a compilation of selected papers presented during both oral and poster sessions at the International Symposium on "Managing Soils for Food Security and Climate Change Adaptation and Mitigation", organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture on 23–27 July, 2012. The objective of this Symposium was to communicate the advances that have been made in using nuclear and conventional techniques to improve land management practices for: (i) enhancing productivity; (ii) increasing soil resilience to climate change; and (iii) reducing GHG emissions. The Symposium also sought to identify current gaps in our knowledge and to discuss ways in which soil (and water) resources can be better managed to meet the challenge of promoting food security through the dual approach of climate change adaptation and mitigation.

Approximately 400 delegates from 80 Member States and representatives of international organizations including the FAO attended. Overall 85 oral and 136 poster papers were presented during the 5-day Symposium. These covered a wide range of topics during the plenary and six thematic sessions, including (i) managing soils for crop production and on-farm and area-wide ecosystem service efficiency; (ii) preserving and protecting soil resources; (iii) establishing soil and water conservation zones for pollution control; (iv) managing soils for climate change adaptation and mitigation through increasing soil carbon stocks (C sequestration) and reducing greenhouse gas emissions; (v) managing agricultural water for climate change adaptation; and (vi) recent advances in nuclear techniques and applications in land management research.

It is hoped that the information presented in these Proceedings provides valuable guidance to scientists and land managers in both the public and private sectors, as well as to government and institutional policy- and decision-makers involved in addressing land management issues for climate smart agriculture and the conservation of natural resources for agricultural productivity and food security.

Qu Liang
Director
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

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The Editors
May 2014

PLENARY SESSION

Integrated Soil Fertility Management (ISFM) in sub-Saharan Africa: Concepts and Practice

B. Vanlauwe[1,*]

ABSTRACT

Agricultural intensification is a necessity in the densely populated areas of sub-Saharan Africa (SSA). The integrated soil fertility management (ISFM) paradigm has been accepted by the research and development community as a viable set of principles to foster agricultural intensification. Integrated soil fertility management involves a set of soil fertility management practices that include the use of fertilizer, organic inputs and improved germplasm combined with the knowledge on how to adapt these practices to local conditions, aiming at maximizing agronomic efficiency (AE) of the applied nutrients and improving crop productivity. All inputs need to be managed following sound agronomic principles. An alternative approach being promoted in SSA is conservation agriculture (CA). In this paper, the major concepts underlying ISFM are highlighted with a focus on nitrogen (N) fertilizer on maize across Africa through a meta-analytical study consisting of published papers with data on AE as affected by specific ISFM components. The summary data confirm that the components underlying ISFM do result in increased AE values for N fertilizer on maize. Application of ISFM principles within important farming systems is then considered with a focus on dual purpose grain legume–maize rotations. It is proposed that ISFM could be the initial trigger towards the production of sufficient amounts of crop residues to engage in CA. Due to the lack of organic resources within smallholder farms, when promoted in SSA, CA should be accompanied by a fourth principle: the appropriate use of inorganic fertilizer, applied in the right formulation at the right time, place and rate.

Key words: *agronomic efficiency, N fertilizer, conservation agriculture, maize–legume systems, organic resource quality, soil fertility gradients.*

INTRODUCTION

The need for intensification of agriculture in sub-Saharan Africa (SSA) has recently gained support, in part because of the growing recognition that enhanced farm productivity is a major entry point to break the vicious cycle underlying rural poverty. Recent events include the launching of the Alliance for a Green Revolution in Africa (AGRA), which aims at increasing fertilizer use from the current 8 kg to 50 kg fertilizer nutrients/ha (Abuja Fertilizer Summit, 2006), thereby acknowledging that sustainable intensification needs to rely on the sensible use of external nutrient sources. Since fertilizer is an expensive commodity and because the overuse of fertilizer can lead to undesirable environmental degradation, integrated soil fertility management (ISFM), aiming at maximizing the agronomic efficiency of nutrient inputs (Vanlauwe *et al.*, 2010), has been increasingly adopted by the research and development community as a framework for boosting crop productivity with minimal environmental impacts.

In recent years, conservation agriculture (CA) has also been promoted intensively as a paradigm towards sustainable intensification of smallholder farming systems in SSA, in some extreme cases infringing on the domain of religion (Baudron *et al.*, 2012). Conservation agriculture is commonly defined around a set of three principles: minimum tillage, soil surface cover, and crop rotations. One of the main justifications for 'pushing' CA is its impressive adoption under large-scale farming conditions in various parts of the world (Landers, 2001). Although some of the initially hypothesized benefits of CA, including soil C sequestration and higher yields are not unequivocally confirmed (Govaerts *et al.*, 2009), CA is usually observed to result in more stable and economically favourable yields, usually after a number of years after conversion from conventional agriculture to CA (Rusinamhodzi *et al.*, 2011). Also widely observed is the fact that minimal tillage without surface mulch usually results in depressed yields (Paul *et al.*, 2013), partly because mulch provides the necessary conditions for soil biota to thrive and ensure that the physical conditions of the topsoil are conducive for seed germination and initial crop growth. The CA revolution started in the 1970s with large-scale farmers in Brazil (Landers, 2001), and spread to countries in Latin America, Europe, and certain parts of South Asia (e.g. the Indo-Gangetic basin) (Rockstrom *et al.*, 2009). Another important benefit of minimum tillage was the energy saved by eliminating several ploughing operations. Fertilizer use was already a common practice in these systems where high yields were common. In a sense, reduced tillage automatically provided the required crop residues to provide sufficient soil cover due to the high crop productivity under these high input systems.

Any pathway to agricultural intensification needs to be adapted to smallholder farming conditions. Smallholder farming conditions in SSA are different from large-scale farming situations in many aspects. First of all, yields are low due to the limited use of agro-inputs and sub-optimal agricultural practices with the limited availability of crop residues as a consequence. Secondly, in many systems, several competing uses exist for available crop residues, e.g. for livestock feed (Giller *et al.*, 2009). Thirdly, under high population densities where fallow land is virtually absent, management-induced soil fertility gradients are created due to a concentration of scarcely available organic resources, either direct or processed, e.g. as manure or compost, in small areas, usually around the homestead (Tittonell *et al.*, 2005), further degrading more remote plots through nutrient mining and reduced organic matter recycling. Fourthly, farmer resources includ-

1 International Institute of Tropical Agriculture (IITA), Nairobi, Kenya
* E-mail address of corresponding author: b.vanlauwe@cgiar.org

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ing land, cash or labour, are most often in limited supply thus limiting the opportunities farmers have to invest in agriculture.

Concepts underlying integrated soil fertility management

Definition

Integrated soil fertility management has been defined as "A set of soil fertility management practices that necessarily include the use of fertilizer, organic inputs and improved germplasm combined with the knowledge on how to adapt these practices to local conditions, aiming at maximizing agronomic use efficiency of the applied nutrients and improving crop productivity. In ISFM, all inputs need to be managed following sound agronomic principles (Vanlauwe *et al.*, 2010). The goal of ISFM is optimized crop productivity through maximizing interactions that occur when fertilizers, organic inputs and improved germplasm and the required associated knowledge are integrated by farmers (Figure 1).

The definition focuses on maximizing the efficiency with which fertilizer and organic inputs are used since these are both scarce resources in the areas where agricultural intensification is needed. Agronomic efficiency (AE) is defined as incremental yield return to applied nutrient inputs:

$$AE\ (\text{kg/kg}) = (Y_F - Y_C) / (F_{appl})$$

where Y_F and Y_C refer respectively to yields (kg/ha) in the treatment where nutrients have been applied and in the control, which did not receive nutrient, and F_{appl} is the amount of fertilizer and/or organic nutrients applied (kg/ha).

Note that maximal AE also leads to maximum value:cost ratios since both indicators are linearly related for specific input and output prices.

Important components

The ISFM definition has a number of concepts including the use of fertilizer and improved germplasm, combined application of fertilizer and organic inputs, adaptation to local conditions and rehabilitation of degraded soils (Figure 1). In terms of response to fertilizer, two types of soil are distinguished: (i) soils in which crop productivity responds to fertilizer — 'responsive soils' — (Path A, Figure 1), and (ii) soils in which crop productivity responds minimally or not at all to fertilizer — 'poor, less-responsive soils' — due to other constraints besides the nutrients contained in the fertilizer (Path B, Figure 1). Investment in overall soil fertility rehabilitation through, for example, organic resource management will be required before AE will increase on non-responsive soils (Path C, Figure 1). A third type of soil, 'rich, less-responsive soils', is not included in the graph since such soils are sufficiently fertile to supply most or all of the nutrients needed by a crop. Inclusion of such soils in Figure 1 would result in a line with N-AE close to 0 across all ISFM components.

The application of fertilizer to improved germplasm on responsive soils will boost crop yield and improve the AE relative to current farmer practice in SSA, which is characterized by traditional varieties receiving little nutrient inputs that are often inappropriately managed. Combining organic and inorganic inputs has been advocated as a sound management principle for smallholder farming in the tropics since neither of the two inputs is usually available in sufficient quantities and both are needed in the long-term to sustain soil fertility and crop production (Figure 1) (Vanlauwe *et al.*, 2001). Inorganic inputs are often too expensive for smallholders to be applied at optimal rates and organic inputs applied at rates that are feasible for smallholder farmers seldom release sufficient nutrients for optimum

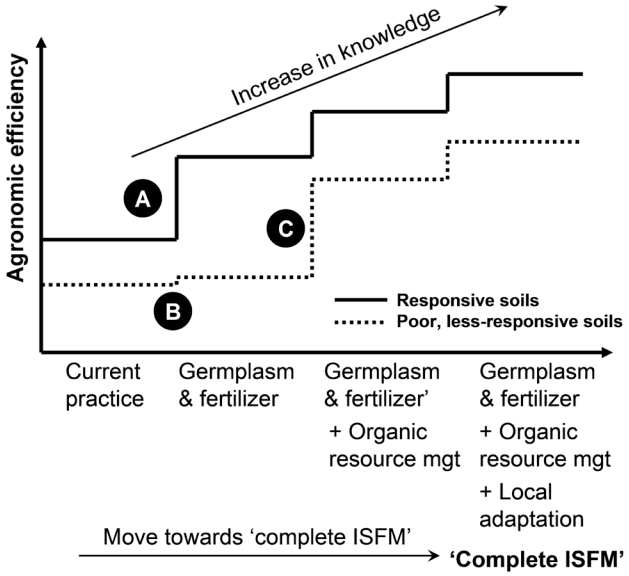


FIGURE 1. Conceptual relationship between the agronomic efficiency of fertilizers and organic resources and the implementation of various components of integrated soil fertility management (ISFM). "Current practice" assumes the use of the current average fertilizer application rate in sub-Saharan Africa of 8 kg per ha. Adapted from Vanlauwe *et al.* (2010).

crop yield (Vanlauwe *et al.*, 2001). Moreover, the combination of the two input types can provide added benefits if managed correctly.

Adjusting for site-specific soil conditions is a last requirement for maximizing AE because of the variability found in farming systems at different scales. Constraints to crop production can vary substantially between different fields within a single farm, creating what is often referred to as "soil fertility gradients" (Tittonell *et al.*, 2005; Vanlauwe *et al.*, 2006). Nutrient deficiencies related to soil type can occur at regional levels, but deficiencies related to cropping history and management can differ within short distances on a single farm. Such fertility gradients can have a substantial impact on fertilizer response and adjustment of inputs used along existing soil fertility gradients is one important aspect of local adaptation (Vanlauwe *et al.*, 2006). Often, within-farm gradients of soil fertility are dissected by considering fields close to the homestead, referred to as "infields", separately from fields furthest away from the homestead, referred to as 'outfields' (Tittonell *et al.*, 2005; Zingore *et al.*, 2007). Adaptation to local conditions also includes accompanying measures that are needed to address constraints that are unlikely to be resolved by fertilizer and/or organic inputs. These measures include the application of lime to acid soils, water harvesting techniques on soils susceptible to crusting, or soil erosion control in hillsides. Again, for poor, non-responsive soils, investment in overall soil fertility rehabilitation will be required before fertilizer AE will be enhanced (Path C, Figure 1). Zingore *et al.* (2007), for instance, demonstrated that responses to fertilizer on degraded outfields were only obtained after application of 17 $Mg \cdot ha^{-1} \cdot y^{-1}$ of farmyard manure during three consecutive years.

Towards complete ISFM

Complete ISFM comprises the use of improved germplasm, fertilizer, appropriate organic resource management, and local adaptation. Several intermediary phases have been identified that assist the practitioner's move towards complete ISFM, starting from the current average practice of applying eight kg/ha fertilizer nutrients to local

TABLE 1. Summary of selected properties related to the various integrated soil fertility management components considered in the meta-analysis, after removing data with low N fertilizer agronomic efficiency (N-AE) values due to relatively high N application rates or relatively high no-input control yields. The total number of data points retained is 721. Source: Vanlauwe *et al.* (2011).

Property	Farmer-lead fertilizer management	Researcher-lead fertilizer management	Improved varieties + researcher-lead fertilizer management	Organic inputs + researcher-lead fertilizer management	Researcher-lead fertilizer management on infields across soil fertility gradients
No. cases	24	324	73	272	28
N-AE mean	19	23	34	32	33
N-AE std. dev.	15	19	23	29	22
N-AE min.	–4	–23	–15	–26	6
N-AE max.	61	128	83	146	95
Upper quartile	20	30	52	46	39
Median	14	21	37	26	32
Lower quartile	11	9	13	12	20

varieties. Each step is expected to provide the management skills that result in an increase in yield and improvements in AE (Figure 1). Figure 1 is not intended to prioritize interventions but rather suggests a stepwise adoption of the elements of complete ISFM. It does, however, depict key components that lead to better soil fertility management. In areas, for instance, where farmyard manure is targeted towards specific fields within a farm, local adaptation is already taking place, even if no fertilizer is used.

Proof of the ISFM concept through meta-analysis of nitrogen (N) fertilizer response to maize in Africa

Data sources

A meta-analytical study was conducted using data obtained through specific searches within several agricultural databases (Vanlauwe *et al.*, 2011). Only peer-reviewed literature in journals and conference proceedings with information on control yields, yields after N fertilizer application and fertilizer N rates in maize-based cropping systems in SSA was included in the database. Considered papers included data from farm surveys, multi-locational on-farm trials and replicated on-station trials. A total of 90 peer-reviewed publications fulfilled all the criteria. Available data from different sites in Ethiopia, DR Congo, Botswana, Somalia, Rwanda and Tanzania recorded in the nutrient response database (Fertibase) of FAO (www.fao.org) were also included provided that they fulfilled the criteria.

Nitrogen agronomic efficiency (N-AE) under farmer managed conditions and researcher management as affected by improved maize germplasm

The average N-AE value for farmer-managed plots (24 cases) was 19 kg·grain·kg^{-1}·N (Table 1). The average N-AE value for researcher-managed plots (324 cases) was 23 kg·grain·kg^{-1}·N (Table 1). With improved hybrid maize varieties, an average N-AE value of 34 kg·grain·kg^{-1}·N (73 cases) was found (Table 1). Least square means calculations from studies that reported N-AE values for both local and improved germplasm showed that improved hybrid maize varieties increased N-AE significantly from 17 to 26 kg·grain·kg^{-1}·N, with no differences between local and improved, open-pollinated varieties (OPV) (Figure 2).

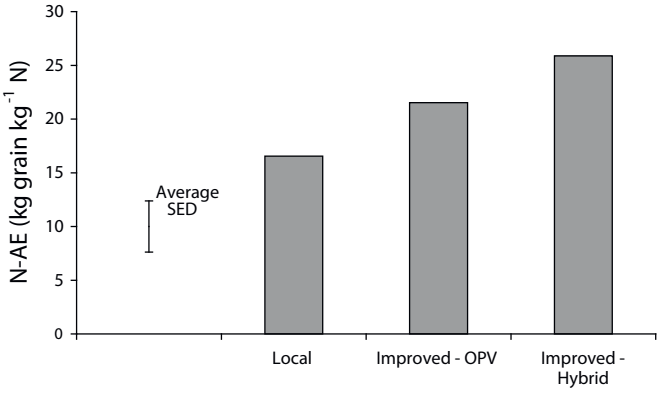


FIGURE 2. Agronomic efficiency of fertilizer N (N-AE) for local, improved open-pollinated (OPV), and improved hybrid maize varieties across the three regions. A total of 11 trial-years was included in the analysis (two from East/Central Africa, five from Southern Africa, and four from West Africa). SED refers to standard error of the difference. Source: Vanlauwe *et al.* (2011).

N-AE as affected by mixing fertilizer with organic inputs

Organic resources can be classified following their quality with consequences for their most appropriate use. Palm *et al*. (2001) proposed four classes of organic resources. Class I contains materials with high N (> 25 g/kg), low soluble polyphenol (< 40 g/kg), and low lignin (< 150 g/kg) content and are proposed to be applied directly to the crop. Class II organic resources have a high N (> 25 g/kg) and a high polyphenol (> 40 g/kg) or a high lignin content (> 150 g/kg), whereas class III organic resources have a low N (< 25 g/kg), a low polyphenol (< 40 g/kg), and a low lignin content (< 150 g/kg). Resources of Class II and III are proposed to be mixed with either fertilizer or class I organic resources to obtain optimal yields. Class IV organic resources have a low N (< 25 g/kg) and a high lignin content (> 150 g/kg) and are advised to be applied as surface mulch. Application of organic resources in combination with N fertilizer resulted in an average N-AE value of 32 kg·grain·kg^{-1}·N (272 cases) (Table 1). Following formal statistical testing including all retained data-points, N-AE values were significantly higher for the treatments where fertilizer was combined with manure or compost (38 kg·grain·kg^{-1}·N), but all other organic resources did not affect N-AE values significantly

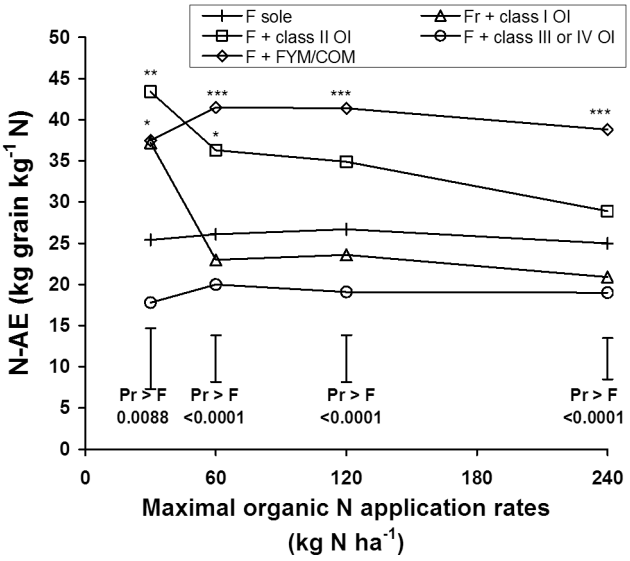


FIGURE 3. Agronomic efficiency of fertilizer N (N-AE) as affected by combination with different classes of organic inputs (Classes I, II, III + IV, and manure + compost) for organic N application rates ≤ 30 kg·N/ha (125 observations), ≤60 kg·N/ha (238 observations), ≤120 kg·N/ha (305 observations), or ≤240 kg·N/ha (352 observations). The classes are described in detail by Palm *et al.* (2001). Error bars are average standard errors of the difference. The symbols "*", "", and "***" indicate a significant difference with the sole fertilizer treatment at the 0.1, 1, and 5 percent level. In the legend, "F", "OI", "FYM", and "COM" refer respectively to fertilizer, organic inputs, manure and compost. Source: Vanlauwe *et al.* (2011).**

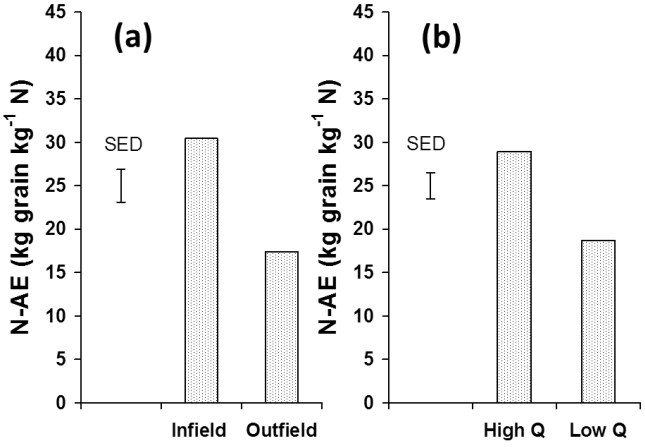


FIGURE 4. N fertilizer agronomic efficiency (N-AE) for paired comparisons between infields and outfields within single studies (a) and N-AE of fields with the maize control yields belonging to the lower (low Q) and upper quartile ranges (high Q) (b) across the three regions. SED refers to standard error of the difference. Source: Vanlauwe *et al.* (2011).

compared with the sole fertilizer treatment (25 kg·grain·kg^{-1}·N). However, when performing the statistical analysis on the data with maximum organic N application rates of 30 or 60 kg·N/ha, organic inputs belonging to Class II and manure/compost had significantly higher N-AE values than the sole fertilizer treatment or the Classes I and III/IV organic inputs (Figure 3). At higher organic N application rates, only the treatment with manure/compost gave significantly higher N-AE values than the sole fertilizer treatment (Figure 3).

N-AE as affected by targeting within-farm soil fertility gradients

The average N-AE for infields (28 cases) was 33 kg·grain·kg^{-1}·N (Table 1). When analysing studies where infields and outfields were included in the same study, average no-input control yields were 2 300 kg/ha for the infields and 1 400 kg/ha for the outfields and average N-AE values were 31 kg·grain·kg^{-1}·N for the infields and 17 kg·grain·kg^{-1}·N for the outfields (Figure 4a). When considering the lower and upper quartiles for the no-input control yields from all the sole fertilizer data, thereby assuming that the yield in the no-input control plots is a good indicator for soil fertility status, N-AE values were found to be consistently higher for the upper (29 kg·grain·kg^{-1}·N) than for the lower quartile (19 kg·grain·kg^{-1}·N) (Figure 4b).

Proof of concept

The above evidence from maize producing areas in Africa supports the main concepts underlying ISFM. Inclusion of improved maize germplasm, combining organic inputs of Classes II or manure/compost in combination with fertilizer and targeting fertilizer to responsive infields was shown to enhance N-AE substantially compared with N-AE values obtained under farmer management. Consequently, the basic principles underlying ISFM are well founded and good entry points for substantial improvements in N-AE. However, due to the high levels of variation inherent to meta-analysis and the lack of experimental designs that include all components embedded in ISFM, a consistent, multi-locational design is required, involving all ISFM components in an unbiased way, to obtain the optimal N-AE adapted to specific biophysical conditions across agricultural landscapes and to develop site-specific recommendations for fertilizer management.

Integrated soil fertility management in practice

Principles embedded within the definition of ISFM need to be applied within existing farming systems. Two examples clearly illustrated the integration of ISFM principles in existing cropping systems: (i) dual purpose grain legume–maize rotations with phosphorus (P) fertilizer targeted at the legume phase and N fertilizer targeted at the cereal phase in the moist savannah agro-ecozone (Sanginga *et al.*, 2003), and (ii) micro-dose fertilizer applications in legume–sorghum or legume–millet rotations with retention of crop residues and combined with water harvesting techniques in the semi-arid agro-ecozone (Bationo *et al.*, 1998; Tabo *et al.*, 2007).

As for the grain legume–maize rotations, application of appropriate amounts of mainly P to the legume phase ensures good grain and biomass production and biological N_2 fixation, the latter in turn benefiting a subsequent maize crop and thus reducing the need for external N fertilizer (Sanginga *et al.*, 2003). Choosing an appropriate legume germplasm with a low harvest index will favour accumulation of organic matter and N in the non-harvested plant parts and choosing adapted maize germplasm will favour a matching demand for nutrients by the maize. Application of a sufficient amount of legume crop residues can also improve other soil conditions, thus leading to enhanced fertilizer N use efficiency (Sanginga *et al.*, 2003). Selection of fertilizer application rates based on local knowledge of the initial soil fertility status within these systems would qualify the soil management practices as complete ISFM.

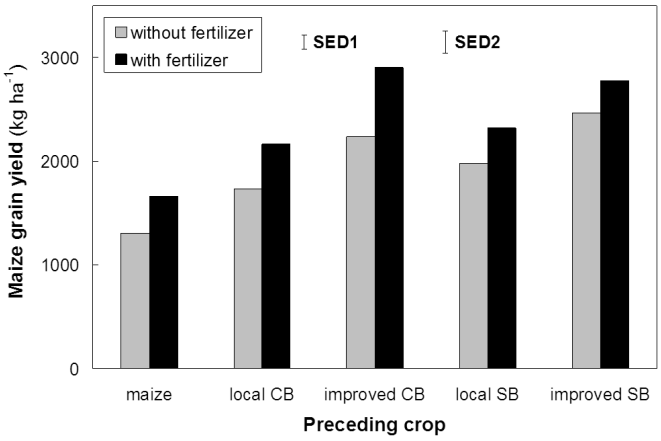


FIGURE 5. Maize grain yield as affected by application of compound fertilizer (NPK, 17:17:17) at 100 kg/ha and the crop grown in the preceding season (maize, climbing beans (CB) or soybean (SB) in South Kivu, DR Congo. SED — standard error of difference; * — significant at p < 0.05; ** — significant at p<0.01. There was no significant fertilizer × preceding crop interaction. Source: adapted from Pypers *et al.* (2012).

Pypers *et al*. (2012) observed a significant effect of previous cropping on maize yields both with and without fertilizer in field demonstrations in South Kivu, DR Congo (Figure 5). Yields of maize following soybean or climbing beans were 27–57 percent higher than those of maize following maize. Rotational benefits were also greater when improved, dual-purpose legume varieties with a low harvest index were grown. These legumes gave similar grain yields to local varieties (not shown), but grain yields of following maize crops were 20–34 percent higher than those of maize following local legume varieties. These yield improvements were related to greater biological N_2 fixation in the improved legumes, which derived a greater proportion of N from the atmosphere (due to their longer growing period relative to local varieties) and gave a higher biomass yield. Independently, application of compound fertilizer (N:P: potassium [K], 17:17:17) increased maize yields by between 22 and 39 percent. Combining crop rotation and fertilizer application resulted in yield increases up to 120 percent relative to the unfertilized maize–maize rotation, and a mean fertilizer value:cost ratio of 2.7.

As for the micro-dose technology, spot application of appropriate amounts of fertilizer to widely spaced crops such as sorghum or millet substantially enhances its use efficiency with further enhancements obtained when combined with physical soil management practices aiming at water harvesting. Recycling crop residues can reduce wind and water erosion (Bationo *et al*., 1998), and thus further benefit growth and nutrient demand of a following cereal. Rotating a legume such as cowpea, with sorghum or millet has been shown to further increase cereal yields (Aune and Bationo, 2008).

Integrated soil fertility management versus conservation agriculture: Different steps along an intensification gradient?

Adoption of conservation agriculture in Africa and the quest for organic inputs

Success with adoption of CA under smallholder farming conditions in SSA has been limited by a number of important constraints to widespread adoption, with the lack of organic resources to provide sufficient surface mulch consistently ranking amongst the top constraints, often related to low crop productivity and thus a low amount of crop residues and/or competing uses for organic resources (Giller *et al.*, 2009). The consequence of the above phenomena is that at planting of a subsequent crop, the area covered with crop residues from the previous crop is often below the 30 percent required, even if all crop residues are recycled. In central Kenya, for instance, Guto *et al*. (2012) showed that with maize yields below 2.5 t/ha, surface cover was less than 30 percent at the onset of the season. Aggregated maize yields in SSA are below 2 t/ha (www.fao.org), and assuming a harvest index of 45 percent, stover yields are thus expected to be usually below 2.5 t/ha.

Even before the current widespread promotion of CA, several attempts were made to enhance the availability of organic resources in smallholder farms, mainly driven by the search for low-input agricultural practices in the realm of "organic" or "green" agriculture and the widespread belief that fertilizer use was unrealistic for African smallholder farmers. Examples include alley cropping systems (e.g. Kang *et al.*, 1985), integration of herbaceous legumes (e.g. Carsky *et al.*, 2001), and biomass transfer systems (e.g. Gachengo *et al.*, 1999). Over time, adoption of the above practices has been disappointing, commonly due to the lack of immediate benefits to farmers who adopted them. With the recent interest in CA, some of these options are being reconsidered to provide the organic resources required to engage in CA, often placing CA amongst "green technologies". Obviously, earlier constraints to adoption of these practices are still valid when tested within the context of CA. Moreover, CA is not a green technology since fertilizer and herbicides are commonly used agro-inputs in areas were CA has taken off.

Fertilizer use as a fourth principle for conservation agriculture in Africa

Since the Abuja Fertilizer Summit in 2006, fertilizer use has regained emphasis in the context of agricultural intensification in Africa, but with a specific focus on maximizing the use efficiency and profitability of these agro-inputs. Consequently, one of the major arguments that led to the focus on low-input agriculture has lost relevance. Fertilizer, when applied appropriately, has been demonstrated to increase crop yields substantially in smallholder farming conditions. "Appropriate" refers to application of the right type of fertilizer at the right rate, time, and place (Zingore and Johnston, 2013), and accompanied by the appropriate agronomic practices (e.g. time of planting, crop spacing, weed control). "Appropriate" also refers to avoiding non-responsive soils or soils on which crops do not respond to the application of standard fertilizer due to other constraints limiting crop growth besides the nutrients contained in the fertilizer (Vanlauwe *et al*., 2010).

If the lack of organic resources is a major constraint to adoption of CA by smallholder farmers and if the appropriate use of fertilizer results in substantial increases in crop productivity and the availability of crop residues, then a logical consequence is to advocate the use of fertilizer in the context of CA activities. More crop residues will also allow alternative uses of these residues while retaining the minimally required soil cover. Since crop productivity in smallholder systems is generally low, adding the appropriate use of fertilizer as a fourth principle of CA will ensure that the appropriate approaches are taken when promoting CA and that valuable farmers' time — which is a limited resource in "hoe and cutlass" agriculture — is prevented from being spent on interventions with limited scalability. Obviously, the models for promoting CA would need to change if fertilizer is considered as a principle equal to the other three principles with input supply and market output value chains becoming necessary

conditions for success. In this context, alternative paradigms for intensification such as ISFM, which aims at assimilating best practices to maximize the use efficiency of fertilizer, could serve as the initial steps to take towards the application of CA, thus moving the latter from a 'competitive' to a 'complementary' paradigm.

The use of isotopic and nuclear techniques in integrated soil fertility management and conservation agriculture

Due to the complexity of different management factors such as crop residues, soil organic matter status and tillage that may affect nutrient and water use efficiency and hence agronomic efficiency, isotopic and nuclear techniques are important tools in ISFM and CA to elucidate the roles of these factors and their interactions and to assess N use efficiency of fertilizer and organic inputs, quantify biological N_2 fixation and the effects of crop residues on soil evaporation–plant transpiration, soil organic carbon and nitrogen dynamics (Nguyen *et al*., 2011).

CONCLUSIONS

Intensification of agricultural systems is a must in many areas in SSA. With fertilizer use back on the agenda, the ISFM paradigm, aiming at maximising the agronomic efficiency of fertilizer and organic inputs, is valid for intensifying agriculture. The principles of ISFM have been demonstrated to increase the AE of fertilizer, and these principles have been shown to be amenable to major cropping systems in SSA with AE values relatively easily exceeding 30 kg·grain per kg·N applied — far above commonly observed values under farmer management of 19 kg·grain per kg·N. This paper also argues that there is an urgent need to advocate a fourth principle which is the appropriate use of inorganic fertilizer for operationalizing CA under smallholder conditions to enhance crop productivity and thus produce the required crop residues to ensure sufficient soil cover.

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Towards Sustainable Land Management for Enhancing Food Security While Mitigating Climate Change Impacts: The Role of Nuclear and Isotopic Techniques

M. L. Nguyen[1]

ABSTRACT

The continuing need to enhance food security and reduce the impacts of climate change demands an action plan that leads to sustainable soil and water management. Nuclear and isotopic techniques (NITs) are used as tracers to understand the processes occurring in soil-water-plant systems and their complex interactions, and to provide comprehensive soil-water management technologies tailored to specific agro-ecosystems. One of the missions of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture is to assist IAEA and FAO Member States to establish their capacities to use nuclear-based methods to develop land (soil and water) management technologies for sustainable food security and conservation of natural resources. This paper provides an overview of the strategies/approaches and relevant NITs used by national agricultural research and development institutes in projects supported by the Soil and Water Management & Crop Nutrition (SWMCN) Subprogramme of the Joint FAO/IAEA Division to increase natural (soil and water) and agricultural (e.g. applied fertilizers) resource use efficiency, enhance soil quality and its resilience against degradation (e.g. through salinization and erosion) and minimize the impacts of climate change on soil and agricultural water resources. These activities are grouped into: (i) managing soils for enhancing crop production; (ii) preserving and protecting soil resources; (iii) managing soils for climate change adaptation and mitigation; and (iv) agricultural water management under climate change. This overview highlights several advances in the development and use of NITs and the successful transfer of these to Member States for sustainable management of soil and water resources in agroecosystems. Although NITs offer comparative advantages of high specificity, accuracy and sensitivity over conventional techniques, neither should be used in isolation; rather they should be integrated to maximize their potential in unravelling processes that influence the complexity of soil-water-plant interactions at scales ranging from field plots to the catchment (area-wide) level.

Keywords: *land management, integrated soil-water management, climate change adaptation, climate change mitigation, isotopic techniques.*

INTRODUCTION

Background Situation Analysis

The world is facing the unprecedented dual challenge of enhancing food security while ensuring environmental sustainability, in particular, the conservation of soil and water as well as plant and animal genetic resources. The present world population of seven billion is projected to exceed nine billion by 2050. Worldwide, land degradation is currently estimated at 1.9 billion ha and it is increasing at a rate of five to seven million ha each year (Lal, 2006). This degradation reduces productivity and biodiversity, damages natural resources and ecosystems, resulting in long term socio-economic and environmental impacts and leading ultimately to human migration and socio-political unrest (Bruinsma, 2003; UNEP, 2010). Besides these urgent issues, several environmental problems that can impact on land productivity and sustainable agricultural development also need to be addressed. These include: (i) increasing risks and impacts of climate change (CC) and variability in crop yield; (ii) rising energy demands, in particular non-renewable energy sources; (iii) expanding urbanization and industrialization; and (iv) deteriorating water and air quality.

To enhance the vital function of soil productivity and ensure adequate provision of soil-water ecosystem services, the following main strategies are required: (i) agricultural intensification on the best arable lands which are already being farmed to enhance food security with minimal environmental degradation; (ii) rational utilization of marginal lands; and (iii) combating land degradation and restoring degraded soils (Lal, 2000). A key element across all land types and an integral part of sustainable agriculture is the need to enhance soil quality for the environmental sustainability of agro-ecosystems (Karlen, Andrews and Doran, 2001). In this context, there is a strong necessity for innovative research to develop specific technologies that address the most strategically important issues of sustainable soil/land and water management in agro-ecosystems.

Objectives

In 1964, two United Nations Organizations, the Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA) established the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture at the IAEA Headquarters in Vienna, Austria. The aim of this strategic partnership was to help Member States solve practical agricultural problems with nuclear technology through international co-operation in research, capacity building, labora-

1 Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy, PO Box 100, A-1400 Vienna, Austria
E-mail address of corresponding author: m.nguyen@iaea.or

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tory support and information dissemination (IAEA, 2014a; FAO/IAEA, 2014a and b). To achieve this, its activities span five separate but interrelated areas: Soil and Water Management and Crop Nutrition (SWMCN), Plant Breeding and Genetics, Animal Production and Health, Insect and Pest Control and Food and Environmental Protection. Activities in each area are planned and implemented through a Headquarters-based Section and a Laboratory Unit located in Seibersdorf near Vienna, Austria (FAO/IAEA, 2014a and b).

Through Co-ordinated Research Projects (CRPs) involving scientists from developing countries, international institutions (e.g. Consultative Group on International Agricultural Research, CGIAR) and advanced research organizations, agricultural issues of regional or global significance are studied. Technologies obtained from CRPs and from the Laboratory are then transferred to IAEA and FAO Member Countries through Technical Co-operation Projects (TCPs) (FAO/IAEA, 2014a).

This paper provides an overview of the SWMCN Subprogramme's activities involving the use of nuclear and isotopic techniques (NITs) to address challenges/issues related to: (i) managing soils for enhancing food production; (ii) preserving and protecting soil resources; (iii) managing soils for counteracting the adverse effects of climate change; and (iv) agricultural water management under climate change. In particular, it highlights the main findings and lessons learned from these activities and outlines future trends for the way forward. It is not an exhaustive review and therefore only key publications are included in the reference section. Further information can be found at http://www-naweb.iaea.org/nafa/swmn/index.html (FAO/IAEA, 2014b).

MANAGING SOILS FOR ENHANCING FOOD PRODUCTION

In the late 1990's, CRPs using NITs were initiated to enhance soil fertility and the productive capacity of selected cropping systems in the main agro-ecological zones (AEZs) of the world (Nguyen *et al*., 2011). The underlying philosophy in the implementation of these CRPs was to adopt an integrated approach to soil, water and nutrient management in addressing issues of major concern and relevance for intensifying crop production under dryland and irrigated conditions. The development of an integrated nutrient management package (involving both manufactured fertilizers and natural sources of nutrients such as rock phosphates, biological nitrogen fixation [BNF], animal and green manures, etc., along with the recycling of crop residues), resulted in a greater demand for the use of nitrogen-15 (^{15}N), phosphorus-32 (^{32}P) and sulphur-35 (^{35}S) isotopes as tracers, to develop efficient agronomic practices tailored to local conditions and specific cropping systems that improve nutrient use efficiency and enhance soil fertility (Chalk, Zapata and Keerthisinghe, 2002; Nguyen and Zapata, 2006; Nguyen, Zapata and Dercon, 2010). Significant advances were also made during the past decade in developing and applying variations in the natural abundance (isotopic signatures) of stable isotopes (deuterium (^{2}H), carbon-13 (^{13}C), ^{15}N, oxygen-18 (^{18}O) and sulphur-34 (^{34}S) to assess the dynamics of nutrients and water in soil-plant systems. These developments were possible due to advances in automated online systems for stable isotope ratio measurements in soil, plant, water and gas samples (Chalk, Zapata and Keerthesinghe, 2002).

One lesson learnt was the need to move away from discipline-oriented research and adopt more holistic croppping systems approaches involving combinations of nutrient sources and nutrient-water interactions (Nguyen *et al*., 2011). In this way, the Subprogramme significantly improved both the development and transfer of results to beneficiaries (scientific community) and end users (farmers). Another valuable lesson was the importance of targeting cropping systems in the main AEZs. This facilitated the identification of management practices designed to mitigate major soil-related constraints to crop production. A major outcome of this approach was the realization that use of the best adapted crop genotypes to local soil/climate conditions was a key requirement for improving the productivity and sustainability of the cropping systems in question. These studies using NITs, which focused initially on the search for crop genotypes with superior nutrient use efficiency, later demonstrated the great potential of such genotypes for identifying suitable germplasm with tolerance to particular abiotic stresses (drought, flooding, soil N or P deficiency, soil salinity, aluminum [Al] toxicity, etc.). In this area, two CRPs were successfully implemented: one on selecting wheat and rice genotypes with increased plant-water use efficiency under water stress (drought and salinity) using the carbon (C) isotope discrimination (^{13}C versus ^{12}C) technique (IAEA, 2012), and the second on identifying food crop (cereal and legume) genotypes tolerant to soils of low N and P fertility status using ^{15}N and ^{32}P isotopic techniques (IAEA, 2013).

PRESERVING AND PROTECTING SOIL RESOURCES

Soil erosion and the associated deposition-sedimentation are natural landscape-forming processes. However, they can be accelerated by human activities (land use change, farm mismanagement, deforestation and overgrazing), resulting in negative impacts on agricultural production and the environment (IAEA, 2002). Land degradation through soil erosion is associated with the irretrievable loss of basic soil resources and it is therefore a major threat to ecosystem services such as water and biogeochemical cycles, biodiversity and plant primary productivity. While soil erosion is the predominant land degradation process occurring worldwide, more than 75% of the total agricultural land area affected by erosion is situated in the developing world (Lal, 2000). Globally, the economic costs of soil loss by erosion have been estimated at US$ 400 billion per yr (Pimentel *et al*., 1995). However, it is also important to recognize that effective soil conservation measures can successfully counter soil erosion losses and make a significant contribution to environmental sustainability. Measuring and identifying sources of soil erosion play the key role in designing effective soil conservation measures. Reliable data on the rates and patterns of soil redistribution (erosion/sedimentation) are required to provide a comprehensive assessment of the magnitude of the erosion problem and to underpin soil conservation measures, including the assessment of their economic and environmental impacts. Existing conventional techniques for monitoring soil erosion are capable of meeting some of these requirements, but they have a number of important limitations including lack of spatial soil distribution and time-consuming measurements (Nguyen *et al*., 2011). The quest for alternative techniques to complement existing conventional methods for both assessing soil erosion and to meet new requirements has directed attention to the use of fallout radionuclides (FRNs) and in particular caesium-137 (^{137}Cs) as tracers for documenting rates and spatial patterns of soil redistribution within landscapes (Ritchie and Mc Henry, 1990; IAEA, 1995).

Initial CRPs using FRNs focused on the refinement and standardization of the ^{137}Cs technique for its worldwide application in agricultural landscapes under a range of environmental conditions (Zapata, 2002 and 2003; Nguyen *et al*., 2011). Results from these CRPs paved the way to extend the use of both the ^{137}Cs technique and other FRNs as tracers for soil erosion/sedimentation investigations. In a follow-up CRP conducted between 2002 and 2009 (Nguyen *et al*., 2011), FRN techniques involving beryllium-7 (^{7}Be), ^{137}Cs and lead-210 (^{210}Pb) as soil/sediment tracers were further developed to

document short-term (<30 d), medium-term (~40 yr) and long-term (~100 yr) average soil redistribution rates and patterns in the landscape under different local conditions (climate, soil, topography and land uses). This combined application demonstrated that these are powerful tools to assess the relative impacts of soil conservation measures on soil erosion and land degradation (Zapata and Nguyen, 2010; Dercon *et al.*, 2012).

The IAEA is assisting developing Member States to establish and strengthen their human and institutional capacities for using FRNs reliably to minimize land degradation and enhance sustainable agriculture through both national and regional TCPs which provide expert services and laboratory quality assurance support. Currently there are 37 Member States using FRN techniques to address issues relating to sustainable land management. For example, a major regional Asia-Pacific TC project involving 14 countries has recently been completed. Here, FRN methodologies were used successfully to assess soil erosion and evaluate soil conservation measures as well as to better understand the link between soil redistribution and soil quality (e.g. soil organic matter) in the landscape. Similarly, since 2009, the IAEA has supported a regional Latin America TC project using environmental radionuclides as indicators of land degradation to enhance soil conservation and environmental protection in different ecosystems in order to ensure sustainable agricultural production and reduce the impacts of land degradation (Dercon *et al.*, 2012).

In order to target cost-effective soil conservation measures, it is important to determine not only the extent but also the source of soil erosion. In an ongoing CRP entitled "Integrated isotopic approaches for area-wide precision conservation to control the impacts of agricultural practices on land degradation and soil erosion", isotopic and conventional approaches are being integrated to support the implementation of precision conservation at catchment scales (Nguyen *et al.*, 2011). The FRN techniques are also being applied to develop sediment budgets on an area-wide basis (catchment) over different timescales. Furthermore, compound specific stable isotope (CSSI; e.g. ^{13}C and ^{15}N in amino acids) techniques (Gibbs, 2008) are being used to identify sources of soils in sediments (fingerprints) and apportion their relative contribution from different land uses (FAO/IAEA, 2008; Dercon *et al.*, 2014). Such integrated applications will help identify critical areas (hot spots) of soil loss and assist extension workers, policy makers, land managers and farmers to target appropriate soil conservation measures, thus providing effective guidelines for area-wide sustainable management of land and water resources in agro-ecosystems (FAO/IAEA, 2008).

Other widespread soil degradation processes severely affecting the productivity and ecosystem services of agricultural lands are soil salinization and acidification. In these cases, a win/win option is needed to enhance the sustainable management of the natural resource base and mitigate climate change impacts on land degradation. This can be achieved through the development, pilot testing and adoption of integrated approaches to soil-water-plant/animal management. A strategic analysis of the components of the system and identification of major constraints are required to formulate appropriate interventions. A core element is the search for plant genotypes tolerant to the major soil constraint, e.g. salt-tolerant trees, shrubs, forage or crop cultivars in salt-affected lands, whereas P-efficient and Al-tolerant crop genotypes are required in tropical acid soils. Nuclear and isotopic techniques play a key role in developing specific and cost-effective management practices and for monitoring changes in nutrient, water and soil quality as well as overall productivity in the crop-livestock farming production systems under consideration. This integrated approach has been successfully applied in several CRPs and TCPs implemented by the Subprogramme (IAEA, 2006; Sakavedan and Nguyen, 2010).

MANAGING SOILS FOR CLIMATE CHANGE

Sustainable intensification of agricultural production systems demands a combined approach to improve crop productivity for food security and at the same time restore soil quality and enhance its resilience against degradation and risks associated with climate change (CC) impacts. Conservation Agriculture (CA), which seeks to meet such requirements, is based on the following principles: (i) minimum mechanical soil disturbance by tillage/cultivation; (ii) the retention of a permanent organic cover on the soil surface; and (iii) the use of crop rotations/plant associations, including cover/green manure crops (FAO, 2014a and 2014b). Such CA systems are currently used in about 100 million ha to enhance the food security of smallholders in the developing world (Derpsch and Friedrich, 2009).

In a CRP entitled "Integrated soil, water and nutrient management in conservation agriculture", the influence of soil, water and crop management practices on soil organic matter (SOM) accumulation and its subsequent impacts on soil water, nutrient and C dynamics were investigated using NITs in various cropping systems worldwide. The results demonstrated that CA can bring benefits such as increased soil moisture retention, BNF, N retention and soil C sequestration. However, these effects were highly variable and site-specific, in some cases the benefits being negated by the influence of crop residues on plant diseases which could reduce crop yields and quality. One of the major lessons, with great implications for adoption strategies, is that CA can only be sustainable and successfully implemented if local constraints such as soil compaction, low soil fertility and lack of SOM are first removed (Dercon et al., 2010). Further research is therefore needed to develop and pilot test specific packages of integrated technologies and practices tailored to targeted agro-ecological zones and local agronomic management. Furthermore, this information is essential for a comprehensive assessment of socio-economic and environmental benefits and the development of appropriate policies to facilitate and encourage the adoption of CA by farmers. With this in mind, a new challenging CRP was initiated in 2011 (FAO/IAEA, 2014b) to investigate the potential of mulch-based cropping systems to enhance soil resilience against degradation and climate change risks and to increase soil fertility for sustainable food production in sub-Saharan Africa (SSA). Stable isotope techniques (15N and 13C) at enriched and/or natural abundance levels enabled an in-depth analysis and understanding of basic soil biological-physical processses, including soil C and nutrient cycling in mulch-based cropping systems. The selection and characterization of benchmark sites will provide a platform for extrapolation of results to other relevant agro-ecological zones in SSA (FAO/IAEA, 2014b).

AGRICULTURAL WATER MANAGEMENT UNDER CLIMATE CHANGE

Although the main focus of this Symposium was on soils and food security and climate change adaptation/mitigation, it is of utmost importance to also pay due attention to water because of soil-water interactions (Nguyen *et al.,* 2011) and their influence not only on food production but also on the provision of essential ecosystem services (UNEP, 2011). At present, agriculture uses around 70% of the total fresh water resources in the world. Rainfed agriculture accounts for 60% of world food production while the remaining 40% comes from irrigated agriculture. Managing agricultural water to enhance crop water productivity (more crop per drop) and water use efficiency in crop production systems is therefore of paramount importance. Studies conducted in CRPs and TCPs using NITs and related techniques have demonstrated that there is considerable scope to improve water use efficiency and crop productivity in rainfed agriculture. This can be achieved through the appropriate integration

of soil-water-plant technological options such as water conservation practices (e.g. zero or reduced tillage, mulching, crop residue retention, crop rotation, inter-cropping), water harvesting techniques, use of crop varieties adapted to drought and saline conditions and improvement of soil fertility (IAEA, 1998). In a recently completed CRP entitled "Managing irrigation water to enhance crop productivity under water limiting conditions: A role for isotopic techniques", integrated soil-water-plant approaches and recent advances in isotopic techniques were used to better manage irrigation water for enhancing crop productivity under water limiting conditions. Stable isotopes of water (^{18}O and ^{2}H), soil moisture neutron probes and related conventional techniques (e.g. micro-lysimetry) were used to quantify soil evaporation (E) and plant transpiration (T) fluxes at different stages of crop development. Significant advances were made using NITs to partition crop evapo-transpiration (ET) into its E and T components for developing management practices that reduce E and improve the amount of biomass produced per unit of transpiration (Heng *et al.*, 2014). Data generated in the project were also used to validate FAO's AquaCrop model for developing improved irrigation water, soil and crop agronomic practices to enhance crop water productivity and increase water use efficiency (Heng *et al.*, 2009; Steduto *et al.*, 2009; FAO/IAEA, 2014b). In addition, a CRP using ^{2}H, ^{15}N and ^{18}O and related techniques is being implemented to investigate the use of water conservation zones (wetlands, riparian buffer zones and farm ponds) to optimize water and nutrient storage, to reduce agricultural pollutants from runoff and deep drainage and to increase food/water security within agricultural catchments (FAO/IAEA, 2014b; Sakadevan, Heng & Nguyen, 2014).

THE WAY FORWARD: TOWARDS SUSTAINABLE LAND MANAGEMENT IN AGRO-ECOSYSTEMS

Recognizing that environmental degradation, poverty reduction and food security are strongly intertwined, one of the key elements in the strategic planning documents of UN organizations such as the FAO Strategic Framework (2010–2019) and IAEA's Mid-term Strategy (2012–2017) is to support environmental sustainability (FAO, 2014c; IAEA, 2014b). The overall aim is to develop and implement activities which will help countries and regions achieve sustainable management and utilization of natural resources and thereby contribute to achievement of the Millennium Development Goals (MDGs) for poverty reduction, food security and environmental sustainability (UN, 2012).

One strategic objective of the FAO (FAO, 2014c) is the sustainable management of land, water and genetic resources and improved responses to global environmental challenges affecting agriculture. The underlying philosophy in developing strategies for the SWMCN Subprogramme is, therefore, to use NITs to address global issues of major concern and relevance to sustainable land and water management in agro-ecosystems (FAO/IAEA, 2007 and 2008; Nguyen *et al.*, 2011), taking holistic and ecosystem approaches (Table 1).

Conceptual Framework of the Ecosystem Approach

Soil is a thin layer covering the earth's surface, which acts as an interface between the biosphere, lithosphere, hydrosphere and atmosphere. The biogeochemical processes occurring in the soil are essential driving forces for the cycling of elements/nutrients and other chemicals, thus influencing key ecosystem functions such as plant primary productivity and water and air quality (UNESCO-SCOPE, 2006; Totsche *et al.*, 2010). Interactive processes affecting coupled cycling of these elements (C, N, P and S) are strongly influenced by increasing global energy demand. However, present knowledge of global cycling of the elements, particularly the global C cycle remains limited by uncertainties in relation to quantitative aspects of soil C storage, loss and dynamics. In this regard, NITs offer considerable potential to both address process-level topics for a better understanding of biogeochemical cycles and to assess the relative value and effectiveness of novel management technologies designed to remove constraints/limitations to soil quality for productivity and/or sustainability in agro-

TABLE 1. Trends and outlook in developing and adopting holistic and ecosystem approaches to agricultural production systems by the SWMCN Subprogramme of the Joint FAO/IAEA Division

Description	Initial	Transitional	Future
Goals	Food security	Food security Preservation/protection Natural resource base Climate change (CC) response strategy	Food security Water security Poverty reduction Environmental sustainability
Objectives	Food production	Food and feed/fodder production CC adaptation/mitigation Soil, land and water, genetic resources conservation	Food and biomass production CC preparedness (risk assessment), response and rehabilitation Combating land and water degradation Promoting biodiversity
Activities	Agriculture (crops)	Agriculture/Livestock	Agriculture/Livestock Horticulture/Forestry/ Aquaculture/Fisheries Linkage to other economic sectors
Soil-water functions	Agricultural production inputs	Production and a range of ecosystem services (resilience and sustainable use)	Production and a full range of ecosystem services
Main issues	Integrated soil fertility management	Soil quality/soil health. Agricultural water management	Sustainable land and water use and management
Scale	Farmer's fields/plots	Farm/landscape/area-wide approach	Agricultural watersheds/catchments
Systems	Crop production	Conservation agriculture Cropping/farming systems in targeted AEZ	Climate smart agro-ecosystems and neighboring natural ecosystems

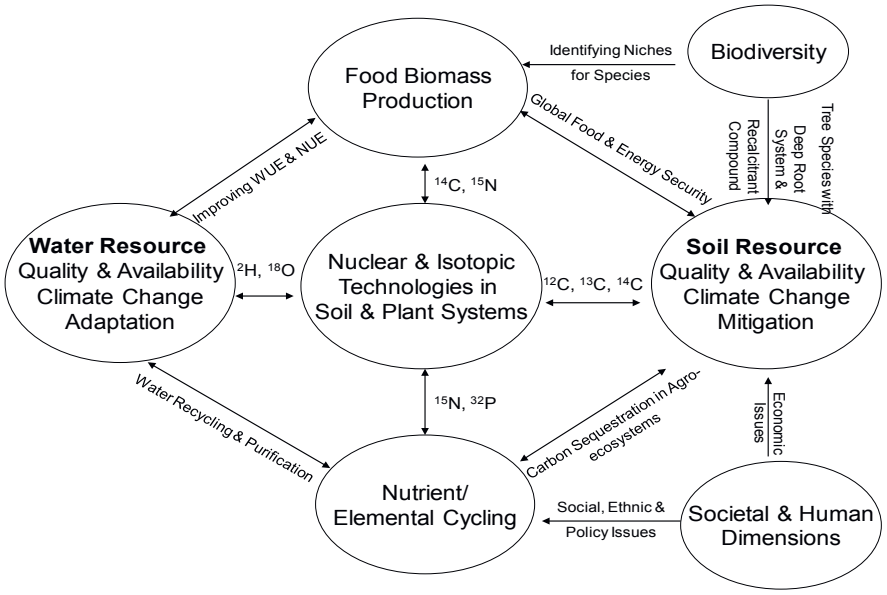


FIGURE 1. The role of nuclear and isotopic techniques in agro-ecosystems to address global issues of carbon sequestration to advance food security and mitigate climate change.

ecosystems (Nguyen *et al*., 2011). Moreover, this research is needed to provide technologies backed by scientific evidence so that land care practitioners/climate change policy makers can be informed of the merits of adopting such technological practices. Figure 1 shows the central role of NITs in addressing the important inter-related issues of food and biomass (feed, fodder, fibre and fuel) production, soil/land use and soil C sequestration and resilience of agro-ecosystems, nutrient/elemental cycling, and the declining availability and quality of water resources, as well as other relevant interactive factors (FAO/IAEA, 2007; Nguyen *et al*., 2011).

Ensuring food security and reducing the impacts from CC and variability necessitates a framework of actions for a multifunctional agriculture, addressing the urgent global issues of C sequestration (CC mitigation), greenhouse gas (GHG) emission minimization (CC mitigation), the agricultural system's resilience (CC adaptation) and food production. This approach represents a key requirement for the sustainable management of land and water resources (Lal, 2004). The primary focus of this multifunctional agriculture is to develop and adopt more holistic approaches, by integrating natural (soil and water) and genetic (plant and animal) resources to assess the impacts of food and biomass production (above-ground plant and below-ground root growth) on soil C sequestration and C budgets, while mitigating GHG emissions and enhancing resilience (adaptation) in agro-ecosystems.

Carbon Sequestration and Nutrient/Water Cycling Process-level Studies in Agro-ecosystems

Because of the need to stabilize the C stored in the soil, key mechanistic studies are required to obtain a better understanding of the processes and driving factors that control the dynamics (transformations/turnover) of specific compounds in soil organic carbon (SOC), as well as the functions of soil biota (biological component of soil quality). These studies demand the refined quantification of C, nutrient and water pools and fluxes in a given agro-ecosystem. Measurement of variations in the natural abundance of stable isotopes (^{2}H, ^{13}C, ^{15}N, ^{18}O, and ^{34}S) in components of the agro-ecosystem (soil organic matter, standing biomass, ground and surface water, atmospheric gases) can provide unique information on such pools and fluxes. This information is essential for the management of SOC sequestration in agricultural lands. For detailed information on the application of NITs in C sequestration studies, the reader is referred to selected publications (see Nguyen *et al*., 2011).

Recent developments also include novel isotopic techniques such as CSSI and advances in instrumental analytical chemistry and computational data acquisition/processing for isotope ratio measurements in soil, plant, water and gas samples (Crosson, 2008). Currently such techniques have been used to investigate the transformations and turnover of specific C compounds in soils and ecosystems (Glaser, 2005). Stable isotope probing (SIP) is a powerful technique in microbial ecological research. It allows the identification of *in situ* active microbial populations in the ecosystem that have been treated with ^{13}C or ^{15}N - labelled substrates, based on the incorporation of ^{13}C or ^{15}N into cellular biomarkers such as nucleic acids (DNA or RNA). The combined use of stable isotope techniques with biomarkers thus increases the potential to unravel the role of biodiversity in soil C cycling and improve understanding of the relationship between soil and vegetation in C modelling (Staddon, 2004; Amelung *et al*., 2008). However, this advanced technique needs to be further refined and the protocols harmonized for worldwide application in agricultural research. The advent of synchrotron facilities that accelerate electrons to almost the speed of light and the continuing development of synchrotron-based techniques (such as X-ray absorption, fluorescence and tomography) to improve spatial resolution and sensitivity offers exciting opportunities to unravel key processes and factors influencing soil-water-nutrient-plant-rhizosphere interactions (Lombi and Susini, 2009).

Applications for Sustainable Land Management in Agro-ecosystems

The design and implementation of successful soil and water management practices aimed at advancing food security and mitigating climate change requires integrated strategies (win/win options) to enhance the net rate of C sequestration in soils by increasing biomass production, enhancing soil quality and reducing GHG emissions from farm lands (Lal and Spruce, 1999). Conservation Agriculture has been proposed by FAO as an essential component of the action programme of the FAO-Global Soil Partnership to promote sustainable management of soil resources and improved governance for soil protection and sustainable productivity (FAO, 2014a and 2014b). Some of the benefits claimed for employing CA include increased SOM accumulation, enhanced soil fertility, improved soil water storage, reduced soil erosion and N leaching, better soil aggregation, enhanced resilience of farming systems to climate change and reduced GHG emissions (Lal, 2007; Govaerts et al., 2009). All of these interact to improve vital soil functions and ecosystem services, ensure agricultural productivity and promote systems sustainability, which consitute the basic principles of climate smart agriculture.

While initial CA investigations have focused on improving soil fertility and increasing agronomic crop yields, much research is still needed to assess CA benefits in terms of resource (water and fertilizer inputs and energy) use/conservation efficiency as well as its environmental impacts (SOC sequestration, GHG emissions and ecosystems services) to develop and promote novel climate-smart agricultural ecosystems in both rural and urban/peri-urban farming environments.

At present, area-wide (watershed scale) agricultural water management in both rainfed and irrigated agriculture and water quality are the most challenging issues for agricultural production systems worldwide. Integrating land and water resources management in agro-ecosystems is an essential requirement to tackle these issues (FAO/IAEA, 2007). The development and adoption of integrated water resources management in climate smart agro-ecosystems includes the design and implementation of a series of watershed studies on: (i) soil-water-plant interactions across the landscape; (ii) area-wide assessment of water use efficiency; and (iii) enhancement of water ecosystem services such as water capture-storage and reuse, wetlands construction/management, drought/flooding risk assessment and rehabilitation and non-point source (diffuse) pollution control. This approach involves a suite of NITs and conventional techniques and requires the formation of multi-disciplinary and often inter-institutional teams, thus demanding more effort in terms of networking, co-ordination and information technology.

Techniques that offer great potential include variations in the natural abundance of water stable isotopes, CSSIs, cavity ring-down laser absorption spectroscopy (CRD-LAS) and the cosmic ray soil moisture observing system (COSMOS). Area-wide agricultural water management also calls for the use of databases to integrate large and complex sets of data obtained under a range of agro-ecological conditions. Such data can also be used to validate and refine existing models, as well as to develop decision support systems to make better decisions about the technologies suitable for specific farming and catchment systems. Because of the nature of sustainability issues, long-term experiments are needed to develop and pilot test appropriate technologies over time. In view of the large spatial variability over short distances across the landscape, innovative area-wide (catchment/watershed) upscaling approaches and related geo-information systems and techniques (GIS and geo-statistical tools) are required to assess land and water connectivity issues and also the interactions between neighbouring natural ecosystems and agro-ecosystems (Nguyen *et al.*, 2011).

In all cases it is envisaged that a core element of climate smart agro-ecosystems will be the development of soil-water-plant approaches for the identification/selection of best adapted crop genotypes to climate change impacts. In this context, there is great scope for interdisciplinary research between plant breeders and soil/water management specialists in the area of crop resilience to climatic change and resulting abiotic stresses (drought, flooding, salinity, variable nutrient/water availability, etc.). Research is needed to predict the evolutionary response of different species to climate change, i.e. extinction versus adaptation, and to develop best-fit genotypes for particular environments. Soil type matching plus appropriate "external" nutrient and water inputs could be the key to climate change adaptation. Isotopic techniques can be used in crop improvement programmes to examine the physiological response/adaptation of plant genotypes to climate variations and extreme events.

CONCLUSIONS

This overview describes relevant strategies/approaches and NITs used in the main project areas of the SWMCN Subprogramme to address soil and water management issues in crop production systems for food security and climate change adaptation/mitigation. These are outlined in terms of managing soils for enhancing crop production, preserving and protecting soils and managing soils and agricultural water under climate change. A conceptual framework for adopting an ecosystem approach, the potential strategies and the role of NITs as cutting edge tools for sustainable land and water management in agro-ecosystems are outlined. In view of the complexity of the systems and scale (area-wide) of the studies, a suite of approaches/strategies and NITs and related conventional techniques, databases and modeling will be needed. In addition, the adoption of more holistic approaches calls for more inter-disciplinary research, networking and co-ordination.

Like all other techniques, NITs have advantages and limitations. They offer the comparative advantages of high specificity, accuracy and sensitivity over conventional non-nuclear techniques. Because of these advantages, they generate quantitative data, providing direct answers to the questions posed and thus saving time and effort. However, certain preconditions have to be met to take advantage of the NITs chosen — in particular the availability of skilled and trained human resources and access to adequate instrumental analytical facilities. These techniques cannot be used in isolation, but rather must be integrated with others in national and/or regional research activities to maximize their potential in terms of achieving successful outcomes and people-centred benefits.

Human and institutional capacity-building, networking, co-ordination, information exchange and communication technologies are important tools in the development and application of NITs for sustainable land and water management in agro-ecosystems. Partnerships and innovative collaboration modalities such as involvement in the FAO Global Soil Partnership play important roles in fostering technology dissemination and enhancing developmental efforts. In this context, greater advocacy is needed for the key role of sustainable land management in achieving the MDGs of food security/poverty alleviation and environmental sustainability and their overall contribution to sustainable development.

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SESSION 1

MANAGING SOILS FOR CROP PRODUCTION AND ECOSYSTEM SERVICES

Contributions of Fertilizer Nitrogen in Global Cereal Production, Soil Organic Matter Status and Nitrogen Balance

J.K. Ladha[1,*]

ABSTRACT

Presently, 50 percent of the human population relies on synthetic nitrogen (N) fertilizer for food production. In the subsistence agriculture of the pre-chemical era, biological N_2 fixation (BNF) was the primary source of reactive N but, in recent years, chemical N fixation (synthetic N) has become more important in global agriculture. Today, the Haber-Bosch and cultivation-induced BNF processes of converting N_2 from the atmosphere to ammonia introduce reactive N of over 100 Tg N/year into the global environment to increase food production (Galloway *et al.*, 2004). Although this has sustained the large human population of Earth in meeting dietary needs, a large population in the world still lacks available N to sustain crop production. This together with increasing population obviously means that the future global demand for reactive N is bound to grow substantially (Cassman *et al.,* 2003; Wood, Henao and Rosegrant, 2004). However, since a substantial amount of N created for food production is lost to the environment, this has also greatly increased the contribution of reactive N to a wide variety of environmental problems (Galloway *et al.,* 2004; Vitousek *et al.,* 2010). Unlike nonreactive gaseous N_2, reactive N has magnified the adverse effects because the same atom of N can cause multiple effects in the atmosphere, in terrestrial ecosystems, in freshwater and marine systems, and on human health. This paper (i) analyses the global consumption and demand for fertilizer N in relation to cereal production, (ii) evaluates the nitrogen-15 (^{15}N) and N difference methods to determine synthetic N recovery efficiency in current and succeeding crops grown across agro-climatic regions, (iii) examines long-term use of N on the sustenance of soil organic matter (SOM), (iv) constructs global N balances, and (v) analyses various strategies available to improve the overall use efficiency of N.

Key words: *cereals, N fertilizer, recovery efficiency, biological N_2 fixation, use efficiency, soil organic matter.*

GLOBAL N CONSUMPTION AND DEMAND FOR MAJOR CEREALS

During and after the Green Revolution, synthetic nitrogen (N) fertilizer has played a crucial role in increasing crop productivity to alleviate the ever-increasing food insecurity caused by a worldwide increase in population. Since the 1960s, the application of synthetic N fertilizer to fertilizer-responsive and lodging-resistant short-stature cultivars of cereals boosted food production by about 260 percent or an average growth of about 6.4 percent per year. Today, fertilizer N supplies approximately 45 percent of the total N input for global food production and world use is around 100 million metric tons (Mt) (FAO, 2010). Currently, about 52 percent of global N fertilizer consumed worldwide is used for the world's three most important cereal (rice, wheat and maize) production (Ladha, 2010). The N application rate (kg/ha) showed a curvilinear time trend with averages of 80.4, 65.7 and 52.4 in maize, rice and wheat, respectively (Figure 1). In 2009, maize and rice approached similar N application rates (109.4 vs 98.3 kg/ha) followed by wheat (83.5 kg/ha). It is projected that to meet the global cereal demand of three billion tons (t) by 2050 and with projected increase of 7 percent in harvested area, fertilizer application rates to the three cereals will have to increase on average by about 54 percent assuming no change in N use efficiency. In terms of total global synthetic N use, it would increase from 51.8 Tg in 2009 to 85.6 Tg in 2050 (Ladha *et al.,* unpublished).

FERTILIZER N RECOVERY EFFICIENCY BY MAJOR CEREALS

Nitrogen fertilizers are expensive inputs, costing agriculture more than US$50 billion per year. Most agricultural crops use fertilizer N inefficiently. Trials with major cereals (maize, rice and wheat) show recovery efficiencies of fertilizer N varying from 44 to 55 percent (Table 1). Our review of published data showed that the average

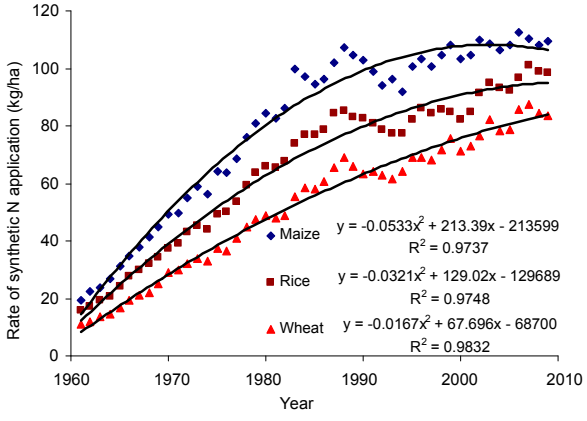


FIGURE 1. Trends in global averages of fertilizer N application rates to maize, rice and wheat.

1 International Rice Research Institute, DPS Marg, Pusa, New Delhi - 110 012, India

* E-mail address of corresponding author: j.k.ladha@irri.org

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Table 1. Range of N use efficiency for cereals in various regions

Region / Crop	RE* Mean	RE^{15}N** Mean
Africa	0.63	0.37
Australia	0.46	0.41
Europe	0.68	0.61
America	0.52	0.36
Asia	0.50	0.44
Average / total	0.55	0.44
Maize	0.65	0.40
Rice	0.46	0.44
Wheat	0.57	0.45
Average / total	0.55	0.44

* RE = recovery efficiency of fertilizer N based on total plant N (kg N taken kg^{-1} N applied)

** RE ^{15}N = recovery of ^{15}N-labelled fertilizer N based on total plant N (kg N taken kg^{-1} N applied)

recovery efficiencies across all regions and crops was seven percent lower when estimated by the nitrogen-15 (^{15}N) dilution method than by the N-difference method.

The amount of fertilizer N that remains available for subsequent crops can only be quantified with the use of labelled ^{15}N fertilizer. In a limited number of studies, the uptake of residual fertilizer was monitored for several growing seasons. The IAEA (2003) reported that the average accumulated recovery of ^{15}N fertilizer by subsequent crops during five growing seasons amounted to 6.5 percent, which is equal to 16 percent of the total fertilizer N recovered during the first growing season. With an average ^{15}N fertilizer recovery (RE^{15}N) of 44 percent in the first growing season (Ladha *et al*., 2005), the total recovery of ^{15}N fertilizer, including the recovery by the five subsequent crops, is approximately 50 percent.

ROLE OF N FERTILIZER IN SUSTAINING SOIL ORGANIC MATTER IN CEREAL CULTIVATED SOILS

Soil organic matter (SOM) is essential for sustaining the food production and maintaining ecosystem services and is a vital resource base for storing N. The impact of long-term use of synthetic-N fertilizer on SOM, however, has been questioned recently. We tested the hypothesis that long-term use of synthetic fertilizer-N results in a decrease in SOM. We analysed peer-reviewed data from 100 long-term field experiments with controlled N fertilizer treatments representing a wide range of climatic zones, soil types, crops, and management practices. Results demonstrate dramatic world-wide declines of 7–16 percent of SOC and 7–11 percent of SOM with no N amendments. In soils receiving synthetic fertilizer N, the rate of SOM loss decreased (Ladha *et al*., 2011). There was an average increase of respectively 8 percent and 12 percent for SOC and SOM following the application of synthetic fertilizer N treatments compared with no N fertilizer applied (Figure 2). On the other hand, long-term application of an organic source either alone or in combination with synthetic fertilizer N consistently increased both soil carbon (C) and N ranging from 9–34 percent over control (Ladha *et al*., 2011). Among the land types, flooded dryland (i.e. rice–wheat rotation) responded better to organic amendment than dryland–dryland (i.e. maize–wheat) or flooded–flooded (i.e. rice–rice). Although synthetic N fertilizers

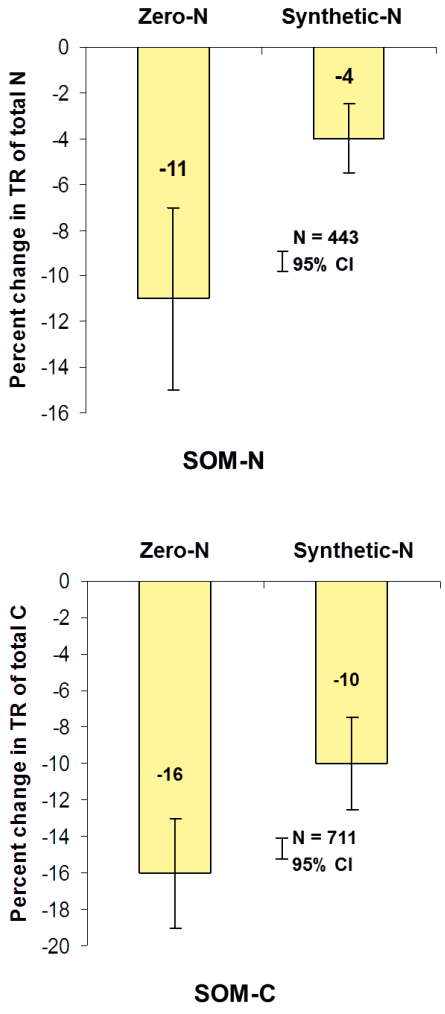


FIGURE 2. Meta analysis of change in time response ratio of SOM-N and SOM-C: A global analysis of 114 long-term experiments.

showed a lower potential to improve the long-term N-supplying capacity than organic matter, both amendments are vital to increase the intrinsic capacity of agricultural soils to sustain crop productivity. The flooded soils showed higher SOM content than the soils going through flooding and dry conditions. Increases in SOC and SOM content in flooded soils is attributed to increased C input (Tanji *et al*., 2003; Kogel-Knabner *et al*., 2010) in combination with flooded conditions, leading to a reduction in the rate of decomposition of plant material caused by a lack of oxygen (Neue *et al*., 1997). Increased non-symbiotic BNF in these flooded rice systems also leads to higher SOC and SOM (Ladha *et al*., 2000; Pampolino *et al*., 2008). When rice is grown in rotation with wheat (lowland–upland system), soil is exposed to an increase in aeration, leading to a higher loss of SOM, which can ultimately lead to a reduction in the cropping systems' sustainability (George *et al*., 1992; Ladha *et al*., 2011). As these cropping systems developed inherently low SOM content, they also became more responsive to organic amendments.

GLOBAL N BALANCE IN MAIZE, RICE AND WHEAT CROPPING SYSTEMS

Since there is a continual loss of reactive N in an agro ecosystem, an important question arises as to whether the system is reaching

N disequilibrium. An agro ecosystem would be in N equilibrium if the sum of N inputs equaled the sum of N outputs. Among various inputs and outputs, inputs from BNF and synthetic N sources and outputs through crop harvest and losses are the most important. Therefore, constructing N balance sheets is the key to both increasing our understanding of N transformation and N transfers, and to quantifying the size of various N reservoirs that ultimately are needed to conserve N in various transformations and biological processes of the system (Legg and Meisinger, 1982). Many efforts have been made to construct N balances but they were limited to small scale greenhouse, lysimeter, and field studies. Our ability to integrate various loss and gain processes and to construct N balances at a higher level such as a food production system on a global scale continue to be an obvious gap in our knowledge. Greenland and Watanabe (1982) identified three difficulties associated with the origin of the enigma: the difficulty in measuring the change in total N content of a given mass of soil, the difficulty in measuring the amount of N added to the soil–plant system by BNF and the difficulty in measuring losses of N from a soil–plant system. However, since then, major progress has been made in all three areas in terms of developing methodologies and generating relatively accurate numbers of various components of N gains and N losses required to construct N balances in different agricultural systems. Using a wealth of knowledge accumulated over the years, global N balance sheets covering 49 years (1961–2009) were constructed for rice, wheat, and maize production systems (Ladha *et al*., unpublished). Results show that a significant amount of crop N demand by the world's major cereals is met from sources other than synthetic fertilizer N and inherent soil N reserves. It appears that the majority of this third source of N input is from non-symbiotic N fixation and recycling of N. The results highlight the need to consider all sources of N (synthetic, soil organic reserve and non-symbiotic N fixation) when designing strategies to improve N use efficiency.

STRATEGIES TO IMPROVE THE N FERTILIZER USE EFFICIENCY

Reducing N losses through achieving synchrony between crop demand and supply from soil and other sources is most crucial to achieving high levels of N use efficiency. There is an inverse relationship between loss and synchrony of N (Figure 3). Compared with plant genetic improvement, the resource management approach is likely to have more losses of N and less synchrony between supply and demand. If N losses are to be eliminated completely from a soil–plant system, a regulated N supply through a plant fixing its own N is an ideal approach. If BNF could be assembled in cereals, it would amplify the potential for N supply because fixed N would be available to the plant directly, with little or no loss. However, management of fertilizer N which has played and will continue to play important role in meeting the N demand of food crops would be of primary importance.

Removing plant growth-limiting factors would increase crop demand for N, leading to a greater use of available N and consequently higher efficiency (Ladha *et al.,* 2005). This is possible by adopting fertilizer, soil, water, and crop management practices that will maximize crop N uptake, minimize N losses and optimize indigenous soil N supply including non-symbiotic N fixation. Management decisions that increase fertilizer N use by crops can focus on two approaches: increase fertilizer N use during the growing season when the fertilizer is applied, and decrease fertilizer N losses, thereby increasing the potential recovery of residual fertilizer N by the subsequent crops. Approaches suggested for increasing NUE include optimal time, rate, and methods of application for matching N supply with crop demand; the use of specially formulated forms of fertilizer, including those with urease and nitrification inhibitors;

TABLE 2. Comparative evaluation of tools/tactics of enhancing fertilizer N use efficiency

Tools/tactics	Benefit:cost	Limitations
Site-specific N management	High	Has to be developed for every site, infrastructure required
Chlorophyll meter	High	Initial high cost
Leaf colour chart	Very high	None
Plant analysis	High	Facilities need to be developed
Controlled-release fertilizer	Low	Low profitability and lack of interest by industry
Nitrification inhibitors	Low	Low profitability and lack of interest by industry
Fertilizer placement	High	Lack of equipment, labor-intensive
Foliar N application	High	Lack of equipment, risk involved
Breeding strategy	Very high	Varieties yet to be developed
N-fixation in non legumes	High	Technology yet to be developed for field scale
Models and decision support systems	Medium	Tools are not available
Remote-sensing tools	Low	Technology needs to be fine-tuned
Geographic information systems	Low	Technology needs to be fine-tuned
Precision farming technology	High	Technology needs to be fine-tuned
Resource-conserving technologies	High	Technology needs to be evaluated for long-term impacts
Integrated crop management	High	Technology needs to be evaluated for long-term impacts

Source: modified from Ladha *et al.* (2005)

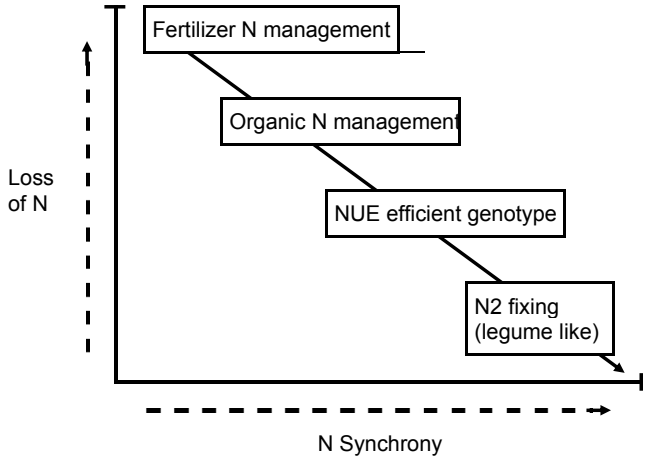


FIGURE 3. N loss – N synchrony relationship for various strategies.

the integrated use of fertilizer, manures, and/or crop residues; and optimizing irrigation management. In addition, some modern tools such as precision farming technologies, simulation modeling, decision support systems, and resource-conserving technologies also help to improve NUE. A full account of these strategies can be found elsewhere (Ladha *et al.,* 2005). In large-scale agriculture practised in industrialized countries, precision farming studies have demonstrated that variable-rate N fertilizer application has the potential to reduce significantly the amounts of N required to achieve yields similar to those obtained with standard uniform management practices. In agriculture with small to medium farm sizes in developing countries, the use of a simple and inexpensive leaf colour chart assists farmers in applying N when the plant needs it. The use of this simple tool has been shown to reduce misuse of fertilizer N. An analysis of various strategies to improve NUE together with their cost/benefit assessment and limitations is provided in Table 2.

CONCLUSIONS

The primary function of synthetic fertilizer N is to provide the crop with an immediately available source of available N, often the most limiting nutrient for plant growth. The secondary function is to reduce the decline in SOM content, a function which has long-term consequences on the sustainability of the systems as SOM plays multiple roles in maintaining soil quality and ecosystem services. Recent N balance studies also show that although about half of the synthetic N leaks out resulting in adverse impacts on the environment, a positive augmentative role of synthetic N in the overall economy of cereal production is to be recognized. The results therefore highlight the need to consider all N sources (synthetic, soil organic reserve and non-symbiotic N fixation) when designing strategies to improve N use efficiency.

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Ammonia Volatilization Losses from Urea Fertilizer Applied on Wheat

M.M. Terrada[1,*], L. Benavides[1] and S.C. López[1]

ABSTRACT

Ammonia (NH_3) emissions represent a major loss of nitrogen (N) in agricultural production. A field experiment was performed in the Pampean region to evaluate the effect of different urea rates applied to wheat (*Triticum aestivum* L.) on NH_3 losses through volatilization using a direct method (^{15}N as tracer) and an indirect method (N-difference). Volatilization was quantified using static semi-open N-NH_3 collectors during eight d after fertilization with urea N at rates of 0, 50 and 100 kg/ha (W0, W50 and W100) in 2008, and W0 and W100 during 52 d in 2009. In both years, NH_3 volatilization correlated with soil accumulated temperature during the first eight d after fertilization and the percent of N derived from fertilizer (%Ndff) increased markedly between 24–48 h after fertilization, especially in W100, where %Ndff reached 100 percent. The total NH_3-N losses during the whole wheat growing season were 6.3, 7.4 and 9.6 kg/ha for W0, W50 and W100 in 2008, and 7.3 and 13.4 kg/ha for W0 and W100 in 2009, being significantly higher in W100. The N-NH_3 losses, in proportion to the fertilizer applied, were 2.2 and 3.3 percent for W50 and W100 using the indirect method, and 0.9 percent and 3.3 percent for W50 and W100 by the direct method, and 6.2 percent for W100 by both methods in 2009. There were no significant differences between methods. Volatilization under wheat increased with rate of urea application but the losses were less than 6.5 percent of the applied fertilizer.

Key words: *nitrogen-15, ammonia volatilization, urea, %Ndff, wheat, Pampean region.*

INTRODUCTION

Nitrogen (N) is a dynamic and mobile element which can undergo substantial losses in agricultural systems. Nitrogen is lost by leaching of nitrate (NO_3^-), surface runoff, volatilization of ammonia (NH_3), emissions of nitrous oxide (N_2O), nitric oxide (NO) and nitrogen dioxide (NO_2) and dinitrogen (N_2) gas (Delgado, 2002). Ammonia emissions also represent a major loss of nutrient N (Rochette *et al*., 2009) and also result in degradation of air and water quality (Galloway *et al*., 2003).

Wheat is one of the most important crops in Argentina, but efficient use of fertilizer and conservation tillage are required to increase or maintain productivity without damaging the soil and the environment (Melaj *et al*., 2003). The most popular fertilizer used for wheat production is urea, which is transformed to NH_3 in the soil and can be volatilized to the atmosphere. After surface application, urea is quickly hydrolyzed within 1–2 d by urease to ammonium (NH_4^+), hydroxyl (OH^-) and carbonate (CO_3^{-2}) ions, leading to a high pH and very high concentrations of NH_4^+ around the urea granule; consequently, it increases the gaseous NH_3 losses to the atmosphere (Zaman *et al.,* 2008). The extent of NH_3 losses depends mainly on soil factors and the season of year (Videla *et al.*, 1994).

The aims of this study were to evaluate the effect of different urea rates applied to wheat on the losses of NH_3 through volatilization using two different methods, namely, a direct method (using ^{15}N as tracer) and an indirect method (N-difference) and to evaluate the relation between NH_3 volatilization and soil temperature.

MATERIALS AND METHODS

A field experiment was performed during the 2008 and 2009 spring wheat (*Triticum aestivum* L.) growing seasons in an experimental field at the Centro Atómico Ezeiza, Comisión Nacional de Energía Atómica, Buenos Aires, Argentina (34°49´S, 58°34´W). The soil was classified as a typical Argiudoll with 13.7 percent clay, 47.5 percent silt and 38.8 percent sand. Within the 0–10 cm layer the soil bulk density was 1.07 g/cm^3, pH was 6.8, organic matter was 4.5 percent and extractable P was 46 $\mu g \cdot P \cdot g^{-1}$. The experimental site was located in the Pampean region.

The experimental design was fully randomized blocks with four replications. Different N fertilization rates were applied to wheat grown under conventional tillage on each block and receiving: 0, 50 and 100 kg N-urea per ha (W0, W50 and W100) during 2008; and 0 and 100 kg N-urea (W0 and W100) during 2009. The wheat (Baguette 13, Nidera) was sown in August 2008 and in July 2009 with a row spacing of 15 cm and a sowing density of 6×10^6 seeds/ha, and was harvested in December 2008 and 2009. The N fertilizer was applied at tillering in September of both years.

Ammonia volatilization was measured over eight d in 2008 and over 52 d in 2009. To quantify the N volatilized derived from fertilizer (Ndff) and from soil (Ndfs), a micro-plot of 5 × 5 m was established on each fertilized plot and received nitrogen-15 (^{15}N) enriched urea (5% ^{15}N atom excess) as a tracer.

Volatilization of NH_3 was quantified using static semi-open NH_3-N collectors, adapted from Nömmik (1973), Videla *et al.* (1994) and Lara Cabezas *et al*. (1999). The collectors consisted of PVC cylindrical structures 0.5 m high and 0.15 m in diameter. They were inserted into the soil at a depth of 0.10 m and had two absorber foams soaked with 35 ml of 0.25M sulphuric acid (H_2SO_4) solution with 3% (v/v) glycerine. The upper foam absorbed atmospheric NH_3 coming from outside the collector and protected the lower foam, which retained the NH_3 volatilized from the soil surface inside the collector.

In each plot, an NH_3 collector was inserted into the soil immediately after fertilization and periodically the lower foam from each

1 División Agronómica, Centro Atómico Ezeiza, Comisión Nacional de Energía Atómica, Presbítero González y Aragón 15, CP B1802AYA, Ezeiza, Provincia de Buenos Aires, Argentina

* E-mail address of corresponding author: maltert@cae.cnea.gov.ar

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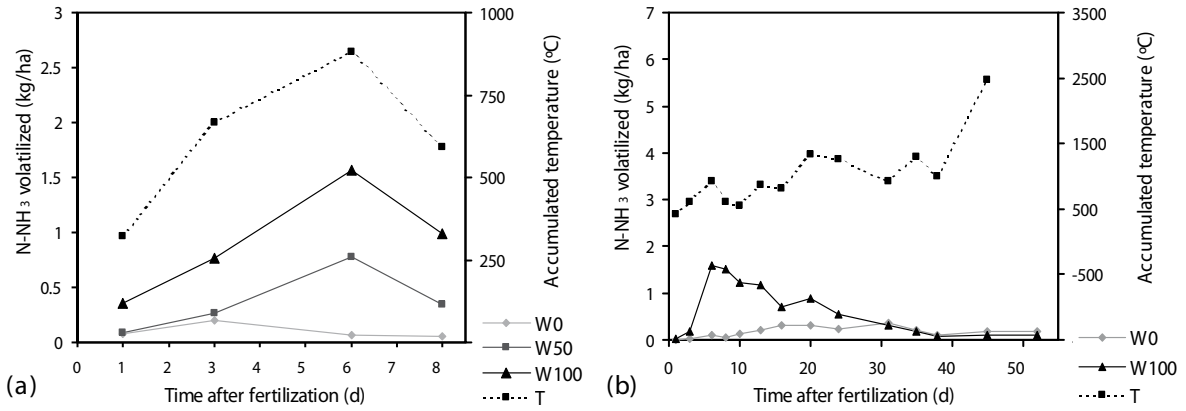


FIGURE 1. Ammonia volatilized and soil accumulated temperature (T) between sampling after fertilization in W0, W50 and W100 in 2008 (a) and in W0 and W100 in 2009 (b).

collector was removed to measure NH_3 retained; it was replaced immediately by new foam. In fertilized plots, the NH_3 collector was inserted inside the ^{15}N labelled micro-plots. The same methodology was used to recover NH_3 in unfertilized plots or $^{15}NH_3$ in fertilized plots. The NH_3 or $^{15}NH_3$ retained was removed by two washings with 200 ml of deionized water using a shaker and shake for 30 min. The extract was acidified with 1 ml of 0.1 M H_2SO_4 and concentrated at 50ºC to a volume of 50 ml. The NH_3 in the extract was determined by Kjeldahl distillation and titration. The ^{15}N-NH_3 was analysed for N isotope ratios with an optical emission spectrometer NOI 6PC (Fisher Analysen Instrumente GmbH, Leipzig, Germany).

The percent N derived from fertilizer (% Ndff) was calculated as follows (IAEA, 2001):

$$\%\,\text{Ndff} = \frac{\text{atom } \%^{15}\text{N excess (foam)}}{\text{atom } \%^{15}\text{N excess (urea)}} \times 100$$

The soil temperature of each plot was measured with a soil temperature sensor and data logger (Logger16, Cavadevices, Argentina), to evaluate the relation between NH_3 volatilization and the soil accumulated temperature between sampling dates.

RESULTS AND DISCUSSION

In both growing seasons, NH_3 volatilization in wheat fertilized plots showed an increasing trend during the first six d and then began to diminish (Figures 1a and 1b).

During 2008, NH_3 volatilization correlated (r^2 = 0.92) with changes in soil accumulated temperature between sampling dates, as has been reported by other authors (Barbieri *et al.,* 2010). Total N-NH_3 volatilized during the first eight d after fertilization were 0.4, 1.5 and 3.7 kg·N-NH_3·ha^{-1} for W0, W50 and W100, respectively, with significant differences between W0 and W100 ($p < 0.01$) and between W50 and W100 ($p < 0.05$). Cumulative NH_3 losses were calculated using these values. In order to estimate volatilization during the whole wheat growing season the baseline volatilization measured in W0 was considered to be occurring in all treatments before and after the measurement period.

The total N-NH_3 losses were 6.3, 7.4 and 9.6 kg/ha for W0, W50 and W100, respectively, being significantly higher in W100 plots ($p < 0.01$).

The %Ndff increased markedly between 24 and 48 h after fertilization, especially in W100, with all volatilized N derived from applied fertilizer reaching values of 100 percent 72 h after fertilization (Figure 2). Sainz Rozas *et al.* (1997) described a similar situation in a soil with an initial pH of 5.8, because the highest rate of volatilization would occur when the increase in soil pH due to urea hydrolysis reaches its maximum value, two or three d after fertilization. On average, the weighted percentages of N losses by volatilization derived directly from fertilizer during the eight d were 33 percent and 90 percent for W50 and W100, respectively, with significant differences due to fertilization rates ($p < 0.05$). These values were the equivalent of 0.4 and 3.3 kg·N-NH_3·ha^{-1} as Ndff during the eight d after fertilization.

In the 2009 season, the NH_3-N losses by volatilization during the first eight d were 3 kg/ha by either the indirect or direct method, without a significant difference between these two methodologies and between different wheat seasons. However, volatilization of NH_3 continued to be higher ($p < 0.05$) in fertilized plots than in non-fertilized plots until 16 d after fertilization (Figure 1b). As NH_3 volatilization in wheat fertilized plots decreased with time, and soil temperature began to increase in relation to external climatic conditions (beginning of spring), accumulated volatilization correlated with changes in soil accumulated temperature between sampling dates only during the first eight d after fertilization (r^2 = 0.73), as in the previous season.

The amounts of N-NH_3 volatilized during the 52 d after fertilization were 2.4 and 8.6 kg/ha for W0 and W100, being significantly higher in the fertilized plots ($p < 0.01$). The total N-NH_3 losses during the wheat season were 7.3 and 13.4 kg/ha for W0 and W100, respectively.

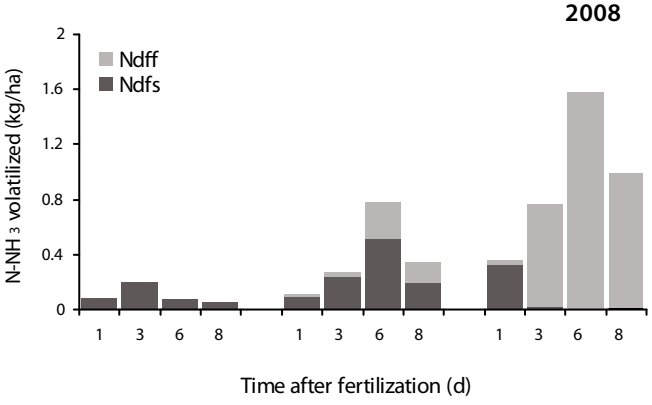


FIGURE 2. Ammonia volatilized derived from fertilizer (Ndff) and from soil (Ndfs) in the 2008 season.

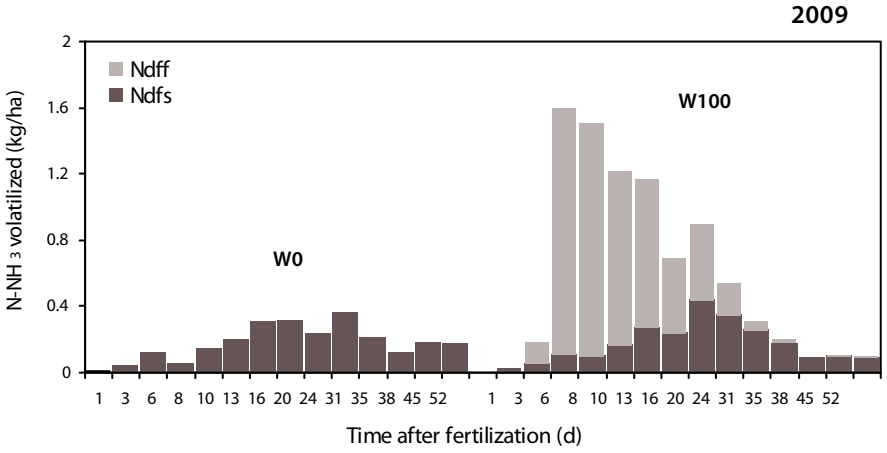


FIGURE 3. Ammonia volatilized derived from fertilizer (Ndff) and from soil (Ndfs) in the 2009 season.

TABLE 1. The N-NH_3 losses by volatilization during eight d (2008) and 52 d (2009) after fertilization in proportion to the fertilizer applied (%), by the ^{15}N method and the indirect method without ^{15}N

2008	^{15}N method	Indirect method
W50	0.9 ± 0.9 aA	2.2 ± 1.9 aA
W100	3.3 ± 1.5 aB	3.3 ± 1.5 aA
2009	**^{15}N method**	**Indirect method**
W100	6.2 ± 2.0 a	6.2 ± 2.0 a

The same lower case letter in a line indicates no significant differences ($p < 0.05$).
The same upper case letter in a column in 2008 indicates no significant differences ($p < 0.05$)

As in the previous season, the %Ndff increased markedly between 24 and 48 h after fertilization and the volatilized N derived from applied fertilizer reached values of almost 100 percent (Figure 3). On average, the weighted percentage of N losses from volatilization derived directly from fertilizer during the 52 d was 71 percent for W100. The Ndff during the 52 d after fertilization was 6.2 kg/ha, almost twice the amount registered during eight d in 2008.

A comparison between the percentage fertilizer lost by volatilization using the indirect method and the ^{15}N method was made (Table 1). The indirect method, also called the N-difference method, evaluated the apparent volatilization in fertilized plots by the difference between NH_3-N volatilized from fertilized and non-fertilized plots. Differences between methods were not significant in either year. During 2008, estimates of NH_3-N losses as a proportion of fertilizer applied using the indirect method were respectively 2.2 and 3.3 percent for W50 and W100. When ^{15}N was used as tracer, the NH_3-N losses as a proportion of fertilizer applied were 0.9 percent and 3.3 percent for W50 and W100, respectively (Table 1).

During 2009, estimates by both methods produced the same value, i.e. 6.2 percent of applied fertilizer was lost by volatilization over 52 d (Table 1) although the ^{15}N dilution method may lead to different results than the indirect or N-difference method when evaluating N use efficiency (Ladha *et al.*, 2005).

Although relatively expensive, ^{15}N techniques usually provide results that have lower variability and are of higher sensitivity, resulting in more precise information in a shorter period of time (IAEA, 2008). In this case, variability was the same with both methods although higher with the lower rate of fertilization. On the other hand, the use of ^{15}N would increase the cost of trials but measurements only in fertilized plots would be enough to assess cumulative NH_3-N volatilization, thereby reducing costs in conventional laboratory analysis.

A longer period of measurement ensured a better estimation of cumulative NH_3-N volatilization. Little NH_3-N volatilization is expected in cold weather (Ladha *et al.*, 2005) as occurs during the sowing of winter wheat. When fertilization is delayed and it is applied at tillering as in this case, it is expected that N uptake by the crop can be improved (Melaj *et al.*, 2003) and use efficiency increased. However, at the same time it is possible that NH_3-N volatilization increases because of rising external temperature. Nevertheless, a correlation between NH_3-N volatilization and temperature was obtained only during the first eight d after fertilization because of the trend of decreasing volatilization with growth period related to N-fertilizer incorporation into crops and N dynamics in soil including physico-chemical and biological processes.

In the south east Pampa region, Sainz Rozas *et al.* (1997) observed N losses through volatilization of 9 percent in a maize crop receiving a lower rate of urea fertilization (70 kg/ha). There is a lower risk of volatilization in winter wheat compared with summer-grown crops, but warm conditions in early spring would increase volatilization losses.

CONCLUSIONS

In the literature, NH_3 volatilization is generally recorded during the first eight d after fertilization, but this study showed that volatilization may continue to be important during a longer period of time. Baseline volatilization was reached 25–30 d after the addition of urea-N at a rate of 100 kg/ha with significant differences between fertilized and unfertilized plots being found until 16 d after fertilization.

Losses of N by volatilization in wheat increased with the rate of urea application but the losses were lower than 6.5 percent of the applied fertilizer. In general, ammonia volatilization in winter wheat is lower than in summer-grown crops. However, its importance in the N budget would not be negligible and must be considered.

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Influence of Fine Particle Suspension of Urea and Urease Inhibitor on Nitrogen and Water Use Efficiency in Grassland using Nuclear Techniques

M. Zaman[1,*], M.M. Barbour[2], M.H. Turnbull[3] and L.V. Kurepin[4]

ABSTRACT

Controlled environment experiments were conducted to assess the effects of urea applied as fine particle suspension (FPA) together with urease inhibitor, (N-(n-butyl) thiophosphoric triamide (nBTPT), trade-name Agrotain® to ryegrass swards on nitrogen use efficiency (NUE) and water use efficiency (WUE). Perennial ryegrass was sown 7–10 seeds per plastic pot. After eight weeks of sowing, the ryegrass sward pots (36 pots) were transferred to a growth cabinet which was maintained at 20°C, 70% relative humidity (RH), 700 $mmol \cdot m^{-2} \cdot s^{-1}$ photosynthetic available radiation (PAR) during the 16-h light period, and 15°C, 70% RH during the 8-h dark period. After two weeks in the growth cabinet, ryegrass from each pot was trimmed to 6 cm height to achieve uniformity followed by applying six treatments that include: (1) control (no N), (2) control + leaf spray irrigation (equivalent of 10 mm of rain to wash applied urea) after d-1, (3) urea applied as fine particle suspension (FPA) on d 0, (4) urea applied on d 0 + leaf irrigation one d after of urea application, (5) Agrotain treated urea in FPA form, and (6) Agrotain treated urea + leaf irrigation after d-1. Each treatment had three replicates. To determine herbage ^{15}N uptake, chosen swards of ryegrass were treated with ^{15}N labelled urea (10% atom excess) with or without Agrotain at a rate equivalent to 25 $kg \cdot N \cdot ha^{-1}$. Twenty-eight d after initial treatment application, ryegrass plants were harvested, separated into new (newly grown) and old (tissue grown after uniformity cut before application of treatments) plant tissue, weighed and analysed for ^{15}N content. For intrinsic water use efficiency (WUE_i) measurements, 18 additional pots of ryegrass receiving the above six treatments were chosen. Pre- and post-treatment measurements of leaf-level gas exchange and on-line photosynthetic carbon and oxygen isotope discrimination were carried out to calculate WUEi. Urea applied with Agrotain in FPA form significantly increased herbage dry matter yield and WUE_i compared to urea alone. The herbage ^{15}N data showed that Agrotain improved NUE. Ryegrass receiving the urea+Agrotain+irrigation treatment took up 49% of N from applied urea, compared to 38% by the urea+irrigation treatment. Irrigating the leaves one d after applying urea + Agrotain further increased ryegrass growth and also resulted in the highest WUEi compared to no leaf irrigation.

Key words: *Urea, Agrotain, Fine Particle Suspension, Pasture, N uptake, N and water use efficiency, stable isotopes.*

INTRODUCTION

Increasing demand for dairy and meat products in fast-growing economies like China and India is encouraging countries, such as New Zealand, to intensify dairy production. In New Zealand, the major farming system is animal grazing, with mixed ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) as the pasture for animals. Nitrogen (N) is an important plant nutrient for increasing pasture productivity. According to the International Fertilizer Industry Association (IFA), world N fertilizer consumption is estimated to grow at an average annual rate of 2.5% and is projected to reach 112.4 Mt·N in 2015 (IFA, 2011). However, the increase in N use in pastoral systems will have a high environmental cost.

Urea [$(Co(NH_2)_2$] is the predominant form of N fertilizer worldwide (>50%) (Watson 2000; IFA 2011) and also in New Zealand (ca. 80%), mainly because of its lower cost per unit N and higher N content (46% N), in comparison to other N based fertilizers. Furthermore, urea is easy to transport, store, blend and spread. However, urea has been reported to have lower N use efficiency (NUE) than ammonium- and nitrate-based fertilizers (Black *et al.,* 1985; Zaman *et al.,* 2008). Here, NUE is defined as kg of additional dry matter produced per kg of applied N. This reduced NUE is partly due to N losses (10% to 30% of the applied N) from urea as ammonia (NH_3) (Zaman *et al.,* 2013a). Some of these N losses like gaseous emission of NH_3, nitric oxide (NO) and nitrous oxide (N_2O) will of course be unavoidable. That's because gaseous emissions of NH_3, N_2O and NO are part of the natural N cycle and can even occur after application of any ammonium-based fertilizer. Hence, controlling N losses, especially gaseous N losses, is critical for improving the NUE of all N-based fertilizers to minimize negative effects on the environment and decrease costs.

Granular urea efficiency can be considerably improved by coating it with a urease inhibitor (UI), such as Agrotain® (N-(n-butyl) thiophosphoric triamide (nBTPT, Watson *et al.,* 2008; Zaman *et al.,* 2008, 2013b). After application to soil, nBTPT is quickly converted to its oxygen analog N-(n-butyl) phosphoric triamide (nBTPTO) (Byrnes and Freney, 1995), which temporarily blocks the action of the urease enzyme (Christianson *et al.,* 1990), thereby slowing the process of urea hydrolysis (Zaman *et al.,* 2008). This delayed urea hydrolysis by nBTPTO reduces the rate of increase of soil pH (around the fertilizer

1 Ballance Agri-Nutrients Limited. Private Bag 12503, Tauranga Mail Centre, Tauranga 3143, New Zealand
2 Faculty of Agriculture and Environment, the University of Sydney, Australia.
3 School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand.
4 Department of Biology, Western University, London, Ontario, Canada, N6A 5B7.
* E-mail address of corresponding author: zamanm_99@yahoo.com

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granule) as well as decrease the amount of ammonium (NH_4^+) in the soil, all of which reduces the potential for NH_3 volatilization and other N losses. Also, the NUE of applied urea is reported to be improved further if urea is applied in fine particle application form (Dawar *et al.,* 2012). Fine particle application of urea results in a more even distribution of the urea on a per plant basis, thus minimising localized hot spots for N losses. However, there is limited information on fine particle suspension applications of urea applied together with Agrotain in grazed pastures.

Plant WUE, calculated as the amount of plant biomass produced per unit of water used depends on irrigation techniques, soil fertility, crop variety, and soil and water conservation practices. Leaf intrinsic water use efficiency (WUEi) defined as the ratio of instantaneous photosynthetic and transpiration rates, has been shown to be positively related to the stable ^{13}C isotope composition of plant (Brugnoli and Farquhar 2000). We anticipated that plants with increased N supply (through the combined effects of urea and Agrotain) will increase leaf photosynthetic rate leading to increased WUE, increased $\delta^{13}C_p$, combined with more modest changes in stomatal conductance and $\delta^{18}O_p$. We further anticipated that spray irrigation after urea applied with Agrotain in FPA form will increase both WUE and NUE. With both fertilizer and irrigation representing a substantial cost to the farmer, a major aim of our study was to assess the effect of urea with UI "Agrotain" on ryegrass water- and N-use efficiency, using stable isotopic techniques ^{15}N and ^{13}C.

MATERIALS AND METHODS

We carried out two controlled environment experiments in a growth cabinet. The soil used for the study was collected (0–7 cm depth) from a dairy pasture site near Lincoln, Canterbury, New Zealand. It was Paparua silt loam, Typic Haplustepts (Soil Survey Staff, 1998), had a silt loam texture with a pH of 5.65, total N of 0.38%, organic matter of 7%, Olsen P of 20 mg/L, CEC of 14 $cmol_c$ /kg. The soil was sieved (4 mm) to remove plant litter and roots. Sieved soil was adjusted to 80% of field capacity and 1.5 kg of moist soil was transferred to plastic pots (140 mm in diameter) to a depth of 150 mm. Each pot also received a basal dose of phosphorus and sulphur at an equivalent rate of 40 kg/ha. Perennial ryegrass cv. "Nui" was sown in each pot at 7-10 seeds per pot. Eight weeks after sowing, the ryegrass sward in each pot was cut to 6 cm height to achieve uniformity and then transferred to a growth cabinet. The growth cabinet was maintained at 20°C, 70% relative humidity (RH), 700 $mmol \cdot m^{-2} \cdot s^{-1}$ photosynthetic available radiation (PAR) during the 16-h light period, and 15°C, 70% RH during the 8-h dark period. After two weeks in the growth cabinet, ryegrass from each pot was trimmed again to 6 cm height followed by applying the six treatments (1) control (no N), (2) control + leaf spray irrigation (equivalent of 10 mm of rain to wash applied urea) after one d, (3) urea applied as fine particle suspension (FPA) on d 0, (4) urea applied on d 0 + leaf irrigation one d after of urea application, (5) Agrotain treated urea in FPA form, and (6) Agrotain treated urea + leaf irrigation after one d. Each treatment had three replicates. To determine herbage ^{15}N uptake, chosen swards of ryegrass were treated with ^{15}N labelled urea (10% atom excess) with or without Agrotain at a rate equivalent to 25 $kg \cdot N \cdot ha^{-1}$. Twenty-eight d after treatment application, ryegrass plants were harvested separated into new and old (previously cut before application of treatments) plant tissue, weighed fresh, washed with deionized water, and then dried at 60°C for 7 d. Dried herbage weight was recorded to calculate herbage dry matter yield. The dried herbage samples were then ground.

Total N and ^{15}N in herbage were measured using a Dumas elemental analyser (Europa Scientific ANCA-SL) interfaced to an isotope mass spectrometer (Europa Scientific 20-20 Stable Isotope Analyser; Europa Scientific Ltd, Crewe, U.K.). Total N uptake in each treatment was determined using the product of herbage N content and herbage dry matter. Calculations of ^{15}N recovery in plant were carried out as described by the International Atomic Energy Agency (IAEA 1976).

$$\textit{Percentage N derived from fertilizer (\%Ndff)} = \left(\frac{\%^{15}N \textit{ excess in sample}}{\%^{15}N \textit{ excess in fertilizer}}\right) \times 100$$

$$\textit{Percentage uptake of applied N} = \left(\frac{\%N\,dff \times \textit{Yield of N}}{\textit{Rate of N application}}\right)$$

For intrinsic water use efficiency (WUE_i) measurements, 18 additional pots of ryegrass receiving the above six treatments were chosen. Pre- (one d before treatment application) and post-treatment (one d before harvest) measurements of leaf-level gas exchange and on-line photosynthetic carbon-13 and oxygen-18 isotope discrimination (Barbour & Farquhar, 2000; Barbour *et al.,* 2000) were carried out. After post-treatment measurements of leaf-level gas exchange and on-line photosynthetic carbon and oxygen isotope discrimination, ryegrass from each pot was harvested to determine ^{13}C in plant tissue.

RESULTS AND DISCUSSION

Herbage Dry Matter Yield and N Uptake

Urea applied with Agrotain-in FPA form produced significantly ($P<0.05$) higher herbage dry matter yield than did urea alone (Figure 1). Applying irrigation to ryegrass leaves after one d of applying treatments further increased growth in all cases. This resulted in the highest growth for the urea with Agrotain + irrigation treatment. Similarly, the new plant tissues (new tissue emerged after treatment application) contributed more toward plant dry matter production than the old plant tissue (the leaves which were previously trimmed). Considering both new and old plant tissues, urea applied with Agro-

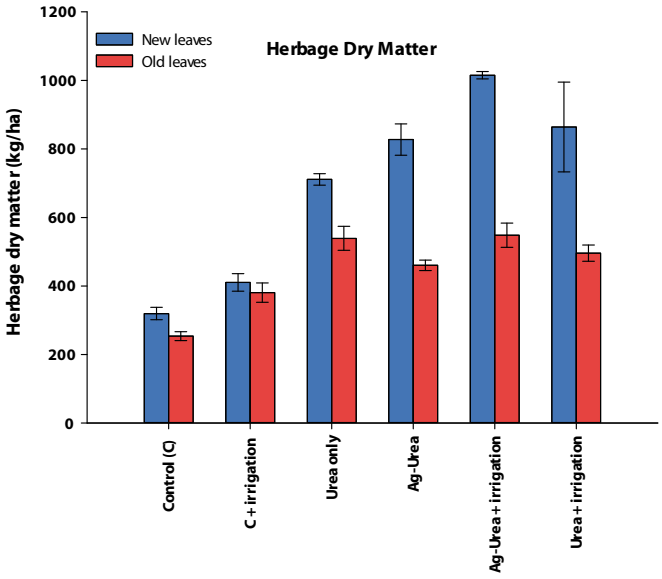


FIGURE 1. Herbage dry matter yield of new and old plant tissues of ryegrass as influenced by application of urea with the urease inhibitor (Agrotain) by the fine particle suspension method. Values are means (n = 3). Vertical bars represent one standard error of the mean.

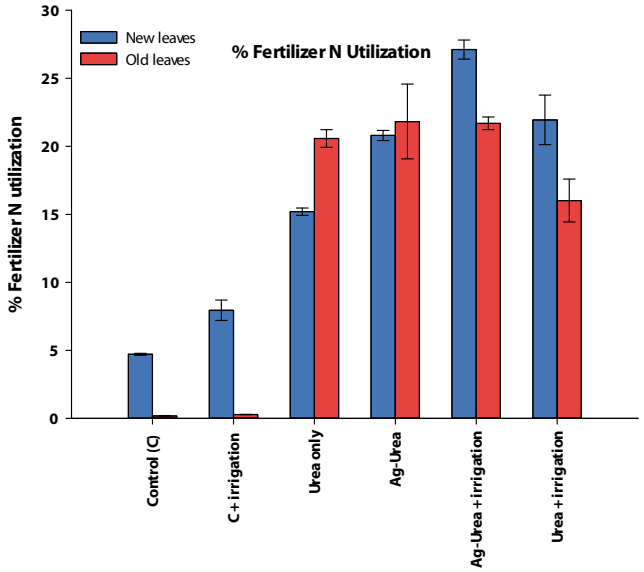


FIGURE 2. Percentage recovery of applied ^{15}N by new and old plant tissues of ryegrass as influenced by urea applied together with the urease inhibitor (Agrotain), using the fine particle suspension method. Values are means (n = 3). Vertical bars represent one standard error of the mean.

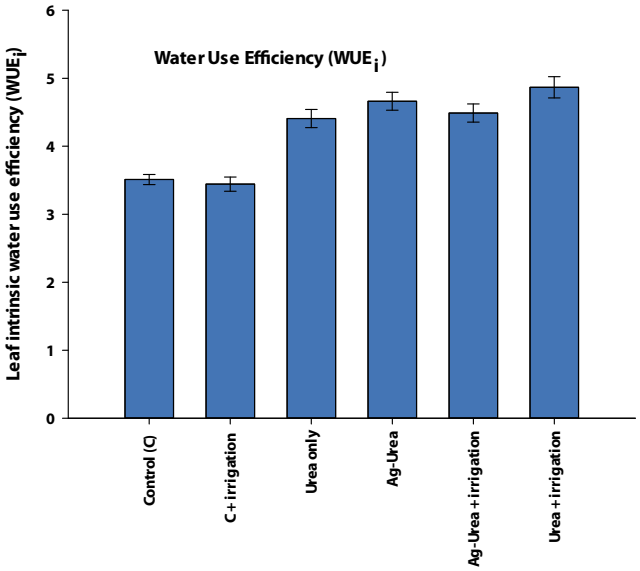


FIGURE 3. Leaf intrinsic water use efficiency (WUEi) of ryegrass as influenced by application of urea with the urease inhibitor (Agrotain) applied via the fine particle suspension method. Values are means (n=3) of a dimensionless ratio between assimilation rate and transpiration rate. Vertical bars represent one standard error of the mean.

tain in FPA form increased herbage dry matter by 14% over urea alone. Such increases in herbage dry matter yield can be attributed to the positive effects of Agrotain on soil microbial, chemical, and physical processes and plant physiological processes. Among the soil microbial process, Agrotain delays soil urease activity which minimizes the rate of NH_3 losses (Zaman *et al.,* 2008, 2013a; Soares *et al.,* 2012). Urea is an uncharged particle and can thus move both laterally and downward in soil. Since Agrotain retains urea in the soil for 7 to 10 d, this facilitates urea dispersion in sub-surface soil layers where it is likely to make good contact with the plant under moist condition (Dawar *et al.,* 2011). Urea applied with Agrotain is also reported to reduce nitrification (Sanz-Cobena *et al.,* 2012), which conserves N against denitrification and nitrate leaching. Since urea with Agrotain was applied to plant leaves, which is likely to delay plant urease activity and facilitate direct uptake of applied urea N through plant leaves. In our earlier field trial we observed that urea applied to ryegrass with Agrotain by the fine particle application method, followed by light irrigation (5 mm), significantly improved NUE, compared to granular urea (Zaman and Blennerhassett., 2009). Light irrigation after one d also washed urea from plant leaves and the surface soil, thus potentially minimizing the risk of leaf burning and NH_3 volatilization (Zaman *et al.,* 2013a).

There were significant treatment differences in the proportion of plant N derived from the labelled urea (Figure 2). Urea applied with Agrotain exhibited significantly ($p < 0.05$) higher N uptake than did urea alone; while irrigating the leaves after one d further increased the proportion of N derived from labelled urea. The higher N uptake in the treatment receiving both irrigation and Agrotain treated urea in FPA form suggests that the inhibitor needs a "dewfall" in order to gain the maximum benefits from the applied urea. Over all, plants receiving the urea+Agrotain+irrigation treatment took up 49% of N from applied urea, compared to 38% by the urea+irrigation treatment. Similarly, the two treatments of control (control only and control + irrigation) also showed that plants took up a small amount (between 5 and 8%) of plant N derived from the labelled urea. This was probably due to the dry deposition of ^{15}N-NH_3 onto the ryegrass sward leaves in the growth cabinet. Also, the control plants that were spray irrigated (artificial dewfall) after one d, had taken up more labelled N than the control plants without irrigation, suggesting that wet leaves are better sinks for atmospheric NH_3.

Intrinsic leaf water use efficiency

Pre-treatment measurements (one d before treatment application) showed that ryegrass height, photosynthesis parameters and isotopic values were not significantly different between pots (data not shown). As shown above, urea applied with Agrotain to the pasture leaves significantly increased growth of the sward as well as increasing N uptake (Figures 1 and 2); which was strongly related to an increase in photosynthetic rate (data not shown), probably due to higher N availability for Rubisco. The higher photosynthetic rate in response to applied urea, combined with a smaller increase in stomatal conductance and thus transpiration, resulted in higher leaf intrinsic WUE (photosynthetic rate divided by transpiration rate, as measured in the leaf chamber) (Figure 3). Again, leaf irrigation one d after urea treatment resulted in higher WUE, and Agrotain presence gave a small (non-significant) increase. On-line (Barbour *et al.,* 2000) and tissue carbon isotope discrimination analysis was strongly correlated to WUE_i suggesting that discrimination may provide a good record of changes in WUE_i.

CONCLUSIONS

Applying urea together with Agrotain as fine particle suspension method showed appreciably improved NUE and WUE_i compared with urea alone. This is likely due to increase in N assimilation rate. Applying light leaf irrigation (10 mm) one d after applying N treatments further improved both NUE and WUE_i. The ^{15}N data showed that ryegrass plants receiving the urea + Agrotain + irrigation treatment took up 49% of N from applied urea, compared to 38% by the urea+irrigation treatment. Our results suggest that applying urea with Agrotain followed by light irrigation is a useful management

tool for enhancing both NUE and WUE_i and has a good potential for improving profitability.

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Stabilized Nitrogen Fertilizers to Reduce Greenhouse Gas Emissions and Improve Nitrogen Use Efficiency in Australian Agriculture

H.C. Suter[1,*], D. Chen[1] and D. Turner[1]

ABSTRACT

Loss of nitrogen (N) from applied fertilizer is a major cause of inefficiency in N fertilizer utilization. This loss of N can occur through many pathways including ammonia (NH_3) volatilization, nitrate (NO_3^-) leaching and emissions of gases such as nitrous oxide (N_2O). One way of addressing these losses is to amend N fertilizers with compounds that slow the production of the forms of N that can be lost. Two such compounds are urease inhibitors, designed to reduce NH_3 loss, and nitrification inhibitors, designed to reduce NO_3^- leaching and gaseous emissions. However, the impact of these compounds on N loss is variable across soil type, region, cropping system and temperature. An examination of where these compounds may be beneficial requires detailed laboratory investigation plus field validation. This paper reports on the results of experiments performed under both laboratory and field conditions and draws conclusions regarding the suitability of these compounds for improving N use efficiency in Australian agriculture.

INTRODUCTION

Nitrogen (N) use efficiency from applied fertilizer in a variety of Australian agricultural systems ranges from 8 to 88 percent with an average of around only 50 percent of the N recovered in the plant (Chen *et al.*, 2008). Losses from applied N fertilizers occur mostly as gaseous emissions (ammonia [NH_3], nitrous oxide [N_2O] and other gases [N_2, NO_x]) or nitrate (NO_3^-) leaching. Some fertilizer N is retained by the soil where it is either immobilised or remains available for later crop use. Losses of up to 30 percent of applied N as NH_3 have been reported from pasture systems in Australia (Eckard *et al.,* 2003; Prasertsak *et al.,* 2001). Denitrification, can also be a major pathway of N lost as N_2O, N_2 and NO_3^- during periods of irrigation or waterlogging, with losses of up to 80 percent of applied N recorded in irrigated cotton (Chen *et al.,* 1994). In Australia, N_2O emissions from agriculture account for 71 percent of the national total. Fertilizer usage is a major contributor, with current fertilizer emission factors for N_2O ranging from 0.05 to 2.8 percent (DCCEE, 2011).

Use of stablized N fertilizers, those treated with urease and/or nitrification inhibitors, is one approach to reduce N losses from fertilizers and to mitigate both direct (N_2O) and indirect (NH_3) nitrogenous greenhouse gas emissions from agriculture. The urease inhibitors work by slowing the pathway of N transformations through inhibition of the activity of the urease enzyme which is involved in hydrolyzing urea. The nitrification inhibitors suppress the activity of NH_3 oxidizing bacteria and hence slow the conversion of NH_3 to nitrite and subsequently NO_3^-. Research has found that the impact of these inhibitors in reducing N loss and improving crop yield is highly variable with temperature and soil type (Carmona *et al.*, 1990; Barth 2006).

Australia has a wide range of climatic conditions and soil types and hence the few studies examining the use of stabilized fertilizers are not sufficient to assess comprehensively their potential use across Australian agricultural systems.

This research presents findings from laboratory and field experiments investigating the impact of stabilized fertilizers on N transformations and losses on a range of soil types from different regions in Australia, covering pasture, cropping and sugarcane production systems. The stabilized fertilizers used were urea treated with the urease inhibitor *N*-(n-butyl) thiophosphoric triamide (NBPT), and the nitrification inhibitors dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP) and nitrapyrin (N-serve).

MATERIALS AND METHODS

Laboratory incubation experiments

Air-dried and sieved (< 2mm) top soil (0–20 cm depth from sugarcane sites and 0–10 cm from all others) was placed into an incubation vessel (250–500 mL capacity) and pre-wetted to 60 percent water filled pore space for all experiments.

Soils of varying physical and chemical properties were collected from both pasture (dairy) and cropping sites in Victoria, Australia (Table 1) for assessing the impact of the urease inhibitor NBPT on urea hydrolysis rates. The methodology followed that described in Suter *et al.* (2011) with slight modifications for soil mass (40 to 150 g of oven-dried equivalent used), N application rate, temperature of incubation (5, 15, 25 and 35 ^oC) and extraction soil:solution ratio (1:5 for all except those reported in Suter *et al.,* 2011). Urease activity was measured following the method of Douglas and Bremner (1971). The minor variations in methodology as described above were not considered to have a major influence on the results achieved because the trends observed for different soil types were consistent, and so it is possible to compare results from all experiments.

For experiments on the impact of nitrification inhibitors (DCD, DMPP and N-serve) on nitrification rates and N_2O emissions, urea with or without the nitrification inhibitors was applied either as a buried granule or as a liquid. Five different soils, three from Victoria and two from Queensland were examined. The selected properties

1 Department of Agriculture and Food Systems, Melbourne School of Land and Environment, The University of Melbourne 3010, Victoria, Australia
* E-mail address of corresponding author: helencs@unimelb.edu.au

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TABLE 1. Selected properties of soils used for the urease inhibitor incubation experiments

Soil	Soil texture	Industry	pH_w	Clay (%)	Silt (%)	Sand (%)	Organic C (%)	Urease activity (mg urea-N/g soil/h)
1	Clay loam	Dairy	5.5	20	25	55	3.9	134
2	Fine sandy clay loam	Dairy	5.4	21	28	51	10*	186
3	Silty loam	Dairy	5.5	22	38	40	2.4	97
4	Clayey sand	Cropping	7.8	9	2	89	1.3	54
5	Medium clay	Cropping	8.1	37	24	39	1.0	78
6	Medium clay	Cropping	8.1	39	21	40	1.3	90

*Note: this soil included the pasture thatch layer and hence a large organic component

TABLE 2. Selected properties of the soils used for nitrification inhibitor incubation experiments

Soil no.	Soil texture	Region	Industry	pH_w	Clay (%)	Organic C (%)
7	Clay loam	Queensland	sugarcane	5.3	39	1.9
8	Loam	Queensland	sugarcane	4.8	13	1.5
9	Fine sandy loam	Victoria	dairy	5.4	21	10
10	Medium clay	Victoria	dairy	5.5	33	2.4
11	Clay loam	Victoria	grains cropping	7.8	30	1.3

*Note: this soil included the pasture thatch layer and hence a large organic component

of these soils are provided in Table 2. The methodology followed for the cropping soil from Victoria is provided in Chen *et al.* (2010) and the studies on the pasture and sugarcane soils followed a similar method with the modification of soil mass used (40 and 80 g of oven dried equivalent for the sugarcane and pasture soils, respectively) and soil:solution ratio of the extraction (1:5).

Field experiments

A field experiment was conducted on a ryegrass seed crop in Victoria, Australia where NH_3 loss, N_2O emissions, soil mineral N transformation and biomass production were measured regularly over an eight-month period where regular fertilization occurred. Details of the soil properties at the field site and the methodology used for the NH_3 volatilization study are reported in Suter *et al.* (2013). Another NH_3 volatilization field experiment was conducted on cropping soils from southern Australia and details of the methodology are reported in Turner *et al.* (2010).

The N_2O emissions from surface applied granular urea with and without the addition of the nitrification inhibitor DMPP were investigated using manual chambers (23 cm diameter, 25 cm height) in a small (2 x 1 m) plot trial in the ryegrass seed crop experiment. Fertilizer was applied every 1 to 2 months while the pasture was cut to five cm height above ground level to simulate grazing in the same period. A nitrogen-15 (^{15}N) micro-plot (25 cm internal diameter × 20 cm depth) study using ^{15}N granular urea to determine the fate of applied fertilizer N was also conducted at the ryegrass seed crop site and is described in Suter *et al.* (2013).

RESULTS AND DISCUSSION

Urease inhibitors

In the laboratory incubation experiments, use of NBPT reduced urea hydolysis across all soil types examined, with the level of reduction dependent upon soil properties including soil urease activity and temperature. Typical responses from pasture and cropping soils are shown in the data provided for two of the soils (Soils 1 and 4) in Figure 1. In the cropping soils (Soils 4, 5 and 6), NBPT was found to reduce urea hydrolysis rates markedly under cooler conditions, but increasing temperature reduced the level of inhibition. In the more organic pasture soils (Soils 1, 2 and 3), the trend was for rapid urea hydrolysis for both urea and urea with the added NBPT. The differences in urea hydrolysis and in the impact of NBPT between the soils are attributed mainly to the urease activity (54–90 mg urea-N g·soil/h measured in cropping soil compared with 134–186 mg urea-N g·soil/h in pasture soils).

In field experiments on cropping soils, NBPT reduced NH_3 loss from urea by 89 percent compared with no NBPT (from 10 to 1 percent of applied N lost) (Turner *et al.,* 2010), and on pasture soils by between 42 and 67 percent depending upon the season (reducing losses from 30 to 9 percent of applied N in autumn and from 2 to 1 percent of applied N in spring) (Suter *et al.*, 2013). These results show that the urease inhibitor NBPT can reduce N losses in high urease activity systems. Despite the large N saving with the use of the urease inhibitor, biomass production was not altered in the pasture site. This is likely due to the presence of sufficient N in the soil to support the pasture growth, as indic ated by the results obtained from the ^{15}N micro-plot study which showed that of the total N taken up by the plant (52 kg·N/ha in the biomass) the fertilizer supplied only around 30 percent (17.7 kg·N/ha) of the required N to the plant (Suter *et al.,* 2013). Of the applied ^{15}N, 27 percent was unaccounted for and considered to be lost as NH_3 based on measured loss of NH_3 on the site of 30 percent of applied N, 42 percent was taken up by the plant and the remainder (31 percent) was found in the soil.

Nitrification inhibitors

Nitrification inhibitors (DCD, DMPP and N-serve) were found to reduce N_2O emissions across a range of soils, temperatures and moisture contents by between 15 and 98 percent in laboratory experiments, whilst their impact on NO_3^- production was variable (Table 3). The reason for the variability between soil types could not be clearly identified from the dataset developed to date. It is hypothesized that

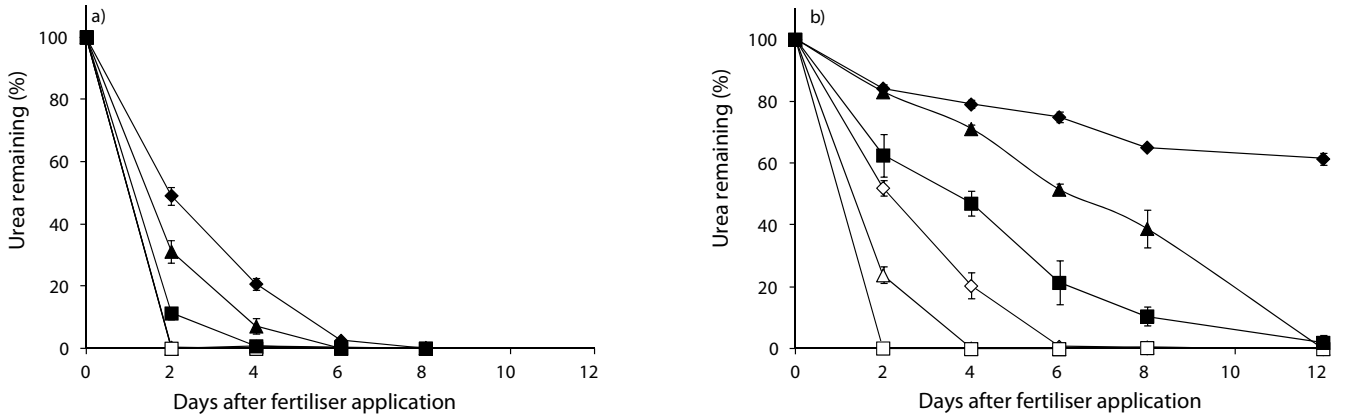


FIGURE 1. Urea remaining in soil during incubation for: (a) clay loam dairy pasture soil (Soil 1), and (b) clayey sand wheat cropping soil (Soil 4), with application of granular urea with and without NBPT. Urea at 15°C (◇), 25°C (△)and 35°C (□); Urea + NBPT at 15°C (◆), 25°C (▲) and 35°C (■)

TABLE 3. Summary of incubation experiments with Queensland and Victorian soils showing the effect of nitrification inhibitors on NO_3^- formation and cumulative N_2O emissions over the indicated range of temperatures. Note the values presented are percent reduction relative to the control.

Soil texture	Region	Temp. (°C)	NO_3^- reduction (%)			N_2O reduction (%)		
			DMPP	DCD	N-serve	DMPP	DCD	N-serve
Clay loam	Queensland	25–35	> 85	—	—	> 94	—	—
Loam	Queensland	25–35	0	—	—	19–64	—	—
Fine sandy loam	Victoria	5–25	0	0	—	36–68	15–83	—
Medium clay	Victoria	5–25	0–51	0–81	—	31–76	44–88	—
Clay loam	Victoria	5–25	4–70	—	83–98	> 95	—	> 95

the soil microbial community may have an important role in determining whether the inhibitors work as they are only considered to target the autotrophic NH_3 oxidizing bacteria whilst other microbes such as heterotrophic bacteria or archaea can perform the same function and are not affected by the inhibitors (Amberger 1993; Di *et al.,* 2010). Differences in soil properties such as texture, carbon, pH and nutrient status, will impact on the microbes found in the soils and consequently the response to the use of the inhibitors (Nicol *et al.,* 2008).

In the field experiment on the ryegrass seed crop, the use of the nitrification inhibitor DMPP reduced the fertilizer induced N_2O emissions over an eight-month period by 64 percent. The greatest impact was seen during spring when soils that were saturated over winter were drying and soil temperatures were warming. The saved N from reduced N_2O emissions did not translate into increased biomass as the amount lost was small relative to the amount applied. No other nutrient limitation to plant growth (such as phosphorus, sulphur and potassium deficiency) was observed on-site. At this site, NO_3^- leaching was considered to be minimal due to the nature of the soils (texture contrast) and the site topography (flat to slightly undulating). This helps to explain the lack of observed difference in biomass production with the use of the nitrification inhibitor.

CONCLUSIONS

Stabilized fertilizers, those amended with urease or nitrification inhibitors, show promise for reducing N losses from applied fertilizers and mitigating emissions of both N_2O and NH_3 gases in targeted Australian agricultural industries. Further research is required into the productivity effects of the use of urease inhibitors, the variable response seen with the nitrification inhibitors and the role of the soil microbial community in nitrification inhibition.

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Selection and Evaluation of Maize Genotypes Tolerant to Low Phosphorus Soil

J.C. Yang[1,*], H.M. Jiang[1], J.F. Zhang[1], L.L. Li[1] and G.H. Li[1]

ABSTRACT

Genotype selection for tolerance to low phosphorus (P) stress is an important strategy for the development of maize cultivars growing on soils low in available P. In this study, 15 maize genotypes were selected from 116 inbred lines during a three-year field experiment based on their 100-grain weight at maturity. Some morphological and physiological changes associated with low P stress were investigated at the seedling stage. In order to understand the mechanisms used by genotypes tolerant to low P soil to utilize P from the sparing soluble P forms, five typical maize genotypes selected from the inbred lines, were used in a phosphorus-32 (^{32}P) isotope tracer experiment. The results showed that root growth of the low P tolerant genotypes was accelerated under low P conditions, leading to wider exploration of soil space for the uptake of nutrients. Low P tolerant genotypes had higher P uptake efficiencies and could capture more available soil P under P deficiency than low P sensitive ones. A higher rate of transformation for water-soluble P to slowly available P in P deficient soil than in soil with sufficient P. L-values showed that different cultivars had different soil P use efficiencies and low P tolerance mechanisms. The low P tolerant cultivar DSY-32 regulated soil P use efficiency and plant P content according to the L-value under P fertilizer application, but another low P tolerant cultivar, DSY-2, utilized soil P more efficiently regardless of the L-value.

Key words: *phosphorus-32, L-value, maize, phosphorus use efficiency, phosphorus fractions, root traits.*

INTRODUCTION

Phosphorus (P) deficiency limits plant growth and crop productivity in many regions of the world, especially in developing countries (Tomscha, *et al.,* 2004). To increase crop productivity, farmers often apply substantial amounts of phosphate fertilizers. However, applying large amounts of fertilizer not only reduces limited P resources, but also leads to environmental pollution. Maize is an important grain and forage crop in the world after wheat and rice. It is both sensitive to P and grown widely in areas where P, although abundant, is largely unavailable to plants. Consequently, an approach that has received increasing attention is to adapt crops to unfavourable soil conditions by selecting and evaluating genotypes with enhanced nutrient use efficiency in soils with a low nutrient status, and/or requiring moderately low external inputs to induce expression of their genetic potential for adequate production. This strategy is now considered a promising, energy-efficient, eco-friendly and socio-economically feasible approach (Guo *et al.*, 2002).

Plants differ greatly in their ability to grow on low P soil because they have developed specific physical, chemical and biological mechanisms to utilize P compounds under such conditions (Hiradate *et al.*, 2007). Naruzzaman *et al.* (2006) showed that the evaluation and identification of crop plants for genotype variation in their ability to access and utilize sparingly soluble forms of soil P (Ca–P, Al–P, Fe–P) is a possible means to overcome P deficiency in soils and optimize P fertilizer use in cropping systems where P is poorly available.

In a pre-screening experiment, 116 maize inbred lines with various genetic backgrounds were employed in a field experiment in 2007 which resulted in the selection of 15 maize inbred lines for developing an index system for assessing low P tolerant maize genotypes in a two-year field experiment in 2008 and 2009. To confirm P uptake from sparingly soluble P soils by different maize genotypes, a ^{32}P tracer technique was used as an additional criterion to evaluate maize tolerance in five lines selected from the 15 maize inbred lines.

MATERIALS AND METHODS

Field experiment

Plant materials

A total of 116 maize inbred lines with various genetic backgrounds were collected from several agricultural universities and institutes in China and used in a field experiment (experiment 1) in 2007. Of these, 15 maize cultivars were selected based on differences in 100-grain weight at the mature stage when grown in a P deficient soil and P sufficient soil. Eleven low P tolerant genotypes (DSY-30, DSY-2, DSY-31, DSY-20, DSY-21, DSY-39, DSY-101, DSY-33, DSY-32, DSY-23 and DSY-93), and four low P sensitive genotypes (DSY-113, DSY-79, DSY-129 and DSY-48) were selected for experiments 2 and 3, respectively in 2008 and 2009.

Experimental site

The study was carried out in 2007, 2008 and 2009 at Langfang experimental station, located in Hebei Province (E116°35′41, N39°36′). The general soil characteristics were pH 8.49, available P 4.9 mg/kg, available nitrogen (N) 55.8 mg/kg, available potassium (K) 92.9 mg/kg and organic matter 14.4 g/kg. These parameters were measured as described by Lu (1999). The soil was characterized as a typical P deficient soil.

Experimental design

The experiment involved two treatments, namely, P0 (P deficient) receiving no P fertilizer and P1 (P sufficient) receiving P at

1 Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China
E-mail address of corresponding author: jcyang@caas.ac.cn; yangjuncheng@caas.cn

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52 kg·P ha^{-1}. Urea, superphosphate, and potassium chloride were applied with N (225 kg·N·ha^{-1} in total, half of which was applied basally and the remainder as a top-dressing during the bell-mouthed or pre-anthesis stage), P (applied basally) and K (87 kg·K·ha^{-1} applied basally). To prevent cross pollination, maize cover package pollination was utilized.

Measurements and statistical analyses

In 2007, ten representative plants were sampled at maturity and the 100-grain weights of the inbred lines were determined. During 2008 and 2009, the plants were harvested at both the seedling and maturity stages. Plants collected during the seedling stage were separated into roots and shoots to assess morphological traits, dried at 65°C in an air-forced oven for 48 h and weighed to determine root and shoot dry weights. The roots were placed in a transparent water-filled tray (15 cm × 25 cm) on a scanner (Epson Perfection 4990 Photo; Model J1318) to facilitate root spreading. The system was used to scan all fine root fragments and the program calculated the traits of all fine roots such as length, volume and surface area. The data obtained were then analysed using the digital image analysis system, WinRHIZO Pro 2007a. After harvesting, the 100-grain weight was determined. Phosphorus uptake efficiency was measured as the amount of P in the plant materials including the roots and shoots at the seedling stage (mg P per plant) (Morris and Garrity, 1993). The P content of the plant materials was determined using the Mo-Sb-Vc colorimetric method (Barry and Miller, 1989) after digestion in sulphuric-perchloric acids.

The inhibition (percent) (due to P limitation) was defined as:

$$\text{Inhibition (percent)} = (P1 - P0) / P1 \times 100 \text{ percent}$$

where P1 is plant P uptake in the P1 (P sufficient) treatment (52 kg·P·ha^{-1}), and P0 is plant P uptake in the P0 (P deficient) treatment (0 kg·P·ha^{-1}).

^{32}P isotopic tracer experiment

Plant materials

Based on the results of a previous three-year field experimental selection, five typical cultivars were used: genotypes DSY-30, DSY-2 and DSY-32 (low P tolerant genotypes) and DSY-48 and DSY-79 (low P sensitive genotypes).

Preparation of ^{32}P carrier-free solution

Carrier-free ^{32}P solution was prepared (100 mL) with an activity concentration of 1.85 MBq/mL. Four ml (7.4 MBq) of the solution was diluted in 500 mL deionized water, added to the soil of each pot, and the soil with solution homogenized carefully using a stainless steel rod to avoid spilling. The pots were covered with aluminum foil to avoid light during equilibration and analysis of P fractions.

Treatments

To evaluate the P pool of the low P soil for the different maize genotypes, the pots were separated into two groups, one group with plants and the other group without. Each group included treatment 1 (the P0 treatment: no P application but 200 mg·N·kg^{-1} and 166 mg·K·kg^{-1} soil), and treatment 2 (the P1 treatment: included 66 mg P·kg^{-1} soil, 200 mg·N·kg^{-1} and 166 mg·K·kg^{-1} soil). Each treatment was replicated three times. One week after pot preparation, the experiment was conducted in a greenhouse. Soil samples were taken on days 0, 3, 7, 14 and 25 while plant samples were taken only on day 25.

Measurements

Sequential P fractionations of Ca_2–P, Ca_8–P, Al–P, Fe–P, O–P (occluded P) and Ca_{10}–P were performed by methods described by Gu and Jiang (1990). Measurements of ^{32}P radioactivity in the fractions were made using a liquid scintillation counter (LSC, LS-6500, Beckman, USA). Radioactivity in 5 mL extracts was determined by Cherenkov counting, with counting efficiency being determined using the standard ^{32}P solution provided by the HTA Co. Ltd.

The L-value (μg·P·g^{-1} soil) was calculated as follows:

$$\text{L-value} = [^{32}\text{P Bq}\cdot g^{-1} \text{ soil}] / [^{32}\text{P Bq}\cdot\mu g^{-1} \text{ P in plant}]$$

Statistical analysis

All data are presented as the means of three replicates with standard errors. Differences between treatment means were compared by least significant difference (LSD) test at $p < 0.05$

RESULTS

Field experiment

Screening of maize genotypes tolerant of low P soil

Figure 1 shows the reductions in 100-grain weights of the different maize genotypes. Overall, the results were normally distributed (KS test, Z value = 0.928, p value = 0.356). Maize genotypes tolerant to low P soils were defined as those showing a change in inhibition lower than -5 percent under low P conditions, while low P sensitive genotypes were selected when the inhibition was higher than 10 percent.

Four quadrant analyses for the selected typical maize genotypes (Figure 2) showed that maize genotypes tolerant to low P were distributed primarily in the first (responsive and efficient) and fourth quadrants (non-responsive and efficient). However, low P sensitive genotypes were distributed mainly in the second (responsive and inefficient) and third quadrants (non-responsive and inefficient). Based on the depression in 100-grain weight and the four quadrant analysis, the following typical 15 inbred lines were selected for further analysis: DSY-30, DSY-2, DSY-31, DSY-20, DSY-21, DSY-39,

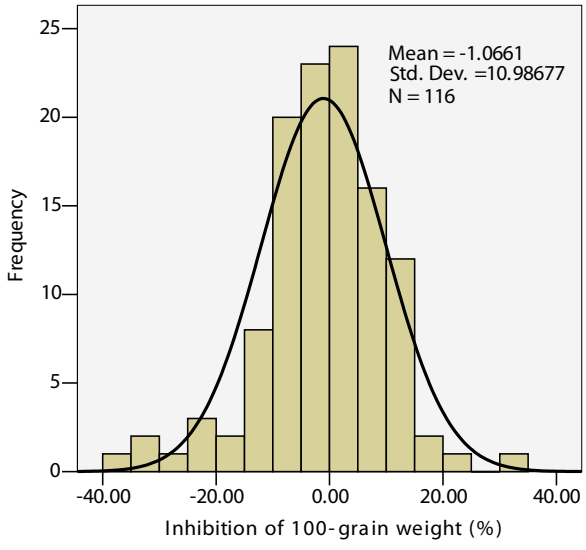


FIGURE 1. Reductions in 100-grain weights of 116 maize inbred lines in field experiment 1.

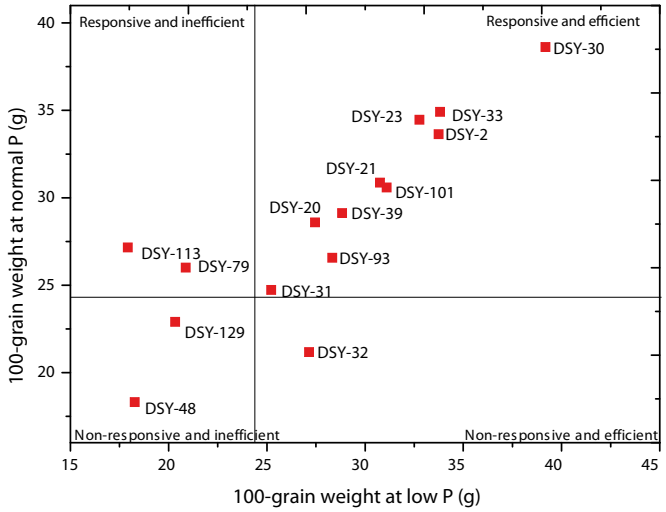


FIGURE 2. Selected P-tolerant and P-sensitive genotypes out of 116 lines. DSY-30 and DSY-2 (responsive and efficient, i.e. tolerant); DSY-32 (non-responsive and efficient, i.e. tolerant); DSY-79 (responsive and inefficient, i.e. sensitive); and DSY-48 (non-responsive and inefficient, i.e. sensitive).

DSY-101, DSY-33, DSY-93, DSY-23 and DSY-32 (genotypes tolerant to low P), and DSY-113, DSY-79, DSY-129 and DSY-48 (genotypes sensitive to low P).

Root architectural traits at seedling stage

Root morphological parameters are shown in Table 1. In 2008, enhancements of root lengths were observed for maize genotypes grown under low P conditions. Specifically, 64 percent of the genotypes that were tolerant to low P showed enhanced root lengths, while only 25 percent of the genotypes that were sensitive to low P displayed this feature under P deficiency. The total root surface areas and root volumes of low P tolerant genotypes were higher than those of low P sensitive genotypes under low P conditions, except for DSY-113.

The data also show that inhibition of root length ranged from around 44 percent to 48 percent in 2009. The total root lengths of low P tolerant genotypes (especially DSY-30, DSY-2, DSY-31, DSY-21, DSY-39, DSY-33 and DSY-32) increased dramatically under P deficiency. Among the low P sensitive genotypes, the inhibition of root length in DSY-21 was significantly lower than those of other genotypes, indicating that low P tolerant genotypes developed longer roots. By contrast, low P sensitive genotypes developed smaller roots under P deficiency compared with P sufficiency. These results indicate that the longer root lengths of low P tolerant genotypes were essential for their higher ability to acquire soil P. Comparing the inhibition of total root surface area with that of total root volume between low P tolerant and sensitive genotypes, the inhibition of root surface area and total root volume ranged from around 45.5–47.0 percent and from 50.0–58.0 percent respectively. Inhibitions of root surface area and total root volume were lower in low P tolerant than in low P sensitive genotypes.

P nutrient characteristics of genotypes at seedling stage in field experiments 2 and 3

There was a close relationship between plant P uptake and the level of soil P supply. The efficiency of P uptake of the different genotypes was lower under P deficiency than under P sufficiency in both field experiments (Figure 3). Under P deficiency, P uptake efficiency in DSY-21 and DSY-23 (both are low P tolerant genotypes) was significantly higher than that of DSY-113 (low P sensitive genotype) in experiment 2 (Figure 3A). Compared with the low P sensitive genotypes, those that were low P tolerant had higher P contents under P deficiency, indicating that the ability of low P tolerant genotypes to take up P was relatively stronger in field experiment 2 (Figure 3B). For example, P uptake efficiencies in DSY-30, DSY-2, DSY-39, DSY-101 and DSY-93 were significantly higher than those of DSY-113, DSY-79, DSY-129 and DSY-48. The level of inhibition of P uptake by low P tolerant genotypes was much lower than that of most low P sensitive genotypes

TABLE 1. Changes in root morphological characteristics of seedlings in field experiments 2 and 3

Genotype	No.	Inhibition of root length (%)		Inhibition of root surface area (%)		Inhibition of root volume (%)	
		2008	2009	2008	2009	2008	2009
P tolerant	DSY-30	–6.9	–6.7	–16.1	–12.2	–25.6	–20.0
	DSY-2	–56.7	–41.6	–10.1	–45.5	22.1	–49.6
	DSY-31	11.0	–29.0	18.1	–28.5	25.0	–27.8
	DSY-21	–17.3	–44.0	5.5	17.3	23.8	52.5
	DSY-39	–7.4	–19.2	8.3	–14.6	21.2	–10.0
	DSY-101	–1.1	29.7	–5.4	27.9	–9.9	26.0
	DSY-33	50.3	–13.0	34.4	–10.7	13.0	–8.3
	DSY-32	34.1	1.4	40.0	–8.0	45.0	–18.0
	DSY-23	11.8	47.7	5.3	50.4	–1.0	53.0
	DSY-93	–127.4	22.0	–53.2	46.5	–3.0	54.3
	DSY-20	–45.5	14.9	–47.6	–1.1	–49.7	–20.1
P sensitive	DSY-113	–68.9	34.8	–56.4	41.0	–45.6	46.7
	DSY-79	17.6	32.9	26.7	32.2	34.5	31.5
	DSY-129	28.2	24.5	22.7	29.1	16.7	33.4
	DSY-48	2.3	32.9	3.3	47.1	4.4	58.3

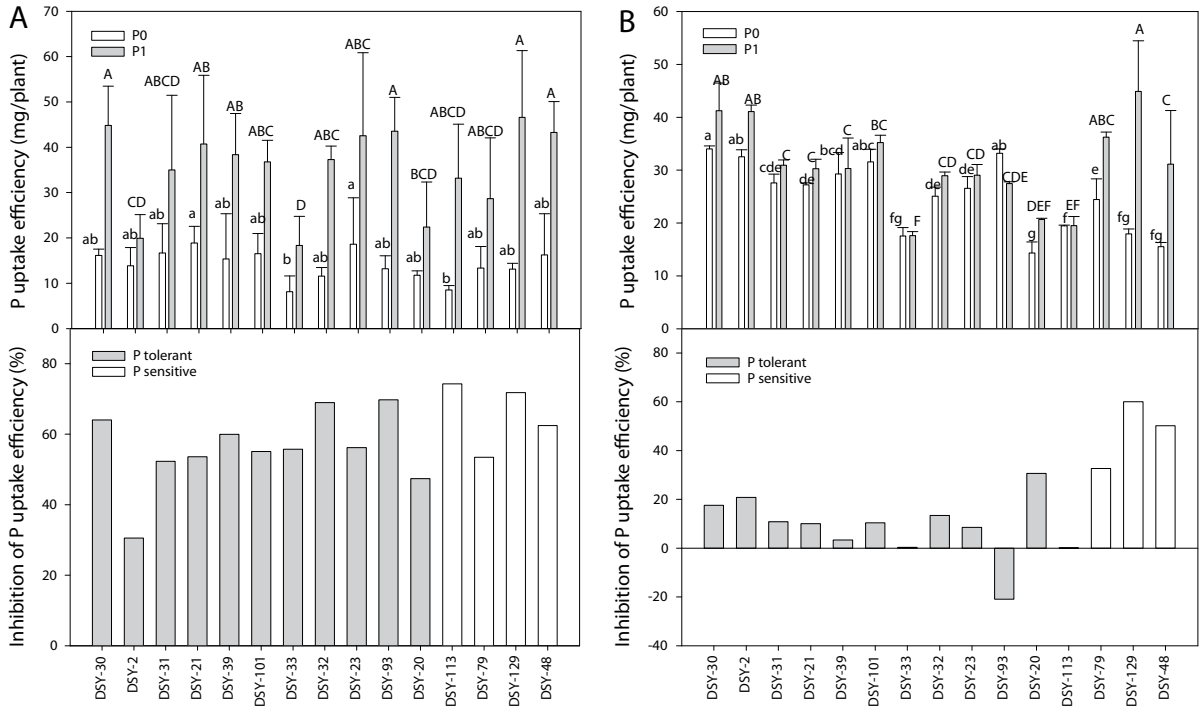


FIGURE 3. P uptake efficiency and inhibition of typical maize inbred lines. A: field experiment 2 (2008), B: field experiment 3 (2009). Different uppercase and lowercase letters mean significant at 5% level.

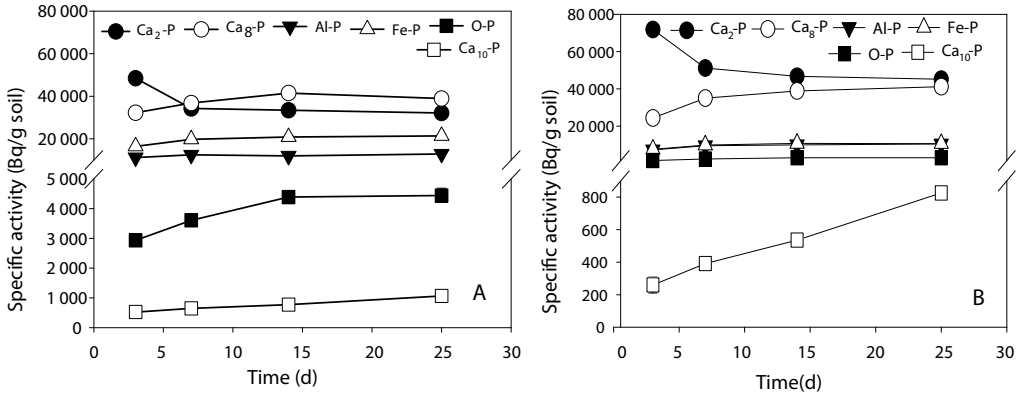


FIGURE 4. Changes over time in ^{32}P specific activities of inorganic P fractions with (A) and without (B) P fertilizer.

in field experiments 2 and 3, indicating that the ability of low P tolerant lines to take up P was relatively strong under low P conditions.

^{32}P isotope tracer experiment

Dynamic variations of P fractions

Without P fertilizer, the ^{32}P activity in Ca_2–P decreased rapidly during the early period. However, the reverse was observed in Ca_8–P, i.e. a rapid increase during the early period was followed by a slow increase. The ^{32}P activities in Fe–P and Al–P increased steadily at a moderate rate and reached a steady state, while those in O–P and Ca_{10}–P increased slowly and steadily during the entire test period of 25 d (Figure 4A). When P fertilizer was added to the soil, the transformation dynamics of ^{32}P in all P soil fractions were consistent with the transformation dynamics without P fertilizer (Figure 4B). Nevertheless, the ^{32}P activities of different fractions showed different characteristics. First, in Ca_2–P the activities were significantly higher than those without P fertilizer in the soil. Second, the ^{32}P activities in both slowly available P (Ca_8–P, Al–P and Fe–P) and unavailable P (O–P and Ca_{10}–P) were significantly lower than without P fertilizer in the soil. These results suggest that the rate at which water-soluble P was transformed to slowly available and unavailable P in the soil deficient in P was higher than in the soil with sufficient P.

Inorganic P pools and L values

As shown in Table 2, the DSY-30 and DSY-2 genotypes had higher L-values and intermediate P uptakes irrespective of whether or not external P fertilizer was applied, indicating slight dependency on insoluble and soluble P and that the two genotypes were likely low P tolerant. The L-value of DSY-32 was the highest under low P limitation, suggesting that this genotype activated and utilized different Pi forms in soil and reduced the dependence on soil soluble P. After P

TABLE 2. L values of different maize genotypes with or without external P fertilizer

	Without external P fertilizer			With external P fertilizer		
Variety No.	P specific activity of plant (Bq/μg)	Specific activity of soil (Bq/g soil)	L value (ug·P·g^{-1} soil)	P specific activity of plant (Bq/μg)	Specific activity of soil (Bq/g soil)	L value (ug·P·g^{-1} soil)
DSY-30	22.4±2.5	4 216.0±144.4	190.0±27.6	16.8 ± 0.9	4 015.3±339.1	239.4±32.3
DSY-2	20.0±0.1	4 483.9±153.6	224.7±6.8	14.0 ± 1.0	3 517.3±120.5	252.2±25.9
DSY-32	7.8±1.0	3 964.6±135.8	513.3±45.4	20.4 ± 1.7	3 792.5±129.9	250.8±5.1
DSY-79	11.4±0.8	3 572.4±122.4	314.7±32.4	8.3 ± 0.4	3 820.9±130.9	459.8±17.1
DSY-48	64.9±2.9	3 622.5±124. 1	55.8±0.6	28. 8±2.7	5 089.3±312.2	133.6±36.2

fertilizer was applied, the L-value of DSY-32 decreased rapidly, indicating that more P was utilized from the fertilizer. Therefore, DSY-32 is a typical low P tolerant genotype. Although the L-value of DSY-79 was higher than DSY-30 and DSY-2, the radioactivity of its shoot was also higher (data not shown), indicating that DSY-79 required a much higher P content in the plant to maintain normal crop growth. Therefore, DSY-79 is a typical low P sensitive genotype. The L-value of DSY-48 was significantly lower than that of the remaining four maize genotypes with or without external P fertilizer. One probable explanation is that this genotype had a high dependency on, and was sensitive to the supply of soil available P.

DISCUSSION

The primary objective of most maize breeding programmes is to develop high yielding and well adapted cultivars. The data reported here support these findings since the 100-grain weights of low P tolerant genotypes were higher than those of the low P sensitive genotypes under low P conditions. This suggests that 100-grain weight could be an indicator for screening maize genotypes tolerance to low P. A similar "specific mechanism(s)" has been described by Gourley, Allan and Russelle (1993).

A larger root system provides greater root-soil contact, which is particularly important for uptake of P. Mobile nutrients such as nitrate can be depleted at low rooting density, while for less mobile ions like P, uptake is often closely related to root length. There are a number of reports concerning root length under P deficient conditions. For example, root elongation associated with P deficiency was observed in *Arabidopsis* (Ma *et al.*, 2003), barley (Steingrobe, Schmidt and Claasen, 2001), and rice (Kirk and Du, 1997). The screening of maize germplasm in the present study also revealed root elongation induced by P deficiency since low P tolerant germplasm groups were found to elongate their roots specifically under P deficiency. This could result in roots exploring more soil space for uptake of nutrients (Table 1). This finding is consistent with previous reports of maize root system growth and development being influenced by P deficiency (Mollier and Pellerin, 1999).

Zhang *et al.* (2005) reported that during the seedling stage, P uptake efficiency was the main contributor to P tolerance because the high P uptake and P accumulation features at the seedling stage of P tolerant genotypes were the nutritional foundation for producing more biomass. In this study, low P tolerant genotypes were able to maintain higher uptake efficiencies and absorb more P from the soil to satisfy their growth requirements under low P conditions while low P sensitive genotypes were less able to do so.

Elucidating the transformation dynamics of P fractions is important for better understanding the availability of different P fractions. Jiang and Gu (1989) reported that Ca_2–P was the most rapidly available P source to plants, with Ca_8–P, Fe–P and Al–P being slowly available sources, and P in Ca_{10}–P and O–P being unavailable. Previous studies have shown that after applying P fertilizer to a calcareous soil, the initial P form was Ca_2–P (Dai *et al.*, 2006). The data reported here indicate that the ^{32}P activity in Ca_2–P reached a maximum within three days and then decreased rapidly between 3 to 7 days after ^{32}P-labelled fertilizer P was applied to the Langfang low P soil. It is therefore proposed that Ca_2–P in Langfang low P soil was a fast transformation process in the soil and is the main P fraction available to the plant. A continuously increasing trend in ^{32}P activity was observed with Ca_8–P, Fe–P and Al–P during the entire 25-d test period, and this increasing rate was moderate. Therefore, Ca_8–P, Fe–P and Al–P can be considered as a moderate transformation process. Among these three P fractions, the ^{32}P activity and its increasing rate in Ca_2–P and Ca_8–P reached maximum values, possibly in part because the concentration of Ca is greater than that of Fe and Al in calcareous soils. Therefore, Ca_8–P might be another important fraction of available P in Langfang low P soil. Nevertheless, the ^{32}P activity in Ca_{10}–P and O–P increased very slowly, suggesting that these forms represented a slow transforming phase (Figure 4). Additionally, after fertilizer P application the ^{32}P activity and its increasing rate in O–P decreased radically, indicating that a larger proportion of water-soluble P in P deficient soils without external P fertilizer would be transformed to the plant-unavailable O–P fraction, i.e. the plant would have more difficulty in acquiring P from a P deficient soil. The results presented here are further evidence that external P fertilizer was especially essential for optimum crop growth in the Langfang low P soil.

In low P soils, the P efficiency of plants differs between genotypes within a given plant species (Ozturk *et al.*, 2005). The L-value has considerable theoretical advantages as a measurement of plant available P from the soil P. In this study, the L-values were determined to evaluate the P efficiency of different maize genotypes using a ^{32}P tracer technique after the maize plants were grown in the Langfang low P soil for 25 d. The results showed that the P efficiency of five maize genotypes differed significantly. DSY-32, which is a typical low P tolerant genotype, actively regulated the P use ratio between the soil and exogenous fertilizer P. When exogenous P was supplied, DSY-32 absorbed exogenous P preferentially; otherwise, it would try to exploit soil P when no exogenous P was supplied. However, another low P tolerant genotype, DSY-2, exhibited a different low P tolerant pathway, utilizing soil P efficiently regardless of exogenous P application. This result suggests that different maize genotypes have very different low P tolerance pathways and therefore that it would be useful to fully exploit the limited P resources in low P soils by screening and planting low P tolerant crop varieties. Additionally, the results confirmed the L-value as a very useful parameter for evaluating plant P efficiency.

CONCLUSIONS

This field study clearly demonstrated that maize germplasm differ in their ability to take up P from a low P soil and that the differences were attributed to plant morphological and physiological features. Based on these results, an effective method for enhancing P efficiency is to develop P tolerant cultivars for achieving high yields under conditions of P deficiency. The results indicated that soil P availability during maize seedling development is critical for the early growth and grain yield. Inhibition of root length, surface area, root volume and P uptake efficiency under P deficiency were preliminarily defined as screening indexes for rapid selection of low P tolerant genotypes during the seedling stage. In addition, the 100-grain weight was defined as the screening index for low P tolerant genotype during the mature stage.

The ^{32}P tracer technique was a powerful adjunct for better understanding soil P availability and sources of P pools in a low P soil–plant system. Results indicated that the rate at which water-soluble P was transformed into slowly available and unavailable P in the P deficient soil was higher than in the soil with sufficient P. Ca_2–P was a fast transformation process, while Ca_8–P might be another important available P fraction and Ca_{10}–P and O–P were transformed slowly. The L-values showed that low P tolerant cultivars regulated soil P use efficiency and plant P content.

ACKNOWLEDGEMENTS

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Overview of FAO/IAEA Coordinated Research Project on Crop Genotypes Tolerant to Low N and P Soils

J.J. Adu-Gyamfi[1,*] and G. Dercon[1]

ABSTRACT

A five-year coordinated research project (CRP) entitled "Selection and Evaluation of Food (Cereal and Legume) Crop Genotypes Tolerant to Low Nitrogen and Phosphorus Soils through the Use of Isotopic and Nuclear Related Techniques" that supports Member States in their efforts to optimize crop yields and soil productivity in low nitrogen (N) and phosphorus (P) environments was initiated in 10 developing countries in Africa, Asia and Latin America. The overall objective of the CRP was to develop integrated crop, soil and nutrient management practices to increase crop production in marginal lands by identifying and promoting the development of food crop genotypes with enhanced N and P use efficiency and greater productivity. The studies conducted within this CRP concerned two major food security cereal crops namely upland rice (*Oryza sativa* L.) and maize (*Zea maize* L.), and three legumes, namely common bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* L.) and cowpea (*Vigna unguiculata* L.). Protocols for characterization of root traits contributing to enhanced N and P acquisition from low fertility soils with emphasis on rapid root phenotyping methods were developed and used to evaluate and select crop genotypes with superior N and P acquisition and/or utilization. Results showed that out of 150–200 genotypes from the five crops that were tested, 3–5 cultivars identified as N and P efficient in low N and P soils, had better root architecture and produced 15–20 percent higher yields than those with poor root architecture. Branching angle interval was identified as a suitable root selection parameter for soil N use efficiency, while adventitious rooting and root hair formation were identified as suitable plant parameters for selecting P use efficiency. The genotypes of rice, common bean, maize, soybean and cowpea identified in a number of cases provide valuable resources for plant breeding programmes aimed at enhancing P and N use efficiency in crops. The CRP created a database on how cereals and legumes can acquire N and P in low nutrient soils, and this database could be further expanded and interpreted using multivariate analysis on how cereal and legume crops can acquire N and P in low nutrient soils.

Key words: *genotypes, low N and P soils, N use efficiency, P use efficiency, root architecture, root traits.*

INTRODUCTION

Global climate change is likely to exacerbate plant abiotic stresses in the coming decades by increasing water stress and by accelerating soil fertility degradation (St Clair and Lynch, 2010; Lynch and Brown, 2013). To respond to this set of challenges, there is a need to develop agricultural systems with significantly greater productivity and resilience while at the same time use limited natural resources more efficiently. Low phosphorus (P) and nitrogen (N) availability are primary limitations to productivity in low-input agriculture and fertilizers are primary resource inputs in intensive agriculture. A critical feature of future agricultural systems will be new crop varieties with improved conversion of soil resources to yields. These new cultivars would have improved productivity in low-input systems and decrease input requirements in high-input systems.

There are two distinct avenues to improve crop resource use efficiency: (i) improve resource acquisition, and (ii) improve physiological utilization of acquired resources. While both avenues merit exploration, improving resource acquisition represents the greater opportunity for crop breeding (Lynch, 2011). For example, over half of N fertilizer applied in intensive agriculture is not taken up by the crop; crops typically acquire only 5–8 percent of applied fertilizer during the growth season. Improved N and P recoveries by crops would therefore translate into significant economic and environmental benefits along with reduced production of greenhouse gases from denitrification and fertilizer production, and reduced water pollution from leaching and runoff (Cordell, Drangert and White, 2009). Scientists are currently turning their attention to roots — the hidden half of the plant — as central to their efforts to develop crops with greater yields without causing environmental damage. Root biology and the mechanisms of taking up nutrients from the soil are therefore central components of efforts to develop crops with improved soil resources acquisition. Roots are most efficient when their architecture is tailored to their environment. For example, deep, shallow and fine roots can exploit soils in which limiting nutrients are trapped in the soil. Several root traits are known to be associated with P and N acquisition efficiency in low P and N soils. Several of these have the potential to be deployed in crop breeding programmes including: rhizosphere modification to mobilize nutrients (Ryan, Delhaize and Jones, 2001), enhanced symbioses with N_2-fixing bacteria and longer, denser root hairs to enhance the acquisition of N and P and other immobile resources (Lynch and Brown, 2013). Possible root system architecture (RSA) and morphological traits include root hair length, root length, root branching angle and root density and basal root whorl number. Nitrate is highly mobile in soil and is readily lost to leaching and therefore a crop root system with lateral root development and branching may be useful for capturing N as it

1 Soil and Water Management & Crop Nutrition Laboratory, International Atomic Energy Agency, Seibersdorf, Austria
* E-mail address of corresponding author: J.Adu-gyamfi@iaea.org

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FIGURE 1. (a) A growth chamber cigar roll method, (b) field sampling and (c) transparent glass tube for evaluating genotypic variations in roots (root architecture and morphology) and plant growth traits associated with N and P acquisition efficiencies in maize at the vegetative growth stage.

moves deeper through the soil. Crop species differ in their ability to take up P and N from the soil, and these differences are attributed to the root system architecture, morphology and physiology of the crops relative to their germplasm base. The identification of root traits for enhanced P and N acquisition is enabling crop breeders to develop new genotypes with better yield in low fertility soils of Africa, Asia, and Latin America.

However, in order to use a trait as a selection criterion for crop improvement through either direct phenotypic selection or marker-assisted selection, it is necessary to develop protocols to measure accurately root traits that enhance N and P acquisition in the glasshouse and in the field.

THE COORDINATED RESEARCH PROJECT

This five-year coordinated research project (CRP) entitled "Selection and Evaluation of Food (Cereal and Legume) Crop Genotypes Tolerant to Low Nitrogen (N) and Phosphorus (P) Soils through the Use of Isotopic and Nuclear-Related Techniques" established a research network and supported the efforts of teams of scientists in sixteen Member States (Australia, Benin, Burkina Faso, Brazil, Cameroon, China, Cuba, France, Germany, Ghana, Kenya Malaysia, Mexico, Mozambique, Nigeria and the United States). The aim of the CRP was the development of integrated crop, soil and nutrient management practices to increase crop production in marginal lands by identifying and promoting the development of food crop genotypes (cereal and legume) with enhanced N and P use efficiency and greater productivity. n Studies were conducted along four main areas of investigation to (i) develop and validate screening protocols for plant traits that enhance N and P acquisition and utilization in major food cereal and legume crops grown in low fertility soils, (ii) employ validated screening protocols to identify genotypes with superior N and P acquisition and/or utilization, (iii) identify mechanisms for adaptation and high productivity of selected legumes and cereals to low N and P soils using isotopic techniques (stable ^{15}N and radioactive phosphorus-32 [^{32}P] and phosphorus-33 [^{33}P], and (iv) assess the selected genotypes with different enhanced root architecture and morphology that capture and/or utilize nutrients from different soil depths under field conditions from the standpoints of yield and productivity.

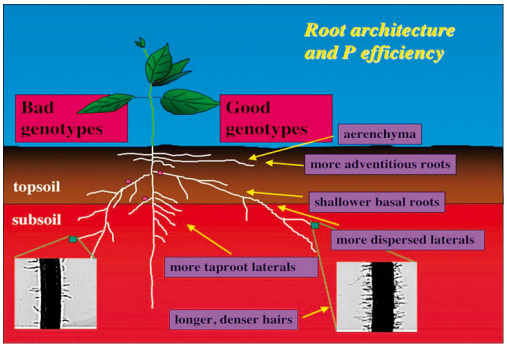
The studies concerned two major food security cereal crops, namely, upland rice (*Oryza sativa* L.) and maize (*Zea maize* L.), and three legumes, namely common bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* L.) and cowpea (*Vigna unguiculata* L.). They were conducted across a wide geographical area in both the northern and southern hemispheres under a wide range of environmental and edaphic conditions. Experiments were conducted in the laboratory or in glasshouses for rapid screening at the early seeding stage using the paper-rolled cigar method, and under field conditions for final evaluation and selection of the genotypes. The main results and recommendations arising from the CRP are summarized below according to the following four main outputs.

Protocols for evaluating root traits (architecture and morphology) contributing to enhanced P acquisition from low-P soils.

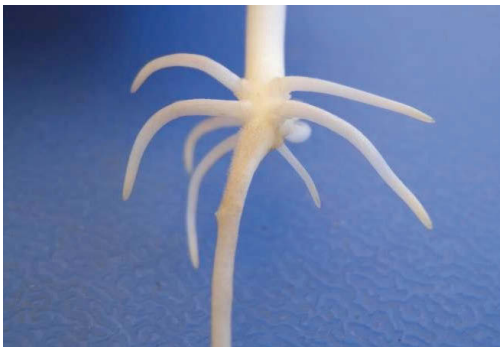
Root architectural phenotype influences P and water acquisition from the soil. Crop genotypes with shallow roots, many basal root whorls, adventitious roots and basal roots have advantages in acquiring P from low P soils, while genotypes with deeper basal roots and longer primary roots will acquire water from deeper soil horizons. Developing protocols which can provide robust and rapid evaluation of RSA traits that enhance N and P acquisition by different crops in the glasshouse and in targeted production environments is vital. A simple visual method to evaluate crop root phenotypes at the early seedling stage using the paper-rolled cigar method and at the late growth stage in the field using shovelomics were developed and validated. This method is available in three different languages at http://roots.psu.edu or http://www.naweb.iaea.org/nafa/swmn/news-swmcn.hmtl. Field phenotyping using shovelomics should be useful for evaluating food crop genotypes for low P and drought tolerance in developing countries. During the CRP, a new version of the SIMROOT model was created for simulating large diversity of root systems. In addition, protocols for fractionation of soil P using ^{32}P to elucidate the mechanisms of P acquisition from different soil P pools were developed and fine-tuned at the Seibersdorf Laboratories to support the CRP.

Validation of screening protocols to select genotypes with superior N and P acquisition and/or utilization

Germplasm of maize, upland rice, common bean, cowpea and soybean were exchanged among the participants or were acquired from four centres belonging to the Consultative Group on International Agricultural Research (CGIAR): soybean and cowpea lines from the International Institute of Tropical Agriculture (IITA), maize from the Centro Internacional de Mejoramiento de Maiz y Trigo (CYMMT), common bean from the Centro Internacional de Agricultura Tropical (CIAT) and upland rice from The African Rice Center [formerly West



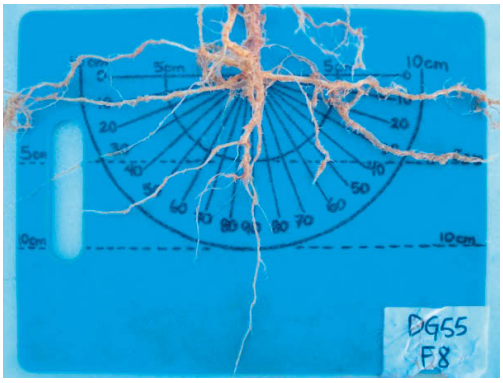
Root Architecture



Basal Root Whorls Number (BRWN)



Root Hairs



Basal Root Growth Angle (BRGA)

FIGURE 2. Root phenotypes associated with genotypic differences in adaptation to low nitrogen and phosphorus.

Africa Rice Development Association (WARDA)]) and other advanced research institutes. One hundred and fifty to two hundred genotypes were screened at the early seedling stage for enhanced N and P acquisition using the paper-rolled cigar method using PVC tubes of length 15.0 cm and 3.4 cm inner diameter (Figure 1). Twenty five genotypes with different abilities to grow in low P and N conditions selected from the different crops at the seedling stages were further evaluated under field conditions (Figure 1) at two or more sites under diverse agro-ecological environments. Data on environmental variables (latitude, longitude, altitude, rainfall and temperature), soil classification and soil physico-chemical characteristics and systems studied were recorded.

Root characteristics evaluated included: basal root whorls number (BRWN), root hair length density (RHLD), basal root growth angle (BRGA), root length (RL), root length density (RLD), root angle (RA), root branching (RB), adventitious root length (ARL), adventitious root number (ARN), adventitious root branching (ARB), basal root length (BRL) basal root number (BRN), basal root branching (BRB), basal root depth (BRD), primary root depth (PRD), primary root branching (PRB), seminal root length (SRL), lateral root length (LRL), lateral root number (LRN), seminal root elongation (SRE), root AM colonization (RAMC) and root biomass (RB). In addition, data were obtained on: shoot biomass (SHB), grain yield (GY), stem diameter (STDIA), leaf area index (LAI), plant height (PLHT), leaf chlorophyll (LCHL) and nodule weight (NODWT) for legumes (Figure 2)

The results from the 16 countries for the five crops showed that:

- branching angle interval and seminal root length were suitable root selection parameters for soil N use efficiency, while adventitious rooting and root hair formation were identified as suitable parameters for selecting P use efficiency.
- P efficiency correlated strongly with genotypic differences in root hair length, root hair plasticity and lateral root length.
- genotypes with more root cortical aerenchyma (RCA) had deeper roots and produced twice the amount of shoot biomass in low N conditions than genotypes with less (RCA). For beans, RHLD, BRGA, BRWN, ARL and BRN were identified as the most suitable traits (Figure 3 and Table 1).

It is concluded that (i) seedling screening tools demonstrated significant genotypic variation for root traits. These included root length, angle, number of axial roots and branching as well as root hair parameters (length and density), (ii) cultivars identified with some of these traits proved superior for uptake of P and N under conditions of nutrient stress, and (iii) cultivars with superior growth, nutrient acquisition and efficiency obtained good yields of grain under conditions of nutrient stress (Figure 3).

Effects of selected genotypes on cropping system performance

The assessment and selection of crop genotypes with different enhanced root characteristics that explore nutrients from different soil depths under field conditions is relevant for promoting food security and long-term sustainability of soil fertility. Five to ten genotypes were further assessed for their productivity in low-input systems. Five rice and maize genotypes were selected that had the highest N use efficiency (66–80 percent) and the highest P use efficiency (6–8 percent), and which also had 15–30 percent greater yields than

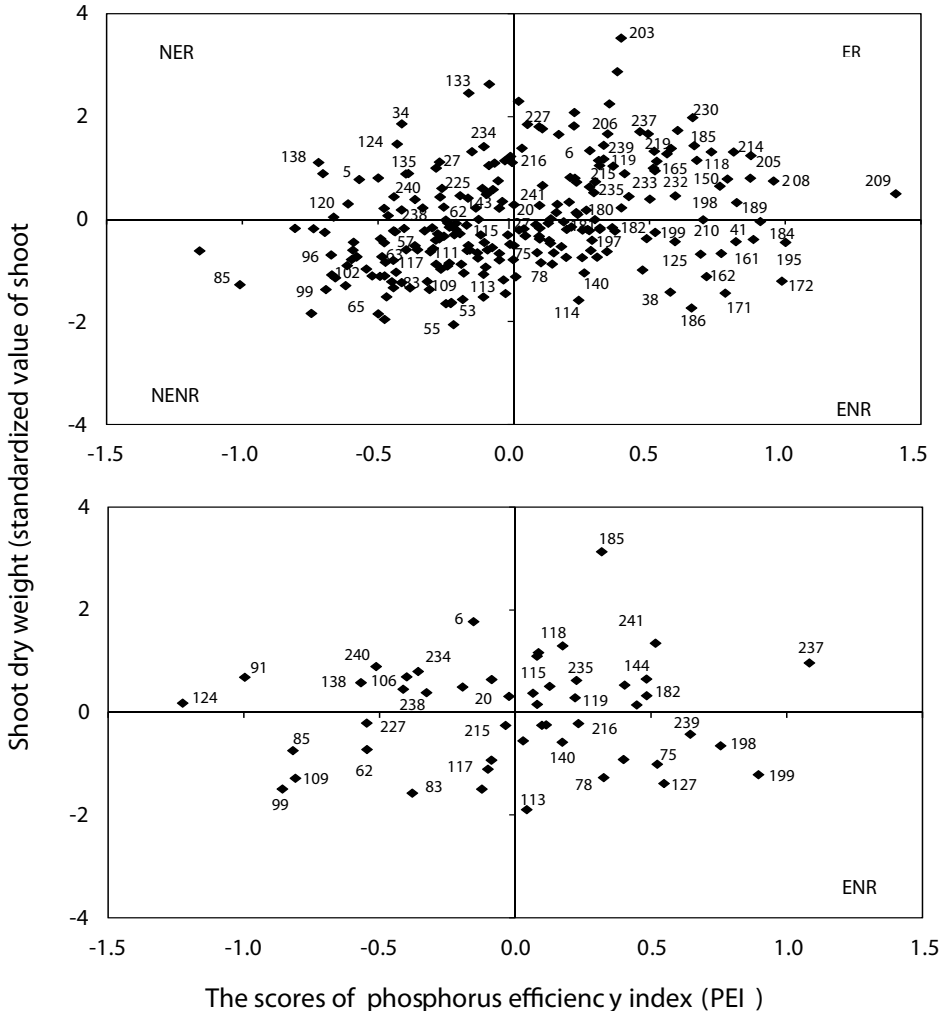


FIGURE 3. Division of charts of 242 and 50 common accessions according to P efficiency and standardized value of shoot dry weight under high P conditions. Phosphorus efficiency is expressed as PEI, which is an assessment index calculated from principal component analysis. Standardized values of shoot dry weight were estimated as the following function: $Xs = (X - \bar{X})/SD$. Categories represented by efficient and responsive (ER), non-efficient and responsive (NER), non-efficient and non-responsive (NENR), and efficient and non-responsive (ENR). Accession numbers are indicated (Bayuelo-Jiménez *et al.*, 2011).

the others. For common bean, soybean and cowpea genotypes were identified with deep root systems produced 20–40 percent better yields, 45 percent increases in biological N_2 fixation (BNF), and 40 percent less soil erosion in low P soils. Phosphorus efficient legumes contributed to soil fertility by enhanced biological BNF, which is quite sensitive to P supply. Economically, the greater productivity of P- and N- efficient genotypes would give third world farmers greater flexibility in soil management options, purchasing fertility inputs etc., in addition to greater household incomes and food security.

Mechanisms for adaptation and high productivity to low soil N and P using isotopic techniques

Nuclear, isotopic and related conventional techniques were employed to obtain quantitative estimates on optimization of plant nutrient (N and P) uptake and utilization from fertilizers and soils. For instance, stable N-15 and radioactive P-32/P-33 techniques were employed to obtain quantitative estimates for optimization of plant nutrient (N and P) uptake or utilization by N and P efficient crop genotypes in low N and P environments. In order to understand the mechanisms of the genotypic tolerance to low P soil to utilize P from the sparing soluble P forms, five maize genotypes selected from 116 maize inbred lines were used in a ^{32}P isotope tracer experiment to follow the recovery of ^{32}P in soil P fractions. The L-value and P availability of soil was also assessed.

The ^{32}P tracer results showed that after the addition of ^{32}P-Pi to the soil with no P fertilizer applied for 25 d, 29.0 percent of ^{32}P was quickly transformed into Ca_2–P (rapidly available P), and 66.1 percent was transformed into Al–P, Fe–P and Ca_8–P (slowly available P). Only 5.0 percent of ^{32}P was transformed into O–P and Ca_{10}–P (plant-unavailable P). Moreover, in the soil with P fertilizer applied, ^{32}P transformation into Ca_2–P increased, and the transformation into Ca_8–P, Fe–P, Al–P, O–P and Ca_{10}–P decreased significantly compared with the soil with no P fertilizer applied ($p < 0.05$). This result suggested a higher rate for water-soluble P transformation to slowly available and plant-unavailable P in P deficient soil than in soil with sufficient P. Low P tolerant cultivar DSY-32 regulated soil P use efficiency and plant P content according to exogenous P fertilizer application. How-

TABLE 1. Phenotypic correlations among nodal root traits and shoot biomass: number of nodal roots (Nodal_No.), nodal root length (Nodal_RL), nodal branching (Nodal_Br), nodal root angle (Nodal_Ra), shoot dry weight (ShDW), and grain yield (Gy)

HP/LP [a]	Nodal_No.	Nodal_RL	Nodal_Br	Nodal_Ra	ShDW	Gy
Experiment 1 (n = 242)						
Nodal_No	0.49***	0.25***	0.39***	0.37***	0.38***	0.10
Nodal_RL	0.42***	0.39***	0.50***	0.48***	–0.09	0.24**
Nodal_Br	0.55***	0.51***	0.49***	0.51***	0.10	0.21**
Nodal_Ra	0.33***	0.31***	0.46***	0.32***	–0.02	0.07
ShDW	0.49***	–0.03	0.21**	–0.02	0.50***	0.09
Gy	0.18*	0.07	0.28**	0.10	0.16	0.68***
Experiments 1 + 2 (n = 50)						
Nodal_No	0.32**	0.36**	0.32**	0.13	0.41**	0.09
Nodal_RL	0.19	0.39***	0.52***	0.31**	0.00	0.21*
Nodal_Br	0.25*	0.54***	0.26*	0.24	–0.03	0.18
Nodal_Ra	0.05	0.21	0.45**	0.32**	0.04	0.25*
ShDW	0.48***	–0.01	–0.01	–0.24*	0.36**	0.05
Gy	0.01	0.38***	0.35**	–0.06	0.02	0.54***

[a] For each environment, values below the diagonal represent correlations within the low P treatment; values above the diagonal represent correlations within the high P treatment; values on the diagonal (italic) correspond to across-phosphorus treatment correlations.

*, **, *** Significant respectively at $p < 0.05$, $p < 0.01$, $p < 0.001$

TABLE 2. L-values of maize genotypes without external P fertilizer

Variety no.	Specific activity		L-value
	Plant (Bq/μg)	Soil (Bq/g · soil)	(μg P/g · soil)
DSY-30	22.4±2.5†	4 216 ± 144	190 ± 28
DSY-2	20.0 ± 0.1	4 484 ± 154	225 ± 7
DSY-32	7.8 ± 1.0	3 965 ± 136	513 ± 45
DSY-79	11.4 ± 0.8	3 572 ± 122	315 ± 32
DSY-48	64.9 ± 2.9	3 623 ± 124	56 ± 1

† Values following means are ± standard errors

TABLE 3. ^{15}N enrichment, amount of N derived from fertilizer and percentage nitrogen use efficiency of upland rice

Variety	^{15}N (atom % excess)		N derived from fertilizer (kg/ha)			% N use efficiency
	Plant	Grain	Plant	Grain	Total	
Nabawan	0.671 a	0.553 a	40.2 de	23.0 bc	63.2 bc	42.1 bc
Tenom	0.721 a	0.614 a	31.3 e	29.7 abc	61.0 c	40.7 c
WRDA 20	0.629 a	0.684 a	27.7 e	41.5 ab	69.2 bc	46.2 bc
WRDA 99	0.731 a	0.696 a	48.3 cde	51.8 a	100.1 abc	66.7 abc
Sintok	0.711 a	0.654 a	64.0 bcd	38.8 ab	102.7 ab	68.5 ab
Pulut Petai	0.770 a	0.758 a	70.6 bc	43.0 ab	113.6 a	75.7 a
Merah	0.729 a	0.714 a	109.3 a	11.7 c	121.0 a	80.7 a
Kuku Belang	0.753 a	0.685 a	78.6 b	21.1 bc	99.7 abc	66.4 abc

Data within a column followed by the same lower case letter are not significantly different ($p < 0.05$)

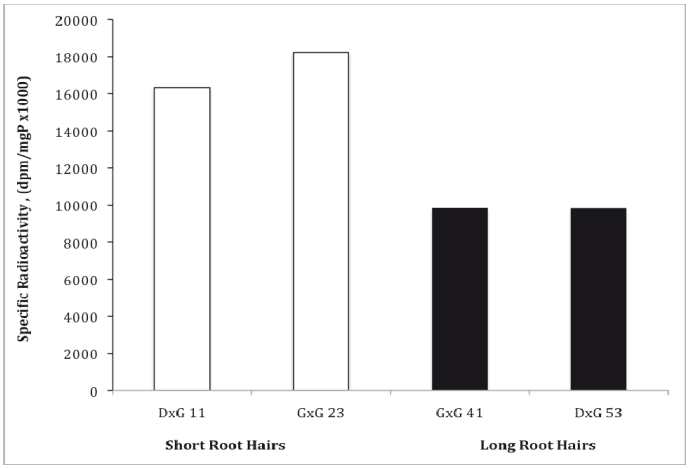


FIGURE 4. Specific radioactivity in plant tissue of four genotypes with contrasting root hairs: Genotypes with short root hairs (open bars) and genotypes with long root hairs (full bars). Genotypes G x G 41 and D x G 53 have long root hairs, while genotypes G x G 23 and D x G 11 have short root hairs.

ever, another low P tolerant cultivar, DSY-2, used soil P more efficiently, regardless of the application of exogenous P (Yang *et al.,* 2012). It was therefore concluded that the ^{32}P tracer technique was a valuable tool for understanding the physiology behind superior genotype performance (Table2).Similar results were reported by Adu-Gyamfi, Aigner and Gludovacz (2009) who showed that in a low-P soil, maize was more efficient than soybean in taking up soil P. The available P (Bray II) and the Ca–P were the fractions most depleted by plants followed by the Fe–P fractions. For common bean, the results showed that efficient genotypes with long root hairs had lower specific activity values compared with inefficient genotypes, since these were able to take up P from two different pools with a greater total P accumulation (Figure 4). For upland rice, ^{15}N and ^{32}P were employed to obtain quantitative estimates for optimization of plant N and P by N and P efficient crop genotypes in low N and P environments. The results presented here showed that (i) the ^{15}N enrichment in plants ranged between 0.629 and 0.753 atom % excess, while the ^{15}N enrichment in upland rice seeds ranged from 0.553 to 0.757 atom % excess. Variety Merah showed the highest N use efficiency in upland rice (80.7 percent) while the lowest N use efficiency was obtained from variety Tenom (40.7 percent). Nitrogen use efficiency by these upland rice genotypes was high (40–80 percent of applied N), with good grain yield (Table 3), while P use efficiency was similar to the other crops (2.4–8 percent) (Data not shown).

CONCLUSIONS

The main conclusions from the CRP are summarized as: (i) seedling screening tools demonstrated significant genotypic variation for root traits; these included root length, angle, number of axial roots and branching as well as root hair parameters (length and density); (ii) cultivars identified with some of these traits proved superior for uptake of P and N under conditions of nutrient stress; (iii) cultivars with superior growth, nutrient acquisition and efficiency had good yields of grain under conditions of nutrient stress; (iv) in some cases, positive agro-ecological outcomes were identified that were related to the performance of cultivars selected for favourable root traits; (vi) nuclear tools, specifically the use of ^{15}N and ^{32}P as tracers, proved valuable in studies that sought physiological explanations for superior genotype performance; and (v) some genotypes of rice, common bean, maize, soybean and cowpea were identified that provide valuable resources for plant breeding programmes aimed at enhancing P and N use efficiency.

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Using Boron Isotope (^{10}B) to Study Boron Uptake and Translocation by Peach and Plum Trees Grown in Sandy and Calcareous Soils Under Different Levels of Calcium

R.A. El-Motaium[1,*], S.H. Badawy[2] and P.H. Brown[3]

ABSTRACT

The objective of this study was to investigate boron (B) uptake and translocation using boron-10 (^{10}B), in response to calcium (Ca) supplementation of the substrate in an attempt to understand B function in plant metabolism. A pot experiment was designed using two-year old Marianna plum and Lovell peach trees. They were grown in 20 litre (L) pots filled with sandy or calcareous soil. Treatments included two concentrations of B (2.5 and 5.0 mg/kg) and three concentrations of Ca (80, 200 and 400 mg/kg). Boron was applied as H_3BO_3 enriched in ^{10}B and Ca as $(CH_3COO)_2$ $Ca.H_2O$. The pots were arranged in a completely randomized design. Leaf, stem, fine root, root tip and root tip cell wall samples were collected at the end of the growing season for determination of total B, ^{10}B and Ca. Plant samples were extracted using 0.20 N HNO_3 and analysed using an inductively coupled plasma mass spectrometer (ICP/MS). Cell wall extraction was performed on 0.5–1.0 g fresh weight of the root tips. Results indicated that in sandy soil, there was an inverse relationship between B concentration in the nutrient solution and plant dry weight. However, as Ca concentration in the nutrient solution increased the dry weight increased. Analysis of variance showed that both B and Ca in the irrigation solution had significant effects on B partitioning in the three plant organs. Calcium application at 200 and 400 mg/kg reduced the B concentration significantly in the leaf and stem but linearly increased B concentration in the fine root. Under calcareous soil, less B was recorded in the leaf and stem but more in the fine root compared with its concentrations in the plants grown in sandy soil. The root tips maintained the highest B concentration. In both peach and plum the ^{10}B:^{11}B ratio increased as B concentration in the nutrient solution increased. However, Ca application decreased the ^{10}B:^{11}B ratio in the leaf indicating less B was translocated to the leaf. There were significant differences between B concentrations in Lovell peach and Marianna plum trees. Marianna accumulated less B than Lovell in the leaf and stem but more B in the root tips. Under calcareous soil, less B was translocated to the shoot than under sandy soil. The lower B translocation to the shoot system could be explained by the high fixation capacity (precipitation and adsorption) of calcium carbonate to B under calcareous soil. Boron and Ca levels in the root tip cell wall showed that Ca followed the same pattern as B. As Ca concentration in the irrigation solution increased (400 mg/kg), significant increases occurred in the B concentration in root tip cell walls. The percent increase of B in the cell wall reached about five- and seven-fold of its concentration at low Ca level (80 mg/kg) in sandy and calcareous soil, respectively.

Key words: *stable isotope, boron-10, ICP/MS, cell wall, boron partitioning, root tip.*

INTRODUCTION

Egypt is one of the countries most affected by climate change. It is located in an arid/semi-arid zone, north of the Equator, characterized by low erratic rainfall, high temperature and periodic drought. As a consequence, levels of boron (B) are often high in soils and groundwater and can reach toxic levels in plants causing crop yield reduction. Reclamation of high B soils requires about three times as much water as reclamation of saline soils (Keren and Bingham, 1985).

Since 1923, B was established as an essential element (Warington, 1923) and significant advances have been made in understanding its functions in plant metabolism (Brown *et al*., 2002). These include synthesis of plant cell walls, involvement in reproductive growth and development (flowering and fruit set), in phenol metabolism, influence on photosynthesis, role in membrane structure and function, involvement in membrane electron transport (Brown *et al*., 2002) and role in cross-linking of pectin substances in the cell wall (Kobayashi, Matoh and Azuma, 1996; O'Neill *et al.,* 2004).

The functions of calcium (Ca) in plant metabolism are: maintenance of the integrity of cell membranes including permeability, stability and selective ion uptake (Mengel and Kirkby, 1982). Calcium is also required for cell elongation, cell division (Burstrom, 1968), pollen germination and growth of pollen tubes. Calcium increases the rigidity of the cell wall by binding with pectin (Burstrom, 1968).

Functional similarities between Ca and B have also been established, the main one being their absence from the phloem sap and their extracellular apoplastic function (Marschner, 1995). Boron and Ca move along the transpiration stream and they share the same deficiency symptoms (Crisp *et al.,* 1976). They both exist in the same location in the plant cell wall (Marschner, 1995) and play similar roles in lignification. Both are involved in the basipetal transport of the plant hormone indole acetic acid (IAA; Tang and Dela Fuente, 1986).

There have been difficulties in studying B uptake and mobilization in higher plants due mainly to the lack of radioactive isotope for

1 Plant Research Department, Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt
2 Soil Sciences Department, Faculty of Agriculture, Cairo University, Egypt
3 Department of Plant Sciences, The University of California, Davis, USA
* E-mail address of corresponding author: elmotaiumr@yahoo.com

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B. Several researchers have shown the possibility of using boron-10 (^{10}B) as a tracer to study B uptake and transport in plants (Martini and Thellier, 1980). Also, the development of inductively coupled plasma mass spectrometry (ICP-MS) and the use of the enriched ^{10}B isotope have made it possible to study B uptake and movement within the plant.

Boron adsorbing surfaces in soils include: aluminum and iron oxides, clay minerals, calcium carbonate and organic matter (Goldberg, 1997). Calcium carbonate acts as an important B adsorbing surface in calcareous soils (Goldberg and Forster, 1991). Retention of B on calcium carbonate results from an adsorption mechanism (Ichikuni and Kikuchi, 1972) which could involve exchange with carbonate groups.

A limited amount of work has been carried out on the internal distribution of B in plants although it has been shown to be present in the free space, cytoplasm, vacuole and cell wall (Thellier, Duval and Demarty, 1979).

The objectives of this research were to study: (i) the uptake, translocation and partitioning of B in plants at the organ and cellular levels (compartmentation of B) using ^{10}B, (ii) the interaction between B and Ca in plants, and (iii) the use of Ca supplementation approach as a substitute for leaching to reclaim soils high in B. It was anticipated that the results might shed light on the functions of B in plant metabolism.

MATERIALS AND METHODS

Two-year-old Mariana plum (local variety) and Lovell peach (imported variety) plants were grown in 20 L pots filled with either sandy or calcareous soil. Lovell peach and Marianna plum were chosen because they have proven to be the most sensitive rootstocks to high B concentrations in the substrate (El-Motaium *et al.,* 1994). The general properties of the soils used are given in Table 1. The original concentrations of hot water extractable B and available Ca were determined in the two soils. These were respectively 1.3 and 1.4 mg/kg, and 0.25 and 20 mM for the sandy and calcareous soils.

Treatments included two levels of B (2.5 and 5.0 mg/kg) and three levels of Ca (80, 200 and 400 mg/kg). Boron was applied as H_3BO_3 with enriched ^{10}B and Ca as $(CH_3COO)_2$ $Ca.H_2O$. Modified half-strength Hoagland (Johnson *et al.,* 1957) solution was applied as the irrigation solution.

The pots were arranged in a completely randomized design, a treatment being assigned randomly to each pot and each treatment was replicated eight times. Plants were grown for six months (March–August). Plants were separated into leaf, stem, fine roots and root tips. Leaves of Marianna plum were separated from the stem in three different categories: young leaves, at the top portions of the stems, mature leaves, at the middle portions of the stems, and old leaves, at the bottom portions of the stems.

Determination of total B, ^{10}B and Ca of the three organs was performed after oven drying at 65°C for 24 h. Root tips were collected from the fine roots for determination of total B, ^{10}B and Ca. Plant samples were extracted using 0.2 N HNO_3 and analysed using ICP/MS. To test for the accuracy of the method, a standard reference material was used. Cell wall extraction was performed on 0.5–1.0 g fresh weight of the root tips according to Selvendran and O'Neill (1987). The experiment was repeated for two years using new trees each year.

RESULTS AND DISCUSSION

Plant growth

Plant growth was measured as leaf, stem and root dry weight (Table 2). The data showed an inverse relationship between B concentration in the nutrient solution and plant dry weight. As the B concentra-

Table 1. Chemical and physical characteristics of sandy and calcareous soils

Soil	pH	EC (dS/m)	OM (%)	$CaCO_3$ (%)	CEC (meq 100g)	WHC (%)	BD (g/cm)	Clay (%)	HWE (mM)	Ca Avail. (mM)	Texture
Sandy	7.5	0.40	0.45	1.40	1.45	12.5	1.40	1.53	0.13	0.25	Sand
Calcareous	8.4	0.75	0.60	45.0	5.32	26.6	1.30	5.60	0.14	20.0	Loamy sand

SE — saturation extract; EC — electrical conductivity; OM — organic matter (%); CEC — cation exchange capacity; WHC — water holding capacity; BD — bulk density; and HWE — hot water extract

Table 2. Dry weight (g) of plum and peach organs grown in sandy and calcareous soils as affected by B and Ca in the substrate

		Sandy						Calcareous					
		Leaf		Stem		Fine root		Leaf		Stem		Fine root	
	Ca (mg/kg)	B (mg/kg)						B (mg/kg)					
		2.5	5	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5
Plum	80	20	15	55	50	90	75	30	20	60	56	105	90
	200	25	20	65	57	120	100	40	28	70	61	140	120
	400	30	25	70	65	140	125	50	38	80	72	170	150
LSD(0.05)	B	1.88		2.01		2.87		2.01		2.48		2.01	
	Ca	2.30		2.46		3.52		2.46		3.04		2.46	
Peach	80	40	30	50	40	150	130	50	36	56	45	160	140
	200	45	33	60	50	200	160	55	44	65	55	230	180
	400	52	37	70	60	250	200	62	50	75	66	270	220
LSD(0.05)	B	2.01		2.22		5.67		2.01		3.19		6.80	
	Ca	2.46		2.72		6.95		2.46		3.91		8.33	

tion in the nutrient solution increased the dry weight decreased significantly in the three organs. However, as the Ca concentration in the media increased the dry weight increased significantly. The same trends were noted for plants grown in sandy and in calcareous soils.

In both plum and peach trees, B and Ca levels in the substrate had significant effects on the dry weight of leaf, stem and root. Boron had a greater effect on the dry weight of plant organs than Ca. In fine roots, B levels in the substrate increased as root dry weight decreased but Ca increased fine root dry weight significantly at both B levels. The effect of Ca on dry weight was more pronounced in the fine roots. This was not only related to the Ca effect but also to the high pH (8.4) particularly in the calcareous soil. The data indicated some kind of binding between B and Ca that could result in the formation of non-toxic compounds. Trees showed a stunted appearance under high B but vigorous growth when higher amounts of Ca were added to the substrate.

Boron partitioning in the different plant organs

Analysis of variance showed that both B and Ca in the irrigation solution had significant effects on B partitioning in the three plant organs (leaf, stem and fine root). Boron concentrations in leaf, stem and fine root increased as the B concentration in the irrigation solution increased. However, at the same B concentration (2.5 or 5.0 mg/kg), B concentrations in the three organs were considerably modified by levels of Ca in the irrigation solution. Boron was concentrated mainly in the fine root and less was translocated to the shoot under high Ca application rate.

Calcium application at 200 and 400 mg/kg reduced significantly B concentrations in the leaf and stem but linearly increased B concentration in the root (Table 3). Calcium application at 200 and 400 mg/kg reduced significantly the B concentrations in leaf and stem compared with the 80 mg/kg concentration. By contrast, B accumulation in the fine root increased significantly with increasing Ca supplementation. Similarly, as the Ca concentration in the irrigation solution increased its concentration in the fine root increased (data not shown). Calcium in the irrigation solution increased significantly the concentration of B in the fine root. This increase was linearly related to the increase in Ca in the substrate. These results applied to both species.

In the calcareous soil, the concentration of B was lower in the leaf and stem but higher in the root compared with the corresponding values in sandy soil. The root tips maintained the highest B concentration. The lower B content of the shoot system could be explained by the high B fixing capacity of calcium carbonate (precipitation and adsorption) in the calcareous soil. The higher B concentration in the root was presumably due to the higher pH and higher background Ca in the soil.

The correlation coefficients confirmed our previous finding. Highly significant correlation coefficients were found between B concentrations in the plant leaf and fine root ($p = 0.01$) and their dry matter yield ($r = -0.977$ and $+0.959$, respectively) (Figure 1). The cor-

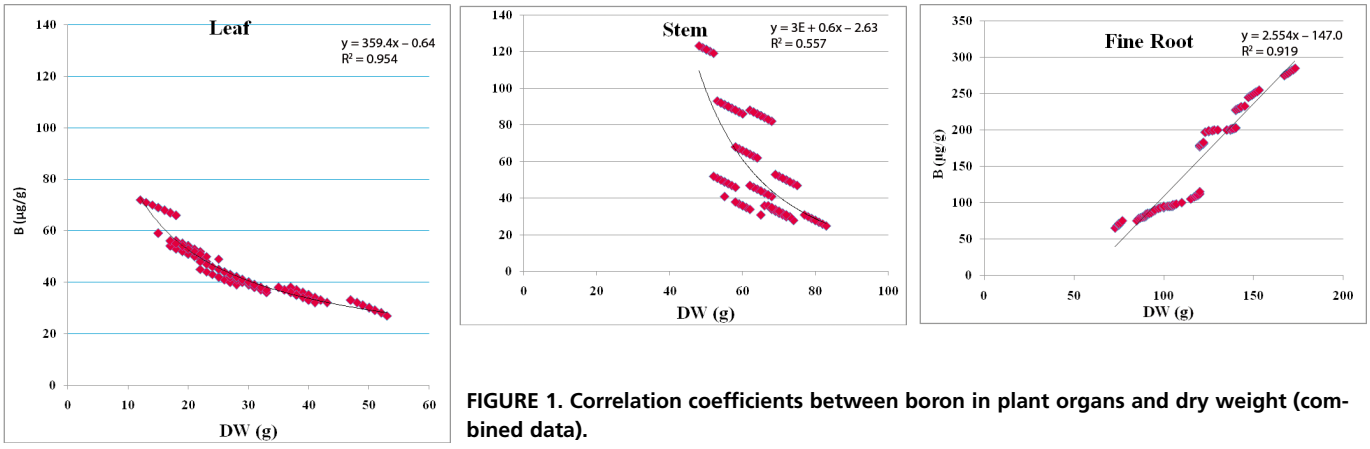


FIGURE 1. Correlation coefficients between boron in plant organs and dry weight (combined data).

Table 3. Total B content (mg/kg) of plum and peach organs grown in sandy and calcareous soils as affected by B and Ca in the substrate

		Sandy						Calcareous					
		Leaf		Stem		Fine root		Leaf		Stem		Fine root	
	Ca (mg/kg)	B (mg/kg)						B (mg/kg)					
Plum		2.5	5	2.5	5	2.5	5	2.5	5.0	2.5	5	2.5	5
	80	54	69	49	121	80	95	40	53	36	90	73	95
	200	42	51	44	89	110	250	35	42	33	65	100	200
	400	39	45	32	85	200	280	30	35	28	50	180	250
LSD(0.05)	B	2.26		2.01		5.75		2.01		2.78		6.86	
	Ca	2.77		2.46		7.04		2.46		3.41		8.41	
Peach	80	80	100	70	130	80	100	53	75	55	100	70	85
	200	60	80	65	100	110	220	45	60	45	75	95	200
	400	50	65	55	80	180	270	38	50	35	60	160	230
LSD 0.05)	B	2.48		3.22		7.89		2.22		1.88		3.75	
	Ca	3.04		3.94		9.66		2.72		2.30		4.59	

Table 4. 10B uptake and long distance transport of plum and peach organs grown in sandy and calcareous soils as affected by B and Ca in the substrate

		Sandy						Calcareous					
		Leaf		Stem		Fine root		Leaf		Stem		Fine root	
	Ca (mg/kg)	B (mg/kg)						B (mg/kg)					
		2.5	5	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5
Plum	80	26	34	25	65	55	58	20	28	18	45	42	50
	200	21	25	22	50	109	143	17	21	16	32	90	100
	400	18	22	16	42	120	150	15	17	14	25	100	125
LSD(0.05)	B	1.62		2.37		3.02		2.18		1.84		4.02	
	Ca	1.99		2.91		3.70		2.67		2.25		4.93	
Peach	80	40	50	35	65	47	55	26	37	27	50	21	25
	200	30	40	31	50	91	130	22	30	22	37	45	50
	400	25	31	26	40	110	150	19	25	17	30	60	80
LSD(0.05)	B	2.22		2.33		3.22		2.65		2.10		2.41	
	Ca	2.72		2.86		3.94		3.25		2.57		2.95	

Table 5. B content (mg/kg) in the root tip cell wall of plum and peach organs grown in sandy and calcareous soils as affected by B and Ca in the substrate

	B:Ca (mg/kg)	Sandy			Calcareous		
		Root tip	Cell wall	% increase	Root tip	Cell wall	% increase
Plum	2.5:80	111	19		120	30	
	2.5:200	218	38	200	230	60	200
	2.5:400	242	100	526	280	150	500
	5:80	130	48		160	50	
	5:200	286	145	322	295	180	360
	5:400	310	270	555	380	350	700
LSD(0.05)		9.27	9.50	27.75	15.41	9.22	39.10
Peach	2.5:80	95	16		100	20	
	2.5:200	190	33	206	200	60	300
	2.5:400	220	80	500	240	105	525
	5:80	110	36		120	40	
	5:200	240	100	278	260	120	300
	5: 400	300	200	555	360	280	700
LSD(0.05)		28.59	6.08	30.14	9.64	6.08	49.14

relation was negative in leaf but positive in root, whereas the stem B concentration correlated significantly with the dry matter yield. The correlation coefficient matched well with B toxicity symptoms which were concentrated mainly in the leaf (leaf margins and midrib) followed by less marked symptoms in the stem. The positive correlation between B content and root dry weight indicates the possibility of the formation of B–Ca complex in the cell wall that is non-toxic.

^{10}B uptake and long distance transport

Boron (^{10}B) uptake and long distance transport was affected significantly by the B and Ca concentrations in the substrate. The uptake and translocation of ^{10}B was shown to be a function of the total B level.

As boron concentration in the substrate increased, high ^{10}B activity was recorded in the three organs, indicating that large amounts of ^{10}B were taken up and translocated. However, as the Ca concentration in the substrate increased, reduced ^{10}B levels were recorded in the leaf and stem indicating that less ^{10}B was translocated to the two organs (Table 4). The lower the Ca concentration in the substrate the higher was the ^{10}B activity, but as Ca supplementation of the substrate increased, ^{10}B levels decreased.

Plum trees accumulated less B than peach trees in leaf and stem but higher levels in the fine root. Significant differences were recorded for boron uptake and translocation between Ca concentrations (200 and 4000 mg/kg) in the irrigation solution. In the leaf, the effect of Ca on B translocation was mainly linear.

In the calcareous soil, less B was translocated to the leaf and stem but more was taken up by the root compared with the corresponding values on sandy soil. The root tips maintained the highest B concentration (Table 5). The lower B content of the shoot system could be explained by the high B fixation capacity (precipitation and adsorption) of calcium carbonate in the calcareous soil.

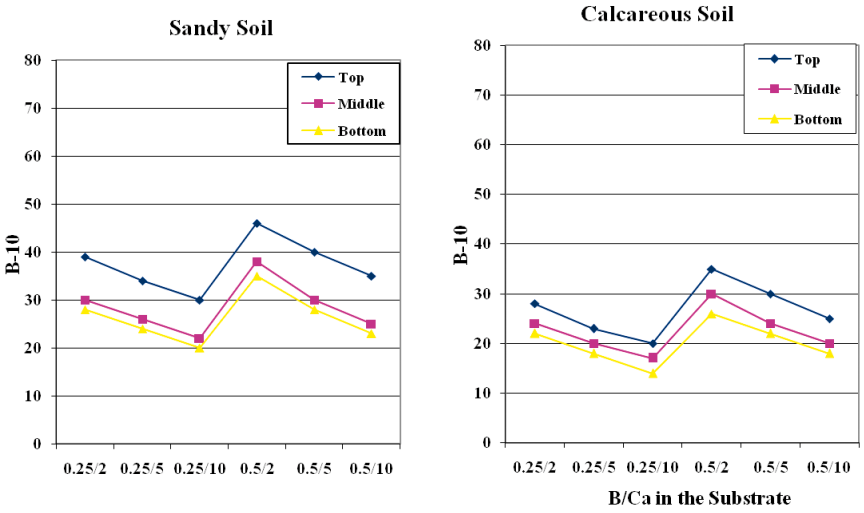


FIGURE 2. Effect of leaf age on B-10 translocation to plum leaf grown in sandy and calcareous soils.

Data for ^{10}B showed that the younger the leaf the more B was translocated. Concentrations of ^{10}B showed the following descending order: top > middle > bottom (Figure 2). ^{10}B activities were higher in the top (young leaf) followed by the middle (mature leaf) then the bottom (old leaf). These date indicate greater B translocation to the young leaf than to the older ones. However, as Ca levels in the substrate increased, the translocation of B was reduced.

These results provide evidence of passive B transportation via the transpiration stream. Young leaves have higher transpiration rates than older leaves due to less lignification, and as a result ^{10}B levels were higher in the young top leaf.

Boron and calcium accumulation in the cell walls of root tips

Boron accumulation in the root tip increased dramatically with Ca supplementation, most noticeably at levels of 200 and 400 mg/kg Ca in the substrate (Table 5).

Concerning B concentration in the root tip and its cell wall, the data indicated that as B and Ca levels in the substrate increased, B in the root tip increased significantly. Calcium in the irrigation solution increased B concentration significantly in the root tip as well as in its cell walls. Calcium in the root tip and cell walls followed the same pattern as B, with levels in the tissue being a function of levels in the substrate. At both B levels, there was a significant increase in B concentration in the cell wall at 400 mg/kg Ca compared with the values at 80 mg/kg Ca. The maximum increases in B and Ca in the root tip and in its cell walls were with the highest Ca application rate.

As the Ca concentration in the irrigation solution increased (400 mg/kg) there was a significant increase in B concentration in the cell wall (Table 5). Calcium in the cell wall of the root tips followed the same pattern as B, the percentage increase of B in the cell wall (at high Ca application rate, 400 mg/kg) reaching respectively about five and seven times its concentration at low Ca application rate (80 mg/kg) in sandy and calcareous soils.

CONCLUSIONS

Boron-10 and ICP-MS are good tools to study B uptake and translocation in plants. Boron concentrations in leaf, stem and root were modified significantly by levels of Ca in the substrate. Under high Ca and B applications, both elements accumulated in the cell walls of the root tip. This confirms that B and Ca have an extracellular function in the cell wall. Both elements could form a complex in the cell wall that prevents B transport to the shoot system and thereby reduces its toxicity

In a calcareous soil, less B was taken up and translocated to the shoot system. This indicates that high levels of B would have less harmful effects on plants grown on such soils. In arid regions, where soil and/or groundwater B levels are high, Ca supplementation could be used to reclaim such soils to reduce the adverse effect of B on plant growth.

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The Use of Isotopic Techniques to Quantify the Potential Contributions of Legumes to the Mitigation of Climate Change and Future Food Security

M.B. Peoples[1,*], D.F. Khan[2] and I.R.P. Fillery[3]

ABSTRACT

The large number of diverse legume species used to produce food for humans and livestock hold considerable promise as a means of adaptation to climate change. Legume systems have also been implicated as effective means of mitigating climate change through reductions in emissions of greenhouse gases such as carbon dioxide (CO_2) from fossil fuels and nitrous oxide (N_2O) from soil, and by accelerating soil carbon (C) sequestration. However, predicting how elevated concentrations of carbon dioxide (e[CO_2]) and future climates will affect soil nitrogen (N) cycling, soil C accretion and productivity in legume based farming systems remains a challenge. This paper identifies a number of opportunities where the isotopes of N and C could be applied to provide information on the key interaction between e[CO_2] and climate variables on the capacity for legumes to fix atmospheric N_2, influence the above and below ground allocation of C and N, and change the C and N dynamics of soils.

Key words: *legume agroecosystems, climate change, nitrogen and carbon isotopes, N fixation, N_2O emissions, C sequestration.*

INTRODUCTION

Legumes contribute directly to food security by providing protein-rich food for humans and livestock, and indirectly by enhancing the grain yield of following cereal crops (e.g. Espinoza *et al.,* 2012; Seymour *et al.,* 2012). Legumes also provide a renewable source of organic nitrogen (N) input for soils via their ability to fix atmospheric nitrogen (N_2) in symbiosis with rhizobia (Fillery 2001; Peoples *et al.,* 2009a). Annual global inputs of biologically fixed N by legumes in agricultural systems represent between 33–46 million tonnes (t) of N (Herridge, Peoples and Boddey, 2008), and it is widely recognised that the inclusion of N_2 fixing legumes in cropping sequences helps mitigate climate change by decreasing the fertilizer N requirements of subsequent food crops and thereby reducing the net emissions of fossil fuel derived carbon dioxide (CO_2; Jensen *et al.,* 2012). Some circumstantial data also indicate that legume-based systems may be responsible for lowering the emissions of the potent greenhouse gas nitrous oxide (N_2O), and contributing to soil carbon (C) sequestration (Jensen *et al.,* 2012). However, additional research is required to fully define the extent to which legumes can assist in mitigating climate change. There are many uncertainties about how legumes and soil management might need to be adapted to ensure adequate food production in the future when atmospheric concentrations of CO_2 and ambient temperatures are expected to be higher, and rainfall to be more variable, than experienced today (Tuiello *et al.,* 2007; Vadez *et al.,* 2012). The aim of this paper is to review the existing scientific literature to identify gaps in knowledge and to propose research opportunities where the isotopes of N and/or C could be employed to improve our understanding of the potential future role for legume systems.

The role of legumes in food security and adaptation to future climates

There are 20 000 species of legumes, several hundred of which have been used as traditional foods for humans or livestock in different geographic regions of the world. However, much of the 300 million hectares (ha) of crop and forage legumes grown globally each year is dominated by just 20–30 species (Herridge *et al.,* 2008). It has long been recognised that beyond these major species there are many underexploited legumes that hold considerable promise of providing valuable sources of grain, vegetables, fruits, root crops, forage or green manure with further domestication and selection (e.g. ~200 species listed in NAS 1975 and NAS 1979). Many of these underexploited species are already adapted across a wide range of environments, including dry climates and impoverished soils, and represent an untapped pool of genetic material to respond to future climate challenges. In principle there should be enough diversity within the large number of legume species with either the right phenology to minimize exposure to climatic stresses, or with suitable tolerances to temperature extremes, or the incurions of new diseases and pests in regions where climate change threatens the reliability of food supply (Vadez *et al.,* 2012). The indeterminate growth patterns of many legumes and their multi-purpose nature (i.e. grain, forage or green manure) could also assist in adapting to climate change where future farming systems will need to continue to produce food, generate income and protect the soil resource in the face of increased

1 CSIRO Sustainable Agriculture Flagship, CSIRO Plant Industry GPO Box 1600 Canberra, ACT 2601, Australia
2 Agricultural Research Station Bannu, Model Farm Service Centre Bannu, Bannu Township, Khyber Pakhtunkhwa (formerly North West Frontier Province), Pakistan
3 CSIRO Sustainable Agriculture Flagship, CSIRO Plant Industry Private Bag 5 Wembley, WA 6014, Australia
* E-mail address of corresponding author: mark.peoples@csiro.au

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climatic variability (Vadez *et al.,* 2012). Furthermore, it has been demonstrated that legumes tend not to exhibit the marked decline in foliage N content and seed protein that is observed in grasses and cereals growing under elevated concentrations of atmospheric CO_2 (e[CO_2]; Rogers, Ainsworth and Leakey, 2009; Lam *et al.,* 2012b), so in respect to nutritional security, legumes could help offset the anticipated reduction in protein intake in cereal-based human diets and forage provided to livestock.

Since many of the benefits derived from including legumes in farming systems arise from their ability to fixed atmospheric N_2 and to contribute to soil N fertility (Fillery, 2001; Peoples *et al.,* 2009a) it will be necessary to confirm that these benefits will continue to accrue under changing environmental conditions. The following sections consider how nitrogen-15 (^{15}N) techniques might be used to provide new information on the impact of future climates, particularly e[CO_2], on biological N_2 fixation and N cycling in legume-based farming systems. Since the amounts of N_2 fixed by legumes are generally closely related to biomass production (Unkovich *et al.,* 2010), discussion will also be included on whether carbon-13 (^{13}C) discrimination can be utilized as a means of comparing the efficiency of water use by different legume germplasm.

Legume inputs of fixed N

There are two main stable isotopes of N, nitrogen-14 (^{14}N) and ^{15}N. Of these, the lighter isotope, ^{14}N, is naturally more abundant than ^{15}N. If the ^{15}N concentration of the two main sources of N used by legumes for growth — namely atmospheric N_2 and soil N differ sufficiently then it is possible to calculate the proportion of the legume N accumulated during growth that was derived from N_2 fixation on the basis of the comparison of the ^{15}N composition of the legume with that of a non-N_2 fixing reference plant which is assumed to provide a measure of the ^{15}N signature of the plant-available soil N (Peoples *et al.,* 2009b). With appropriate analytical procedures and a suitably precise mass spectrometers it is possible to utilise the slight elevation in ^{15}N abundance of plant-available soil N (0.3673–0.3733 atom% ^{15}N) relative to atmospheric N_2 (0.3663 atom% ^{15}N) that occurs naturally in many soils. It is also possible to obtain or generate different sources of N that are artificially enriched in ^{15}N (5–99 atom% ^{15}N) that can be added to the soil to expand the difference in ^{15}N compositions of soil N and atmospheric N_2 to facilitate measures of N_2 fixation (Peoples *et al.,* 2009b). Both ^{15}N approaches have been applied to quantify N_2 fixation in many legume studies comparing legumes grown with ambient or e[CO_2]. In the majority of cases e[CO_2] was found to result in a stimulation of N_2 fixation as the result of the increased legume demand for N associated with enhanced plant growth (Roger *et al.,* 2009). Meta-analysis of the results of 127 studies suggests that N_2 fixation could be increased by 35–40 percent on average under e[CO_2] equivalent to twice current levels (Lam *et al.,* 2012b).

However, much of the data reviewed by Roger *et al.* (2009) and Lam *et al.* (2012b) came from short-term CO_2 enrichment studies, generally with only one legume variety and often with only one of the dominate legume species currently used in agriculture. Generally, little is known about the degree of genetic variation in the potential magnitude of response either within, or between, legume species, and certainly almost nothing is known about the symbiotic capacity of undomesticated species which may need to be exploited in future. The only data we are aware of which examine genetic differences in legume responses to e[CO_2] come from recent comparisons of:

(i) Five field pea varieties at an Australian FACE (Free Air CO_2 Enrichment) field facility which indicated considerable differences in productivity gains between varieties (from < 10 to > 40 percent) under e[CO_2] (550 ppm) compared with the ambient conditions (380 ppm; Fitzgerald, Brand and Mollah, 2012);

(ii) Two soybean cultivars at a FACE system in northern China which showed no significant effect of e[CO_2] on N_2 fixation by one cultivar (~80 kg fixed N/ha under both treatments), but a 66 percent stimulation (from 166 to 275 kg fixed N/ha) by the other (Lam *et al.,* 2012c);

(iii) The impact of soil type and levels of nutrition on the performance of the crop legumes field pea and chickpea, and the pasture legume barrel medic (*Medicago truncatula*). This glasshouse study showed a large influence of soil type on the relative responses by the three species with e[CO_2] with N_2 fixation increasing by 20–86 percent for chickpea, 44–51 percent for field pea, and 114–250 percent for barrel medic (Lam *et al.,* 2012a).

To complicate matters the climate changes projected for future decades could well modify, and may often limit, the direct CO_2 effects on crop and pasture productivity and N_2 fixation (Tubiello, Soussana and Howden, 2007). For example, there is evidence that higher N_2 fixation experienced under e[CO_2] can be lost in the presence of higher temperatures (Lilley *et al.,* 2001), or if plant growth is resticted by low soil phosphorus availability (Edwards, McCaffery and Evans, 2006; Lam *et al.,* 2012a). Several Free Air CO_2 Enrichment studies have also now observed "progressive N limitation" and longer-term "acclimation responses" to e[CO_2] which result in a decline in plant response to CO_2 and the amount of N harvested in biomass over time so that yield responses are much smaller than previously expected (Leakey *et al.,* 2009; Newton *et al.,* 2010). However, the effects on soil N availability may not necessarily occur if e[CO_2] is accompanied by a 2°C warming (Hovenden *et al.,* 2008).

Isotopic methodologies need to be employed to fully evaluate adaptation strategies. Especially to: (a) explore genetic variation in N_2 fixation response to e[CO_2] across a much wider range of legume species, (b) study the interdependence of C and N metabolism in legumes under different combinations of nutrition, temperature and water regimes, (c) quantify the impact of the timing of environmental stresses on N_2 fixation, and (d) determine the role of indeterminate growth patterns may play in the resilience of legume productivity and capacity to fixed N in the face of climate change.

Identifying more efficient use of variable rainfall

Most assessments of anticipated climate change impacts on agriculture predict more variable rainfall and an increased frequency of climatic extremes (Tubiello *et al.,* 2007). The major function of stomata is to maximize the rate at which CO_2 can diffuse into the leaf for photosynthesis while minimizing the simultaneous loss of water vapour. There is overwhelming evidence that e[CO_2] decreases both stomatal conductance and plant transpiration, and consequently is expected to improve plant water use efficiency (WUE; i.e. kg plant dry matter accumulated per mm rainfall; Leakey *et al.,* 2009). Nonetheless, it will still be necessary for researchers to be able to evaluate what effect management and environmental variables might have on the WUE of different legume germplasm when developing the most appropriate strategies to adapt legumes to climate change (Vadez *et al.,* 2012).

It has been demonstrated that plant species with the C_3 photosynthetic pathway discriminate against carbon-13 (^{13}C)O_2 during photosynthesis, and that there is less discrimination delta carbon-13 ($\Delta^{13}C$) in plant species or varieties which have a greater transpiration efficiency (Farquhar and Richards, 1984). The improved WUE results from lower stomatal conductance, greater photosynthetic capacity, or a combination of the two effects. The close inverse relationship between $\Delta^{13}C$ and transpiration efficiency has been successfully utilized in cereals to identify genetic material with intrinsically high WUE

TABLE 1. Examples of estimates of the below-ground partitioning of legume N derived from ^{15}N shoot-labelling techniques (derived from Khan et al., 2002; Unkovich, Baldock and Peoples, 2010)

Species	Common names	% Total plant N below-ground
Cajanus cajan	Pigeonpea	32–47
Cicer arietinum	Chickpea	48–68
Glycine max	Soybean	38
Lupinus angustifolius	Narrow leaf lupin	28–35
Ornithopus compressus	Yellow serradella	30–46
Pisum sativum	Field pea	32
Trifolium subterraneum	Subterranean clover/subclover	42
Vicia faba	Faba bean	24–40
Vigna radiata	Mung bean	20–39

TABLE 2. The use of ^{15}N methodologies to quantify the fate of faba bean N remaining in shoot residues or nodulated roots after grain harvest over a subsequent cropping cycle in an Australian rainfed system where wheat was grown following the faba bean crop (unpublished data, Khan, 2000)

Crop component	Faba bean N remaining after harvest (kg N/ha)	Faba bean N recovered by following wheat[1] (kg N/ha)	Faba bean N recovered in the soil (kg N/ha)	Faba bean N assumed to be lost after 1 year (kg N/ha)
Shoot residues	44	2 (4%)[2]	22 (50%)[2]	20 (45%)[2]
Below ground	51	4 (8%)[2]	43 (84%)[2]	4 (8%)[2]

[1] Total wheat N content at maturity represented 97 kg N/ha.

[2] Values in brackets represent the percentage of the faba bean N originally present.

(e.g. Condon *et al.,* 2004; IAEA, 2012). The $\Delta^{13}C$ approach has also been used as: (a) supporting data for better WUE by tall conventional field pea to semi-dwarf types (Armstrong, Pate and Tennant, 1994), (b) a means to compare WUE by five cowpea (*Vigna unguiculata*) genotypes grown in different densities on mixed culture with sorghum (*Sorghum bicolor*; Makoi, Chimphango and Dakora, 2010), and (c) a tool to select cowpea (Hall, Thiaw and Krieg, 1994) and peanut (groundnut, *Arachis hypogoea*; Hubick, Farquhar and Shorter, 1986; Wright, Rao and Farquhar, 1994) germplasm with more drought tolerance. Unfortunately, the use of leaf ^{13}C discrimination to screen for variations in plant WUE does not appear to be applicable to chickpea, lentil, or narrow leaf lupin (Turner *et al.,* 2007).

Before genetic variabilty in WUE can be explored further using the $\Delta^{13}C$ technique it will be necessary to explain the apparent inconsistencies observed between legume species. At very least it would be instructive to compare species where $\Delta^{13}C$ seems to be a useful guide to WUE with those where it apparently does not under the same conditions. This could help define the experimental and sampling protocols where the technique is likely to be most reliable.

The partitioning of legume N and the subsequent fate of legume N

Various ^{15}N-enrichment approaches have been used for many decades to investigate the fate of legume shoot N following a legume phase in a cropping sequence and to quantify the subsequent uptake of legume N by following cereal crops (e.g. Thompson and Fillery, 1997; Russell and Fillery 1999; Peoples *et al.,* 2009a). It has only been in recent years that different *in situ* ^{15}N shoot labelling techniques have been developed to quantify the above- and below-ground partitioning of legume N in the glasshouse (McNeill, Zhu and Fillery, 1998; Khan *et al.,* 2002) and field (Russell and Fillery 1996; Khan *et al.,* 2003; McNeill and Fillery, 2008). Collectively, these studies indicate that between 24 and 68 percent of the total N accumulated by crop or pasture legumes over a growing season may be associated with, or derived from, the nodulated roots (Table 1). This pool of below-ground N has often been ignored in previous research. Although such ^{15}N shoot labelling methodologies could also be utilized to follow the fate of above- and below-ground legume N separately, there have been relatively few attempts to do so. Data available from rainfed cropping systems in Australia using ^{15}N shoot labelling methods suggest that between 8 and 15 percent of the below-ground N from crop legumes may be recovered by a subsequent wheat crop (*Triticum aestivum*) which represented a 2–5 fold greater uptake than the N contributed from above-ground residues in the same trials (Table 2; McNeill and Fillery, 2008; Peoples *et al.,* 2009a). There is also some evidence that the below-ground pool of N could be less susceptible to loss processes than shoot residues (Table 2). These observations contrast with data collected from agroforestry systems which, while reporting comparable levels of partitioning of below-ground N for a range of woody perennial legume species (34–51 percent of total plant N; IAEA, 2008) as determined for crop and pasture legumes (Table 2), found lower recoveries of below-ground legume N by maize (*Zea mays*) than from legume shoot prunings (Seiter and Horwarth, 1999; IAEA, 2008). It was concluded that in agroforestry systems below-ground legume N played a more important role in contributing to soil structural stability than nutrient supply (IAEA, 2008).

Few studies have ascertained what effect e[CO_2] may have on the below-ground partitioning of legume N. Increased root nodulation has often been observed under e[CO_2] (Roger *et al.,* 2009; Lam *et al.,* 2012b), although circumstantial evidence based on comparisons of soil ^{15}N natural abundance following soybean grown under ambient and e[CO_2] suggests that e[CO_2] could result in a net decrease in below-ground allocation of biologically fixed N (Decock *et al.,* 2012), and no significant effect of atmospheric CO_2 concen-

tration was observed in the proportion of total plant N partitioned below-ground in a recent pot study with field pea (Lam *et al.,* 2013).

It is also not clear what influence high CO_2 environments might have on the availability of legume N from either shoot or root residues for the benefit of other crops. It has been concluded from a number of (predominantly) non-legume studies that the gross N mineralization rates in soil tend to be unaffected by e[CO_2], but N immobilization by the microbial biomass can be 30 percent greater than under current ambient conditions (van Groenigen *et al.,* 2006). This is consistent with observations of progressive N limitations in long-term e[CO_2] treatments (Newton *et al.,* 2010). We are aware of only one study that has specifically investigated the effect of e[CO_2] on the recovery of legume N, and in this case wheat derived much less of its N supply from a previous field pea crop under e[CO_2] than ambient conditions (11 percent *cf* 20 percent, respectively; Lam *et al*., 2013).

Clearly there are opportunities to apply *in situ* ^{15}N shoot labelling techniques to a wider range of legumes and systems to assess the above- and below-ground partitioning of legume N, and to quantify the contribution of both sources to the N assimilated by following crops under both ambient and e[CO_2] conditions.

The role of legumes in mitigation of climate change

Contributions of legume N to N_2O emissions

The soil emissions of N_2O from legume systems collated from the published literature (1.29 kg N_2O-N/ha on average) tend to be lower than measurements from N fertlized crops and pastures (4.49 kg N_2O-N/ha on average; Table 3). Jensen *et al.* (2012) concluded from such data that legume systems emit less N_2O than where fertilizer N is used, and that emissions under legumes may be comparable with those derived from native soil organic N (1.20 kg N_2O-N/ha; Table 3). Yet only a few of these data came from direct comparisons of legume or fertilizer sources undertaken within the same study, and many experiments did not include appropriate controls so it was not always possible to identify conclusively the actual source of the N_2O (i.e. endogenous soil N, legume or fertilizer N). Neither is there much information on N_2O emissions from legume residues or unutilized N fertilizer beyond the first subsequent growing season (Jensen *et al.,* 2012). Legume residues enriched in ^{15}N could be used to address this shortcoming and to allow the specific losses of legume N as N_2O to be quantified.

There are also very few measures of the impact of e[CO_2] on N_2O emissions from legume systems. A number of e[CO_2] trials supplied with N fertilizer indicate higher N_2O emissions (Lam *et al.,* 2012b). However, in the absence of additions of fertilizer N there are several potentially counteracting factors that can influence the extent of N_2O losses. Emission could be higher with e[CO_2] because of an association between microbial mediated N_2O losses and enhanced availability of soil C substrate (Lam *et al.,* 2012b), and the improved WUE by plants growing with e[CO_2] resulting in higher soil water contents and possibly resulting in an increased risk of denitrification (Leakey *et al.,* 2009). But N_2O emissions due to denitrification could also be lower due to e[CO_2] enhanced microbial immobilization of available forms of soil N reducing the likelihood of nitrate accumulation (van Groenigen *et al.,* 2006). A recent report from the SoyFACE facility in Illinois in the USA indicated no major effects of e[CO_2] on field measures of N_2O emissions from soybean growing in a a soybean-maize rotation (Decock *et al.,* 2012). The question remains whether this result is widely representative of other legume systems.

Contributions to soil C sequestration

Several long-term studies have demonstrated significant increases in soil C stocks where legumes have been included in farming systems (Jensen *et al.,* 2012). However, it is not clear whether the additional C had come directly from the legume component, or whether the soil organic C was present in labile or stable forms. Whether or not the soil organic C is vulnerable to short-term losses has important repercussions for land sustainability and the success of climate change mitigation strategies (Tuiello *et al.,* 2007). The well described differences in discrimination of ^{13}C during photosynthesis in C_4 plants ($\delta^{13}C$ ~ –13‰) compared with C_3 plants ($\delta^{13}C$ ~ –27‰) can be used to determine the proportion of soil C derived from C_4 or C_3 species. The advantage of this approach is that the effect of C_3 plants on C sequestration in mixed or rotation systems can be assessed using existing land use systems. For example, a study comparing continuous maize with a maize–lucerne (alfalfa, *Medicago sativa*) system showed that maize residues contribute < 15 percent and lucerne > 50 percent of the soil C (Gregorich, Drury and Baldock, 2001). Nevertheless, it should be noted that isotopic fractionation of ^{13}C during decomposition of different plant residues could confound the interpretation of data derived from analyses of the ^{13}C natural abundance of soil organic matter (Schweizer, Farr and Cadisch, 1999).

Pulse-labelling legumes with C isotopes is another approach that can be used to quantify the contribution of legume biomass to soil C sequestration. Although it is simpler to use ^{13}C, the comparatively high natural abundance of this stable isotope typically requires use of expensive highly enriched ^{13}C compounds to improve the precision of measurements of changes in ^{13}C content in soil C pools. Because of its very low natural abundance it is often more convenient to use ^{14}C to quantify the above- and below-ground allocation of C, describe the effect of the presence or absence of legumes on soil C sequestration and estimate flow of C through different soil OM pools (e.g. Bhupinderpal-Singh, Hedley and Sagger, 2005; Sanaullah *et al.,* 2012).

TABLE 3. Summary of field measurements of N_2O emissions from legume and N fertilized systems (derived from data presented by Jensen *et al.,* 2012)

Land use	Number of site-years of data collated	kg N_2O-N/ha per growing season or year	
		Range	Mean
Legume-based pasture	25	0.10–4.57	1.38
N fertilized grass pasture	19	0.30–18.16	4.49
Crop legume	46	0.03–7.09	1.02
N fettilized crop	48	0.09–12.67	2.71
No legume or added N	33	0.03–4.80	1.20

The effect of e[CO_2] on soil C has proved to be quite variable across different studies, but it appears that the rate of N supply is a key factor influencing changes in total soil C (van Groenigen *et al.,* 2006). No significant difference in soil C content between ambient and e[CO_2] treatments tend to occur with annual N inputs < 30 kg N/ha, but an 8 percent increase in soil C can result under e[CO_2] where > 150 kg N/ha was applied (van Groenigen *et al.,* 2006). Therefore, inputs of fixed N and high N legume residues should encourage C accretion (Jensen *et al.,* 2012). However, inputs of nutrients such as phosphorus, and sulphur in addition to N could be crucial in determining the rate of change in soil C stocks (Kirkby *et al.,* 2011). In many non-legume systems much of the increase in soil C with e[CO_2] seems to be in labile pools of C rather than stable forms (van Groenigen *et al.,* 2006). When determining the role legumes can play in mitigating climate change it will be important to resolve how vulnerable the accumulated legume organic C in soil may be to short-term losses, and this is a significant gap in current knowledge.

Isotopic methods could be applied to a range of legume crops or pasture legumes under different management scenarios to: (a) quantify the below-ground partitioning of C and the change of distribution of that C in nodulated roots, soil microbial biomass and different soil organic C fractions over time, (b) identify genotype x management combinations to accelerate the rate of soil C sequestration, (c) determine how management influences whether legume C is predominantly accumulated in labile (e.g. particulate organic matter) or stable (humus) soil C pools, and (d) investigate the impact of the C-rich legumes residues generated under e[CO_2] on soil microbial activity, and the ability of soils to store C.

CONCLUSIONS

The use of N and C isotopes could greatly assist in resolving some of the uncertainties concerning how e[CO2] and future climates could affect soil N cycling in legume agroecosystems and enable a better informed assessment of the potential for legumes to contribute to global food security.

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Contribution of Nitrogen from Biofertilizer Inoculum to Young Oil Palm under Field Conditions

F.A.A. Zakry[1,2,*], K.A. Rahim[3], Z.H. Shamsuddin[1], Z.Z. Zakaria[4] and A.A. Rahim[1]

ABSTRACT

Continuous and excessive use of chemical fertilizers leads to deterioration of soil and environmental health especially under tropical climates which could accelerate losses of nutrients in the soil through leaching and other processes. The use of plant growth–promoting rhizobacteria (PGPR) as a biofertilizer inoculant could potentially mitigate the problem. However, the effectiveness of microbial inoculants in the field is still questionable. The present study was conducted on young oil palms to compare the contribution of nitrogen (N) through N_2 fixation from a PGPR biofertilizer with that from chemical fertilizer using the nitrogen-15 (^{15}N) isotopic dilution method. The results indicated that inoculated young oil palm accumulated 63.4 percent N through the activities of biofertilizer microorganisms, similar to uninoculated young oil palms which were fertilized totally by chemical fertilizer and accumulated 58.8 percent N. Therefore, biofertilizer inoculant can be potentially exploited for improving food crop productivity and environmental health.

Key words: *biofertilizer, biological nitrogen fixation, Elaeis guineensis Jacq., fertilizer, inoculation, ^{15}N isotope dilution, PGPR.*

INTRODUCTION

Improper and excessive use of synthetic fertilizers have damaged the environment, bringing about water pollution from algal blooms which are detrimental to aquaculture (Shumway, 1990; Tanga *et al*., 2003), increased greenhouse gas emissions (Zou *et al*., 2005; Kim and Dale, 2008), groundwater pollution which negatively affects water quality for human consumption (Johnson, Adams and Perry, 1991) and soil acidification which makes soil less fertile for agricultural production (Campbell *et al*., 1995; Barak *et al*., 1997). These impacts are cascading, difficult to manage, and cost billions of dollars to rehabilitate. Oil palm is highly traded on international markets and generates large incomes for countries like Malaysia and Indonesia which are major oil palm producers. An estimated 74 percent of global palm oil usage is for food products and 24 percent is for industrial purposes (USDA, 2010). Since the 1990s, the area occupied by oil palm cultivation has expanded by around 43 percent worldwide driven mainly by demand from India, China and the European Union (RSPO, 2011). However, the cultivation of oil palm contributes to ecosystem imbalances through activities such as manuring since oil palm is a nutrient-demanding crop.

Application of biofertilizer containing plant growth-promoting rhizobacteria for agricultural production is seen as one of the potential solutions to mitigate the harmful effects of synthetic fertilizers (Mohamed and Babiker, 2012; Khan *et al.,* 2012) since biofertilizers could reduce dependency for synthetic fertilizer and may even be an alternative. Plant growth-promoting rhizobacteria (PGPR) belong to several genera namely *Azospirillum*, *Bacillus*, *Pseudomonas* and several others (Esquivel-Cote *et al*., 2010; Mia *et al*., 2010; Prasanna *et al*., 2011; Aziz *et al*., 2012). Plant growth promotion by rhizobacteria involves several direct and indirect mechanisms such as biological nitrogen (N_2) fixation, phosphate solubilization, phytohormone production and antagonism against plant diseases (Mia *et al*., 2010; Aziz *et al*., 2012; Sayyed *et al.,* 2012; Zakry *et al.,* 2012). However, the value of biofertilizers for commercial agricultural production especially in oil palm is still in doubt. Questions have been raised, for example, about their cost effectiveness in delivering nutrients to the crop as compared with synthetic or inorganic fertilizer applications and also due to inconsistent results obtained from field experiments (El-Sirafya *et al*., 2006).

The present study was conducted using the nitrogen-15 (^{15}N) isotope dilution method to estimate N accumulation by field grown young oil palms from a biofertilizer containing diazotrophic plant growth promoting rhizobacteria and to compare this with amounts accumulated from a conventional nitrogenous fertilizer in delivering N nutrition

MATERIALS AND METHODS

The experiment was conducted in a field plot at Tangkah Estate, Sime Darby Plantation Berhad (formerly Golden Hope Plantation Berhad), Tangkak, Johor, in southern Peninsular Malaysia (2° 21′ N, 102° 40′ E). Soil chemical data are presented in Table 1. Fourteen-month-old GH500 cloned oil palms were established for five months after transplantation in the field. The upkeep and maintenance of the trial plots included a normal estate fertilizer application schedule of inorganic fertilizers, comprising N as ammonium sulphate, phosphorus (P) as Christmas Island rock phosphate, potassium (K) as muriate of

1 Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
2 Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus, 97008 Bintulu, Sarawak, Malaysia
3 Agrotechnology and Biosciences Division, Malaysian Nuclear Agency, Bangi, 43000 Kajang, Selangor, Malaysia
4 Biology Division, Malaysian Palm Oil Board, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia
* E-mail address of corresponding author: zakryfitri@upm.edu.my

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TABLE 1. Chemical properties of the soil[2] (Ultisol) from the oil palm experimental field

pH (KCl)	Available (mg/kg)				
(1:2.5)	Total N	P[1]	K	Ca	Mg
4.7	16.0	22.3	19.0	55.0	15.0

[1] Extracted with an aqueous solution of 0.05 M HCl and 0.0125 M H_2SO_4

[2] Bungor sandy clay loam soil with 1.2 percent total carbon content

potash, magnesium (Mg) as kieserite and boron (B) as borate (Goh and Härdter, 2003).

Bacillus sphaericus UPMB-10, isolated in Malaysia from oil palm roots (Amir *et al.,* 2003), was sub-cultured on tryptic soy agar (TSA) (Merck KGaA) to produce a pure mother culture for inoculum production as described by Zakry *et al*. (2012). The minimum population of strain UPMB-10 was ≥ 10^9 colony-forming unit (cfu)/g during field inoculation.

In the field, the plants were laid down in a randomized complete block design with four treatments and four replicates (Figure 1).

The four treatments include (1) the un-inoculated + 0 percent N + ^{15}N, (2) un-inoculated + 100 percent N + ^{15}N, (3) inoculated + 67 percent N + ^{15}N, and (4) un-inoculated + 67 percent N + ^{15}N. Treatments (1) and (2) served as negative and positive controls and also as benchmarks for deficient N (negative control) and optimum N (positive control). The treatment (3) involved inoculation with *B. sphaericus* strain UPMB-10 inoculum. Treatment (4) had a similar N rate (67 percent) to the inoculated treatment. All un-inoculated treatments were provided with killed inoculum (gamma-irradiated at 50 kGy) per palm. The "100 percent N" and "67 percent N" refer respectively to the full and 67 percent recommended inorganic N fertilizer application rates as described by Zakry *et al*. (2012).

Recordings were made from 16 palms for each of the 16 plots. Palms in the two outermost rows served as a buffer. The ^{15}N-labelled fertilizer used was $(^{15}NH_4)_2SO_4$ (ammonium sulphate) with 10.13 atom percent ^{15}N excess serving as a tracer. The field experiment was initiated by the application of ^{15}N-labelled fertilizer five months after transplanting. Within the 16 recording palms, two palms (micro-plot) received labelled ^{15}N fertilizer, with 10.13 at. percent ^{15}N ammonium sulphate at a rate of 1 g·N/m^2. The ^{15}N-labelled fertilizer was applied uniformly in liquid form using 2 L distilled water per isotopic plot of 1 m^2 area. The plots were then covered evenly with black polythene sheets to reduce ^{15}N-labelled fertilizer loss. A week later, the black polythene sheets at the ^{15}N isotopic micro-plots were removed after the inner surface of each sheet was rinsed with water prior to inoculum application. The black polythene sheets were used once only for all inoculated and un-inoculated ^{15}N isotopic micro-plots. Inoculum for the first inoculation was then applied followed by the second inoculation four months later. The inoculated +67 percent N + ^{15}N treatment was carried out at a rate of 2 kg inoculum (containing more than 10^9 cfu/g *B. sphaericus* UPMB-10) by raking the surface of the soil to a depth of approximately 5 cm within an area of 1 m^2, and at a rate equivalent to 296 kg/ha.

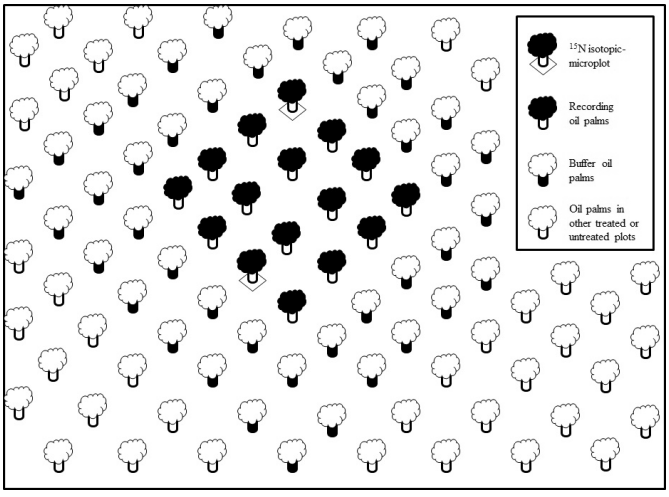


FIGURE 1. A plot with 16 recording oil palms (including two randomly selected palms receiving ^{15}N-labelled fertilizer) and two outermost rows serving as a buffer. The buffer oil palms help to prevent cross contamination between plots. They were treated the same as the recording palms in the ^{15}N isotopic-micro-plot. Recording palms were also used to conduct vegetative growth measurements (Zakry *et al.,* 2012).

Harvesting was carried out 240 days (8 months) after application of the ^{15}N-labelled fertilizer. Four palms from each treatment were harvested destructively, and separated into leaflets, rachis, stems and roots. The major roots were extracted with a backhoe tractor, and the remaining roots were excavated by shovelling and sieving the soil within the area occupied by the harvested palm. Fresh biomass and oven-dried (70°C for 72 h) sub-samples were weighed and recorded. Samples were ground to pass through 0.5 mm sieves and analysed for total N by the semi-micro Kjeldahl method (Bremner, 1996) and ^{15}N excess using an NOI-6PC emission spectrometer at the Malaysian Nuclear Agency, Bangi. The ^{15}N abundance found in palm tissue was corrected for the atom percent^{15}N excess present in the atmosphere (0.3663 at. percent^{15}Ne) (Warembourg, 1993).

Nitrogen fixation in the whole palm was calculated from weighted atom excess (WAE) in the inoculated palm (inoculated + 67 percent N + ^{15}N) and un-inoculated palm (un-inoculated + 67 percent N + ^{15}N), using the following formula (Zakry *et al.,* 2012):

$$WAE = \frac{AE(Lf) \times TN(Lf) + AE(Rc) \times TN(Rc) + AE(St) \times TN(St) + AE(Rt) \times TN(Rt)}{TN\ (Lf + Rc + St + Rt)} \times 100$$

where AE, TN, Lf, Rc, St and Rt refer respectively to atom percent ^{15}N excess in total N, leaflets, rachis, stems and roots.

To determine the proportions of N from the unlabelled fertilizer (percent Ndff [normal fertilizer]), labelled fertilizer (percent Ndff [^{15}N fertilizer]) and from the soil (percent Ndfs), the following formula was used:

$$\%Ndff\ (^{15}N\ fertilizer) = \frac{atom\ \%^{15}N\ excess\ in\ plant\ tissue}{atom\ \%^{15}N\ excess\ in\ labelled\ fertilizer} \times 100$$

$$\%\ Ndfs = 100 - \%Ndff\ (^{15}N\ fertilizer)$$

$$\%\ Ndff\ (normal\ fertilizer) = 100 - \%Ndff\ (^{15}N\ fertilizer) - \%Ndfs$$

RESULTS AND DISCUSSION

After 240 days of plant growth, the inoculated young oil palm had accumulated 0.23 percent N from the ^{15}N-labelled fertilizer (%Ndff) and 36.40 percent N from the soil (%Ndfs), besides the 63.37 percent from N_2 in the atmosphere (% Ndfa) (Figure 2). The un-inoculated young oil palm fertilized at the usual rate accumulated 0.20 percent Ndff (^{15}N fertilizer), 40.97 percent Ndfs and 58.82 percent Ndff (normal fertilizer). On average, the inoculated young oil palms had accumulated slightly higher amounts of N from the atmosphere than the fully fertilized young oil palms that received fertilizer-N at 58.82 percent (Figure 2). These results indicate that young oil palms that received biofertilizer containing *B. sphaericus*

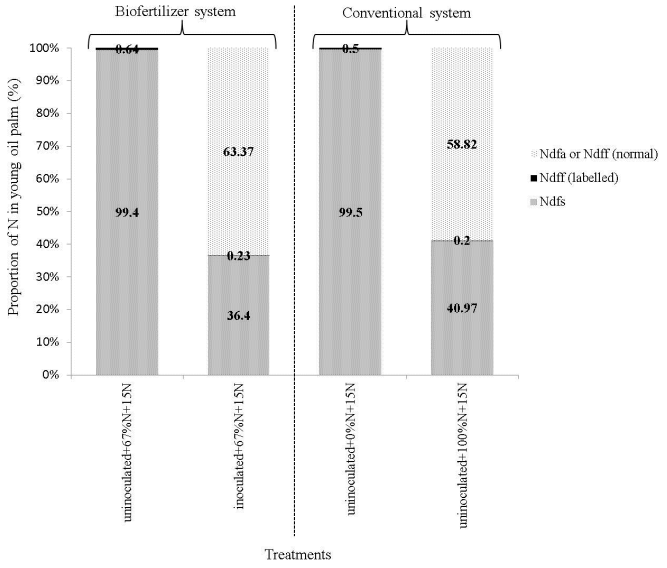


FIGURE 2. Percentages of N derived from atmosphere (%Ndfa [normal]), N derived from normal fertilizer (%Ndff), N derived from labelled fertilizer (%Ndff [labelled]) and N derived from soil (%Ndfs) after 240 d inoculation. Comparison of two systems: biofertilizer system (biofertilizer containing diazotroph *B. sphaericus* strain UPMB-10) and conventional system (the recommended and commonly practiced fertilizer programme at the oil palm estate).

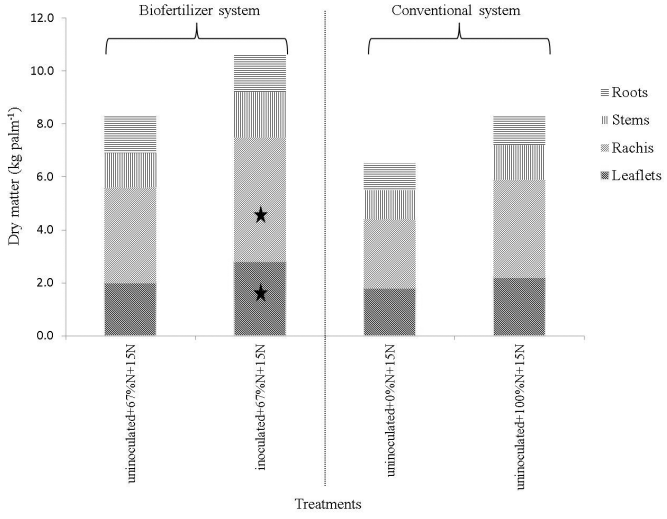


FIGURE 3. Dry matter yield and its distribution in immature young oil palm. Black star refers to $p < 0.05$ versus un-inoculated+67%N + ^{15}N (control) using one-way ANOVA and Dunnett's *post-hoc* test. Rachis and leaflet dry matter increased significantly in inoculated young oil palm compared with un-inoculated young oil palm. Leaflets, rachis, stems and root dry matter levels of inoculated young oil palm were similar to fully fertilized young oil palm and may be even better. Adapted from Zakry *et al.* (2012).

UPMB-10 had accumulated around the same percentage of N from the atmosphere as trees given normal fertilizer.

Values for the former may be an underestimate since the biofertilizer containing *B. sphaericus* UPMB-10 also improved dry matter and N yields and its distribution in immature young oil palm (Figures 3 and 4).

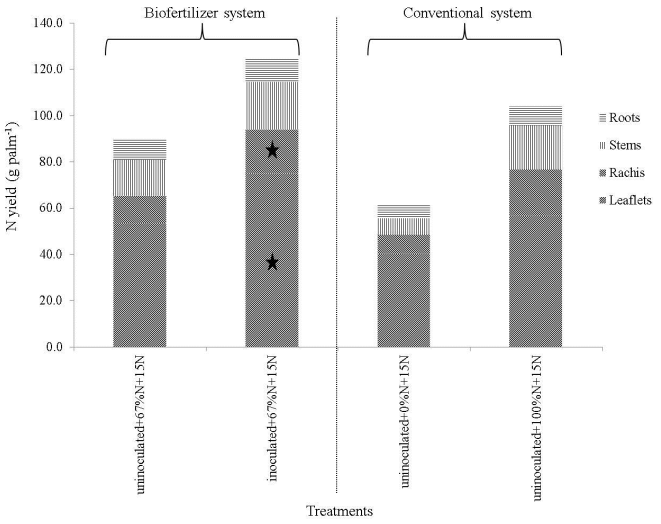


FIGURE 4. Total N yield and its distribution in immature young oil palm. Black star refers to $p < 0.05$ versus un-inoculated+67%N + ^{15}N (control) using one-way ANOVA and Dunnett's *post-hoc* test. Rachis and leaflet N yields increased significantly in inoculated compared with un-inoculated young oil palm young oil palm. Leaflet, rachis, stem and root N yield of inoculated young oil palm were similar to fully fertilized young oil palm and may be even better. Adapted from Zakry *et al.* (2012).

For example, rachis and leaflet dry matter levels increased significantly in inoculated compared with un-inoculated young oil palm, while stem and root dry matter accumulation of inoculated and un-inoculated young oil palm were similar. Increments in dry matter accumulation were paralleled by similar and consistent increases in total N yields of young immature oil palm. Rachis and leaflet N uptake were significantly higher in inoculated than in un-inoculated plants. These results indicate that increased N uptake by oil palms especially to rachis and leaflets which are the most nutrient demanding parts of oil palm and where photosynthetic activity is most active. Photosynthetic rate correlated positively with total leaf N content and subsequently contributed to vegetative growth (Cassman, Peng and Kropff, 1995). In addition, Shaobing, Daniel and Fekade (1991) reported that leaf photosynthetic rate in sorghum correlated significantly with biomass and grain production.

These results suggest that biofertilizers hold great promise for commercial application and this is supported by other studies which demonstrate their effectiveness in promoting nutrient uptake and plant growth. For example, Ahmad *et al.* (2008) conducted two-year pot and field trials which revealed that the organic fertilizer supplemented with 88 kg N/ha was as effective as the full dose of N fertilizer (175 kg/ha) in increasing root weight, fresh biomass, and ear and grain yields of maize. Interestingly, biofertilizer supplemented either with 88 or 132 kg N/ha significantly increased the growth and yield of maize over the full recommended rate of N fertilizer and was superior to organic fertilizer. Adesemoye, Torbert and Kloepper (2009) reported that application of a mixture of PGPR strains of *Bacillus amyloliquefaciens* IN937a and *Bacillus pumilus* T4 (a formulated PGPR product), and the arbuscular mycorrhiza fungus (AMF) *Glomus intraradices* and supplemented with 75 percent of the recommended fertilizer rate produced plant growth rates, yields, and N and phosphorus (P) uptakes that were equivalent to the full recommended fertilizer rate without inoculants.

The advantage of biologically fixed N_2 over fertilizer N may be related to the characteristics of N supply by the two modes of application. *Bacillus sphaericus* UPMB-10 fixes N_2 directly from the atmosphere within the plant itself, so "uptake" is almost complete (100 percent) with no losses to the environment. However, much of the fertilizer N applied may be lost (volatilized, denitrified and leached) (Bijay-Singh, Yadvinder-Singh and Sekhon, 1995) or simply remain in the soil unabsorbed. For example, applying urea will generally only lead to a recovery of less than 50 percent by the plants (Halvorson *et al*., 2002). Moreover, biofertilizer application has been shown to be more cost effective in terms of fertilizer management than conventional inorganic fertilizer application. The present study indicated that the cost of fertilizer N is potentially reduced by approximately 63 percent of total plant N requirement through biological N_2 fixation (Figure 2). Biofertilizer is an organic input containing living microorganisms that act with "flexibility" and differ from chemical fertilizers which deliver nutrients to the plant in a direct and inflexible manner. In effect, biofertilizers act according to plant need and insufficient nutrient in the soil will be rectified or rebalanced through biochemical–microbial action, i.e. biological N_2 fixation, biodegradation of organic material etc. This is different from the chemical fertilizer mechanism which can be ineffective and even harmful to the plant ecosystem. For example, inappropriate application of chemical fertilizers, especially excessive applications, will acidify the plant–soil ecosystem.

Therefore, the use of biofertilizers in agricultural production can be exploited for promoting environmentally friendly and sustainable agricultural crop production and environmental health. Although the present study demonstrated that biofertilizer containing PGPR *Bacillus sphaericus* strain UPMB-10 can deliver nutrients efficiently, its effect on crop productivity is still unclear. Thus, future field trials involving mature palms need to be conducted to evaluate the effect of biofertilizer inoculation on growth, yield and oil productivity in oil palm.

CONCLUSIONS

Comparison of N uptake from N_2 fixation using PGPR biofertilizer and chemical fertilizer showed that inoculated young oil palm accumulated 63.4 percent N through the activities of biofertilizer microorganisms compared to 58.8 percent N in un-inoculated and applied with chemical fertilizer. Therefore, biofertilizer inoculant can be potentially exploited for improving food crop productivity and environmental health. Further field studies are required to assess and quantify the role of biofertilizers on N uptake by mature oil palm leaves.

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Legume Nitrogen Derived from Different Sources as Affected by Rhizobial Inoculant in a Bangladesh Soil

M.E. Haque[1,*] and M.A. Sattar[1]

ABSTRACT

Field experiments were conducted to study the effect of rhizobial inoculant on the quantity of legume nitrogen (N) derived from different sources using nitrogen-15 (^{15}N) tracer. Three different legumes (lentil, mungbean and soybean) were tested with and without rhizobial inoculant in ^{15}N isotope treated micro-plots laid out in a split-plot arrangement and a randomized complete block design with three replications and a non-nodulated wheat crop included as reference in a separate plot. Results showed that soybean produced the maximum number of nodules (33.4 nodules per plant) and the highest nodule dry weight (190.5 mg/plant) under inoculated conditions. The maximum total N recorded in inoculated soybean seed and stover were 112.6 and 77.0 kg N/ha, respectively, while the minimum values of 23.0 and 18.1 kg N/ha were obtained from un-inoculated mungbean. N derived from the atmosphere (Ndfa) ranged from 25.9 kg N/ha in un-inoculated mungbean to 159.6 kg N/ha in inoculated soybean. Due to inoculation, Ndfa increased by 123.87, 118.52 and 212.18 percent in lentil, mungbean and soybean, respectively. The maximum N derived from the soil (Ndfs) was 34.3 kg N/ha in un-inoculated soybean, while the inoculated mungbean derived the lowest N (10.4 kg N/ha) from the soil. The study revealed that all legumes received more N from fertilizer sources under un-inoculated than inoculated conditions. However, under inoculated conditions most (80.7–84.1 percent) of total plant N came from the atmosphere. With inoculation, comparatively less N was taken by the legume plants from soil (12.2–14.8 percent) and fertilizer (3.6–4.3 percent) sources.

Key words: *legume, nitrogen sources, rhizobial inoculant.*

INTRODUCTION

Managing nitrogen (N) inputs in crop production systems to achieve economic and environmental sustainability is a major challenge facing agriculture. Relying less on commercial fertilizer N and more on biological N_2 fixation by legumes has been suggested as a way to meet this challenge (Keeney and Nelson, 1982). The N benefits of legume-rhizobium symbiosis include N_2 fixation and mineralization, sparing of soil inorganic N and reduced immobilization of soil inorganic N (Fedorova *et al.*, 2005). Symbiotic N_2 fixation enhances soil fertility and productivity and increases carbon sequestration and nutrient conservation (Morgan, 1997). Efficient utilization of symbiotic N_2 fixation in agricultural practice is one of the important strategies for establishing sustainable agriculture in the 21st century. Using rhizobia inoculants can be a key part of accelerating rehabilitation of degraded land and ecosystem functions, enhancing survival and growth of plants and reducing costs in establishment and maintenance.

Declining soil fertility, particularly N, is recognized as a major threat to continued rice/cereal cropping in Bangladeshi soils, especially on the Tista Meander floodplain. It is widely believed that legumes improve soil fertility because of their N_2 fixing ability. Legumes vary in the amount of N_2 fixed and in the proportion of plant N derived from different sources – atmosphere, fertilizer and soil. We need therefore to identify legumes and genotypes that yield more and derive a large part of their N requirements from fixation (Wani, Rupela and Lee, 1995). This study was therefore conducted to quantify the amount of N_2 fixed by three important legumes (lentil, mungbean and soybean) with and without inoculation and to estimate the amount of N they derived from different sources.

MATERIALS AND METHODS

Field experiments were conducted during 2008–09 and 2009–10 at the sub-station farm of the Bangladesh Institute of Nuclear Agriculture (BINA), Rangpur, located at 25°43′ N latitude and 89°16′ E longitude in the north-west part of Bangladesh. The soil of the experimental site was silt loam (19 percent clay, 51 percent silt and 30 percent sand), having pH 7.2 (in water) with carbon (C) 0.73 percent, total N 0.074 percent, Olsen's phosphorous (P) 5.03 ppm and exchangeable potassium (K) 0.12 cmol/kg. Three different legumes – lentil (*Lens culinaris* Medik; cv. Binamusur 1), mungbean (*Vigna sinensis*; cv. Binamoog 4) and soybean (*Glycine max* L.; cv. Sohag) were tested with and without rhizobial inoculant by following a randomized complete block design with a split-plot arrangement having three replications.

For quantification of biological N_2 fixation by the different legumes, isotope labelled ammonium sulphate ($[^{15}NH_4]_2SO_4$) with 10.48 percent atom excess (a.e.) @ 20 kg N/ha was sprayed uniformly onto the 1 m^2 area of the legume plots. The reference crop wheat (*Triticum aestivum*) received 100 kg N/ha in the isotopic sub-plot. Unlabelled urea was applied to the remaining part of each plot to keep the N doses uniform for whole of the plot and as per treatment plan. The P, K and S doses for wheat were respectively 20 kg P/ha, 50 kg K/ha and 5 kg S/ha. The size of each main plot was 6 m x 5 m and each plot contained one ^{15}N micro-plot (1m x 1m), which was assigned for the isotopic study. Crops were harvested at physiological maturity. For total N and ^{15}N estimation, the aerial parts of the plants were dried in an oven at 65°C and the percent N determined using a Micro-

1 Soil Science Division, Bangladesh Institute of Nuclear Agriculture (BINA)
2 Bangladesh Agricultural University Campus, Mymensingh-2202, Bangladesh
* E-mail address of corresponding author: ekrambina@yahoo.com

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Kjeldahl digestion apparatus. Nitrogen-15:Nitrogen-14 ($^{15}N/^{14}N$) ratios were determined using an NOI-7 emission spectrophotometer (IAEA, 2002) and ^{15}N related calculations were performed using the equations of IAEA (1990) and Toomsan *et al.* (1995). Analysis of variance was performed and means were classified following Duncan's new multiple range test ($p < 0.05$).

RESULTS

Effect of rhizobial inoculant on nodulation and legume yield

The rhizobial inoculant had significant effects on the number of nodules per plant. Most nodules (25–41 per plant) were found in the plots where soybean seeds were inoculated with *Bradyrhizobium* and the least (2–3 per plant) in un-inoculated soybean (Table 1). On average, the highest nodule dry weight (190.5 mg/plant) was recorded in the plot where soybean seeds were inoculated with *Bradyrhizobium* before sowing and the lowest dry weight of 5.5 mg/plant was recorded in un-inoculated lentil.

Seed and stover yields varied significantly with the legume irrespective of whether inoculation took place with rhizobia. These yields were higher when the legume was inoculated (Figure 1), with the highest seed yield (2.1 t/ha) being recorded in inoculated soybean, and the lowest (0.8 t/ha) in un-inoculated lentil. Increases in seed yield associated with inoculation were respectively 69.4, 41.3 and 44.0 percent for soybean, mungbean and lentil. Inoculation also had a significant effect on stover yields, with the average highest yield (4.5 t/ha) being recorded from inoculated soybean and the un-inoculated mungbean showing the lowest yield (1.6 t/ha). Stover yield increases associated with inoculation were 50.8, 53.4 and 62.5 percent, respectively in lentil, mungbean and soybean. On average the total yield (seed plus stover) increased by 42, 37 and 61 percent in the crops lentil, mungbean and soybean, respectively.

Effect of rhizobial inoculation on legume nitrogen content

The experimental results showed that the N content in legume seed and stover were influenced significantly by the type of legume and inoculant (Table 2). The highest N content was in inoculated soybean (111.6 and 113.7 kg/ha during 2008–09 and 2009–10, respectively) and increased by 89.3, 76.3 and 78.8 percent in lentil, mungbean and soybean, respectively compared to un-inoculated conditions. The maximum stover N content (72.0–81.9 kg/ha) was obtained from inoculated soybean while the minimum N content (17.5–18.7 kg/ha) was obtained from un-inoculated mungbean. The highest average total legume N (189.6 kg N/ha) was found in inoculated soybean and this differed significantly from other legumes, with the lowest amount (41.0 kg N/ha) of total legume N being recorded in un-inoculated mungbean. Due to inoculation, the total legume N uptake increased by 77.4, 71.0 and 98.4 percent, respectively in lentil, mungbean and soybean plants.

TABLE 1. Nodulation in different legumes as affected by rhizobial inoculant

Legume x Inoculant	Nodules per plant			Nodule dry wt. (mg/plant)		
	2008-09	2009-10	Mean	2008-09	2009-10	Mean
Lentil un-inoculated	10.4b	05.7c	08.1	04.7e	06.2e	05.5
Lentil inoculated	15.5b	15.2b	15.4	15.3d	11.7d	13.5
Mungbean un-inoculated	09.8b	06.6c	08.2	22.5c	26.6c	24.6
Mungbean inoculated	12.0b	16.5b	14.3	55.4b	55.3b	55.3
Soybean un-inoculated	03.2c	02.0c	02.6	14.6d	12.2d	13.4
Soybean inoculated	25.5a	41.3a	33.4a	183.3a	197.7a	190.5
% CV	23.8	20.3	—	38.0	17.6	—

Values in a column under a factor/ interaction treatment having same letter do not differ significantly at 5 percent level of probability.

Table 2. Nitrogen content in different legumes as affected by rhizobial inoculant

Legume × Inoculant	Nitrogen in legume seed (kg/ha)			Nitrogen in legume stover (kg/ha)		
	2008-09	2009-10	Mean	2008-09	2009-10	Mean
Lentil un-inoculated	26.07e	26.84e	26.46	22.98c	18.30c	20.64
Lentil inoculated	50.01c	50.17c	50.09	36.44b	30.40b	33.42
Mungbean un-inoculated	22.98e	22.93e	22.96	18.68c	17.49c	18.09
Mungbean inoculated	38.10d	42.86d	40.48	32.12b	27.25b	29.69
Soybean un-inoculated	62.56b	63.77b	63.17	33.18b	31.66b	32.42
Soybean inoculated	111.56a	113.73a	112.65	81.94a	72.03a	76.99
%CV	7.35	3.60	-—	3.49	5.46	—

Values in a column under a factor/ interaction treatment having same letter do not differ significantly at 5 percent level of probability.

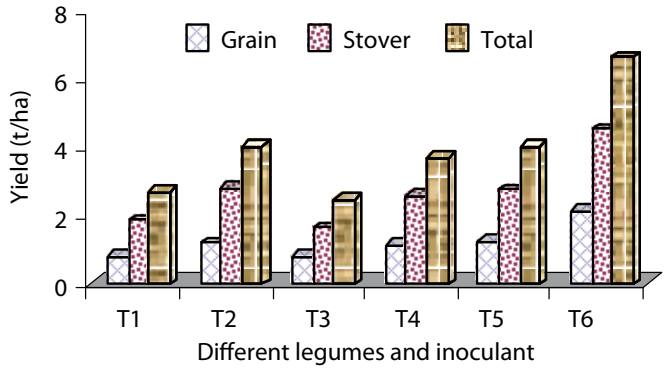


FIGURE 1. Seed, stover and total yield of legumes as affected by rhizobial inoculant (average of results from two years).
T_1 — lentil un-inoculated; T_2 — lentil inoculated; T_3 — mungbean un-inoculated; T_4 — mungbean inoculated; T_5 — soybean un-inoculated; and T_6 — soybean inoculated.

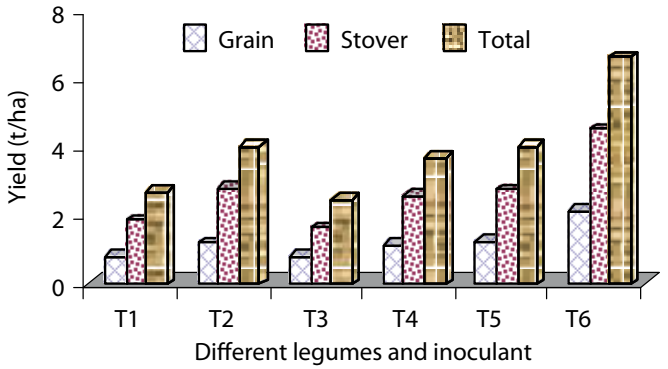


FIGURE 2. Total N, Ndfa, Ndfs, and Ndff of legumes as affected by rhizobial inoculant (average of two years' results).
T_1 — lentil un-inoculated; T_2 — lentil inoculated; T_3 — mungbean un-inoculated; T_4 — mungbean inoculated; T_5 — soybean un-inoculated; and T_6 — soybean inoculated.

TABLE 4. N derived from different sources in legume stover as affected by rhizobial inoculant

Legume x Inoculant	N derived from different sources in legume stover (kg/ha)					
	Ndfa		Ndfs		Ndff	
	2008-09	2009-10	2008-09	2009-10	2008-09	2009-10
Lentil un-inoculated	14.22d	12.35e	6.90c	4.54c	1.86c	1.41c
Lentil inoculated	29.18b	25.30b	5.72d	3.87e	1.54c	1.20c
Mungbean un-inoculated	11.15e	11.20e	5.93d	4.80c	1.60c	1.49c
Mungbean inoculated	25.63c	21.59c	5.11d	4.32d	1.38c	1.34c
Soybean un-inoculated	16.22d	17.34d	13.37a	10.93a	3.60a	3.39a
Soybean inoculated	67.45a	60.78a	11.42b	8.59b	3.07b	2.67b
%CV	3.84	5.13	5.81	4.39	5.75	7.48

Values in a column under a factor/ interaction treatment having same letter do not differ significantly at 5 percent level of probability.

TABLE 3. N derived from different sources in legume seed as affected by rhizobial inoculant

Legume x Inoculant	N derived from different sources in legume seed (kg/ha)					
	Ndfa		Ndfs		Ndff	
	2008-09	2009-10	2008-09	2009-10	2008-09	2009-10
Lentil un-inoculated	16.87d	18.31d	7.16c	6.47c	2.04c	2.06c
Lentil inoculated	41.38b	42.36b	6.71c	5.93c	1.93c	1.86d
Mungbean un-inoculated	14.50d	15.00d	6.60c	6.02c	1.88c	1.91d
Mungbean inoculated	31.00c	35.05c	5.52c	5.93c	1.57d	1.89d
Soybean un-inoculated	33.63c	35.08c	22.51a	21.77a	6.42a	6.93a
Soybean inoculated	94.30a	96.71a	13.43b	12.91b	3.83b	4.11b
%CV	8.60	4.54	10.46	4.34	9.80	4.65

Values in a column under a factor/interaction treatment having same letter do not differ significantly at 5 percent level of probability

Quantity of legume nitrogen derived from different sources

Legume N (in seed and stover) and the relative proportions derived from different sources (atmosphere [Ndfa], soil [Ndfs] and fertilizer [Ndff]) were estimated using the ^{15}N tracer technique (Tables 3 and 4 and Figure 2). The quantity of legume N derived from different sources varied and was influenced significantly by the legume species and inoculation. Maximum Ndfa values for both legume seed and stover were obtained for inoculated soybean and minimum values for un-inoculated mungbean. With inoculation, soybean derived the highest N from the atmosphere (84.1 percent of its N amounting 159.6 kg N/ha), followed by lentil (82.7 percent and 69.1 kg N/ha) and mungbean (80.7 percent and 56.6 kg N/ha). The lowest N (25.9 kg N/ha) derived from atmosphere was recorded from un-inoculated mungbean (Figure 2).

Legumes received a considerable amount of N from the soil under un-inoculated conditions although some inefficient natural rhizobia were present. The highest amount of Ndfs (34.3 kg N/ha) was recorded in un-inoculated soybean plots (in both seed and stover) and the lowest amount was observed in inoculated mungbean (10.4 kg N/ha). The mean total Ndfs for the un-inoculated soybean plot was about more than three times greater than the total Ndfs found from inoculated mungbean (Figure 2). Due to inoculation a considerable amount of total N was obtained from the soil (32.4 percent in inoculated soybean, 10.6 percent in inoculated mungbean and 11.2 percent in inoculated lentil).

All legume plants also received more N from fertilizer sources under un-inoculated compared with inoculated conditions. The highest total Ndff was recorded from the un-inoculated soybean plot (10.2 kg/ha), whereas the lowest value was obtained from the inoculated mungbean plot (3.1 kg/ha).

DISCUSSION

Total N content differed significantly between legumes and with and without inoculant, with the highest and lowest N uptakes being recorded in inoculated soybean and un-inoculated mungbean, respectively. With inoculation, the average plant N content increased by 70.9–98.3 percent, with the greatest increase being obtained in soybean (98.3 percent) followed by lentil (77.3 percent) and mungbean (70.9 percent). Results of a similar nature have been reported previously by other authors for soybean (Hoque, Sattar and Dutta, 1995; Jensen, 1997; Molla *et al.,* 2001; Hungria *et al.,* 2003; Shaha, 2007), lentil (Bremer *et al.,* 1990; Whitehead *et al.,* 2000; Shaha, 2007), chickpea and bean (Duc, Mariotti and Amarger, 1988; Rupela, 1994).

Soybean fixed the most N from the atmosphere followed by lentil and mungbean. When the seeds of soybean were treated properly before sowing with *Bradyrhizobium* inoculant, N derived from the atmospheric increased by 2.7–fold in seed and 3.8-fold in stover compared with un-inoculated soybean. Comparatively lower amounts of N were derived by inoculated lentil (2.4 and 2.1 times more for seed and stover, respectively) and mungbean (2.2 and 2.1 times more for seed and stover, respectively) than in un-inoculated plants. Most interesting were the differences recorded between legumes in N_2 fixation, inoculated soybean being the best fixer, followed by the lentil, with mungbean being the poorest.

Selection of legumes with higher yields and N_2 fixation ability is a potential approach for increasing the contribution of N from atmosphere into soil–plant systems. The results from this study clearly demonstrate this. For example, the contribution of biological N_2 fixation from these three inoculated legumes would range from 56.6 kg N/ha for mungbean to 159.6 kg N/ha for soybean, i.e. an almost 2.8–fold increase by substituting inoculated soybean for inoculated mungbean, or a 22.1 percent increase by substituting inoculated lentil for mungbean. Sattar, Islam and Hossain (2000), Alves, Boddey and Urquiaga (2003) and Tien *et al.* (2002) showed that between 56 percent and 89 percent of the N requirements of soybean could be met from the atmosphere if inoculated, while the corresponding figures for inoculated chickpea, lentil and mungbean were 65–75 percent, 66–73 percent and 45–76 percent, respectively, while un-inoculated legumes could meet only between 44 percent and 67 percent of their N requirements from the atmosphere.

The infectiveness and efficiency in N_2 fixation and thus the response to inoculation by commercial *Rhizobium leguminosarum* inoculant showed a high level of plant genus–rhizobium specificity. In this study, lentil and mungbean showed moderate responses under un-inoculated conditions due to the presence of natural rhizobia in the soil while soybeans, by contrast showed little response when not inoculated. Oberson *et al.* (2007) showed that at maturity the total amount of N_2 fixed by soybean was 150–260 kg N/ha and during maturity the amount of Ndfa was 102 kg N/ha, equivalent to 47 percent of the total N assimilated (Zapata *et al.,* 1987).

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Bio-solubilization of Rock Phosphate and Plant Growth Promotion by *Aspergillus niger* TMPS1 in Ultisol and Vertisol

M. Shrivastava[1,*] and S.F. D'Souza[1]

ABSTRACT

The effectiveness of the phosphate solubilizing fungus *Aspergillus niger* strain TMPS1 isolated from the rhizospheric soil of mangrove was evaluated for wheat grown on Ultisol and Vertisol soil types under greenhouse conditions using the phosphorus-32 (^{32}P) isotope dilution technique. The fungus was identified using 18S ribosomal ribonucleic acid (18S rRNA). Two low grade Indian rock phosphates (RP) from Lalitpur (LRP) and Purulia (PRP) were used as phosphorus (P) sources. Plants were grown for six weeks. In both soils dry matter yield and P uptake of wheat increased significantly with fungus inoculation and rock phosphate fertilizer addition compared with the unfertilized control and un-inoculated RP fertilizer treatments. This demonstrates solubilization of the RPs by fungus which increases the relative contribution to plant P uptake. The relative contribution to plant P uptake from the RPs was comparatively higher in Ultisol than in Vertisol, implying higher solubilization of the RPs by fungus in the former. Phosphate derived from the LRP was greater than from PRP in fungus inoculated treatments in both soil types. This demonstrates the potential of *Aspergillus niger* strain TMPS1 isolate as a phosphate solubilizer in Ultisols and Vertisols. Further, the advantages were shown of using ^{32}P to distinguish the contributions of bio-available native soil P and P from RPs to P nutrition in plant–microbe interactions.

Key words: *18S rRNA, Aspergillus niger, ^{32}P, rock phosphates, Ultisol, Vertisol.*

INTRODUCTION

Phosphorus (P) is an important plant nutrient and high P absorbing capacity of soil, P immobilization and/or fixation of P to insoluble mineral complexes results in low availability of this macronutrient for plant uptake (Marschner, 1995). Vertisol and Ultisol soil types constitute a considerable proportion of the cultivable area in India and P deficiency is one of the major stress factors for crop production in both types of soil (Velayutham and Bhattacharyya, 2000).

Precipitation with calcium (Ca) ions in calcareous Vertisols, and with iron (Fe) and aluminium (Al) ions in acidic Ultisols are the main causes of reduced P availability in these soils (Hisinger, 2001). For this reason, the optimal development of crops demands costly inputs of P fertilizers, part of which is utilized by plants and the remainder converted into insoluble fixed forms (Omar, 1998; Vassileva, Vassilev and Azcon, 1998).

The direct application of rock phosphate (RP) is considered as an agronomic and economically attractive alternative to the use of more expensive water-soluble P fertilizer sources (Hammond, Chien and Mokwunye, 1986). In India, it is estimated that about 260 million tons (t) of phosphatic rock deposits are available but most of this material is not suitable for phosphatic fertilizer production due to its low reactivity and the presence of impurities. These RPs could, however, be applied directly for crop production with or without modification (Shrivastava, Bhujabal and D'Souza, 2007).

Sustainable P supply in agroecosystems can be achieved by using RP in conjunction with phosphate solubilizing microorganisms (Vandermeer, 1995). The use of phosphate solubilizing microorganisms, including arbuscular mycorrhizal (AM) fungi, has been proposed as a low cost and low energy approach to help increase the agronomic effectiveness of RP fertilizers (Gyaneshwar *et al*., 2002). Fungi in soil are able to traverse distances more easily than bacteria and thus may be more important for P solubilization in soils (Kucey, 1983), and RP solubilization by various fungi have been reported (Vassilev *et al*., 1998; Goenadi, Siswanto and Sugiarto, 2000; Reddy, Kumar and Khosla, 2002).

The effectiveness of solubilization of phosphate-bearing materials under soil conditions is unclear because of the possible re-fixation of phosphate ions on their way to the root surface. Phosphorus-32 (^{32}P) radiotracer techniques have been used to evaluate the exchange rates between the solution and solid phases of soil, and to measure the bioavailability of P from RP materials (Zapata and Axmann, 1995; Shrivastava *et al.,* 2007). Isotopic techniques can also be applied to determine the extent of microbial biotransformation of RPs and unavailable soil P into bioavailable soil P (Gianinazzi-Pearson and Gianinazzi, 1989; Toro, Azcón and Barea, 1997).

In the present study, the capacity of the fungus *Aspergillus niger* strain TMPS1 isolated from mangrove rhizosphere as phosphate solubilizing bio-fertilizer was assessed under greenhouse conditions using the ^{32}P isotope dilution technique.

MATERIALS AND METHODS

Isolation of phosphate solubilizing fungus

Phosphate solubilizing fungus was isolated from the rhizospheric soils of mangroves after serial dilution of the soil solution on Pikovskaya's agar plates (Pikovskaya, 1948). Formation of a clear halo around the fungal growth after five days (d) of incubation indicated phosphate solubilizing ability. This fungus was selected on the basis of the

1 Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai-400085, India
* E-mail address of corresponding author: manojshrivastava31@gmail.com

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diameter of the halo (≥ 15 mm) around the fungus. The culture was maintained on Pikovskaya agar slants at 4–6°C and sub-cultured every month.

Molecular identification of phosphate solubilizing fungus

Fungal spores were harvested by washing the slants with sterile saline. For the molecular characterization of the fungus, genomic DNA from fungal isolate was extracted using the method of Latha *et al*., 2002. The extracted DNA was used as a template to amplify the 18S rRNA genes using the polymerase chain reaction (PCR). Universal primers (NS5 [5'-AACTTAAAGGAATTGACGGAAG-3'] and NS8 [5'-CCGCAGGTTCACCTACGGA-3']) for the 18S rRNA as described by White *et al.* (1990) were used to amplify a DNA sequence of TMPS1 560bp in length. Primers were synthesized by MWG-Biotech Ltd. (Ebersdorf, Germany). Polymerase chain reaction amplifications were carried out in 50 mL reaction volumes with a Primus thermocycler (Eppendorf) using the following steps: initial denaturation at 94°C for 1 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 40°C for 1 min, and extension at 72°C for 2 min followed by a final extension phase at 72°C for 10 min. The purified PCR products were sequenced in both directions. The nucleotide sequences of strain TMPS1 was deposited in the NCBI GenBank database under accession number DQ316605.

Greenhouse study

Isotope dilution technique

The ^{32}P isotope dilution technique (IAEA, 1990) was used to evaluate RP solubilization by *Aspergillus niger* strain TMPS1 under greenhouse conditions. In this method, ^{32}P measures the exchange rates governing the equilibrium between liquid and solid phase phosphate. The specific activity (SA), i.e. the ^{32}P: phosphorus-31 (^{31}P) ratio is determined in the plant tissue to ascertain the effect of fungus inoculation on RP solubilization and in turn plant P uptake. A lowering of the SA with fungus inoculation compared with no inoculation would indicate that the plant is receiving extra ^{31}P released a result of RP solubilization by the fungus. Therefore, the isotope-based technique can be used to determine the extent of the transformation of the unavailable soil P pool into bioavailable P by the metabolic activity of the phosphate solubilizing microorganisms.

Soils

Greenhouse pot culture experiments were conducted using two soils, an Ultisol from Karnataka and a Vertisol from Maharashtra, India. The characteristics of the Utisol were as follows: $pH_{1:2}$ (soil: water), 4.8; texture: sandy clay; available P (Bray I) 5.9 mg/kg; organic carbon, 20.4 g/kg, total N, 3.9 g/kg; cation exchange capacity, 8 cmol (p^+)/kg; phosphorus fixing capacity, 700 g/kg, and 0.02 M $CaCl_2$ -extractable Al, 2.58 mg/kg. The Vertisol had: pH (water), 8.2; texture clayey; available P (Olsen), 3.7 mg/kg; organic carbon, 5.1 g/kg; total N, 0.6 g/kg; cation exchange capacity, 52 cmol (p^+)/kg, free calcium carbonate, 3.6 percent; and phosphorus fixing capacity, 620 g/kg. Both soils were deficient in plant available P content.

Rock phosphates

Two Indian RPs from Lalitpur (LRP) and Purulia (PRP) containing 22.1 and 33 percent total P_2O_5, respectively, were used in this study. Both were low grade and had low reactivity.

Experimental

The soils were sieved (2 mm) and 2 kg were placed in plastic pots. Both RPs were applied as finely ground (100 mesh) natural products at a rate 500 mg P/kg soil. Soil labelling was done by thoroughly mixing the soil with 100 mL/pot of the solution containing an activity of 10 megabecquerel (MBq) ^{32}P. Soil was inoculated with 25 mL/pot spore suspension of *Aspergillus niger* TMPS1 containing 1×10^7 spores/mL.

All pots were kept at field capacity moisture content and left to equilibrate for two d. In all, there were seven treatments for each soil type: (i) soil alone (absolute control), (ii) soil + ^{32}P (radioactive control), (iii) soil + fungus + ^{32}P, (iv) soil + LRP, + ^{32}P, (v) soil + LRP + fungus + ^{32}P, (vi) soil + PRP + ^{32}P, and (vii) soil + PRP + fungus + ^{32}P. All the treatments were laid out in quadruplicate in a completely randomized block design.

Wheat (*Triticum aestivum* L. cv. PBW343) seeds were sterilized by soaking in 5 percent sodium hypochlorite (NaOCl) solution for 10 min. and washed three times with distilled water before sowing. Ten sterilized seeds were sown in each pot. The pots were weighed and watered to field capacity daily. After one week of germination plants were thinned to eight plants per pot. Plants were harvested after six weeks of growth.

Shoot dry weights were recorded after drying at 70°C to constant weight. Dried plant samples were digested in di-acid mixture (HNO_3: $HClO_4$, 5:1 v/v) and analysed for total P content by the yellow phospho-vanado-molybdate complex method at 420 nm (Koeing and Johnson, 1942). The ^{32}P activity was measured by liquid scintillation counting (Packard Tri-Carb 2100) using the Cerenkov effect. Counts were corrected for counting efficiency (54 percent) and expressed in Bq (disintegrations per second). The specific activity (SA) of P was then calculated by considering the radioactivity per amount of P in the plant material and expressed in Bq/mg P.

Calculations

Isotopic parameters were calculated as follows and as described by IAEA (1990) and Zapata and Axmann (1995):

Total P uptake by plant (mg·P/pot) U_{TP} = DMY (mg/pot) x (%P/100) x DMY

Specific activity (Bq mg/P) = Bq/g plant/mg P/g plant

$$\%PdfS = \frac{\text{SA in plant(Bq·mg P) in presence of RP}}{\text{SA in plant(Bq mg P) in absence of RP}} \times 100$$

where Pdfs is P derived from soil.

$$\%PdfRP = 1 - \frac{\text{SA in plant(Bq·mg P) in presence of RP}}{\text{SA in plant(Bq · mg P) in absence of RP}} \times 100$$

where PdfRP is P derived from rock phosphate
P uptake from labelled soil (mg·P/pot) = U_{TP} x (%PdfS/100)
P uptake from rock phosphate (mg P/pot) = U_{TP} x (%PdfRP/100)

Statistical analyses

All the data were analysed by analysis of variance (ANOVA) and the means compared with least significant difference (LSD) at $p < 0.05$ level (Hoshmand, 1993).

Table 1. Effect of fungus inoculation on shoot dry matter yield (g/pot) and P uptake of wheat in Ultisol and Vertisol

Group	Treatments	Dry matter yield (g/pot)		P uptake (mg/pot)	
		Ultisol	Vertisol	Ultisol	Vertisol
1	Control (No P)	0.40	0.93	0.48	1.35
2	Soil+ ^{32}P	0.42	0.95	0.54	1.36
3	Soil+ TMPS1+ ^{32}P	0.69	1.90	0.88	2.77
4	Soil + LRP+^{32}P	0.81	0.99	1.03	1.38
5	Soil + LRP + TMPS 1+^{32}P	1.36	2.88	1.81	4.12
6	Soil + PRP + ^{32}P	0.73	0.97	0.95	1.37
7	Soil+ PRP+ TMPS 1 +^{32}P	1.12	2.90	1.51	4.14
LSD ($p < 0.05$)		0.23	0.47	0.24	0.61

Table 2. Effect of fungus inoculation on specific activity (Bq mg/P) of wheat in Ultisol and Vertisol

Group	Treatments	Specific activity (Bq mg/P)	
		Ultisol	Vertisol
1	Soil + ^{32}P	255.3	429.1
2	Soil + TMPS1 + ^{32}P	224.0	370.3
3	Soil + LRP + ^{32}P	230.7	422.6
4	Soil + LRP + TMPS1 + ^{32}P	149.4	300.0
5	Soil + PRP + ^{32}P	243.4	426.0
6	Soil + PRP+ TMPS1 + ^{32}P	157.5	306.2
LSD ($p < 0.05$)		22.0	20.2

RESULTS AND DISCUSSION

Molecular identification of phosphate solubilizing fungus

The complete 18S rRNA sequence of the isolate used in this study was obtained and compared with sequences available in the GenBank (NCBI, USA) database. This fungal strain showed a 98 percent similarity with *Aspergillus niger*. The 18S rRNA sequence of this isolates has been deposited at GenBank viz. accession no. DQ316605. The culture has been designated as Trombay mineral phosphate solubilizer 1 (TMPS1).

Dry matter yield and P uptake

In both soil types, dry matter yield and P uptake were significantly higher in fungus inoculated soil with or without RP treatments as compared with the un-fertilized control and un-inoculated RP fertilizer treatments (Table 1). The increases in plant biomass yield and P uptake could be partially due to increased RP solubilization by the fungus TMPS1.

The increased solubilization of P from RP and subsequent P uptake by plants could be attributed to chelation reactions whereby organic acid released by the fungus chelate the cations present in RP and release the P for plant uptake (Whitelaw, 2000). Greenhouse studies with corn (*Zea mays L.*) showed that corn growth increased with the addition of oxalic or citric acid, suggesting that organic acids have potential as amendments for increasing plant-available P in PR-treated soils (Kpomblekou-A and Tabatabai, 2003). Organic acids secreted from the fungus probably bind the free Al^{3+} present in Ultisol and reduce the phytotoxicity of Al and enhance the plant growth. In the case of Vertisols, the high content of exchangeable Ca and alkaline pH affect nutrient availability negatively and thereby influence soil fertility. When water-soluble phosphatic fertilizers (e.g. single super phosphate or di-ammonium phosphate etc.) are applied to Vertisols, they are converted rapidly into insoluble non-available forms of phosphates. Organic acids produced by this fungus solubilized the RP and native insoluble soil P and formed a complex with the free Ca present in the soil, preventing re-fixation of the P. This illustrates the plant growth promotion as well as the phosphate solubilizing abilities of this fungus in two distinct soils types. Various studies have shown that the dry matter yield of wheat plants increased significantly after inoculation of the RP solubilizing fungi *Aspergillus niger, Aspergillus awamori, Penicillium digitatum, Penicillium citrinum* and *Penicillium bilaji* under greenhouse as well as field conditions (Kucey, Jansen and Leggett, 1989; Omar, 1998).

Isotopic data

Specific activity

Results of the SA measurements in wheat plants are presented in Table 2.

Results show that the SA in the Ultisol was significantly lower in RP treatments that were both un-inoculated and inoculated with TMPS1 than those not receiving RPs, and that the reductions in SA were more pronounced in fungus inoculated treatments. In the case of the Vertisol, significant reduction in plant SA was observed only in the treatment of RP inoculated with TMPS1 compared with the control. The acidic nature of Ultisols helps in the solubilization of RP and inoculation of the fungus further enhanced the RP solubilization process, which in turn reduced the SA in plant shoots. The TMPS1 fungus released phosphate ions either from the soil or added RPs, which reduced the ^{32}P:^{31}P ratio (SA) in the plants. Lower ^{32}P:^{31}P ratios of plants due to microbial inoculation were also reported by Toro, Azcón and Barea (1997).

Phosphorus derived from labelled soil (bioavailable P) and rock phosphate

Plant P derived from labelled soil (bioavailable) and RPs are presented in Tables 3 and 4. In both un-inoculated soils, the plant absorbed predominantly the bioavailable endogenous soil P and percentage share of this P was lower under fungus inoculated treatments. Phosphorus derived from the labelled source (bioavailable soil P) and RPs was significantly higher in both soils inoculated with fungus. This demonstrates the solubilization of RP by the fungus which increases the relative contribution to plant P uptake. This was comparatively

Table 3. Effect of fungus inoculation on percentage of P derived from labelled (bioavailable) soil (PdfS) and rock phosphates (PdfRP) in Ultisol

Group	Treatments	Ultisol			
		PdfS		PdfRP	
		%	mg P/pot	%	mg P/pot
1	Soil + LRP + ^{32}P	90.4	0.93	9.6	0.10
2	Soil + LRP + TMPS 1 + ^{32}P	66.7	1.21	33.3	0.60
3	Soil + PRP + ^{32}P	95.3	0.91	4.7	0.04
4	Soil + PRP + TMPS1 + ^{32}P	70.3	1.06	29.7	0.45
LSD ($p < 0.05$)			0.13		0.08

Table 4. Effect of fungus inoculation on percentage of P derived from labelled (bioavailable) soil (PdfS) and rock phosphates (PdfRP) in Vertisol

Group	Treatments	Vertisol			
		PdfL		PdfRP	
		%	mg P/pot	%	mg P/pot
1	Soil + LRP + ^{32}P	98.5	1.35	1.5	0.02
2	Soil + LRP + TMPS 1 + ^{32}P	81.1	3.34	18.9	0.78
3	Soil + PRP + ^{32}P	99.3	1.36	0.7	0.01
4	Soil + PRP + TMPS1 + ^{32}P	82.7	3.42	17.3	0.72
LSD ($p < 0.05$)			0.82		0.09

higher in the Ultisol, which implies greater solubilization of the RPs by fungus in the Ultisol.

Possible mechanisms for RP solubilization in soil by this fungus are production of low molecular weight organic acids such as gluoconic, malic, citric and oxalic acids (Cerezine, Nahas and Banzatto, 1988; Reyes *et al*., 1999), and the release of protons accompanying respiration and/or NH^+_4 assimilation (Illmer and Schinner, 1995; Ahuja, Ghosh and D'Souza, 2007). Low molecular weight organic acids (LMWOAs) possess one or more carboxyl and hydroxyl functional groups that can form complexes with metals in soils and thus play an important role in soil processes such as mineral weathering, nutrient mobilization and Al detoxification (Hue, Caddock and Adams, 1986; Bolan *et al.,* 1994). Kpomblekou-A and Tabatabai (1994) studied the ability of 19 LMWOAs to release P from PRs and showed that reactions involved in the P release process were not only pH-dependent but also related to the structural characteristics of the organic acids.

CONCLUSIONS

The results of the present study demonstrate the potential use of an *Aspergillus niger* TMPS1 isolate as a phosphate solubilizer in Ultisol and Vertisol soil types. In general, RP solubilization in alkaline Vertisols does not occur, but this fungus solubilized native unavailable soil P as well as RP in such a soil. The advantages of using ^{32}P to distinguish the contributions of bioavailable native soil P and bioavailable P from RPs to P nutrition in plant–microbe interactions were also shown. Future work is required to determine the phosphate solubilizing and plant growth promoting potentials of this isolate under field condition in different agro-climatic regions for different crops.

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Country-wide Agro-ICT Infrastructure for Supporting Assessment of Soil Carbon Sequestration, Soil Nutrient Balance, Soil Water Status and Appropriate Crop Rotation

W.H. Mayer[1,*]

ABSTRACT

High returns on investment in agriculture and forestry come from integrating the newest technologies and cooperation between public and private stakeholders. With the help of ortho-images, a network of agro-sensors (weather, soil), geographic information systems (GIS), a land parcel information system (LPIS), farm and/or forest management systems, advisory services covering regions or countries farmers can be supported in terms of logistics, precision, or virtual farming needs and good agricultural practices (GAP). These integrated technologies can also help to implement environmental risk management tasks. In general, integration of local information with large-scale images, agro-sensor stations and information and communication technology (ICT) that provide tools for managing agriculture and forestry requires 2 to 3 yr for implementation. Technology integration with geographic information systems and stakeholder cooperation is a win-win model for a bright agro-forest future supporting food/feed production, biomass for energy, environmental caretaking and risk management.

Keywords: *GIS, maps, agro applications, information and communication technology, precision farming, business model.*

INTRODUCTION

Based on the use of precise ortho-images such as those available from Microsoft Bing™ Maps, geographic information systems (GIS)-based agro-information and communication technology (ICT) of PROGIS, data from agro-sensor technology and rural area management consulting services, the AGRO-ICT-Backbone® concept was developed. It provides not only the necessary information technology (IT) tools but is also a holistic model to establish an agricultural infrastructure throughout a whole country for fostering better agricultural development. This information technology tool includes:

- The production of a high resolution 30 cm ortho-image for the whole country as a basis for further planning and control with an update frequency of 3–4 yr.
- The setup, or if available as in Europe, the upgrade of existing land parcel information (LPIS) systems — or a cultivation register and/or a rural open street map (OSM), based on ortho-images and PROGIS GIS software WinGIS® .
- The implementation of a sophisticated farm management information system (FMIS) which supports farm advisory (extension) services and serves the ministry for regional or country-wide statistical needs.
- The installation and integration of a logistic system including mobile solutions to support farmers and their chain partners such as the industry, for timely delivery of seeds, fertilizer, harvested products and for traceability needs.
- The installation of agro-sensor networks, consisting of agro-weather stations and soil sensors for decision support and guidance.
- Value added services for needs like precision and virtual farming, land consolidations, environmental management, carbon calculation, risk management to support the 2013 Common Agricultural Policy (CAP) needs etc. including consulting if needed. A special training concept enables users to develop their own on-top applications for solving local needs.
- Apps for mobile phone solutions for Windows phone with Bing maps, (but also iPhone with Google maps, or Android with Google maps), such as:
  - GIS applications for field identification;
  - access to the logistic system for automated order processing;
  - access to farm management tools for sending and receiving specific orders for cultivation (including precision farming maps via advisors);
  - access to the software developer component for personal and local GIS-based developments;
- Capacity-building including education and training models enables local experts to be ready for a rollout.
- The intelligent business model enables the owner of the ICT infrastructure (public, private or private-private partnership), to generate return on investments (ROI) by supporting stakeholders such as banks, insurance companies, large farms, large forest enterprises, chain partners like the food industry, suppliers of farm equipment, agro-chemicals and other agro-resources as well as international investors.

Beneficiaries are farmers and forest holders, also smallholder enterprises, groups of farmers, cooperatives, advisory/extension services, other service providers, affiliated industries, ministries, banks and insurance companies, researchers, rural populations and the public as a whole.

1 PROGIS Software GmbH, Postgasse 6, A-9500 Villach, Austria
* E-mail address of corresponding author: mayer@progis.com; www.progis.com

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Solutions from PROGIS

The implementation of this agro-ICT backbone has to be realized within a large-scale project together with a range of local partners and experts. It can be done in a public, public-private or private project and is partitioned into the following steps:

Ortho-image

The production of 30 cm ortho-images with a vertical digital surface model (DSM) of <1.5 m resolution and a 60 cm infrared image. Examples of technical specifications of compliant ortho-images are given in Microsoft (MS). "Global Ortho: Rapid, High Efficiency Ortho Update Technologies" (available at office@progis.com).

Preparation of LPIS

The first task is to implement the GIS system WinGIS® and on the base of MS images, set up the LPIS (Figure 1) or cultivation register including the assignment of owners or leaseholders to individual plots and thereby build up a country-wide land parcel database. An OSM technology can be integrated. The LPIS systems are already implemented in most countries of the European Community (EC) and updates can be done directly by farmers or farm advisors to increase precision and reduce land administration costs by data transfer to the existing LPIS/IACS system (see details later).

GIS services

Providing GIS services for non-specialists was a primary aim of PROGIS when developing WinGIS®. It is easy to learn and use GIS software running on a personal computer, with extensive geographic application possibilities and facilities. Due to the ability to integrate online map data such as Microsoft Bing Maps as an "embedded module", the access to worldwide available geographic data like satellite and aerial images, road maps and address databases is already part of the software package. Import and export interfaces support the most common GIS/CAD file formats like the ESRI™ shape files, the AutoCAD™ DXF, MapInfo™ MIF and also text based file formats like CSV or GPX for data import from, e.g. global positioning system (GPS) devices. In a few steps external spatial data can be loaded into the user´s project.

By using the developer component, application developers have the possibility to link their application with WinGIS® in order to visualize, edit and administer any data with a geographic relation. This is very relevant for realizing suggestions to implement local integrated agricultural control system (IACS) applications, to monitor good agricultural practices (GAP)/common agriculture policy (CAP) compliance or for supporting consultancy applications.

With the help of such a software development kit (SDK), local IT experts managing the IACS system of an EC country can easily implement an application to generate a subsidy form out of the farm management information system (FMIS) and transfer it via Internet to the government homepage. The effect would be a "one stop shop software", managed by a trained farmer or by an advisor that in parallel with the subsidy form also manages the business calculation, a nutrient balance, a carbon balance, integrates data for other future documentation needs like food traceability, a business plan, insurance data or after 2013, CAP´s ICT needs. Not only would governments save money, but farmers will save travel and time costs from driving to a subsidy centre. Within a similar timeframe, much more output can be realized on one side and if advisors are supporting farmers within a region (in all negotiations about a CAP reform new advisory concepts are asked for) much more can be achieved in all sectors where single farmers alone can´t reach the targets but in groups they would be able to do so. These targets concern mainly the environment, landscapes and natural risks, but also logistics, precision farming, land consolidation missions etc. are served This is also something the new GAP regulations will support (see later).

Implementation of FMIS

When the European Union (EU) launched the CAP reform to increase food quality and safety for the welfare of its citizens, PROGIS developed DokuPlant™ on top of the GIS software tools for farmers and advisors to manage the many needs which this new legislation brought along. This integrates expert databases (all agricultural data and cultivation recommendations sustainably supported by local experts) and a perpetual calendar and documentation tool, and facilitates planning, calculation, control and traceability. With this, extension officers/advisors are able to aggregate the data from fields, farms or a whole region and to prepare them for a Ministry or other public authority for statistical use or for projects.

The following information is generated from every field and can be accumulated countrywide:

- Activity management;
- Crop rotation;
- Cost calculation;
- Nutrient balance and carbon balance;
- All input/resource needs;
- Harvest estimations

PC-GIS, real time management and the expert data base are integrated. The mapping of plots/fields is supported and a perpetual calendar enables the display of any performed activity: what–when–where (Figure 2). The integrated database is filled with agro-expert data, generated in close cooperation with local agro-forest-environmental scientists/experts and contains (example: agro-Germany) 2 500 agro-machine data (KTBL, costs, time, …), data on thousands of mineral and organic-fertilizers, 850 herbicides with contents, crops including varieties and 400 plants with average yield and seed needs. The complete working process for a year with all activities and relevant data are pre-defined for all crops and enables planning with one click: Where (plot in the map) do I plan what (select crop from the expert data bank). This database is consequently also a knowledge base and know-how transfer from scientists to the base, the farmers and foresters – is carried out daily and sustainably. After planning, the data entry can be done manually or automatically.

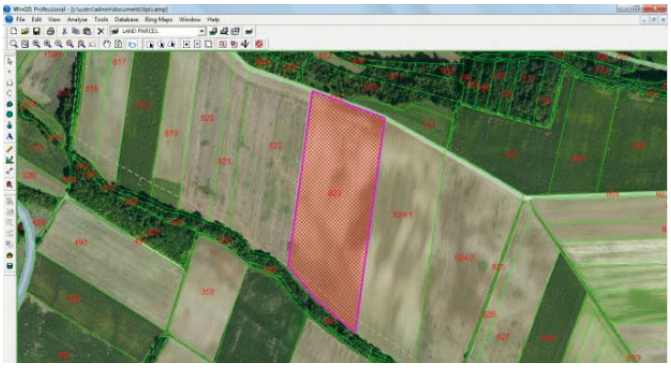


Figure 1. LPIS polygons on ortho-image.

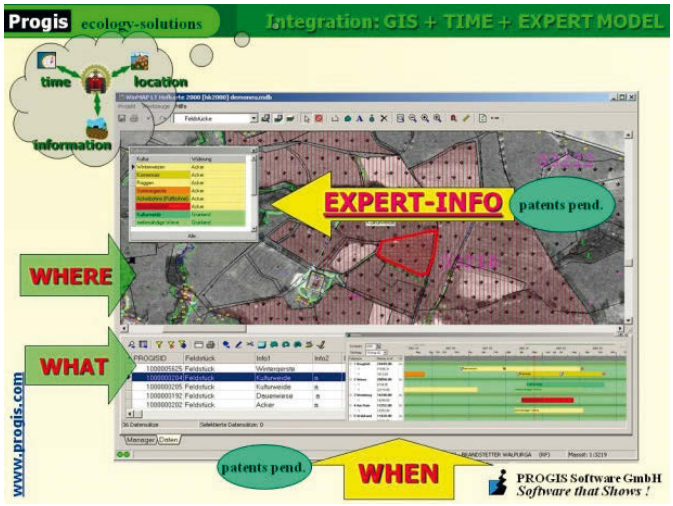


FIGURE 2. Farm management: where– what– when– expert-data.

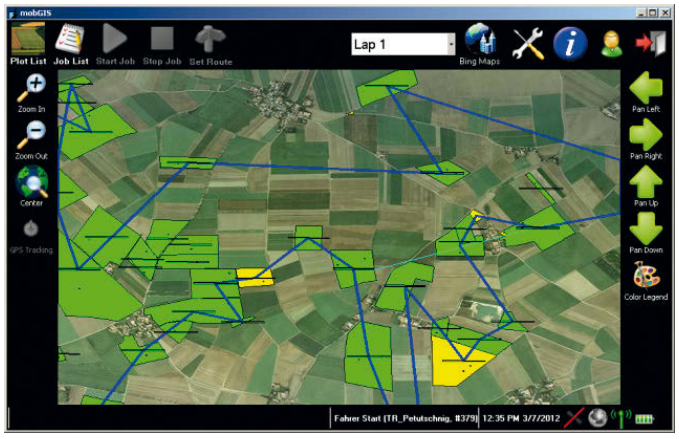


FIGURE 3. Logistics – where to do what.



FIGURE 4. Agro-sensor network.



FIGURE 5. Apps for Windows phone.

Forest management

ForestOffice is FMIS for forest enterprises. It deals with sustainable forestry planning, forest facilities, forest management and forest logistics; the expert database contains local growth tables of different trees. Both agricultural and forest expert data have to be modified by local experts working within a "farmer/forester–advisor–expert" business model.

Logistic services

The protection of the environment and of natural resources is on everyone's lips. Within the agricultural sector group management, activity- based planning and sharing of production facilities contribute to reach these targets. PROGIS developed a smart logistic solution to solve these needs. The base data are those accumulated from the FMIS, and farmers, foresters and the industry deduce therefrom their planning, processing and time optimization answering queries like: "where to deliver what" or "where to pick up what and when" and how to come to a location (with the help of the rural OSM) supports all process related partners (Figure 3).

The system leads to an optimization of daily and seasonal routing, accurate information of harvest status, GPS position data visualization, online two-way communication (GPRS/UMTS) between central and mobile terminals and order processing. The system consists of a central station and a number of mobile units ("mobGIS"). It handles crops for food/feed or biomass production, liquid manure deposits, forest harvesting or any other logistic task. Up to 30 percent cost reductions or even more can be achieved. Environmental pollution is far smaller than with conventional methods and due to the recordings ongoing improvements may occur.

Agro-sensor networks

Sustainable cultivation and protection of soils depend a lot on the application of fertilizers, pesticides and water. Agro-sensor stations help to take decisions and to optimize rates. A network of agro-climate sensors (Figure 4) – one station for every microclimate – and soil moisture sensors are needed. Based on the data and a tool set, experts can provide farmers with tailor made recommendations (e.g. forecasts for weather situations), but also obtain protocols of the climate situation of the past and the related impact for the future; for example, mass reproduction of a fungus or a beetle with an SMS induced decision "start spraying".

The expert models, e.g. based on meteorological conditions collected during the last four weeks concerning which fungi or beetle will tend to outbreak, have to be adjusted or developed and fine-tuned from local phytopathology experts.

With the soil moisture sensors, which are also available in different depths, all necessary data for irrigation can be collected and used to support an automatic controlled irrigation system.

Mobile phone solutions

A range of apps has been developed for "mobile agriculture" to support farm and land management via mobile phones and to develop its own supportive GIS-based apps (Figure 5). It is already possible to digitize, edit and delete polygons, to record GPS positions, to cluster them and to send all recorded positions and digitized polygons via E-mail and import the data into the WinGIS® software for further processing. Also the access to DokuPlant and Logistics is a great advantage for advisors to communicate with their clients and advise them on field-related activities via mobile phone and thereby bridge local distances.

Organizational components

New business models

In the same manner as ICT has supported many other sectors throughout the last decades, ICT is able to support agriculture, but enabling structures and a new form of cooperation are needed. Farmers will also be able to support the new requirements of the CAP reform, but they need better support, assisted by new advisory structures focusing on farmers' needs and not only on the needs of other stakeholders. The farmer is the integrative factor within the food/feed, bioenergy or even environmental or natural risk chain-management and he/she has to be supported. Then all other chain members will also benefit from the ICT structure.

New business models are necessary — and available — that take care of the leverage effect due to integration of technologies and cooperation of structures! Less group egoism in agro-forestry chain management is a must!

A prerequisite to start such an agro-solution is a local infrastructure comprizing local hardware, communication technologies and the whole appropriate personnel organizational structures. It contains both the hardware and software for aggregating data at Ministry level, the countrywide structure for LPIS and farm advisory system (FAS)), the mobile solutions and the communication layout. Access to ortho-images and weather data supporting all farmers' needs is a must in the future. Making data available like Finland´s Cadaster Department did recently is a must, with private ortho-image suppliers like MS-BING being anideal option for future cooperation.

Beneficiaries who are the stakeholders in such a concept are described in the following section and to all mentioned there, the ICT-backbone can provide valuable services. For these services a great deal of ROI finance can be acquired due to the benefits delivered by the ICT, but it always remains a political decision to which extent the Ministry will support the achieved benefits or how much beneficiaries from the use of this ICT backbone will have to pay. (On request ROI calculations can be carried out for single sectors).

The business models may be different: public, private or public-private. A model is imaginable, where public (Ministry of Agriculture) and private (banks, insurance, and investors) share the investment and set up a common structure to support the different beneficiaries with information against a fee.

Beneficiaries

A crucial effect of this agricultural ICT backbone concept is that data will be generated displaying the whole current situation, and for planning the future situation of agriculture and forestry in a country. At a certain point after implementation, the empirical knowledge derived from the storage of the history together with latest R&D leads to further actions. Many stakeholders are interested in these data and need them for their daily work. With an appropriate model for data sharing, this can bring benefits to several businesses. It can be taken for granted that chain partners will be ready to contribute financially to obtain access to this information. The model in detail has to be worked out together with local structures and representatives from different stakeholders, based on a trust centre concept that respects the ownership of information.

A public–private run ICT infrastructure consisting of new ortho-images for the country covering GIS and IT solutions for rural area management in connection with land management and extension services, agriculture management and logistics can be used by different governmental organizations and also by private structures and can support:

- The Ministry of Agriculture's needs for organizing subsidies;
- The Ministry responsible for landscape changes or for the cadaster and ground tax;
- Consultants in their advisory work;
- Food chain partners for traceability and for the documentation of production,
- Logistic service experts to undertake the right actions on the right field, to find the right roads to the field and be there at the appropriate time as well as deliver goods to the food industry "just in time";
- Agro control organization for subsidies;
- Bankers to draw up a business plan for financing farmers/forest holders and obtain output from the LPIS including a calculation of the growth period (costs and expected return);
- Insurance companies to develop the appropriate policies for the relevant crops and fields since an output can also be obtained from an LPIS system that informs on which farmers have which crops and how many ha are grown including a map;
- Ecology experts and natural risk managers for the appraisal of the risks related to field or ecological coherences;
- Medical experts to judge the influence of a farm activity on the public at large;
- And last but not least, support farmers by giving them tools for economic calculations.

CONCLUSIONS

Specialized GIS-software (Geographic Information Systems - WinGIS) and ICT tools for the management of agriculture, forestry, the environment and natural risks can be developed to assist farmers/foresters on their investment in agriculture and forestry. In addition, through a range of apps developed for mobile phone, it enables the management of farm and land via mobile phones. Telemetry and agro-sensors like weather stations or soils sensors offers advisory services and consultation for many rural area management solutions with the help of local experts.

Acquisition Efficiency by Maize Landraces in Acid Soils of Mexico

J.S. Bayuelo-Jiménez[1,*], L.C. Paredes-Gutiérrez[2], J. Pineda-Pineda[3], J.C. Patrón-Ibarra[4] and J.J. Adu-Gyamfi[5]

ABSTRACT

A main constraint to agricultural productivity in the central Mexican highlands is low available soil phosphorus (P) exacerbated by high P sorption capacity of the Andisols. Therefore, substantial amounts of P fertilizers must be applied to obtain optimum crop yields. One cost-effective strategy for this type of soil is to enhance the plant's efficiency to acquire inorganic phosphorus (P_i) from soil and/or to use P more efficiently. The present study was conducted to evaluate genotypic variation in both root architecture and plant growth traits associated with P acquisition efficiency (PAE) and/or P utilization efficiency (PUE) of maize landraces in a P-deficient soil. The results showed that genotypes differed greatly in plant growth, grain yield, root morphology, P uptake, PAE, PUE, and P efficiency defined as growth with sub-optimal P availability. Phosphorus-efficient genotypes not only had greater biomass per unit of absorbed P, but also developed larger root systems, produced more nodal and lateral roots, and had greater root hair density and P uptake per unit root weight than did the P-inefficient genotypes under P deficiency. Genotypes with enhanced nodal rooting and dense root hairs had greater P uptake and growth under low P. The ^{32}P isotope dilution technique was employed to assess the ability of the genotypes tested to utilize P from different P sources. The P-responsive genotypes showed increased P acquisition from fertilizer, whereas P-efficient genotypes accessed soil P not available to P-inefficient ones. These results indicate that maize landraces exhibit variation for several root traits that may be useful for genetic improvement of P acquisition efficiency in maize.

Key words: *^{32}P maize, P acquisition efficiency, P utilization efficiency, root architecture and morphology*

INTRODUCTION

Phosphorus (P) is one of the least available mineral nutrients in many cropping environments (Schaffert *et al.*, 1999). Phosphorus deficiency is more critical in the highly weathered soils common in many developing regions, which may also have limited access to intensive fertilization (Hinsinger, 2001). This has led to the search for more environmentally and economically feasible strategies for improving crop production in low P soils. One approach is to enhance the plant's efficiency to acquire inorganic phosphorus (P_i) from soil and/or use P more efficiently (Lynch, 2007). Nutrient use efficiency of a crop is defined as the ability of a genotype to acquire nutrients from the soil and/or to utilize them for growth and yield (Fageria, 2008). Phosphorus use efficiency can be divided into P acquisition efficiency (PAE) and P utilization efficiency (PUE) (Wissuwa and Ae, 2001). PAE refers to mobilizing P from poorly soluble sources or to take up the soluble P available in the soil solution, while PUE is the ability to produce biomass or yield efficiently using the acquired P. Enhancing P use efficiency by plants can be achieved through improving P acquisition and/or utilization of low available P (Manske *et al.*, 2000; Richardson *et al.*, 2011).

Phosphorus availability for plants is typically greater in the topsoil, so root traits that enhance topsoil foraging tend to enhance P acquisition (Lynch, 2011). Plants have an array of adaptations to low P availability, including increased relative biomass allocation to roots (Bayuelo-Jiménez *et al.*, 2011), architectural traits that enhance topsoil foraging, traits that reduce the metabolic cost of soil exploration such as root cortical aerenchyma (Lynch, 2011), increased production and secretion of P–mobilizing root exudates (Richardson *et al.*, 2011), and increased proliferation and elongation of root hairs (Ma *et al.*, 2001). Plants with improved internal P utilization efficiency (more plant yield per unit of P uptake) can directly reduce the amount of P fertilizer required for agricultural production (Richardson *et al.,* 2011). Greater P utilization is mainly attributed to efficient translocation and use of stored P and low internal P requirements (Akthar, 2007), as well as reduced P-export from farms at harvest (Rose *et al.*, 2010).

The deployment of root architectural traits in plant breeding programmes has great potential to alleviate P deficiency. Evaluation of P-efficient germplasm among existing Mexican landraces is of interest in this regard since Mexico is the global centre of maize genetic diversity (Sánchez, Goodman and Stuber, 2000). In particular, landraces from Michoacan State are well adapted to low P environments and possess traits not common in elite germplasm (Bayuelo-Jiménez *et al.*, 2011). One of the most important traditional maize growing areas in this region is the P'urhépecha Plateau. Over 60 percent of the total arable land is P-deficient in this region (Alcalá, Ortiz and Gutiérrez, 2001).

1 Instituto Nacional de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Km 9 Carretera Morelia-Zinapécuaro. 58800. Tarímbaro. Michoacán, México
2 Instituto Nacional de Investigaciones Nucleares, Carretera México-Toluca s/n, La Marquesa, C.P. 52750, Ocoyoacac, México
3 Universidad Autónoma Chapingo, Departamento de Suelos, km 38.5 Carretera México Texcoco. 56230, Chapingo, Estado de México
4 Benemérita Universidad Autónoma de Puebla. Departamento de Agroecología y Medioambiente, 14 Sur No. 6301, Ciudad Universitaria. 72570. Puebla, México
5 Soil Science Unit, International Atomic Energy Agency, Wagramer Strasse 5, A-1400 Vienna, Austria
* E-mail address of corresponding author: bayuelo@umich.mx

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Andisols contain considerable amounts of P but a large proportion is bound to different soil constituents, forming complexes of limited bioavailability (FAO, 2001). This type of soil is commonly referred to as a P-fixing soil and the concentration of P in the soil solution is suboptimal for crop production. A common strategy for soils with low total P content is regular amendment with small doses of P fertilizer. However, in soils with high total P content that fix most of the P, fertilizer P will also be fixed. In these types of soils, plants respond to P fertilizer application but annual applications of water-soluble superphosphate or ammonium phosphate fertilizers are required to sustain crop yields (Richardson *et al*., 2011). Plants differ greatly in their ability to grow on low P soils because they have developed specific physic-chemical mechanisms to utilize P compounds in these low P fertility soils (Lynch, 2011). Evaluating and identifying crop plants for their ability to access and utilize sparingly soluble P forms in soils has been proposed as a practical means for overcoming P deficiency and optimizing P fertilizer use in cropping systems where P availability is limited (Naruzzaman, Lambers and Bolland, 2006).

The use of phosphorus-32 (^{32}P) isotope as a tracer applied to the soil with P fertilizer permits the detection of exchangeable phosphate ions in the solution and those absorbed by the plant (IAEA, 1990). Isotopic dilution techniques have been widely used to assess the availability of nutrients such as soil P to plants. The objective of this study was to evaluate the adaptation of native landraces to low P soils in the P´urhépecha Plateau, and the expression of root traits that could be important for P acquisition and utilization efficiency. This study also aimed to evaluate the ability of selected maize genotypes grown in an Andisol to use fertilizer P by using the ^{32}P isotope dilution technique.

TABLE 1. Properties of the topsoil (0–20 cm) of the Andisol in Ponzomaran, Juan Tumbio, and Bonilla locations, Michoacán, Mexico

Environmental variables	Pontzomaran	Juan Tumbio	Bonilla
Latitude (N)	19° 24´	19° 31´	19^0 30´
Longitude (W)	101° 38´	101° 36´	101° 41´
Altitude (m.a.s.l.)	2 280	2140	2 240
Rainfall (mm)	800–1 000	700–1 400	900–1 100
Sand (%)	38.6	48.1	55.1
Clay (%)	38.5	18.9	11.6
Silt (%)	22.9	36.0	33.3
Apparent density (g/cm)	0.86	1.08	0.89
pH	5.5	6.1	6.1
Organic matter (%)	7.9	6.2	4.2
Cation exchange capacity (cmol/kg)	18.6	14.7	15.3
Exchangeable aluminium (cmol/kg)	0.09	0.04	0.03
K (mg/kg)	131	444.7	454
Ca (mg/kg)	1 225	1 450	2 254
Inorganic N (mg/kg)	35	27	17.4
Available P Bray 1 (mg/kg)	1.20	4.75	2.74
Low P	1.07	3.06	1.39
High P	3.84	8.69	2.24

MATERIALS AND METHODS

Field studies to assess the genetic variability of maize landraces for root traits that enhance P acquisition in a low P soil

Three experiments were carried out under low and high P fertilization and rain-fed conditions in farmers' fields in Pontzomaran, San Juan Tumbio and Bonilla, in the central highlands of Michoacan, Mexico during the 2008 and 2009 growing season. Soils in the study sites are vitrands (FAO, 2001) and their characteristics are shown in Table 1.

Experiments were arranged in a randomized complete block design with four replications in a split-plot arrangement of treatments where P level was the main plot and genotypes the sub-plots. Each experimental unit consisted of five 5-m long rows for each accession. Experiments were conducted in P-deprived soils. The low (LP) and high (HP) treatments consisted of 23 $kg{\cdot}P_2O_5{\cdot}ha^{-1}$ and 97 $kg{\cdot}P_2O_5{\cdot}ha^{-1}$, applied as single super phosphate at seeding. All plots were supplemented additionally with 60 kg nitrogen $(N){\cdot}ha^{-1}$ as urea at the seedling and silking stages. Maize genotypes were seeded within the optimum sowing dates (around April 17–23, after the beginning of the 2008 and 2009 rainy season).

Plant material

Fifty local maize genotypes were grown. All genotypes were originally from the P'urhépecha region, which had been used recently by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) maize breeding programme; they differed in yield, P responses to fertilization, stalk lodging susceptibility and grain type (floury to flint corn). They were represented by three maturity types according to the number of days (d) required to reach the silking stage: 12 early [(E) 75–85 d], 22 intermediate [(I) 85–95 d] and 14 late [(L) 95–105 d], and by four breeding groups within each maturity type: landraces (C), advanced landraces (AC), hybrids x landraces (HxC) and synthetic hybrids (S). Landraces in the advanced generation were derived from advanced breeding lines of landrace types collected in low P soils from the P'urhépecha Plateau and crossed with an advanced generation of commercial hybrids.

Plant measurements

Root crowns were excavated at the same growth stage by removing a soil cylinder of 30 to 40 cm soil depth and 50 cm from the shoot base between 78 and 89 d after planting (DAP), at the silking stage (R1). The root crowns were immersed in water with P-free soap for about 8 minutes in order to facilitate soil removal. Four crown root traits were scored visually: nodal root length and number, nodal root branching and angle. A rating scale of 1–4 was used to rank root branching and root angle where 1 = first order lateral branching and 4 = multiple lateral branches with up to four orders of branching. For nodal root angle, one indicates shallow root angles (1 = 0° – 22.5°); 2 = 22.5° – 45°; 3 = 45° – 67.5° and four indicates steep root angles (4 = 67.5° – 90°). For root hair evaluation, root fragments were dyed in 0.05 percent trypan blue. Root hairs were evaluated visually using a rating scale of 1–9 to rank the density/length as follows: 1 — no root hairs; 3 — low root hair density/length; 5 — intermediate root hair density/length, 7 —between 5 and 9 rating scale; 9 — abundant root hairs (Vieira, Jochua and Lynch, 2007).

Plant growth and tissue analyses

One plant per plot was harvested between 78 and 89 DAP. Shoot and root biomass were determined after drying at 60°C to constant weight. Root and shoot dry tissue samples were ground and ana-

lysed for P concentration after Murphy and Riley (1962). Phosphorus acquisition efficiency (PAE) is a measure of the P uptake per unit root dry weight (DW) (mg·P/g root DW), and was calculated from total P per plant divided by root dry mass in grams (Fageria, 2008). Phosphorus utilization efficiency (PUE) is a measure of DW return per unit P uptake (g·DW·mg^{-1}·P), and was calculated as total plant DW divided by P content per plant.

Statistical analysis

Phosphorus efficiency of the maize genotypes was determined by the P efficiency index or PEI (Pan *et al*., 2008) and assessed using principal component analysis (PCA) of standardized values for plant growth and grain yield parameters at low P, and relative values at low P to those obtained under high P supply. The principal component analysis was computed using the SAS FACTOR procedure with the PRIN option and the VARIMAX method for orthogonal rotation (SAS, 2000) on all 50 maize genotypes. The relative weight of each principal component was determined by the corresponding contribution rate accounting for variation of all growth traits. Consequently, PEI values of different genotypes were calculated according to the retained principal components and their relative weights, namely $PEI = \sum_{i=1}^{50} PC_i \times RW_i$. The criterion used for classification of maize genotypes was determined by the method of cluster analysis. Genotypes were divided into three categories according to the P efficiency index and four categories according to P efficiency index in combination with growth potentials (shoot DW at high P). A distance matrix was developed with the standardized data using the dissimilarity Euclidian distance coefficient and a Ward's minimum variance clustering method (Romersburg, 1988).

Controlled environmental studies to identify genotypes for superior P acquisition and/or utilization using ^{32}P isotope dilution technique

This study was conducted at the National Nuclear Research Institute (ININ), Mexico, in a greenhouse with a temperature between 14.3 and 26.5°C, a relative humidity of 50 to 70 percent, and a fall photoperiod (13-h day and 11-h night). Artificial light for 12 hour (h) per d from cool-white fluorescent tubes supplemented the fall daylight. Plants were grown in pots with 6 kg of vitric Andisol soil of low available soil P, which was obtained from a plot on Laderas de Bonilla, Michoacan, where maize landraces are cultivated (Table I).

The experiment was set up simultaneously for non-labelled (without ^{32}P) and labelled (with ^{32}P) on two levels of P supply (low and medium available P). The design was completely randomized with a factorial arrangement of (3 × 6) treatments, i.e. three levels of P supply for six genotypes. There were four replications for a total of 72 units. Each experimental unit was composed for one pot and included P additions (ammonium phosphate [$(NH_4)_2HPO_4$] at rates of 0.0, 0.22 and 0.44 g·P/pot. These rates are equivalent to 0, 25 and 50 kg·P_2O_5/ha. The Andisol soil was labelled with $(NH_4)_2H^{32}PO_4$ by applying a high activity (10 mC_i). The tracer was added to the soil at a depth of 10 cm at the plant root zone at concentrations of 0, 0.395 and 0.791 mC_i/pot in 300 mL of water at pH 6.0 to ensure uniform labelling. Plants were irrigated with distilled water applied to the soil to field capacity (33 kPascal) to avoid leaching of the radioactive ^{32}P-labeled marker and other nutrients. Two seeds were planted in every pot. Five d after emergence, seedlings were thinned to one plant per pot. Nitrogen was added as a solution of ammonium nitrate (NH_4NO_3) at 60 kg·N·ha^{-1} to the pots in which maize was grown.

Plant material

The efficiency of P uptake was determined for five maize genotypes represented by two early (Gregorio and M-I-04), two intermediate (PICH-4 and Zacapu), and one late (DP x Tromba) emergence/maturity genotypes, and a commercial variety (Leopardo) was used as a check. These genotypes differed in efficiency and P responsiveness at the early vegetative stages (Table 2).

Plant measurements

One plant from each pot was sampled at 39 d after sowing. The plant shoots and roots were oven-dried at 70°C for two d, ground and digested in a mixture of concentrated nitric (HNO_3) and perchloric ($HClO_4$) acids (4:1). The concentration of total P in the digest was determined as described by Murphy and Riley (1962). The ^{32}P activity in the digest was determined by liquid scintillation counting using a Beckman LS-60000LL liquid scintillation system. Once the activity was quantified, isotopic variables were determined.

Based on the isotopic dilution method, the proportions of P taken up by plants from the fertilizer and soil were calculated according to IAEA (1990):

- The percentage of P derived from the fertilizer (% Pddf) was obtained by dividing the specific activity (SA) in the plant by SA in the fertilizer × 100.
- The percentage of P derived from the soil (% Pdds) was obtained by difference, i.e. 100– (% Pdds).
- The amount of P fertilizer taken up by the crop (P-fertilizer yield) was calculated by multiplying the total P yield by the percentage of P derived from the fertilizer, i.e. P-fertilizer yield (mg·P/pot) = (total P yield x % Pddf)/100.
- The P-fertilizer extracted by the plant relative to the dose of P applied (efficiency P-fertilizer) is known as P use efficiency, i.e. P-fertilizer efficiency (%) = (P – fertilizer yield/dose of P applied) × 100.
- The P content in the plant was calculated by multiplying the P concentration by the dry weight [P concentration in plant (mg·P/g) x dry matter yield (g·P/pot) /100.

Statistical analysis was performed for a completely randomized design and a factorial arrangement: P supply, genotype and interaction P supply x genotype. The average effect of each treatment was determined using the Tukey test (p = 0.05) with SAS (2000) software.

RESULTS AND DISCUSSION

Genetic variability for P efficiency and P responsiveness

Substantial variation in growth for low P soil was recorded among maize landraces from the central Mexican highlands. Genotypes were grouped into three categories of P efficiency based on shoot growth, PAE and PUE parameters at LP and their relative values to those at HP. The results indicated that 12 genotypes across locations had the lowest growth and the highest levels of P efficiency (PEI > 0.54) under low P (Table 2).

When the combination of PEI with P responsiveness at HP is considered, SHUI-2, Paramuén, ZR-6, CB-II, Paso del Muerto, CCHEDE, Macho III-05, M-I-04, and M-IV-03 were the most efficient genotypes for the P-deficient acidic soil of this region (Table 2). These genotypes were categorized as the most P-efficient under LP and as the most responsive to increased P availability. Applications of 97 kg·P_2O_5·ha^{-1} (equivalent to 41.7 kg·P·ha^{-1}) increased the shoot biomass and grain yields of the genotypes. However, the increase was nearly equal to the difference between genotypes in the low P soil (23 kg·P_2O_5·ha^{-1}, equivalent to 9.9 kg·P·ha^{-1}). Thus, in low P soil the P-efficient

TABLE 2. Shoot dry weight, P acquisition efficiency (PAE), and P utilization efficiency (PUE) of 50 common maize genotypes grown in a P-deficient soil with low P (LP) or high P addition (HP), in 2008 and 2009

ID	Genotypes	M	BG[b]	Shoot dry weight (g/plant)					PAE (mg·P ·g^{-1} root DM)				PUE (g·DM·mg^{-1} P)			
				LP	HP	PEI[c]	SdSh[d]	PR[e]	LP	HP	SdPAE	PR	LP	HP	SdPUE	PR
40	Paramuén	Late	C	154	196	0.75	3.38	ER	17.2	18.2	0.55	ER	0.83	0.87	1.24	ER
109	Paso del Muerto	Late	AC	155	174	0.66	1.70	ER	21.8	19.7	0.44	ER	0.72	0.88	-0.16	ENR
78	CB-II	Middle	C	106	134	0.37	0.21	ER	17.4	19.8	0.92	ER	0.78	0.92	-0.48	ENR
236	M-II-03	Early	S	116	139	0.30	0.63	ER	16.2	15.4	-0.18	ENR	0.84	0.91	-0.52	ENR
117	A-7545	Late	HxC	109	132	0.22	0.19	ER	21.8	15.7	-0.43	ENR	0.82	0.94	-0.32	ENR
233	M-I-03	Early	S	105	147	0.20	0.98	ER	18.4	16.3	-0.02	ENR	0.76	0.87	0.31	ER
239	M-IV-03	Late	S	112	132	0.10	0.10	ER	16.2	17.7	-0.01	ENR	0.81	0.89	0.19	ER
75	ZR-6	Late	C	142	147	0.05	1.03	ER	18.7	17.7	0.27	ER	0.90	0.89	0.46	ER
234	M-I-04	Early	S	141	138	0.04	0.58	ER	22.2	20.0	1.05	ER	0.93	0.95	-0.63	ENR
182	M-III-05	Middle	S	130	128	0.64	-0.43	ENR	20.0	19.2	0.57	ER	0.84	0.86	0.45	ER
144	San Gregorio	Early	AC	102	107	0.60	-1.14	ENR	19.5	14.4	-0.62	ENR	0.85	1.18	-2.56	E NR
79	PICH-4	Middle	C	136	124	0.54	-0.12	ENR	17.4	14.9	-0.29	ENR	0.87	0.86	0.03	ER
6	SHUI-2	Late	C	102	117	0.15	-0.45	ENR	15.4	20.2	1.18	ER	0.92	0.89	0.99	ER
127	DP x Tromba	Late	HxC	97	138	-0.95	0.37	NER	23.8	16.9	0.40	NER	1.18	0.85	0.18	NER
215	Zacapu	Late	AC	91	104	-0.36	-1.70	NENR	15.4	17.8	-0.15	NENR	1.10	0.86	0.21	NER
	Average			115	128				17.9	16.7			0.92	0.88		
	LSD (0.05) [a]			52	53				9.00	7.9			0.23	0.21		

a — to compare paired values among genotypes; b BG — breeding groups: landrace (C), advanced landrace (AC), hybrids x landrace (H x C), and synthetic (S); C — P efficiency index (PEI) obtained from the principal component analysis; d — standardized value of shoot dry weight (SdSh), P acquisition efficiency (SdPAE), and P utilization efficiency (SdPUE) under high P conditions. Data from the three individual experiments were standardized by dividing relative values by the standard deviation of the trial. Phosphorus responsiveness (PR) is expressed by shoot biomass under HP level using four categories: efficient and responsive (ER), non-efficient and responsive (NER), non-efficient and non-responsive (NENR), and efficient and non-responsive (ENR).

genotypes seemed to produce the same amount of dry matter and grain yield as genotypes with higher P fertilizer applications (97 kg·P_2O_5·ha^{-1}). These results directly support the added advantage of selection and breeding for P efficiency for maintaining productivity in low P soils.

Higher P efficiency in plants can be achieved by improving P acquisition and/or internal P utilization (Fageria, 2008). Comparisons between common maize genotypes in three soil environments and maturity types showed that differences in P acquisition were due to large variations in plant growth, P content and root architecture (data not shown). In general, genotypes with increasing P efficiency at LP had greater root and shoot dry weights, root to shoot ratios and root and shoot P contents. Genotypes of the P-efficient group had more nodal rooting (64 to 81) and root branching (two orders of branching) under LP, whereas root hair density and length of nodal first-order laterals were significantly greater under LP than in HP (on a rating scale of 5 to 7).

Mechanism involved in P efficiency: root architecture and morphology

Root system architecture and morphology are key traits for optimizing P acquisition, and thus their P use efficiency and responsiveness (Manske, 2000). Architectural traits associated with enhanced topsoil foraging include shallower growth angles of axial roots, enhanced adventitious rooting, a greater number of axial roots and greater dispersion of lateral roots (Lynch, 2007). Genotypes with increased or sustained elongation of axilar roots and lateral root development under P deficiency demonstrated superior ability to acquire P and maintain growth (r^2 = 0.53–0.82). This study confirmed that enhanced nodal rooting and greater nodal branching (nodal root laterals) were indeed important for plant adaptation to low P in maize (Table 3).

Nodal rooting was correlated significantly with plant growth and P uptake in the field among contrasting genotypes (Table 3). Efficient genotypes with greater nodal rooting and lateral branching at low P had greater biomass and P uptake efficiencies (r^2 = 0.53– 0.94)

than did inefficient genotypes with reduced nodal root formation and lateral branching (r^2 = 0.18–0.23). The greater nodal rooting of P-efficient genotypes under low P could be explained by the greater overall biomass and the weak allometric relationship between plant biomass and root biomass (r^2 = 0.04). Therefore, if nodal roots in the topsoil are advantageous for acquiring P under limited P availability as suggested by Lynch (2007), this weak allometric relationship would facilitate the selection of efficient genotypes with high nodal rooting in the field.

Several lines of evidence show that root hairs contribute to P acquisition (Jungk, 2001). Low P availability increases the length and density of root hairs (Ma *et al.*, 2001). In this study large variations were noted for the ability to develop root hairs along the nodal first-order laterals (Table 3). Whereas several inefficient genotypes had

TABLE 3. Relationships between root traits: nodal root length (Nod_RL), number of nodal roots (Nod_No), nodal branching (Nod_Br), root hair density, (RHD_mnr) and length, (RHL_mnr) from the middle region of nodal roots and measures of P efficiency: shoot dry weight (SDW), grain yield (GY) and P acquisition efficiency (PAE) of P-efficient genotypes across locations

Parameter		SDW (g/plant^{-1})			GY (kg/ha)			PAE (mg·P·g^{-1}·root)		
	P	Early	Middle	Late	Early	Middle	Late	Early	Middle	Late
Nod_RL	HP	0.49[a]	0.02	-0.85	-0.08	-0.01	-0.09	-0.04	-0.02	-0.54
	LP	0.50	0.01	0.76	-0.72	0.67	0.88	-0.96	0.25	0.11
Nod_No	HP	0.01	0.13	0.19	-0.21	0.02	0.00	-0.27	0.79	0.35
	LP	0.94	0.53	0.75	-0.21	0.32	0.56	-0.89	0.02	0.43
Nod_Br	HP	0.05	0.28	0.73	0.46	-0.23	0.04	0.52	0.06	0.42
	LP	0.57	0.82	0.31	-0.90	0.35	0.04	-0.82	0.22	0.73
RHD_mnr	HP	0.03	-0.25	-0.26	-0.11	-0.03	0.60	-0.15	-0.14	-0.34
	LP	0.31	-0.52	0.34	0.47	-0.24	-0.30	0.02	-0.05	0.97
RHL_mnr	HP	-0.53	-0.12	0.79	-0.94	-0.04	0.20	-0.97	-0.07	0.55
	LP	0.74	-0.12	0.81	0.46	-0.16	-0.82	0.19	-0.12	0.69

a — regression coefficients at the 5 percent level

shorter and fewer root hairs, efficient genotypes developed much longer and denser hairs on nodal roots. The presence of denser root hairs of nodal first-order laterals (r^2 = 0.95), was associated with plant performance at low P, grain yield and root P acquisition at LP (Table 3). Late P-efficient genotypes can be selected showing a significant relationship between root hairs and root P acquisition, suggesting that the P acquisition ability of genotypes is a decisive factor in expression of high P efficiency.

Phenotypic traits conferring P utilization efficiency

Phosphorus utilization efficiency represents the amount of dry matter produced per unit of P absorbed (g·DM·mg^{-1} P) (Fageria, 2008). Any species able to maintain metabolic activities at low tissue P concentration and produce more dry matter per unit of P absorbed is considered efficient in P utilization. In this study, there were genotypes in which the P content in the shoot had a highly significant correlation with root (r = 0.54**) and shoot dry weights (r = 0.78***) suggesting that genotypes with higher root dry weight accumulated greater amounts of P in the shoot and produced higher shoot dry matter at LP. Thus, under P stress, better P acquisition and PUE by the P-efficient genotypes for biomass synthesis collectively formed the basis of higher shoot dry matter production, evidence that P uptake and PUE are significant plant traits for selecting low P-tolerant genotypes.

Variations in P uptake and use efficiency by maize using ^{32}P

Due to the very low P available soil used in this study (2.2 mg·P·kg^{-1} soil), typical of Mexican volcanic soils, the response of the maize to fertilizer application was evident (Figure 1). From ANOVA data, P levels, genotypes and P × genotype interactions were significantly different (p = 0.01). Phosphorus-responsive San Gregorio, M-I-04, and PICH-4 produced the highest dry weight yields (7.7 and 5.9 g/plant) and Zacapu and DP x Tromba had the lowest yields (3.4 g/plant). With regard to the P treatments, the highest value was obtained in the HP treatment (5 g/plant) followed by LP (2.8 g/plant) and the control (only soil P) treatment (1.9 g/plant). The root and shoot P concentrations and P uptakes by the six maize genotypes under the three P treatments are shown in Figure 1. According to the ANOVA results, the interaction P levels x genotypes was not significant, only the effect of P and genotypes. All maize genotypes grown in the Andisol soil had low root P concentrations (<1.1 mg·g^{-1}·P), suggesting a strong P limitation in the soil (Figure 1b-e). The P concentrations in shoot tissue of the P-responsive Gregorio, Macho I-04, PICH-4 and Leopardo genotypes (0.87–1.0 mg·g^{-1}·P) were low compared with the P-efficient accession DP x Tromba (1.6 mg·g^{-1}·P), suggesting that maize has a higher ability to take up more P from low available P soils than P-responsive ones (Figure 1e).

The P uptake for the HP level (5.9 mg·P·plant^{-1}; 338.6 mg·P/pot) was significantly higher than that for the LP treatment (3 mg·P·plant^{-1}; 164.5 mg·P/pot). Thus, maize genotypes were able to take up significant amounts of P from fertilizer. The P uptake for the P-responsive genotypes was about 2.5-fold greater than that of P-efficient ones. This response to the high application rate confirms the great P demand of this crop and the significance of uptake and usage mechanisms. The data in Table 4 show the proportions of P in samples derived from the P fertilizer treatment (%Pdff) and soil (%Pdds). For the %Pdff the effects of genotypes, P levels and the P x genotype interactions were significant. Comparing the genotypes at each P level, the P-responsive San Gregorio was significantly higher than the other genotypes. From the comparisons among the LP level for each genotype, San Gregorio and PICH-4 showed the lowest %Pdd values. In other words, they were more dependent on the water-soluble fertilizer for their nutrition. By contrast, the P-efficient DP x Tromba with its greater % Pdf value (most efficient in P uptake from soil) had a lower %Pddf.

The P use efficiency of added $(NH_4)_2HPO_4$ ranged respectively from 3.4 to 51.7 percent and from 0.7 to 13.9 percent in the HP and LP levels (Table 5). From the ANOVA, significant differences were found for the effects of treatment or the P x genotype interactions. The range of P use efficiency of the P-responsive genotypes (11.9 to 51.7 percent) was higher than for the P-efficient genotypes (3.4 to 5.8 percent). The P use efficiency from P fertilizer was relatively high for a high P-fixing volcanic soil but maize is a high P demanding crop (Schaffert *et al*., 1999), and the experiment was conducted under well-controlled conditions for a relatively short period (36 d). On the other hand, the P use efficiency from P-efficient genotypes was low in both P treatments, thus confirming the ability of DP x Tromba to take up significantly greater amounts of P from non-labile P in the Andisol. The mean specific activity of P in DP x Tromba was lower than in all other genotypes which had the highest value under HP

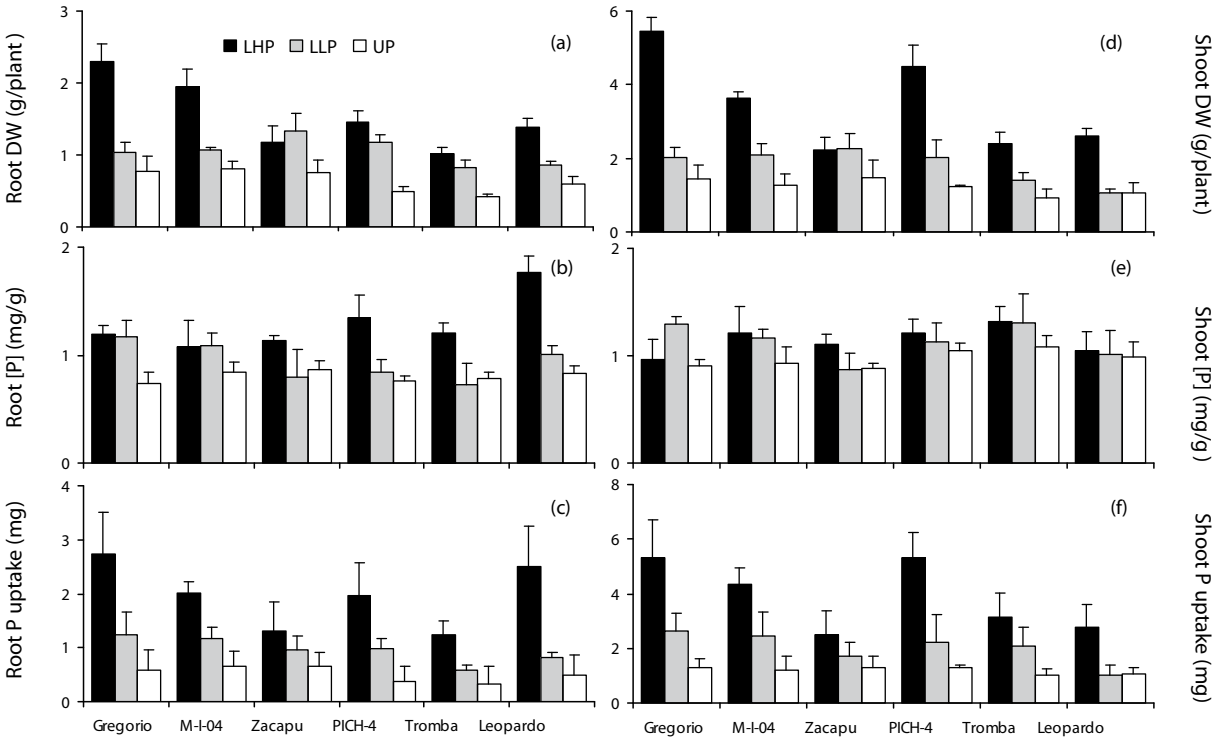


FIGURE 1. Root dry weight (a), root P concentration (b), root P uptake (c), shoot dry weight (d), shoot P concentration (e) and shoot P uptake (f) for maize grown in a soil labelled (LHP, LLP) and unlabelled (UP) with ^{32}P. The error bar represents standard error from the mean (n).

TABLE 4. Effects of the P-fertilizer treatments on total P yield, specific activity and % P derived from fertilizer (%PDFF) in maize

				Total P yield (mg·P·pot^{-1})				Specific activity (Bq·mg^{-1} P)				%Pdff			
				Root		Shoot		Root		Shoot		Root		Shoot	
ID	Accession	M [b]	BG [c]	HP [d]	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP
144	San Gregorio	E	AC	40.9	43.2	466.1	125.5	4 410	5016	26 320	6984	6.2	7.1	37.3	9.9
234	M-I-04	E	S	40.0	24.5	208.7	84.4	5 807	3125	14 362	4911	8.2	4.4	20.3	7.0
215	Zacapu	I	HXC	11.8	14.2	125.6	45.1	2 615	2124	15 037	3672	3.7	3.0	21.3	5.2
79	PICH-4	I	C	37.8	40.9	179.3	84.1	5 760	6182	9 577	5388	8.2	8.8	13.6	7.6
127	DP x Tromba	L	HXC	23.1	11.6	95.7	22.8	5 532	2954	8 903	1847	7.8	4.2	12.6	2.6
247	Leopardo	I	cv	21.5	13.7	74.7	11.2	2 776	2438	8 016	1638	3.9	3.5	11.3	2.3
	LSD $_{0.05}$ [a]			16.8	14.2	105.0	50.9	1 192	1503	5 214	1464	1.7	2.1	7.1	2.1

and LP conditions (Figure 1a). If all the genotypes drew their P from the same pool of available P, then the specific ^{32}P activity of the P in their shoots should be comparable, although concentrations of P in the shoots and the amounts of P accumulated may differ (Larsen, 1952). Consequently, the lower specific activity of the P taken up by DP x Tromba indicates that it was able to access P in the soil that was less available to all other genotypes.

In acid soils, P ions precipitate as iron (Fe) and aluminium (Al) phosphates such as strengite ($FePO_4{\cdot}2H_2O$) and variscite ($AlPO_4{\cdot}2H_2O$), due to the high concentration of trivalent Fe and Al in the soil solution. These types of P compounds are commonly assigned to stable P pools that are considered sparingly available to plants. However, evidence has accumulated that some plant species are efficient in utilizing certain sparingly soluble P sources. For example, Wang *et al*. (2011) found that wheat (*Triticum aestivum* L.) was efficient in using $AlPO_4$ due to its ability to develop high root length density, and canola (*Brassica napus* L.) can access $FePO_4$ due to its ability to excrete protons from the root (Pearse, 2011). Also, recent studies have identified genotypic differences between maize landraces in P efficiency (Bayuelo-Jiménez *et al*., 2011), particularly that the development of an extensive root system with dense root hairs was one of the main strategies of acquiring soil P for P-efficient genotypes. Phosphorus-efficient landraces of the Mexican highlands can therefore be beneficial for improving the use of native soil P and P fertilizers.

TABLE 5. Effects of the P-fertilizer treatments on P derived from soil (%PDFS), fertilizer P yield, and efficiency P fertilizer in maize

				%Pdfs				Fertilizer P yield (mg·P·pot^{-1})				EPF (%)			
				Root		Shoot		Root		Shoot		Root		Shoot	
ID	Accession	M [b]	BG [c]	HP [d]	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP
144	San Gregorio	E	AC	93.8	92.9	62.7	90.1	2.5	3.1	178.8	12.5	0.6	1.5	43.2	6.0
234	M-I-04	E	S	91.8	95.6	79.7	93.0	3.5	1.1	42.7	6.1	0.8	0.5	10.3	2.9
215	Zacapu	I	HXC	96.3	97.0	78.7	94.8	0.4	0.6	26.4	2.6	0.1	0.3	6.4	1.3
79	PICH-4	I	C	91.8	91.2	86.4	92.4	3.1	3.7	25.8	7.0	0.7	1.8	6.2	3.4
127	DP x Tromba	L	HXC	92.2	95.8	87.4	97.4	1.8	0.5	12.3	0.6	0.4	0.2	3.0	0.3
247	Leopardo	I	cv	96.1	96.5	88.7	97.7	0.8	0.5	8.5	0.3	0.2	0.2	2.0	0.1
	LSD $_{0.05}$ [a]			1.6	2.1	7.1	2.0	1.4	1.6	51.9	5.4	0.3	0.8	12.5	2.6

a — to compare paired values among genotypes; b — maturity: early (E), intermediate (I), late (L); c — BG: d — breeding groups: landrace (C), advanced landrace (AC), hybrids x land race (H × C), and synthetic (S); [d] — P additions as $(NH_4)_2HPO_4$ at rates of 0.22 g·P/pot (LP) and 0.44 g·P/pot (HP).

This study demonstrated the ability of maize to absorb significant amounts of P from the inorganic soil P pools. The use of the ^{32}P isotopic technique enabled quantitative measurement of P uptake from P fertilizer sources and differences in P acquisition among genotypes. Although these differences were small, the genotype DP x Tromba showed increased P acquisition from inorganic soil P pools. Phosphorus-efficient genotypes in combination with a high P use efficiency from fertilizers are therefore an attractive strategy for sustainable agricultural production in Mexican Andisols.

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Phosphorus Acquisition from Sparingly Soluble Forms by Maize and Soybean in Low – and Medium – P Soils using ^{32}P

J.J. Adu-Gyamfi[1,*], M. Aigner[1], S. Linic[1] and D. Gludovacz[2]

ABSTRACT

A glasshouse pot experiment was conducted to evaluate the differential ability of maize (*Zea mays*) and soybean (*Glycine max*) to utilize soil phosphorus (P) for plant growth from total P, available P and inorganic (Ca–P, Al–P and Fe–P) soil P pools using a carrier-free ^{32}P solution. A maize variety (DK 315) and a soybean variety (TGX 1910-4F) were grown in pots containing 1 kg of a low available P soil (Hungarian) or a medium available P (Waldviertel) soil labelled with ^{32}P for 42 d or without ^{32}P (unlabelled) for 42 and 60 d. The shoot and root biomass of maize and soybean were significantly greater when grown on the Waldviertel than on the Hungarian soils. The shoot P concentrations were higher for soybean (1.7–2.2 g/kg) than for maize (1.1–1.4 g/kg). The total radioactivity (dpm x 10^6) was higher in plants grown in Waldviertel than in Hungarian soil and the values reflected in the plant P uptake and shoot biomass of soybean and maize. The L-values (μg·P·g·soil^{-1}) of maize and soybean were higher in Waldviertel (72–78) than in Hungarian (9.6–20) soil. No significant differences in L-values were observed for maize and soybean grown on the Waldviertel soil, but for the Hungarian soil, the L-values were higher for maize (20.0) than for soybean (9.6), suggesting that in this low P soil, maize was more efficient than soybean in taking up soil P. The available P (Bray II) and the Ca–P were the fractions most depleted by plants followed by the Fe–P fractions in the two soils, but differences between the crops were not significant. When soil P is limited, maize and soybean are able to access P mainly from the available P (Bray II), and the sparingly soluble Fe– and Ca–P fractions, and not Al–P from the soil.

Key words: *aluminium-P, calcium-P, Hungarian soil, iron–P, maize, soybean, sparingly soluble phosphorus, Waldviertel soil*

INTRODUCTION

Soils characterized by poor phosphorus (P) availability are widespread globally (Raghothama and Karthikeyan, 2005) and for these soils to be agriculturally productive, they require regular application of water-soluble superphosphate or ammonium phosphate fertilizers to either maintain the soil P status of fertile soils or increase that of soils with inherently low P fertility. Soluble phosphates applied to P deficient soils is retained by iron (Fe), aluminum (Al) and calcium (Ca) ions and are virtually unavailable to most plant species.

Plants differ greatly in their ability to grow on low P soils because they have developed specific physico-chemical mechanisms/processes to utilize P compounds in these low P fertility soils. These mechanisms include: (i) alterations (morphological and physiological) to root systems, i.e. mycorhizal plants have better water uptake and Al tolerance in acid soils (Hiradate *et al.,* 2007), (ii) secretion of low molecular weight organic compounds (exudates production), i.e. malonic, oxalic, citric, malic and piscidic acids secreted by roots of pigeon pea help to release low-soluble P compounds in soils (Ishikawa *et al*., 2002), (iii) secretion of enzymatic compounds, i.e. phosphatases, and (iv) molecular changes such as enhanced expression of P transporters (Naruzzaman *et al*., 2006). Intra-specific variations in a crop's ability to use sparingly soluble forms (P associated with Al, Fe and Ca) in low-P available soils have been well documented for pigeonpea (Ae *et al*., 1990) and for soybean, cowpea and maize (Nwoke *et al*., 2007).

Radio-isotopic P techniques, using the principle of isotopic exchange, allow measurement of the amount of orthophosphate that can be transferred from the soil solid to the solution over a given time, and can thus provide a powerful alternative means for characterizing soil P availability and the sources of P, with minimum modifications of soil P forms compared with conventional extraction methods. The technique has been used to measure the quantity of available P in soils for determining the E-value or exchangeable P (Larsen, 1952), the L-value or labile P (Fried and Dean, 1952), using plants grown in a soil labelled with carrier-free ^{32}P or ^{33}P-orthophosphate and the A-value or available P. This study aimed to evaluate the differential ability of maize and soybean to access and utilize P from different soil pools using two soils, one low- and one medium-P availability. The experiment aimed to test the hypothesis that P uptake from sparingly soluble P forms (Al–P, Fe–P and Ca–P) by different crops can be used as a criterion to evaluate and possible select crop plants tolerant to low available P soils.

MATERIALS AND METHODS

Plant growth conditions

Two experiments were set up simultaneously with maize (cereal) and soybean (legume) as test crops to include two treatments consisting of non-labelled (without ^{32}P) and labelled (with ^{32}P) imposed on soils with low and medium available P in a factorial design with four replications. In total, there were 20 pots for the radioisotope (including

1 Soil and Water Management & Crop Nutrition Laboratory, International Atomic Energy Agency, Seibersdorf, Austria
2 Nuclear Material Laboratory, Safeguard Analytical Services, International Atomic Energy Agency, Seibersdorf, Austria
* E-mail address of corresponding author: J.Adu-gyamfi@iaea.org

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TABLE 1. Physical and chemical properties of the soils

Propertiest	Hungarian soil	Waldviertel soil
Sand (%)	83	27.3
Silt (%)	8.8	58.2
Clay (%)	8.2	14.5
Bulk density (g/cm)	–	1.29
Saturated water content (%)	—	47
pH ($H_2$0/KCl)	5.5/4.6	6.5/6.0
Total P (mg/kg)	302	502
Available P (Bray/Olsen) (mg?kg)	21/13.3	44/12.8
Inorganic P (Ca–Al–Fe) (mg/kg)	36–85–65	56–144–68
EC (25°C) (µS/cm)	—	166
Total nitrogen(N) (g/kg)	0.83	1.21
Organic C (g/kg)	7.91	20
Ca (cmol/kg)	1.82	13.69
Mg (cmol/kg)	0.61	3.13
K (cmo/kg)	0.09	0.15
Na (cmol/kg)	0.04	0.06
CEC (cmol/kg)	2.66	23.59

†EC — electrical conductivity; Ca, Mg, K and Na were determined using cobalthexamine; CEC — cation exchange capacity

four pots without plants as controls) and 36 pots (including four pots without plants and two sampling periods) for the unlabelled treatment. A low-P soil from Hungary and a medium-P soil (Waldviertel) from Austria were used. The physical and chemical characteristics of the two soils are given in Table 1.

A maize variety (DK 315) from Austria and a soybean variety (TGX 1910-4F) from IITA, Nigeria, were grown in plastic pots (one plant per pot) containing 1 kg of soil in a naturally lit glasshouse with a temperature of 34/21°C for day/night and relative humidity of 40–70 percent. Each pot received basal fertilizer equivalent to 200 kg·N·ha^{-1} as ammonium sulphate and 50 kg·K·ha^{-1} as potassium chloride. Prior to planting, the weight and P concentration of soybean and maize seeds used for the experiment were determined. The amount of P in seed was 0.98 mg·P·kg^{-1} (0.35 g with 2.8 mg·kg^{-1}·P) for maize and 3.48 mg·P·kg^{-1} (0.59 g with 5.9 mg kg^{-1} P) for soybean. Phosphorus-32 labelled $K_2H^{32}PO_4$ (specific activity 40.7 GBq/mmol) was applied to the pots. A total of 250 ml for the Waldviertel and 150 ml for the Hungarian containing 12.4 MBq (335 µCi) of a $K_2H^{32}PO_4$ solution was applied to each of the 20 pots containing 1 kg soil. Pre-germinated maize and soybean seeds were sown at one per pot immediately after the addition of ^{32}P. Twenty ml of inoculum (*Bradyrhizobium japonicum*) mixture were added to all the soils. Nitrogen was applied at 100 kg·N·ha^{-1} as ammonium sulphate to the maize.

Plant and soil sampling and analyses

The first plant sampling for the labelled and the non-labelled treatments was done at 42 d after sowing (DAS) whereas the non-labelled treatments were allowed to grow until 60 DAS. Soil samples (10–12 g) were taken with a special soil auger (inner diameter 8 mm, outside diameter 10 mm and length 25 cm) at 0, 1, 5, 42 and 60 DAS, oven-dried at 70°C for 18 h, milled and a portion used for analysis. Plants were harvested and separated into shoots (radioisotope-labelled) and shoot and roots (non-radioisotope), chopped into small pieces, oven-dried, weighed, and ground. Total P in soils was determined using the colorimetric method (Murphy and Riley, 1962) after acid digestion, and available P (Bray P2 and Olsen) determined by the colorimetric method after extraction. The inorganic soil P fractions were measured according to a fractionation scheme based on the method described by Sekiya (1983). The ^{32}P radioactivity in all the fractions (total P, available P, Ca–P, Al–P and Fe–P) was measured by liquid scintillation spectrometry (Packard 2000). Phosphorus in the maize

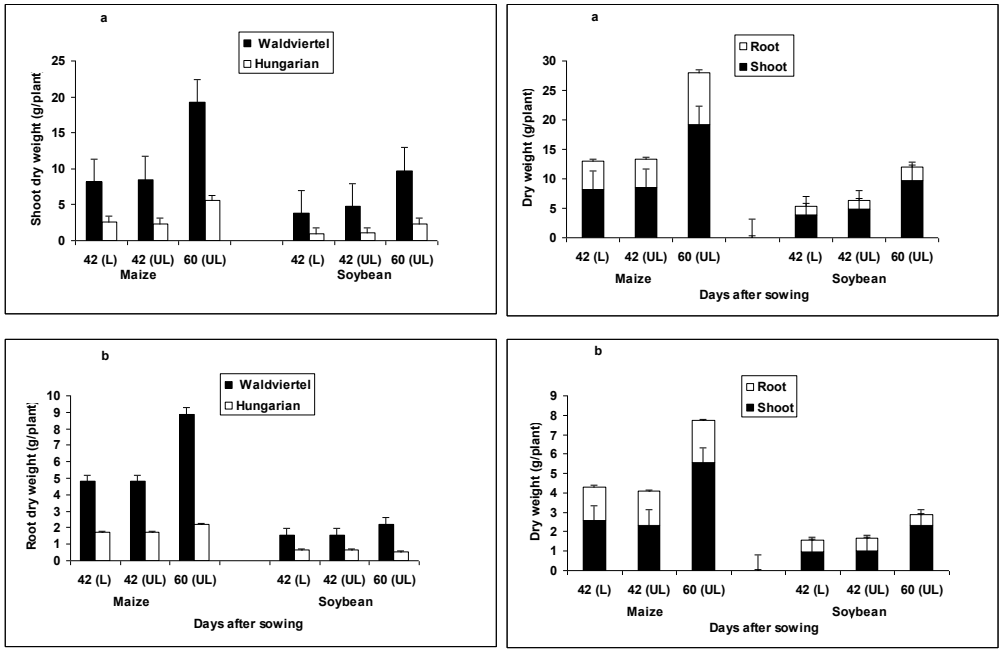


FIGURE 1. Dry weight of shoot (a) and root (b) for maize and soybean grown in Waldviertel and Hungarian soils labelled (L) and unlabelled (UL) with ^{32}P.

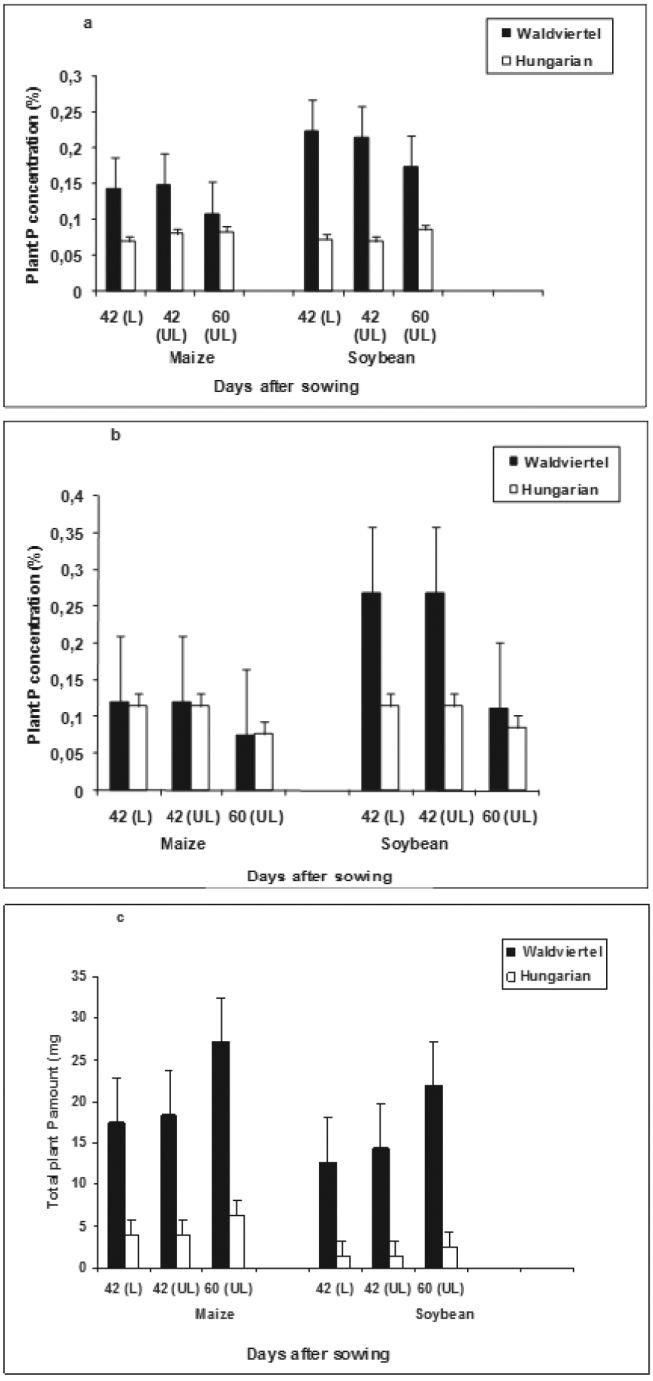


FIGURE 2. Phosphorus concentration of shoot (a) and root (b) and plant P amount (c) for maize and soybean grown in two soils. For legend see Figure 1.

and soybean seeds was determined after five seed samples, each of 100 mg, were ground and acid digested.

RESULTS

Plant growth and P uptake

The shoot and root biomass of both maize and soybean were significantly greater in Waldviertel than in Hungarian soil and there was a significant increase in shoot dry weight per plant from 42 to 60 DAS (Figure 1). Shoot weight of maize increased from 8.1 g at 42 DAS to

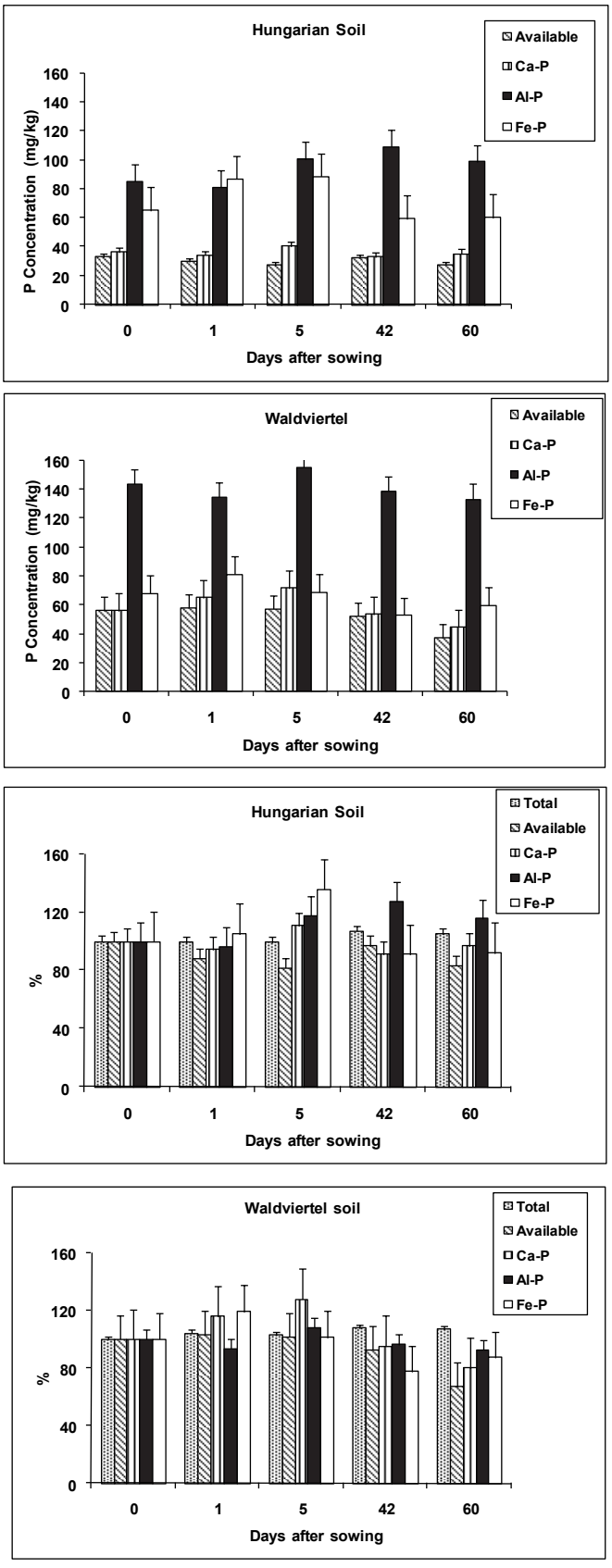


FIGURE 3. Inorganic P pools (Fe–P, Al–P and Ca–P) and available P extracted from the two soils at 0, 1, 5, 42 and 60 DAS.

19.1 g/plant at 60 DAS in Waldviertel, and from 2.2 g at 42 DAS to 5.7 g/plant in the Hungarian soil. For soybean, there was an increase from 4.1 g at 42 DAS to 9.8 g/plant for the Waldviertel and from 1.0 g at 42 DAS to 2.3 g/plant in the Hungarian soil.

The shoot P concentrations were higher for soybean (1.8–2.2 mg/g) than for maize (1.1–1.4 mg/g) and decreased with plant age for maize but not significantly for soybean (Figure 2).

Although the plant P concentrations were high in soybean compared with maize, the total P amount (mg P) was significantly higher in maize than in soybean, reflecting the higher yield in maize than in soybean. The values for maize increased in Waldviertel from 17.6 mg P at 42 DAS to 27.3 mg P at 60 DAS, while for soybean values increased from 13 mg P at 42 DAS to 22 mg P at 60 DAS (Figure 2).

In the Hungarian soil, there was no significant change in Ca–P but a slight decrease in Fe–P and a substantial increase in Al–P (85–98 mg/kg) from 0 to 60 DAS (Figure 3). In the Waldviertel soil, Ca–P decreased (from 56 to 45 mg/kg), Al–P (from 144 to 133 mg /kg) and Fe–P (from 68 to 59.5 mg/kg) between 0 and 45 DAS (Figure 3).

Radioactivity in plants and L-values.

The total radioactivity was higher in plants grown in Waldviertel than in Hungarian soil and the values reflected the plant P uptake and shoot biomass of soybean and maize. To assess the amount of isotopically exchangeable P, the L-value was estimated using the following equation:

$$L = (^{31}P \text{ shoot} - {}^{31}P \text{ seed}) \times {}^{32}P \text{ added to soil} / ({}^{32}P \text{ shoot})$$

where L is the L-value (µg·P·g $^{-1}$soil), the initial applied dose of ^{32}P (Bq·kg^{-1}soil); ^{32}P shoot is activity of shoot mass (Bq·g^{-1} DM); ^{31}P shoot is the total amount of P in shoot biomass (mg·P·g^{-1} DM).

The L-values (µg P g^{-1} soil) in maize and soybean were higher in the Waldviertel (> 70.0) than in Hungarian soil (< 20.0). No significant differences in L-values were observed for maize and soybean grown on the Walviertel soil, but for the Hungarian soil, the L-values were higher for maize (20.0) than in soybean (9.6) suggest ing that in this soil, maize was more efficient in taking up P than soybean (Figure 4). Recovery of radioactivity in shoot was higher for maize (14.5 percent) than for soybean (10.3 percent) and was four times higher in Waldviertel (mean recovery 12.4 percent) than in the Hungarian soil (mean recovery 2.5 percent) (Figure 4).

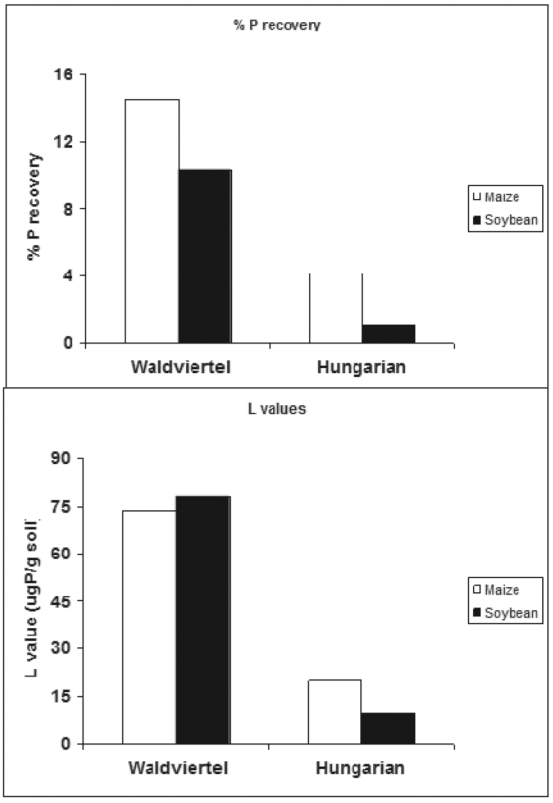


FIGURE 4. Percentage P recovery in plant shoots and the L-values calculated for maize and soybean grown on two soils.

DISCUSSION

Plant growth and P uptake

Maize and soybean grown in the Hungarian soil had low shoot P concentrations (<1 mg·g^{-1}·P) suggesting a strong P limitation. The P concentration in shoot tissue of maize (1.1–1.5 mg·g^{-1}·P) was low compared with that in soybean (1.8–2.2 mg·g^{-1}·P) and this is attributed to a dilution effect as biomass increased (see Figures 2 and 3). However, in the low available P soil, total P accumulation in shoots was higher in maize than in soybean suggesting that maize has a greater ability to take more P from low available P soils than soybean. The Hungarian soil had 21 mg/kg (Bray II) and 13.5 mg/kg Olsen) P, and for 1 kg soil, more than 12 mg P is expected to be available to the plant. This suggests that the available P extraction using Bray II may contain other P forms that are not easily available to maize and soybean. In addition, the fact that nodules were not observed on soybean grown in the Hungarian soil despite the *Rhizobium* inoculation, while soybean grown on the Waldviertel was well nodulated (0.6 mg·DM·plant^{-1}) suggests that nodule formation in the soybean was severely impaired by P deficiency, thereby contributing to the poor growth (Adu-Gyamfi *et al.,* 1989).

Dynamics of P fractions

The Bray P2 and the Ca–P were the fractions depleted by plants followed by the Fe–P fractions in the two soils, and differences observed between the crops were not significant. The results suggest that more P was released in the soil solution (labile) from the available and Ca–P fractions for plant uptake than from the Fe– and Al–P fractions. Among the inorganic pools, Al–P, Fe–P and Ca–P are the three major fractions in the soil. The results reported here imply that when P is not supplied, maize and soybean are able to access mainly Fe– and Ca–P but not Al–P from the soil.

Isotopically exchangeable parameters and P uptake by maize and soybean in low and medium P soils

The high total radioactivity in maize compared with soybean suggests that plant P uptake from soil was greater by maize than by soybean irrespective of the soil used. Whereas there was a 1.4-fold increase in radioactivity in maize over soybean in the Waldviertel soil, there was a 4.3-fold increase by maize over soybean in the Hungarian soil. These data suggest that maize could take up more P under conditions of low P availability than soybean. This is supported by the finding that the L-value (with seed P uptake correction factor) of maize was double that of soybean in the low P Hungarian soil. Maize and soybean grown in medium P (Waldviertel) soil had lower specific radioactivities (dpm x 10^3 mg^{-1} P or kBq x 10^3 mg^{-1} P) in shoot than those grown on the low P Hungarian soil, and the values were lower

in maize than in soybean. Low specific radioactivity indicates that plants were using otherwise unavailable P sources.

CONCLUSIONS

The main finding from this study was that maize was more efficient in taking up P from sparingly soluble inorganic-P sources than soybean in the medium P (Waldviertel) soil; as indicated by the low specific radioactivity (dpm × 10^3 mg^{-1}·P or kBq × 10^3 mg^{-1}·P) in shoots. The L-values of maize were double those of soybean in the low P (Hungarian) soil suggesting the superiority of maize to access sparingly soluble P from soils compared with soybean. When P was not supplied, maize and soybean were able to access P mainly from the available P (Bray P2), Fe– and Ca–P sparingly soluble fractions, but not Al–P from the soil. Maize and soybean showed severe P deficiency when grown on the Hungarian soil and the P concentration in plants was below 1 mg/g suggesting that Bray P2 overestimated the available/labile P fractions in the soil.

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SESSION 2

PRESERVING AND PROTECTING SOIL RESOURCES

Up-scaling the Application of Fallout Radionuclides to Support Catchment Sediment Management Programmes

D.E. Walling[1,*], P. Du[2], P. Porto[3] and Y. Zhang[4]

ABSTRACT

The application of fallout radionuclides in soil erosion and sedimentation investigations has provided a valuable tool for improving the understanding of erosion and sediment transfer processes. However, most studies using fallout radionuclides have focussed on small areas. Increasing acceptance of the important role of fine sediment in degrading aquatic habitats and in the transfer and fate of nutrients and contaminants within terrestrial and fluvial systems has emphasized the need for sediment management programmes in many areas of the world. Such programmes commonly focus on the catchment scale and an understanding of the sediment budget of a catchment represents a key requirement for developing effective sediment management strategies. Although the need for information on catchment sediment budgets is clear, obtaining such information faces many practical problems. While fallout radionuclides offer considerable potential for use in documenting sediment budgets for small areas, there is a need to up-scale their use to provide information at the catchment — and larger scales. To date there have been few attempts to develop and implement such upscaling. This paper describes the results of three studies undertaken by the authors to address this need. These examples include, firstly, the use of caesium-137 (^{137}Cs) measurements to provide the data required for a national scale soil erosion inventory; secondly, the use of areawide-scale spatially distributed sampling of ^{137}Cs inventories to assemble information on catchment sediment budgets; and finally the use of ^{137}Cs and excess lead-210 ($^{210}Pb_{ex}$) measurements to establish sedimentation rates on river floodplains.

Key words: *fallout radionuclides, caesium-137, soil erosion, floodplain sedimentation, catchment-scale, sediment budget.*

INTRODUCTION

In recent years, the potential for using fallout radionuclides to provide an improved understanding of erosion and sediment transfer processes has been clearly and successfully demonstrated (e.g. Zapata, 2002). Particular advantages of the approach include the ability to obtain retrospective spatially distributed data on the basis of a single site visit and without the need to install permanent monitoring equipment and structures (e.g. Walling and Quine, 1995; Mabit, Benmansour and Walling, 2008). The conjunctive use of different fallout radionuclides can provide a basis for assembling information relating to different time windows (Mabit, Benmansour and Walling, 2008), and the ability to generate spatially distributed data has coincided with the increasing need for such data for validating physically-based distributed soil erosion models (He and Walling, 2003). There has been some criticism of the approach directed at the lack of validation of results obtained (Parsons and Foster, 2011), but this ignores the very substantial and convincing body of empirical validation that has now been reported (e.g. Porto, *et al.*, 2003a and b; Belyaev *et al.*, 2008). To date, however, most work in applying fallout radionuclides in soil erosion and sedimentation investigations has focussed on relatively small areas, such as individual fields or sedimentation sites, although there have been some attempts to look at larger areas (e.g. Mabit, Bernard and Laverdière, 2007). The focus on small areas has meant that there have been few attempts to use fallout radionuclides in larger scale sediment budget investigations linked to catchment management and the design and implementation of sediment control strategies for individual catchments. The key role of fine sediment in degrading aquatic ecosytems and in the transfer and fate of nutrients and contaminants in terrestrial and fluvial systems is being increasingly recognized and this means that sediment management is assuming growing importance in many areas of the world, including both areas with high erosion rates and sediment yields and those where such rates and yields are very much lower. The latter areas are frequently the most sensitive to small changes in soil and sediment redistribution rates and represent areas where visual evidence of sediment mobilization and transfer is often limited. It is frequently these areas where fallout radionuclides are most useful. As demonstrated by Walling and Collins (2008) and Gellis and Walling (2011), the catchment sediment budget is commonly the key to designing an effective sediment strategy, since it provides information on the relative importance of different sediment sources, on the deposition and storage of sediment as it moves through the catchment sediment system and on the relationship between the sediment output and internal sources and sinks. Fallout radionuclides are often employed to provide key information needed to construct a catchment budget, and without such information the task of establishing a sediment budget can prove very difficult, if not impossible.

As indicated above, the use of fallout radionuclides to provide information on the functioning of a catchment sediment budget

1 Geography, College of Life and Environmental Sciences, University of Exeter, Exeter, Devon, EX4 4RJ, UK
2 International Research and Training Center in Erosion and Sedimentation (IRTCES), P. O. Box 366, Beijing 100048, P. R. China
3 Dipartimento STAFA, Universersità degli Studi Mediterranea, Località Feo de Vito, Reggio Calabria, Italy
4 ADAS, Pendeford House, Wolverhampton, WV9 5AP, UK
* E-mail address of corresponding author: d.e.walling@exeter.uk

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necessitates an up-scaling of the approach. It is essentially impossible to extend the detailed sampling commonly applied to an individual field across an entire catchment, due to cost and other practical constraints. Even if a large team is available to collect samples, the cost and time involved in analysing large numbers of samples by gamma spectrometry are likely to prove insurmountable problems. As a result, there is a need to develop new approaches that permit such up-scaling without introducing insuperable obstacles. Up-scaling approaches could involve methods of extrapolating the data obtained from a small number of measurements over a much larger area or optimizing the information content of a small number of measurements. The work of Mabit, Bernard and Laverdière (2007) cited above, subdivided the 217 km^2 catchment of the River Boyer in Quebec, Canada into a series of essentially homogeneous units termed isosectors and collected representative data from those units and extrapolated these data across the individual units using a GIS framework.

The authors have also directed attention to the potential for up-scaling the use of fallout radionuclides, and particularly ^{137}Cs measurements, to larger areas, in order to provide the information required for establishing catchment sediment budgets. This paper describes the results of three studies undertaken in recent years to explore different approaches and objectives. These examples include, firstly, the use of ^{137}Cs measurements to provide data required for a national scale soil erosion inventory; secondly, the use of wide-scale spatially distributed sampling of ^{137}Cs inventories to assemble information on the sediment budgets of several small- to medium-scale catchments in Southern Italy and, finally, the use of caesium-137 (^{137}Cs) and excess lead-210 ($^{210}Pb_{ex}$) measurements to establish sedimentation rates on river floodplains, which can represent important sinks in the sediment budgets of larger catchments and therefore need to be considered when the overall perspective is up-scaled.

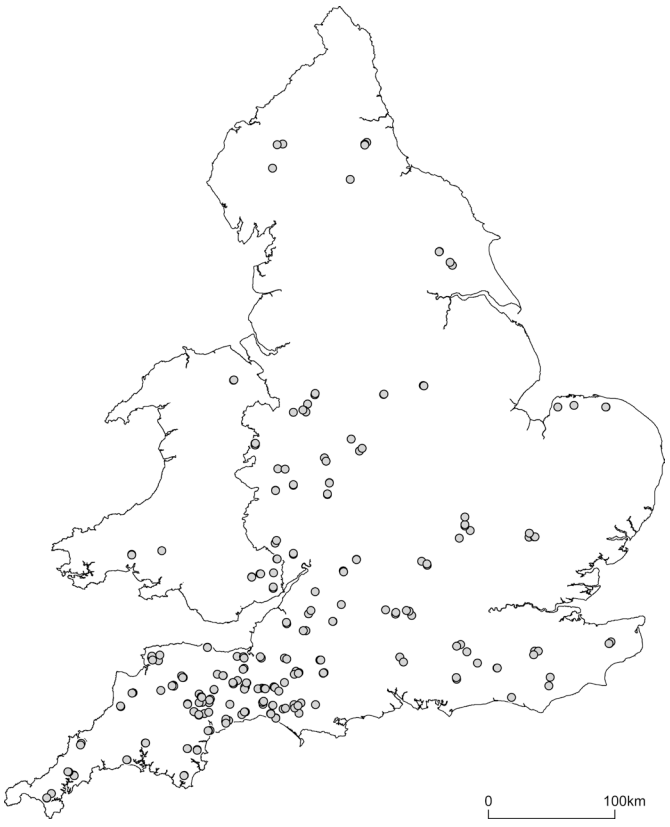


FIGURE 1. The locations selected for field sampling within the project.

A NATIONAL SCALE SOIL EROSION INVENTORY

The national soil erosion inventory study was funded by the Department for Environment, Food and Rural Affairs (DEFRA). It focussed on agricultural land in England and Wales and aimed to use ^{137}Cs measurements to provide national-scale information on the magnitude of soil erosion rates and the sustainability of the soil resource, as well as the importance of soil erosion from agricultural land as a sediment source. In the absence of a national network of erosion plots or other ongoing monitoring, ^{137}Cs measurement were seen as a valuable means of assembling information on mean soil erosion rates over the past ~50 yr and the influence of topography, soil type, land use and other controlling factors on soil erosion rates. The approach taken is described in more detail by Walling and Zhang (2010). In brief, there were three key elements. Firstly, preliminary fieldwork was used to confirm that the collection of a relatively small number of bulk cores (e.g. 10–12) from a small field with a simple topography using a representative transect, could provide a reliable estimate of the longer-term (50 yr) gross and net soil losses from that field using standard conversion models (Walling and He, 1999). Secondly, by sampling a substantial number of such fields, representative of a wide range of slopes and forms, soil texture, land use and hydrometeorological conditions (e.g. mean annual rainfall and hydrologically effective rainfall), and obtaining reliable estimates of

TABLE 1. Summary statistics for the erosion rates for the sample of 248 fields ($t \cdot ha^{-1} \cdot yr^{-1}$)

	Arable			Pasture		
	Eroding area rate	Gross erosion rate	Net erosion rate	Eroding area rate	Gross erosion rate	Net erosion rate
Minimum	1.2	1.2	0.0	0.5	0.7	0.0
Maximum	30.3	29.3	27.3	16.0	13.0	11.7
Average	10.6	8.4	6.7	3.2	2.5	1.8
Median	8.8	6.6	5.2	2.6	1.8	1.2
1st quartile	6.5	4.7	2.0	1.7	0.9	0.2
3rd quartile	13.3	11.2	9.1	3.8	3.0	2.6
CV * (%)	80	99	140	82	118	200

* CV was calculated as: (Q1–Q3) / median × 100

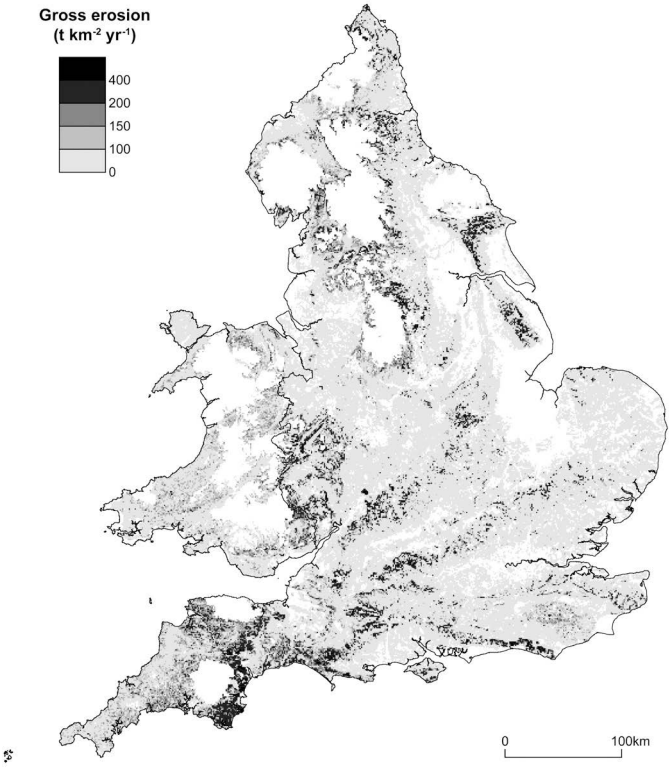


Figure 2. A national map of gross erosion rates for a combination of cultivated and pasture land use, based on spatial extrapolation of the field-scale erosion rates documented by ^{137}Cs measurements.

the reference inventory for such fields, it was possible to assemble information on the range of soil erosion rates found on agricultural land in England and Wales and their key controls. Thirdly, these data were used to populate a typology model which was employed to extrapolate the available estimates of erosion rates over the entire agricultural land area in England and Wales.

Attention was restricted to areas of agricultural land below an altitude of 300 m. National Parks, urban areas and areas of minimal slope (<1°), where water erosion was likely to be negligible, were excluded from the study. In total, 248 fields were sampled, of which 133 were arable fields and 115 were under permanent pasture (Figure 1). The ^{137}Cs measurements undertaken on the cores collected from the 248 fields were used to derive estimates of several measures of the longer-term (ca. 50 yr) soil erosion rate. These included the proportion of the field that was eroding, the erosion rate within the eroding area and the gross and net erosion rates for the field. A summary of the information on soil erosion rates provided by the 248 fields is presented in Table 1.

These data indicate that, as might be expected, both gross and net erosion rates associated with areas of arable cultivation are considerably greater than those for pasture areas. The ratio of net to gross erosion, which provides a measure of the efficiency of sediment transfer out of the field also differed between these two categories of land use and was typically > 0.6 for arable fields and < 0.6 for pasture fields, emphasizing the lower efficiency of sediment delivery from pasture areas. Comparison of the magnitude of the erosion rate estimates obtained from the study with existing erosion hazard assessments based primarily on soil texture, confirmed the broad consistency of the two measures of erosion intensity.

The typology model used to extrapolate the assembled data over the entire study area was based on detailed analysis of the key variables controlling the spatial variability of soil erosion rates at the national scale. The final model involved three primary variables, i.e. slope steepness, soil texture (percent clay, silt and sand) and land use and provided estimates of both gross and net erosion for indivdual grid cells. This model was coupled with a GIS, comprising 1 km × 1 km grid cells and incorporating a 50 m DEM (Digital Elevation Model), for use in deriving information on slope steepness, as well as information on soil texture and land use for each grid cell. The extrapolation procedure permitted estimation of the likely proportion of cultivated and pasture land in each grid cell and the erosion rate reflecting these proportions. National maps of gross and net erosion rates associated with arable, pasture and mixed land use were produced. Figure 2 presents the national map of gross erosion rates associated with the likely combination of arable and pasture land use in each grid cell. Spatial integration of the data presented on these maps was used to generate information on frequency distributions of gross and net erosion rates from agricultural land in England and Wales. These frequency distributions indicated that a soil loss tolerance of 2 $t \cdot ha^{-1} \cdot yr^{-1}$ is exceeded on ~7 percent of the agricultural land in England and Wales for gross erosion and on ~5 percent of the agricultural land for net erosion.

The maps of gross and net soil losses represent soil loss from individual fields and cannot be used directly to represent sediment inputs to river channels, which may be of primary interest when constructing a sediment budget. For this purpose, the national map of net soil loss was coupled with an equivalent map of slope-channel connectivity previously developed by the authors (Walling and Zhang, 2004), and also based on 1 km grid cells, to provide a national map of the sediment input to the local channel system. This map indicated that contributions of surface erosion from agricultural land typically ranged between 5 and 50 $t \cdot km^{-2} \cdot yr^{-1}$ and these values are consistent with available data on suspended sediment yields for catchments in England and Wales. These yields will include contributions from sources other than the surface of agricultural land such as channel or bank erosion, and can thus be expected to be significantly greater than those associated solely with mobilization of sediment from agricultural land.

USE OF SPATIALLY DISTRIBUTED SAMPLING TO DERIVE INFORMATION ON CATCHMENT SEDIMENT BUDGETS

The spatially intensive ^{137}Cs sampling programmes required to assemble information on soil redistribution rates for use in establishing the sediment budget of a medium-sized, or even small, catchment using standard approaches will commonly prove impractical in terms of the vast number of samples that need to be collected and assayed. An alternative wide-scale spatially distributed sampling strategy has been proposed by the authors (see Porto, Walling and Callegari, 2011). Collection of cores for ^{137}Cs measurement from a substantial number of representative points distributed throughout a catchment and using the associated values of ^{137}Cs inventory to estimate soil redistribution rates at the sampling points can provide a representative sample of soil and sediment redistribution rates within the catchment. When combined with information on the sediment output from the catchment, this information can provide a basis for establishing a sediment budget. This approach was applied successfully by the authors in three small- to medium-scale catchments in southern Italy (Porto, Walling and Callegari, 2011).

The three catchments are illustrated in Figure 3 and further information on their characteristics is provided in Table 2. Their drainage areas ranged from 0.015 km^2 to 31.61 km^2 and this substantial range was seen as providing a means of assessing the impact of scale (i.e.

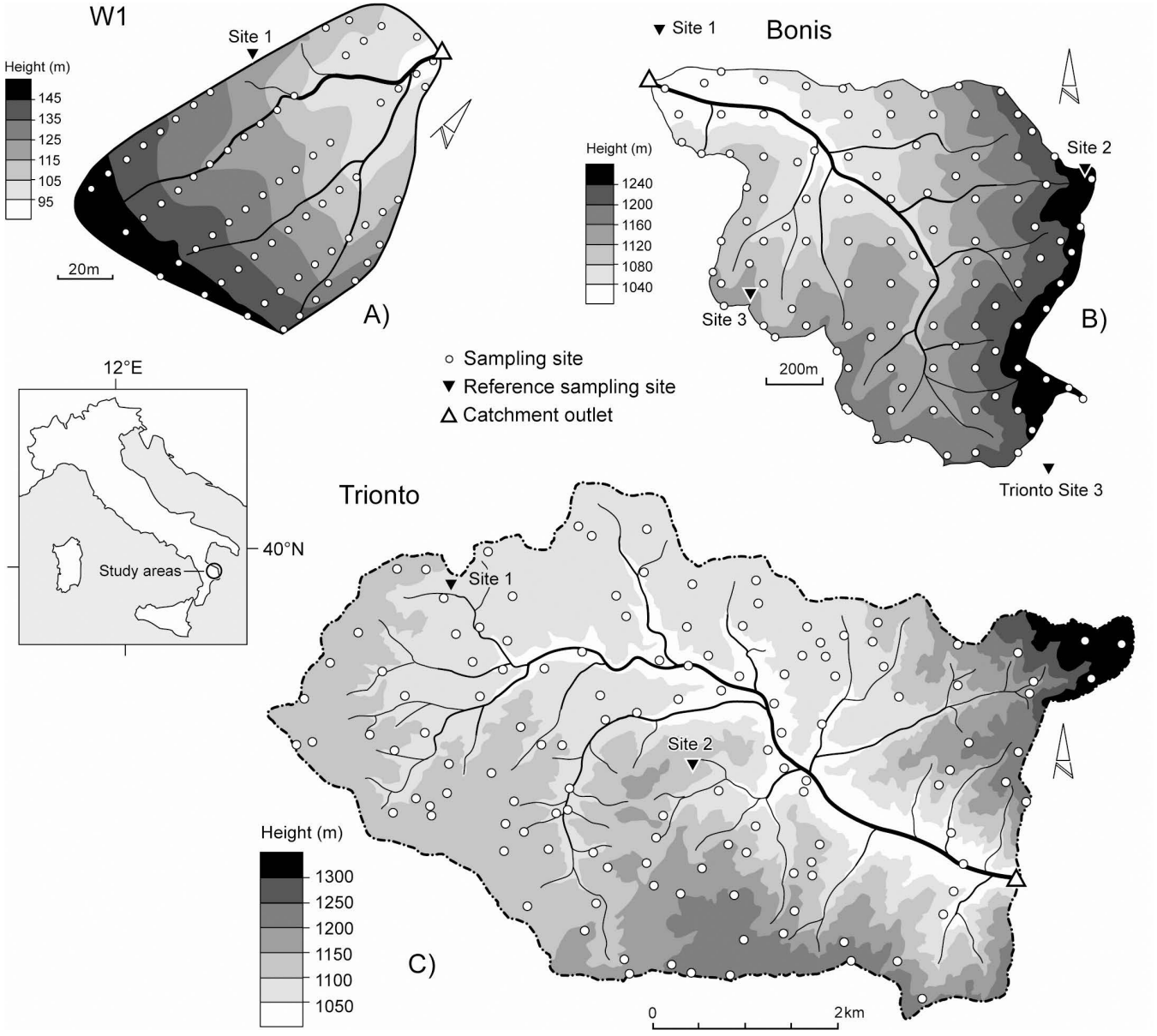


Figure 3. The study catchments: W1 (A), Bonis (B) and Trionto (C).

increasing catchment area) on their sediment budgets. Catchment W1 is uncultivated and has a rangeland cover, whereas the Bonis catchment is largely under forest and the Trionto catchment is largely cultivated. Information on long-term suspended sediment yields was available for the three catchments (see Table 2) based on long-term sediment sampling programmes (W1 and Trionto) and periodic emptying of sediment traps (Bonis).

Bulk cores were collected from each catchment for establishing the ^{137}Cs inventory at individual sampling points. Different sampling strategies were used for each catchment. In the case of catchment W1, this involved the collection of 68 bulk cores along five essentially parallel linear transects (Figure 3A). For the Bonis catchment, 55 bulked replicate cores were collected from the intersections of a 150 m × 150 m grid and these cores were supplemented by a further 55 bulked replicate cores collected from sites selected to improve the coverage of topographic variability (Figure 3B). In the case of the Trionto catchment, the larger size of the catchment and constraints on access precluded the use of a systematic sampling network, and sampling involved the selection of 128 essentially random sampling points across the catchment aimed at providing a representative coverage of the terrain and a relatively uniform spatial coverage (Figure 3C). Additional sampling was carried out in the three catchments to establish the reference inventories and to provide information on the depth distributions of ^{137}Cs in the individual catchments as required by the conversion models used to estimate soil redistribution rates from the measurements of ^{137}Cs inventory. The considerable range of altitude associated with the Trionto catchment meant that three reference inventories, related to three altitudinal zones were established.

The values of ^{137}Cs inventory obtained for the sampling points in the catchments were used to derive estimates of soil redistribution rates using appropriate conversion models. In the case of catchment W1 and the Bonis catchment where the soils were uncultivated, a diffusion and migration model was employed. A mass balance

Table 2. Characteristics of the study catchments

Catchment	Area (km^2)	Mean altitude (m a.s.l.)	Mean slope (%)	Relief ratio	Primary land use	Sediment yield ($t \cdot km^{-2} \cdot yr^{-1}$)
W1	0.015	122	53	0.17	Rangeland	1 16
Bonis	1.39	1131	40	0.14	Forest	25.0
Trionto	31.61	1100	8.4	0.05	Cultivated	21.0

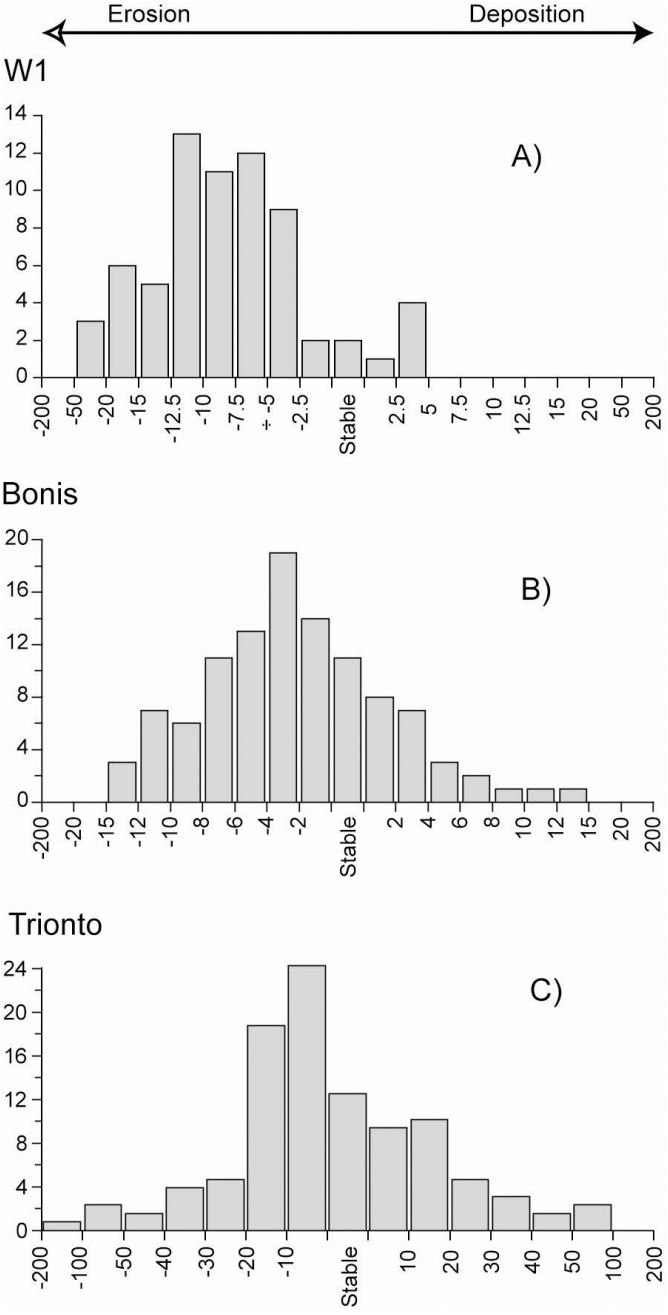


FIGURE 4. Frequency distributions of erosion and deposition rates ($t \cdot ha^{-1} \cdot yr^{-1}$) associated with the sampling points within each study catchment: (A) W1, (B) Bonis and (C) Trionto (based on Porto, Walling and Callegari, 2011).

model was employed for the cultivated areas in the Trionto catchment (Porto, Walling and Callegari, 2011). The resulting estimates of soil redistribution rate are presented in Figure 4, which depicts frequency distributions of erosion and deposition rates for the three catchments. In the case of catchment W1, these data provide little evidence of deposition and suggest that most of the mobilized sediment is transported directly to the catchment outlet. This conclusion was confirmed by field inspection, which indicated that areas of deposition were very limited. In the larger Bonis and Trionto catchments, deposition becomes progressively more important. This is a direct reflection of increasing catchment size and the associated greater opportunities for deposition. Clear contrasts in the magnitude of the soil redistribution rates are also apparent between the three catchments. Redistribution rates, and particularly erosion rates, are, as might be expected, generally lowest in the forested Bonis catchment and highest in the cultivated Trionto catchment, with catchment W1, supporting a rangeland cover, falling between the two.

If the frequency distributions of soil redistribution rates presented in Figure 4 are assumed to provide representative information on medium-term average rates of erosion and deposition on the slopes of the individual study catchments, they can be used to estimate the gross erosion from the slopes and the on-slope deposition and thus the 'slope' component of the sediment budget. The availability of estimates of mean annual sediment yield provides another key component of the sediment budget, namely the output. If the slope component is assumed to represent the input to the channel system from the slopes, the difference between this and the sediment yield at the catchment outlet can be attributed to deposition during conveyance through the channel system. This will provide a minimum estimate of such conveyance losses, since the amount of sediment transported through the channel system could be greater than the input from the slopes, due to additional sediment supply from eroding channel banks. However, this source was judged to be of limited importance in the study catchments. The sediment budgets for the three study catchments established using the above information and assumptions are presented in Figure 5.

These budgets emphasize the increasing importance of sediment deposition, both on the slopes and within the channel system, as catchment scale increases. The catchment sediment delivery ratio (SDR) (Walling, 1983) provides a useful means of assessing the efficiency of sediment delivery from a catchment, and Figure 5 indicates that the SDR declines from 98 percent for catchment W1 to 7 percent for the Bonis catchment and to two percent for the Trionto catchment. This is consistent with existing representations of the inverse relationship between SDR and catchment area (e.g. Roehl, 1962).

DOCUMENTING SEDIMENTATION RATES ON RIVER FLOODPLAINS

A different dimension of the requirement to up-scale the use of fallout radionuclides in order to provide information at the catchment scale, is the need to furnish information on key components of the sediment budgets of larger catchments. Attention must be

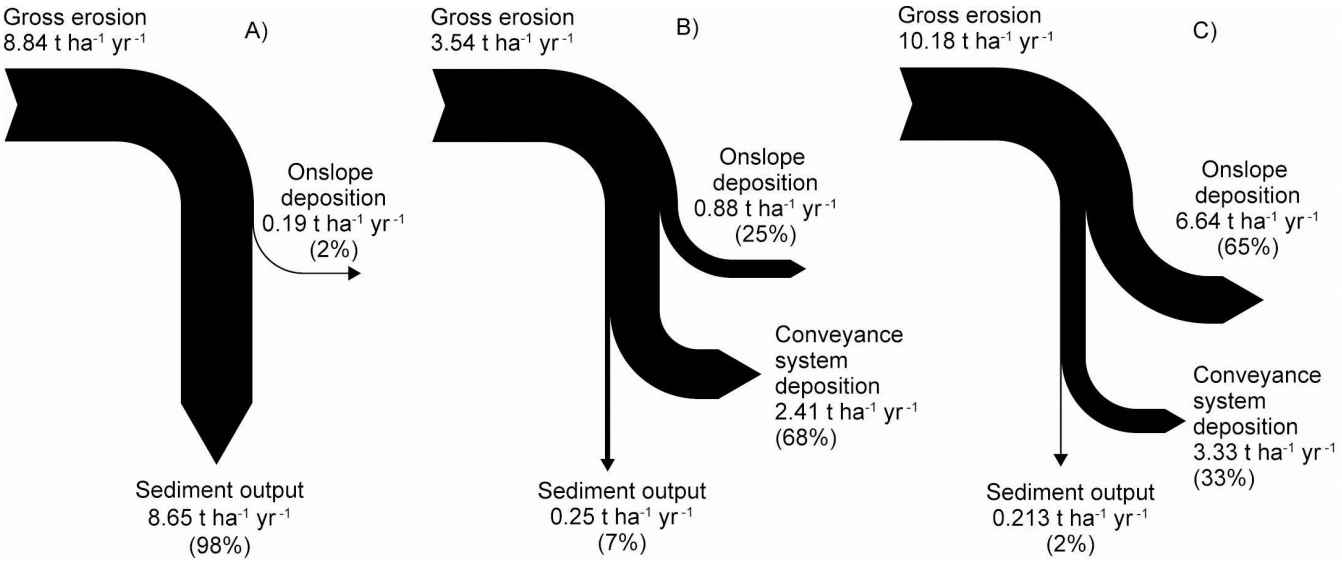


FIGURE 5. Schematic sediment budgets for catchment W1 (A), the Bonis catchment (B) and the Trionto catchment (C). The SDR associated with the sediment output from each catchment is indicated as a percentage (based on Porto, Walling and Callegari, 2011).

directed to the downstream channel system as well as the catchment slopes, since as catchment size increases, conveyance losses associated with the channel system can exert an increasing influence on the sediment output from a catchment and thus the catchment sediment budget. River floodplains are of considerable significance in this context since they can represent very important sediment sinks. The need to obtain estimates of rates of medium-term sediment accumulation or storage within floodplain systems has directed attention to the use of fallout radionuclides and more particularly ^{137}Cs and excess lead-210 (^{210}Pb$_{ex}$) measurements for this purpose. In this application, the ability to identify peaks of ^{137}Cs fallout associated with the peak of bomb fallout in 1963 and the Chernobyl fallout in 1986 (where such fallout occurred) can provide valuable time markers. Because of its essentially continuous fallout, ^{210}Pb$_{ex}$ does not provide peaks of a known age, but information on the downcore decline in ^{210}Pb$_{ex}$ activity can be used to derive estimates of recent sedimentation rates, based on the known half-life of ^{210}Pb of 22.3 yr (Du and Walling, 2012). By studying the depth distribution of both ^{137}Cs and ^{210}Pb$_{ex}$ in floodplain cores, it is possible to obtain estimates of the average sedimentation rate over the past ca. 100 yr and for specific sub-periods. If such data can be spatially extrapolated across a river floodplain, by collecting representative cores in different locations it is possible to derive estimates of the mass of sediment sequestered in a floodplain system and thus establish its importance as a sediment sink within the catchment sediment budget and assess any changes in that importance through recent time.

Figure 6 provides an example of this potential. The data presented relate to a core collected at Welshpool, UK in 2009. In this case, the ^{137}Cs profile provides clear evidence of the 1963 peak of bomb fallout at a mass depth of ~50 g/cm^2 and the 1986 Chernobyl peak at a depth of ~17 g/cm^2. The ^{210}Pb$_{ex}$ profile was used to derive an estimate of the mean annual sedimentation rate over the past ~60 yr and thus back to 1950. These estimates indicate an average sedimentation rate of 1.14 g·cm^{-2}·yr^{-1} over the period 1950–2009, based

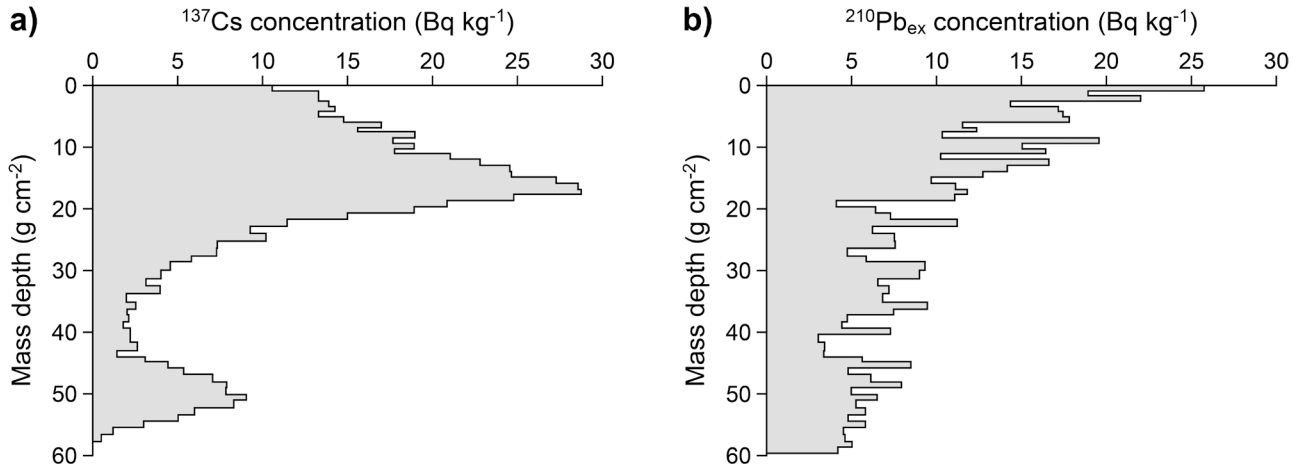


FIGURE 6. The ^{137}Cs (a) and ^{210}Pb$_{ex}$ (b) depth profiles associated with a sediment core collected from the floodplain of the River Severn at Welshpool, UK in 2009.

on the $^{210}Pb_{ex}$ measurements, and sedimentation rates of 1.38 g·cm^{-2}·yr^{-1} for the period 1963–1986 and 0.70 g·cm^{-2}·yr^{-1} for the period 1986–2009. It is beyond the scope of this contribution to speculate about the causes of these apparent changes in sedimentation rate, but such information clearly has considerable potential value for investigating the impact of human activity and particularly land use change, as well as climate change, on floodplain sedimentation and thus the sediment budgets of larger catchments.

CONCLUSIONS

The three examples outlined above serve to demonstrate the potential for up-scaling the use of fallout radionuclide measurements to the catchment scale in order to assemble the information on catchment sediment budgets needed to develop and implement effective sediment management strategies. The first approach described involved extrapolation. Here, alternative statistical models could be employed and scope undoubtedly exists to couple such models with geo-statistical techniques. In the second approach, emphasis was placed on collecting a relatively small dataset that was, nevertheless, representative of a particular catchment. In this case, further work is clearly needed to define the minimum number of samples required to characterize adequately a catchment and the optimum sampling framework for collecting those samples. The use of fallout radionuclides to document floodplain sedimentation rates was introduced to illustrate the need to consider additional components of the sediment budget, as the area of interest is up-scaled from a small to a larger catchment. Here also, further work is clearly required to explore and develop optimum strategies for moving from estimation of the sedimentation rate at a single point to providing an assessment of the mass of sediment sequestered within a given floodplain reach. This emphasises again the need to extrapolate a limited number of measurements of sedimentation rate or to assemble a limited dataset which can be viewed as representative of that reach.

Fallout radionuclide measurements have the potential to provide essentially unique information, which cannot be obtained using other techniques. By understanding the sources and sinks of sediment within a catchment, it is possible to implement sediment control measures aimed at reducing sediment fluxes in the most cost effective manner. However, important challenges undoubtedly remain to ensure that the full potential of using fallout radionuclide measurements to support catchment sediment management programmes is achieved.

ACKNOWLEDGEMENTS

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Use of an Integrated Approach for Assessing Soil Redistribution in the River Vorobzha Basin

V.N. Golosov[1,2,*] ,V.R. Belyaev[1], E.N. Shamshurina[1] and Ju.S. Kuznetsova[1]

ABSTRACT

Significant climate and crop rotation changes have taken place over the last few decades in the Central Chernozem zone of European Russia. These have considerably influenced sediment redistribution rates within agricultural catchments. Quantitative assessments of soil losses from cultivated lands and sediment redistribution intensity have been made using an integrated approach for typical catchments of Central Russia located near the city of Kursk in the River Vorobzha basin. Combined application of the caesium-137 (^{137}Cs) technique, erosion models, the soil morphological method and large-scale geomorphic mapping with detailed evaluation of the area of each typical morphological unit indicated that soil erosion rates on arable hill slopes decreased by about 1.5 times during the last 25 years (yr) compared with rates during the previous quarter century. Sediment delivery from cultivated fields into the River Vorobzha valley also decreased by up to three-fold over the same timeframes. The main reason for these changes was the considerably reduced surface runoff during the spring snowmelt period. In addition, the approach employed made it possible to quantify the effectiveness of soil conservation measures (SCMs) introduced within the small experimental sub-catchments occupying part of the study area. Application of SCMs reduced soil losses from cultivated fields by 2.5 times. Most of the eroded sediment and sediment-associated ^{137}Cs has been redeposited within the cultivated fields and adjacent dry valley bottoms. It can be concluded that taking an integrated approach is the most appropriate strategyfor assessing soil degradation and determining both localized and off-site soil and nutrient sinks.

Key words: *sediment redistribution, agricultural catchments, caesium-137, erosion models, soil conservation.*

INTRODUCTION

Soil loss from cultivated land causes land degradation and water pollution as a result of sediment and nutrient transport into river channels and water reservoirs. Quantitative assessment of sediment redistribution in different parts of fluvial systems can be made using an integrated approach to evaluate sediment budget components (Walling *et al.*, 2002; Polyakov *et al.*, 2004). Evaluation of sediment budgets provides a valuable basis for developing catchment management strategies through reliable identification of areas dominated by soil loss and locations of major sediment sinks. Caesium-137 (^{137}Cs) has been used successfully in different parts of the world as a tracer to construct sediment budgets as well as to assess mean annual erosion rates and the dynamics of deposition rates in areas of sediment storage (Mabit, Benmansour and Walling, 2008), including in European Russia (Golosov *et al.*, 2011). The southern part of European Russia is considered to be one of the most productive agricultural areas worldwide, in particular due to the high organic carbon content of its Chernozem soils. One of the most intensively cultivated areas of the Russian Chernozem zone is the Kursk region, characterized by extended areas of row crops such as sugar beet, potato and corn. Intensive cultivation started in this region in the 17th century, with the maximum percentage of cultivated land being reached after land tenure reform in 1861. Subsequently, the area of arable land decreased due to growth of urban areas and the cessation of tillage on the steepest slopes (Sidorchuk and Golosov, 2003). Intensive gully growth between 1861 and 1970 was a further reason for the abandonment of some cultivated parcels.

Soil conservation measures were first introduced at the beginning of the 1970s in the most eroded parts of the Russian Chernozem zone. Erosion associated with both spring snowmelt and summer rainstorms is responsible for soil degradation in this region. According to calculations based on the universal soil loss equation (USLE) erosion model, mean annual soil erosion rates in the Kursk region vary within the range 5 to 15 tonnes (t)·ha^{-1}·yr^{-1} (Litvin, 2002). Direct observations of snowmelt erosion indicated that the rate of the latter did not exceed 1–5 t·ha^{-1}·yr^{-1}. The highest erosion rates were observed during summer rainstorms, particularly on fields under fallow or row crops. For instance, in the Sovetsky district of the Kursk region, an extreme erosion event occurred on 20–21 August 1976 when about 190 mm of precipitation was recorded, causing soil losses of up to 200 t/ha during that single rainfall event (Gerasimenko and Rozhkov, 1976). More typical rainstorms with precipitation of about 20–40 mm can still cause soil losses of up to 36–44 t/ha for a single event when high intensity rainstorms fall over erosion-prone surfaces such as harrowed fallow or row crops (Belyaev *et al.*, 2008).

The main aim of the present study was to evaluate the dynamics of erosion and sediment redistribution rates for typical catchments of different sizes located in the Chernozem zone (the Kursk region) using an integrated approach. Its importance is underlined by the fact that during the last decades considerable climatic fluctuations in the study area coincided with notable crop rotation changes. In addition, this study illustrates the applicability of the integrated approach used for quantitative assessment of the effectiveness of soil conservation measures (SCMs).

1 Laboratory for Soil Erosion and Fluvial Processes, Faculty of Geography, Lomonosov Moscow State University, Leninskie Gory, 1, 119991, Moscow, Russia
2 Institute of Ecology and Geography, Kazan (Volga Region) Federal University, Kremlevskaya, 18, Kazan, 420008, Russia
* E-mail address of corresponding author: gollossov@gmail.com

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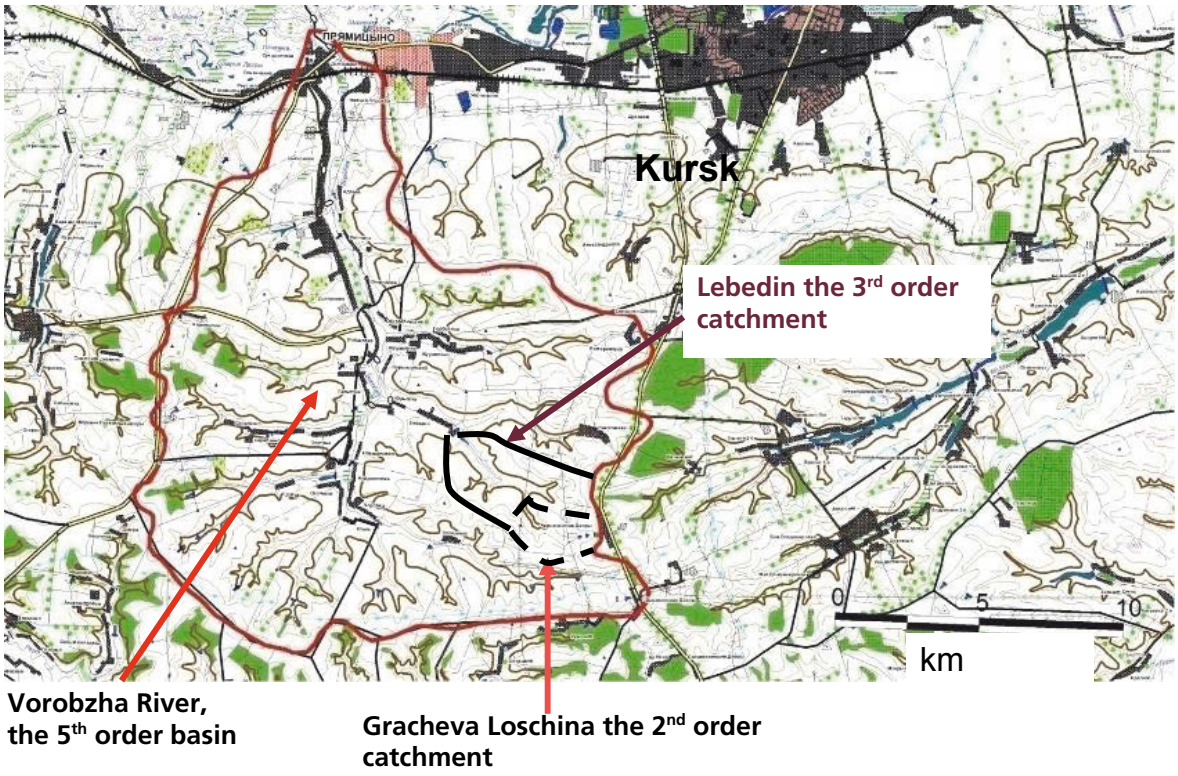


FIGURE 1. Location of the study catchments.

STUDY SITE

The River Vorobzha basin is a typical small river basin for the agricultural area of the Chernozem zone of Central Russia. It is located in the Kursk region 15–20 km SSW from the regional centre Kursk. It has an area of 228 km^2 with about 85 percent of cultivated land. The territory is characterized by a continental climate with relatively cold winters and hot summers. Average annual precipitation is 585 mm (over a 100–yr period of observation), varying from 400–800 mm. About 30 percent of precipitation falls during the cold months mostly as snow. Rainstorms with total precipitation of 10–40 mm and occurring commonly from May to October are the most typical precipitation events. The River Vorobzha basin was contaminated by radionuclide fallout after the Chernobyl accident in 1986. The level of Chernobyl-associated contamination is about ten times higher than the bomb-derived ^{137}Cs contamination which took place between 1954 and the 1970s.

Assessments of soil redistribution rates were undertaken for the Gracheva Loschina catchment (area 1.98 km^2) and the Lebedin catchment (area 15.2 km^2), which are typical 1st and 3rd Hortonian order catchments within the River Vorobzha basin. Both catchments have earthen dams at their outlets which were constructed in 1986 and 1956, respectively. These enabled calculation of detailed sediment budgets for both catchments. In addition, experimental SCMs have been employed on 70 percent of the Gracheva Loschina catchment since the beginning of 1986. Hence it was also possible to evaluate the impact of the introduction of SCMs on soil erosion and sediment redistribution.

METHODS

An integrated approach (involving erosion model calculations, ^{137}Cs budget, and sediment sequence dating, and large-scale geomorphic mapping) was used for quantitative assessment of soil loss/gain on arable hillslopes and in different order catchments over several time periods since the middle of the 20th century. In addition, detailed information about land use and crop rotation changes and meteorological data over a 100 yr period was collected for the study area. Principles of the integrated approach are based on applying at least two independent methods to assess each component of a sediment budget and the possibility of extrapolating the results of more detailed evaluation of soil redistribution obtained for relatively small catchments in order to cover larger areas (Belyaev *et al.*, 2009; Golosov *et al.*, 2011).

During the first stage of the study, large-scale geomorphic mapping was used for area evaluation of different morphological units within the Gracheva Loschina catchment. Mean soil loss/gain within each morphological unit was determined using ^{137}Cs techniques, the soil morphological method (Kiryukhina and Serkova, 2000) and erosion model calculations. As a result, it was possible to check the correctness of each technique applied. These techniques enabled calculation of the Gracheva Loschina catchment sediment budget based on evaluation of soil losses from arable hillslopes, sediment redeposition within cultivated slopes and aggradation of uncultivated valley banks and dry valley bottoms, including deposition in small reservoir upstream from the earthen dam at the catchment outlet. Values for soil redistribution rates and sediment delivery ratios determined for different geomorphic units within a smaller catchment were then used to determine sediment budgets for larger

TABLE 1. The post-1986 ^{137}Cs budget for sub-catchments of the Gracheva Loschina catchment characterized by different types of soil conservation measures or their absence

Sub-catchment	Total area (ha)	Eroded area ^{137}Cs loss, kBq	Deposition area, ^{137}Cs gain, kBq (% from ^{137}Cs loss)		Residual kBq (%)
			Within cultivated areas (including grassed waterways)	In tributary hollow bottoms and main valley bottom	
Sub-catchment with forest shelter belts and grass waterways	52.8	189 606 (100%)	154 620 (82%)	7 954 (4%)	27 032 (14%)
Sub-catchment with forest shelter belts, grass waterways and contour terraces	88.1	926 885 (100%)	834 195 (90%)	24 650 (3%)	68 040 (7%)
Area without soil conservation measures and the main valley bottom	56.9	236 786 (100%)	22 061 (9%)	200 508 (85%)	14 217 (6%)

catchments based on distinguishing similar types of morphological units and estimating erosion rates by the erosion model. The resulting values for sediment delivery into the main valley of the Lebedin catchment were tested by comparison with sedimentation rates and volumes obtained from analysis of the ^{137}Cs-based valley bottom sediment microstratigraphy, including the dry reservoir infill.

RESULTS AND DISCUSSION

Analysis of bomb-derived ^{137}Cs inventories at different geomorphic landscape units can be applied effectively for evaluating sediment budgets in small catchments (Loughran *et al.*, 1992; Owens *et al.*, 1997; Walling *et al.*, 2002). The authors attempted to apply this approach for the Gracheva Loschina catchment and to compare the results with those obtained by other methods.

The catchment topography is characterized by gently rolling interfluve areas and a predominance of convex slopes with maximum gradients of between 5 and 8 degrees. Several hollows dissect the slopes in the upper part of the catchment. Before 1986, most of the catchment area was cultivated. In 1986, the lower parts of the slopes and the hollow bottoms of the tributary were sown with grass and converted into pasture. In addition, SCMs were introduced on 70 percent of the catchment area (sub-catchments of the two upper tributaries) in March 1986 as part of soil conservation field experiments. Two-rowed forest shelter belts were planted parallel to the slope contours at a spacing of about 200–250 m, and grassed waterways were constructed along the bottoms of the hollows within both experimental sub-catchments. Water retention ditches with a depth of about 1 m were dug within each forest shelter belt between the two rows of trees. The bottoms of hollows were sown with perennial grasses, left continuously uncultivated and used as erosion-protected flow pathways for concentrated surface runoff. In addition, contour terraces parallel to the contour lines with a relative height of about 1 m were constructed between the forest shelter belts within one of the sub-catchments with a spacing of about 50 m between the adjacent terraces. Simultaneously, a closed earthen dam was constructed at the main valley outlet. The rest of the catchment slopes downstream from the main tributary confluence remained cultivated in the traditional manner.

The Chernobyl fallout occurred at the end of April 1986, shortly after the introduction of the SCMs. The Gracheva Lochina catchment therefore prov ided a unique opportunity to establish a closed system sediment budget based on the redistribution of the Chernobyl-derived ^{137}Cs, and to test the reliability of the results by comparison with independent approaches. In order to design a representative sampling programme, a detailed large-scale geomorphic map was created based on a combination of already available topographic data (1:10 000 scale map with 1 m contour intervals), and additional differential global positioning system (DGPS) and digital tacheometer surveys carried out in selected parts of the catchment. The sampling programme aimed to characterize all important geomorphic units in terms of ^{137}Cs inventory and, subsequently, sediment redistribution between them. The area of each geomorphic unit was determined based on the geomorphic map constructed. The ^{137}Cs budget was compiled for each of the three sub-catchments distinguished on the basis of different post-1986 land use patterns (Table 1).

Uncertainty with respect to the calculations (represented by the *Residual* column) arises from the presence of bomb-derived ^{137}Cs and redistribution prior to the introduction of SCMs were not accounted in the budget calculations. According to available information (Izrael, 1998), the ratio between the Chernobyl-derived and bomb-derived ^{137}Cs inventories (corrected for radioactive decay) for the case study area is about 6:1–5:1. It is therefore suggested that part of the bomb-derived ^{137}Cs inventory would have been removed through the catchment outlet before construction of the dam in 1986. This can explain some of the differences between ^{137}Cs losses from eroded areas and ^{137}Cs accumulation within deposition areas. However, it is also clear from Table 1 that most of the Chernobyl-derived ^{137}Cs was delivered into the main valley bottom from areas with traditional cultivation. For the two sub-catchments with SCMs the situation is the opposite — more than 95 percent of Chernobyl-derived ^{137}Cs has remained within the slopes. This emphasizes the marked differences in sediment delivery into the main valley between sub-catchments with different land uses. Mechanical soil translocation towards contour terraces during tillage operations in the experimental area can explain the ^{137}Cs redistribution observed within the cultivated area of the northeastern sub-catchment with the SCM applied. In the southeastern SCM sub-catchment with only forest shelter belts, most of the ^{137}Cs redistribution within the slopes can be attributed to the limited erosion and subsequent sediment redeposition within grassed waterways.

The sediment budget was calculated from the ^{137}Cs total inventory data using the following equation based on the simple proportional calibration model:

$$R = \frac{\int_S A\,dS - \int_S A_{ref}\,dS}{C_p \Delta t S}$$

where R is mean annual soil loss/gain, kg·m^{-2}·yr^{-1} (negative values mean erosion, positive mean accumulation); Δt is time elapsed since fallout of Chernobyl-derived ^{137}Cs, yr; C_p is ^{137}Cs concentration in

TABLE 2. Post-1986 sediment redistribution (expressed as t and percent (in brackets)) within the Gracheva Loschina catchment calculated by two different approaches

Method	Time interval (years)	Gross erosion (t/%)	Deposition within cultivated field (t/%)	Deposition within hollows and valley bottom (t/%)	Output from catchment (t/%)
^{137}Cs budget	1986–2006	50 989 (100)	33 778 (82.8)	8766 (17.2)	0 (0)
Erosion model calculation and sediment deposition in the valley bottom (vertical distribution of ^{137}Cs)	1986–2006	22 606 (100)	17 050 (75.4)	5556 (24.6)	0 (0)

TABLE 3. Mean annual erosion rates and total soil losses from cultivated slopes of the Lebedin catchment for three time intervals covering the entire period after dam construction in catchment outlet

Time interval (years)	Mean annual erosion rate ($t \cdot ha^{-1} \cdot yr^{-1}$)	Total soil losses (t)
1956–1964	8.5	83 912
1964–1986	10.6	287 769
1986–2008	6.8	191 848
Mean for 1956–2008	8.6	563 529

plough layer, Bq/kg; A ius ^{137}Cs inventory at sampling point, Bq/m; $\int_S A_{ref} dS$ is total ^{137}Cs fallout inventory within a geomorphic unit, Bq; $\int_S A dS$ is total ^{137}Cs inventory within geomorphic unit, Bq; and S is area of geomorphic unit, m^2.

Data for individual geomorphic units were integrated to provide the sediment budget for the entire catchment over the post-1986 period. Table 2 indicates that according to the sediment budget derived from the ^{137}Cs budget, more than 80 percent of the sediment eroded from arable hillslopes was redeposited within the slopes after introduction of SCMs, while only <20 percent was delivered to valley bottoms.

The higher values of gross erosion and within-slope redeposition provided by the ^{137}Cs budget approach can be explained, using the case study with individual soil transects. In fact the ^{137}Cs technique gives an integral evaluation of soil redistribution including both water erosion and tillage translocation. The latter is especially important in the sub-catchment with contour terraces.

It can be concluded that the application of Chernobyl-derived ^{137}Cs for establishing the sediment budget of a small cultivated catchment produced results comparable with data obtained by independent techniques. In addition, it allows the effectiveness of various SCMs to be evaluated through comparisons with the sediment budgets constructed for earlier periods using methods with different temporal resolutions (Golosov *et al.*, 2008). Application of SCMs in the Gracheva Loschina catchment since 1986 has reduced average soil loss rates by at least a factor of 2.5 based on the ^{137}Cs budget method.

After dam construction in 1956 only about 10 percent of sediment was transported outside the Lebedin catchment (Prytkova, 1981). Gross erosion rates for arable hillslopes were calculated for known cultivated areas and crop rotations using the USLE-based model for the periods 1956–1964, 1964–1986 and 1986–2008 and are provided in Table 3.

Analysis of the vertical distribution of ^{137}Cs at several incremental depths and depositional locations enabled evaluation of deposition rates over different time intervals. The most intensive deposition (about 2 cm per yr for the post-Chernobyl period) occurred in infills of secondary discontinuous valley bottom gullies. However, such valley bottom cuts are typically only about 2–3 m wide. Deposition rates on other parts of dry valley bottoms were within the range of 0.8 to 1.0 cm/yr, while for the 1964–1986 period, rates ranged between-1.0 and 1.2 cm/yr. Analysis of ^{137}Cs data from the sediment section located in the dried reservoir suggest that before 1986 the intensity of sediment transport during the concentrated snowmelt runoff period was much higher than that during the post-Chernobyl period. This conclusion was based on the location of the Chernobyl-associated peak of ^{137}Cs distribution at a depth about 7 cm below the surface. On the other hand, the deposition layer for the 1956–1986 period had a thickness of about 68 cm. Hence, the intensity of sediment delivery to the Lebedin catchment outlet was about 10 times higher during the latter period. Nevertheless, it is necessary to take into consideration the fact that during reservoir infill by sediment, the gradient of the valley bottom decreased considerably, promoting more active sediment redeposition at the upper part of the reservoir. These observations are consistent with the results of monitoring data on spring snowmelt runoff on cultivated slopes at the Novosil experimental station (Orel region) that demonstrated a considerable decrease in runoff coefficients during the last 12 years (Petelko, Golosov and Belyaev, 2007). A similar trend was found during monitoring observations in the upper part of the Lebedin catchment (the Gracheva Loschina experimental catchment) for the 1986–2002 period (Kumani, 2003).

Detailed determination of areas consisting of different morphological units (including main bottom level, 1–2 terrace levels and discontinuous valley bottom gullies) within the main valley bottom (3rd Hortonian order) of the Lebedin catchment and its tributaries of 1st and 2nd Hortonian orders was carried using tacheometric and global positioning system (GPS) surveys. The total volume of sediment deposited was calculated based on morphological unit areas and ^{137}Cs-based aggradation rates for the main bottom of the Lebedin catchment valley (3rd order) and Gracheva Loschina catchment valleys (1st and 2nd order valleys) (Table 4).

In addition to sediment deposition on the valley bottoms, other sediment sink zones exist. These include: redeposition within cultivated fields; redeposition along the lower boundaries of cultivated fields (at plough terraces); steep grassed dry valley sides; and uncultivated parts of slope hollows (infilled valley side gullies). It is difficult to calculate the sedimentation volume for each of these zones accurately. However, it is possible to estimate the percentage of sedimentation in each zone based on observation data during extreme erosion events and detailed measurements undertaken within the Gracheva Loschina catchment. Redeposition within the cultivated fields based on direct measurements after snowmelt and rainstorm runoff events usually varies within a range of 2 to 25 percent of gross soil values for the respective fields. It is also confirmed by detailed evaluation of gross and net erosion rates at slope catchments using the ^{137}Cs technique (Golosov *et al.*, 2011). Average within-field sediment rede-

TABLE 4. Total sediment deposition in valley bottoms of different orders (Lebedin catchment) for different time intervals

Valley	Hortonian order of the valley	Sediment deposition (t)		
		1956–1964	1964–1986	1986–2008
Gracheva Loschina	1	1 260	5 044	250
	2	2 814	11 256	10 553
Other valleys	1	5 591	22 365	16 773
	2	12 665	50 661	39 579
	3	7 156	28 625	28 625
Total volume in valley bottoms upstream from reservoir		29 486	117 951	95 555
Reservoir		46 769		
Total volume		289 761		

TABLE 5. Percentage of sediment re-deposited in different deposition zones of the Lebedin catchment for the 1956–2008 period

Deposition Zone	% sediment re-deposited in given zone (from gross soil losses)	
	Range (based on observation)	Mean value
Within arable lands	2–25	10
Near the lower edge of cultivated fields	5–30	8
Valley banks	2–7	4
Uncultivated part of hollows (former bank gullies)	4–10	6
Bottom of valleys including pond	—	51
Beyond the pond	5–15	10
Residual	—	11

TABLE 6. Sediment redistribution within the Lebedin catchment estimated for different time intervals based on evaluation of sediment deposition in valley bottoms and corrected results of soil loss calculations by empirical models

Time interval	Total gross soil losses (t) (based on empirical model calculations)	Sediment volume in the valley bottoms (t) (based on ^{137}Cs dating)	Hillslope-mobilized sediment redeposited in the valley bottoms (%)	Hillslope-mobilized sediment redeposited along pathways from cultivated slope to valley bottoms (%)
1956–1964	83 912/74 682*	37 858	45/51*	39
1964–1986	28 7769/256 114	151 438	53/59	31
1986–2008	191 848/170 745	100 465	52/59	31
1956–2008	563 529/506 881	289 761	51/57	33

* numerator — results of soil losses calculation without correction; denominator — results of soil losses calculation with correction based of sediment budget calculations

position is around 10 percent of total eroded volume. Acummulation along the lower boundaries of cultivated fields (at plough terraces) is usually more significant and varies between 5 percent and 30 percent of gross soil losses according to different measurements and observations. For the Gracheva Loschina catchment, it was determined that 8 percent of hillslope-mobilized material remained stored in plough terraces.

The most difficult task is to estimate sediment deposition on the grassed valley sides because of the extremely random nature of this process. Data obtained after extreme erosion events and during snowmelt periods showed that between 2 and 7 percent of sediment mobilized from cultivated land was redeposited within these morphological units (Belyaev *et al.*, 2008). Sediment deposition in uncultivated parts of slope hollows (most of which actually represents infilled formerly active valley side gullies) was calculated based on direct measurements of total sediment volumes stored in hollows of the Gracheva Loschina catchment and the total number of uncultivated hollows on the valley sides of the Lebedin catchment (Table 5). By combining all the above data and comparing the results with the USLE-based soil loss calculations, it was concluded that the empirical erosion model overestimated total soil loss from the cultivated slopes of the Lebedin catchment by about 11 percent.

Comparision of total soil losses from cultivated hillslopes with sediment deposition in the valley bottoms also demonstrated that the proportion of sediment deposited in valley bottoms from total soil losses did not differ significantly between the different time intervals considered (Table 6). It can therefore be suggested that empirical models consistently over-estimate soil losses and that it is very likely that actual soil losses are 11 percent lower than those calculated. Based on this assumption, 57 percent of material eroded from cultivated slopes remained redeposited in the valley bottoms, 33 percent along pathways from cultivated slopes to the valley bottoms, and only about 10 percent of sediment was transported further downstream and reached the catchment outlet reservoir (Table 6).

TABLE 7. Proportion of sediment potentially exported from the Lebedin catchment into the River Vorobzha valley for different time intervals (if the catchment outlet reservoir did not exist)

Time interval	Total gross soil losses (t) (based on corrected empirical model calculations)	Sediment volume in valley bottoms (t) (based on ^{137}Cs dating and area of bottoms)	Sediment deposited in pond (t)	Percentage of sediment potentially exported from the Lebedin catchment (%)
1956–1986	330 796	189 296	39 754	12/21*
1986–2008	170 745	100 465	7 015	4/7

*numerator — % from total soil losses on cultivated hillslopes; denominator — % from deposition in valley bottoms

It can be suggested therefore, that the volume of sediment redeposited within the main reservoir at the catchment outlet was proportional to the volume of sediment potentially exported from the Lebedin catchment. In this respect, it is noteworthy that before 1986 about 12 percent of total soil losses or about 21 percent of total deposition in the valley bottoms reached the catchment outlet. After 1986 the percentage of sediment reaching the Lebedin catchment outlet decreased by more than threefold (Table 7), mainly as a result of lower surface runoff and erosion during the spring snowmelt period. Consequently, it can be tentatively suggested that during the last two decades, the volume of sediment delivered into the River Vorobzha valley from its main tributary catchments fell by about one third.

CONCLUSIONS

This study shows that changes in climate and crop rotation reduced significantly the rates of soil loss from cultivated fields within typical catchments of the Chernozem zone of Central European Russia during the last two decades. Most of the eroded sediment and sediment-associated ^{137}Cs were redeposited within cultivated fields and dry valley bottoms. Application of an integrated approach involving several independent techniques allowed detailed and reliable evaluation of soil degradation rates and the contributions of both local and off-site soil sinks.

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Assessment of Soil Erosion and Sedimentation Rates in 'My Bouchta' Watershed in North Morocco using Fallout Radionuclides and Stable Isotopes

M. Benmansour[1,*], N. Amenzou[1], A. Zouagui[1], H. Marah[1], M. Sabir[2], A. Nouira[1], H. Benjelloun[2], A. Benkdad[1] and F. Taous[1]

ABSTRACT

The aim of this work was to combine measurements of fallout radionuclides (FRN's) Caesium-137 (^{137}Cs) and excess Lead-210 ($^{210}Pb_{ex}$) with stable isotopes of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) for investigating land degradation and the origin of sediment deposits in the My Bouchta watershed in northern Morocco. FRN's were used as tools for obtaining quantitative information on soil erosion and sedimentation rates over a range of different timescales whilst the stable isotopes enabled the primary sediment source areas to be identified. Using ^{137}Cs the net soil erosion rate for the My Bouchta watershed was estimated to be about 22.1 tonnes (t)·ha^{-1}·year^{-1} over a period of 50 years with a major contribution from agricultural fields. Net soil erosion rates over a period of 100 years derived from $^{210}Pb_{ex}$ were lower than those estimated by ^{137}Cs. These results indicate that soil erosion has increased significantly during the last 50 years. Sedimentation rates of about 0.50 g·cm^{-2}·year^{-1} (equivalent to 50 t·ha^{-1}·year^{-1}) were obtained for the Talembout water reservoir suggesting additional contributions from gully erosion and mass movement. Similar behaviours of stable isotopes with depth were obtained for forest and shrub fields and a good correlation between $\delta^{13}C$ and total C was obtained for forest and shrub fields. Using the $\delta^{13}C$ profile, sediment deposits in the water reservoir seemed to originate mainly from fields under maize culture (C_4 plant). The sediment profile of $\delta^{15}N$ indicated an increased use of synthetic fertilizers during the last 15 years.

Key words: *fallout radionuclides, carbon, nitrogen, stable isotopes, soil erosion, sedimentation.*

INTRODUCTION

Land degradation by soil erosion is a major concern for Moroccan watersheds especially in the north, leading to considerable on-site and off-site impacts. As reported by Namr and Mrabet (2004), the main on-site impact on the Moroccan agricultural landscape is reduced soil fertility while the main off-site impact is increased siltation in water reservoirs. Out of 22.7 million ha of potentially exploitable land in the northern part of Morocco, 77 percent is estimated to be subject to very high erosion risks (Belkhiri, 1988). However, few reliable datasets are available on the extent of land degradation in many Moroccan basins. Integrated data are therefore needed to develop soil conservation strategies and protect water reservoirs against sedimentation and pollution at the watershed scale. Most previous studies have used classical techniques such as experimental plots or prediction models to estimate soil erosion and its impacts. Although nuclear techniques have been used in Morocco for some years (e.g. Bouhlassa, Moukhchane and Aiachi, 2000; Nouira, Sayouty and Benmansour, 2003; IAEA, 2011), their application to study events at the watershed scale is limited. This paper presents preliminary results of a study which used an integrated approach involving FRNs (Walling, He and Quine, 1995; Zapata 2002; Mabit, Benmansour and Walling, 2008) and stable isotopes (Phillips and Greeg, 2002; Gibbs, 2008) to establish a complete sediment budget and identify soil erosion sources at the My Bouchta watershed.

MATERIALS AND METHODS

The watershed My Bouchta is located in the occidental part of the Rif mountains between the cities of Tetouan and Chaouen (Figure 1). The total area is 76.6 km^2 and the mean annual precipitation is about 800 mm. The watershed is characterized by high slopes (from 20 to 40 percent), and the soils are degraded by different forms of very active water erosion: sheet, rill, gully erosion and mass movement. Based on the information collected and preliminary visits to the study site, a sampling strategy was established for the whole watershed, including upland to lowland areas using a geographic information system tool (ArcGIS). It was based on selecting representative fields taking into account the different classes of land use, topography and soil type. The main classes selected for these three parameters were as follows:

- Three classes for the land use: forest (FR), shrub (SR) and agricultural (AG) areas.
- Four classes for the slope gradient (in percent): from 0 to5, from 5 to15, from 15 to 30 and > 30.
- Four classes for the soil type: vertisols (VE), alfisols (AL), aridisols (AR) and entisols (EN).

All together 48 homogenous units or iso-sectors were defined. To optimise the sampling strategy, only 22 important units covering

1 Centre National de l'Energie, des Sciences et des Techniques Nucléaires (CNESTEN), BP.1382, R.P 10001, Rabat, Morocco
2 Ecole Nationale Forestière d'Ingénieurs (ENFI), B.P. 511, Salé, Morocco
* E-mail address of corresponding author: benmansour64@yahoo.fr

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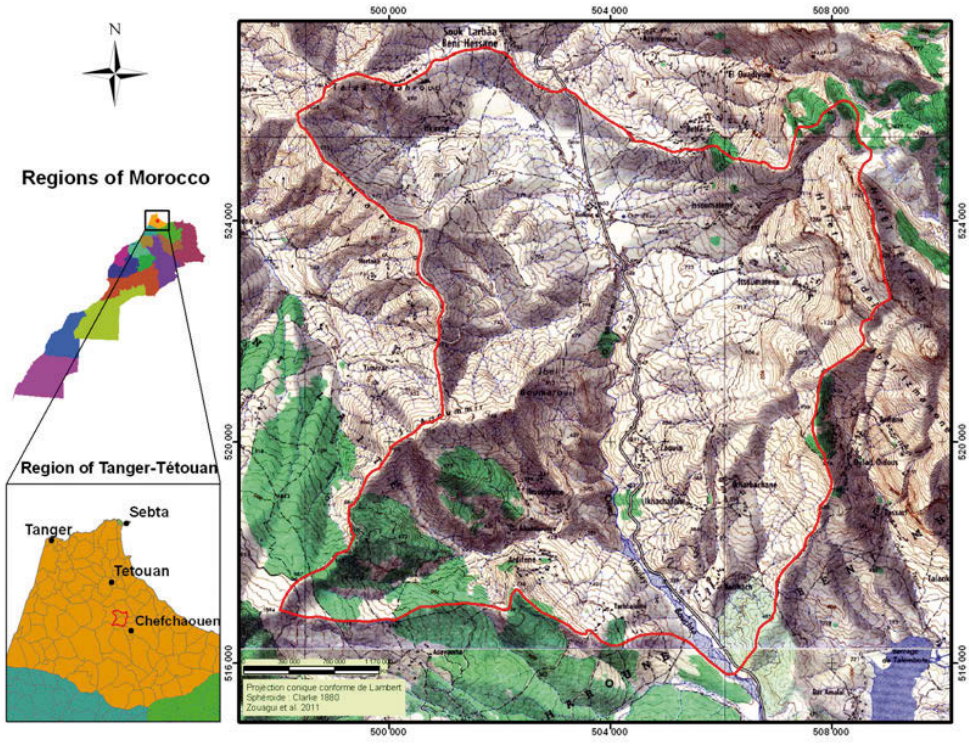


FIGURE 1. Location of My Bouchta watershed.

more than 90 percent of the total watershed area were considered for the study. A transect approach (from 1 to 4 transects) was adopted to collect samples from the top to the bottom of the field. Until now, around 200 soil and sediment samples were collected in various fields representing ten homogenous sectors in upland areas of the watershed, four reference sites (RF1, RF2, RF3 and RF4) and one area of the Talembout water reservoir (WR1, WR2, WR3). A motorized cylindrical tube (diameter ca. 9 cm) or the so-called Column Cylinder Auger was inserted to a depth of between 30 and 40 cm to ensure that all ^{137}Cs or ^{210}Pb were measured. Regarding the use of the stable isotopes (δ^{13}C and δ^{15}N), the transect approach was also used from the top to the bottom, and soil profiles were taken with the same core sampler at six sampling points per transect (distance between sampling points was approximately 20 m). At each sampling point a soil core was taken and divided into four subsamples representing incremental depths (from 0 to10, 10 to 20, 20 to30 and 30 to 40 cm).

The soil and sediment samples taken for radionuclide analyses were dried, lightly ground, sieved (<2 mm) and homogenised prior to the measurement of ^{137}Cs, ^{210}Pb, ^{226}Ra (from ^{214}Bi) by gamma spectrometry using an HPGe detector (Canberra p-type 30 percent). Calibration of the detection systems was done by preparing standards from a certified multi-gamma source (Amersham) and IAEA reference materials (IAEA 327 and 375). Generally, samples were placed in Marinelli bakers (500 ml) or cylindrical containers (200 ml). ^{137}Cs, ^{210}Pb and ^{214}Bi activities were determined from the net gamma ray peak areas at 662, 478 and 46.5 keV, respectively. Counting rates varied from 12 to 24 h which provided a precision of about 5 percent to 20 percent at 95 percent level of confidence. Indirect determination of excess ^{210}Pb activity from total ^{210}Pb and ^{226}Ra gave a low precision for activity values (range 30 percent to 50 percent). To improve the precision of ^{210}Pb activity, measurements by alpha spectrometry through ^{210}Po (daughter of ^{210}Pb), was performed for some soil and sediment samples with low activities. This method required a total digestion of soil samples containing ^{210}Pb, ^{210}Po and tracer ^{209}Po, using HNO_3, HCl and HF acids and spontaneous deposition of polonium isotopes in silver discs.

Counting was carried out using silicon detectors (EG & G, Ortec). Alpha spectrometry provides a good precision for measuring ^{210}Pb but rquires longer counting times than gamma spectrometry. Quality control procedures used control charts (efficiency, resolution and background), certified reference materials and regular participation in inter-comparison exercises and proficiency tests organised by the IAEA.

For the stable isotopes, as even small amounts of inorganic C can lead to significant errors in δ^{13}C, inorganic C was removed by acid fumigation prior to analysis. The samples were stored at approximately 4°C until further processing. Moistened sub-samples were exposed overnight to HCl. Afterwards the samples were rinsed by shaking with 2 ml of distilled water, dried at 40°C and ground in a mortar before measuring the stable isotope ratio. Stable C isotope analysis was carried out using a continuous flow isotope ratio mass spectrometer (Delta Plus) coupled with an elemental analyzer (Carlo Elba, NC2500). Carbon and N stable isotope abundances were expressed as follows as delta (δ) values to indicate differences between the isotopic ratio of the sample and accepted standard materials:

$$\delta X(‰) = \left(\frac{R_{sample}}{R_{std}} - 1\right) \times 10^3$$

where: X — (^{13}C or ^{15}N), R_{sample} — the isotope ratio (^{13}C:^{12}C or ^{15}N:^{14}N) of the sample; and R_{std} = the isotope ratio of the standard Vienna Pee Dee Belemnite (VPDB), and atmospheric N, respectively.

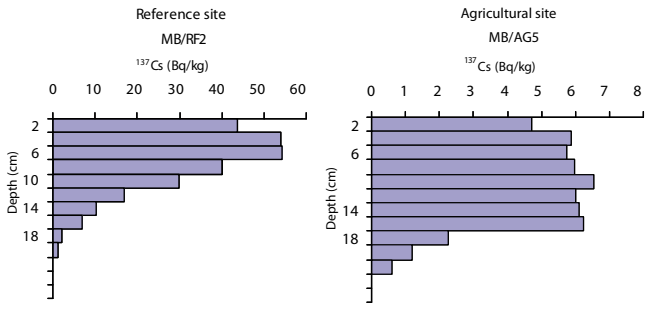


FIGURE 2. Example of ^{137}C depth profiles associated with a reference and an agricultural site at 'My Bouchta' watershed.

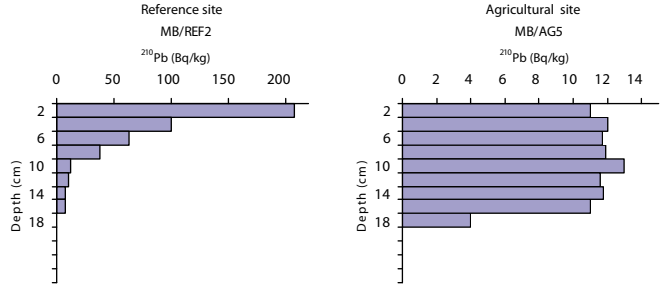


FIGURE 3. Example of $^{210}Pb_{ex}$ depth profiles associated with a reference and an agricultural site at My Bouchta watershed.

RESULTS AND DISCUSSION

Soil erosion and sedimentation rates using ^{137}Cs and $^{210}Pb_{ex}$

Typical depth profiles of ^{137}Cs and $^{210}Pb_{ex}$ activities associated with the reference (forest with no slope) and cultivated sites are shown in Figures 2 and 3. Most ^{137}Cs and $^{210}Pb_{ex}$ were contained in the top 10 cm and, as expected, concentrations decreased exponentially with depth. For the cultivated site, concentrations were almost uniform throughout the plough layer (~16 cm) as a result of mixing caused by cultivation. The ^{137}Cs reference inventories associated with the two identified reference sites (RF1 and RF2) were respectively about 2704 Bq/m^2 and 3554 Bq/m^2. This variation reflects the difference between RF1 and RF2 in mean annual precipitation (about 660 mm and 1700 mm., respectively). ^{137}Cs inventories corresponding to the cultivated sites were the lowest, ranging between 320 and 2998 Bq/m^2, while for the forest and shrub areas, ^{137}Cs activities were found to range between 1340 and 6830 Bq/m^2. For fallout $^{210}Pb_{ex}$, reference inventories associated with the two reference sites were about 3220 Bq/m^2 and 4800 Bq/m^2, respectively for RF1 and RF2. $^{210}Pb_{ex}$ activities were measured for some fields (FR1, SR1, AG1) near to the first reference site (RF1). The mean $^{210}Pb_{ex}$ activity associated with cultivated site AG1 was 2 685 Bq/m^2, while for the forest (FR1) and shrub (SR1) fields the mean inventories were found to be respectively 3 088 and 2950 Bq/m^2.

Medium-term rates (over 50 years) of water-induced soil erosion and deposition were estimated from the ^{137}Cs inventories and using the refined mass balance II model (MBII) for the cultivated site and the diffusion and migration model (DMM) for the uncultivated sites (Walling, He and Appleby, 2002). For the cultivated fields, all the sampling points were found as eroded areas. Consequently, the gross, mean and net soil erosion rates were similar indicating sediment delivery ratios of about 100 percent. Therefore, erosion rates corresponding to agricultural fields ranged between 18 t·ha^{-1}·yr^{-1} and 36 t·ha^{-1}·yr^{-1} (Table 1), while for the forest and shrub areas the net soil erosion rates were found to be low.

Using the DMM model, the net soil erosion rates ranged between 0.8 t·ha^{-1}·yr^{-1} and 2.4 t t·ha^{-1}·yr^{-1} and sediment delivery ratios varied from 52 to 94 percent. In all cases, these results show that soil erosion rates associated with agricultural fields were significantly higher than those associated with the forest and shrub fields. They also demonstrate clearly the role of forest plantations and vegetation cover in protecting the soil against erosion, reducing by a factor of between 4 and 20 the soil loss in the watershed. Until now, net soil erosion rates have been estimated for ten homogeneous units or iso-sectors (Table 1). The extrapolation of data on soil erosion to the watershed scale covering over 72 percent of the total area, indicates an average net soil erosion rate of 22.1 t·ha^{-1}·yr^{-1}. The most vulnerable soils corresponded to the agricultural iso-sector with aridisols and slopes greater than 30 percent (Table 1). These are estimated to have contributed 56 460 t of soil loss annually, representing almost half of the total loss in the area of the watershed investigated. Total annual soil erosion from all agricultural fields was estimated to be

TABLE 1. Net soil erosion rates associated with ten iso-sectors of the watershed

Site	Iso-sector (%)	Area (ha)	% of total area	Net erosion rate (t·ha^{-1}·yr^{-1})	Net erosion rate (t·ha^{-1}·yr^{-1})	% of total erosion
AG1/AG2	AG, VE, 5–15	654	8.8	27.4	17 920	14.0
AG3	AG, AR, 15–30	1 723	23.1	18.5	31 876	24.9
AG4	AG, VE, 15–30	382	5.1	22.5	8 595	6.7
AG5	AG, AR, > 30	1 564	21.0	36.1	56 460	44.1
AG6	AG,AR, 5–15	579	8.4	18.8	10 884	8.5
FR1	FR, AL, > 30	121	1.6	1.6	194	0.2
FR2	FR, AL, 15–30	146	2.0	0.8	117	0.1
SR1	SR, AR, > 30	472	6.3	2.4	1 133	0.9
SR2	SR, AL, 15–30	23	0.3	4.5	104	0.1
SR3	SR, AR, 15–30	119	1.7	6.9	821	0.6
Total		5 783	71.9	22.1	128 121	100

AG — agriculture; FR — forest; SR — shrub; VE — vertisol; AL — alfisol; and AR — aridisol

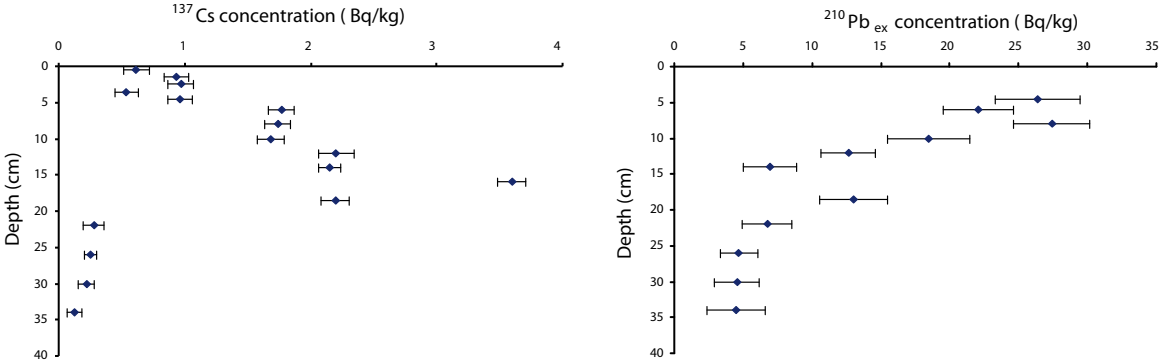


FIGURE 4. ^{137}Cs and ^{210}Pb depth profiles associated with a sediment core collected in the Talembout water reservoir.

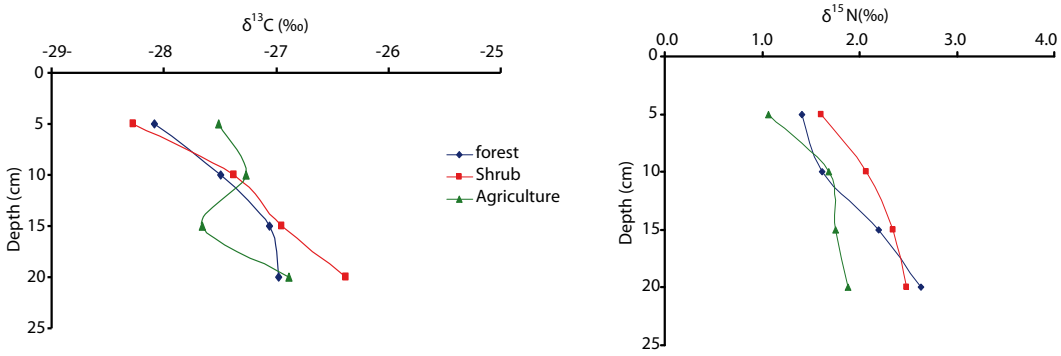


FIGURE 5. Mean depth profiles of δ^{13}C and δ^{15}N associated with forest, shrub and agricultural fields.

114 818 t. Fallout $^{210}Pb_{ex}$ was also used to estimate long-term soil erosion rates over 100 years in the fields AG1, FR1 and SR1. Preliminary results indicated that the gross erosion rates were respectively about 12 t·ha^{-1}·yr^{-1}, 0.61 t·ha^{-1}·yr^{-1} and 1.3 t·ha^{-1}·yr^{-1}. These values are lower than those obtained by ^{137}Cs, a difference that may be explained by the significant increase in soil loss that has taken place in the watershed during the last 50 years.

The mean sedimentation rate in the water reservoir Talembout is provided in Figure 4. The maximum depth of the ^{137}Cs profile was 16 cm which can be attributed to the atmospheric fallout from nuclear weapon tests occured in 1963. The sedimentation rate was estimated approximately at 0.34 cm/yr. Taking into account the soil bulk density, the sedimentation rate in the water reservoir was about 0.503 g·cm^{-2}·yr^{-1} (50 t·ha^{-1}·yr^{-1}). Using fallout $^{210}Pb_{ex}$ and the constant flux and constant sedimentation (CFCS) model (Krishnaswami *et. al.*, 1971; Appleby and Oldfield, 1978), the sedimentation rate was about 0.495 g·cm^{-2}·yr^{-1} (49.5 t·ha^{-1}·yr^{-1}), i.e. similar to the value obtained by fallout ^{137}Cs. The sedimentation rate was therefore higher than the net erosion rate estimated from the upland fields of My Bouchta watershed. This could be explained by the contributions of other possible forms of water erosion such as gully erosion and mass movement including landeslides.

Stable isotopic signatures (δ^{13}C and δ^{15}N) and C and N contents of soil and sediment samples

The soil δ^{13}C and δ^{15}N signatures as well as carbon and nitrogen concentrations were determined for three different fields on the upland areas of the watershed: forest (AG1), shrub (SR1) and agriculture (AG1). The δ^{13}C associated with forest (AG1), shrub (SR1) and agriculture (AG1) along one transect and at six points of each field ranged from –27.26 to –28.80‰, from –25.71 to –28.60‰ and from –26.51 to –28.34‰, respectively. These values are associated with C_3 plants. Similarly, the δ^{15}N corresponding to the fields ranged from 0.74 to 3.90‰, and from 0.58 to 4.76‰ and from 0.36 to 1.85‰, respectively.

The mean depth profiles of δ^{13}C and δ^{15}N for the three fields are given in Figure 5. The mean value for six points was taken for each level. δ^{13}C and δ^{15}N profiles associated with forest and shrub areas seemed to have the same behaviour and differed from the δ^{13}C and δ^{15}N profiles associated with the agricultural field. Stable isotopic signatures increased with depth especially for the forest and shrub areas. Comparable results were obtained in a study conducted in lower Montane, Ecuador (Rhoades *et al.*, 2000) who recorded a 2–3 percent enrichment of soil δ^{13}C with soil depth. According to these authors, the greater isotopic enrichment at depth was associated with burial of organic matter. The mean depth profiles of C and N showed that the average C content decreased with depth in forest, shrub and agricultural fields, whereas with the N depth profile, mean values deacreased only in the forest and shrub areas, being almost constant for the agricultural field (Figure 6).

Mean values for the stable isotopes δ^{13}C and δ^{15}N as well as for C and N content and C:N ratios including all points for the three fields are given in Table 2. Carbon and nitrogen concentrations and C:N ratios are in general indicative of soil organic matter quality and provide metrics of fertility. Differences were observed especially for

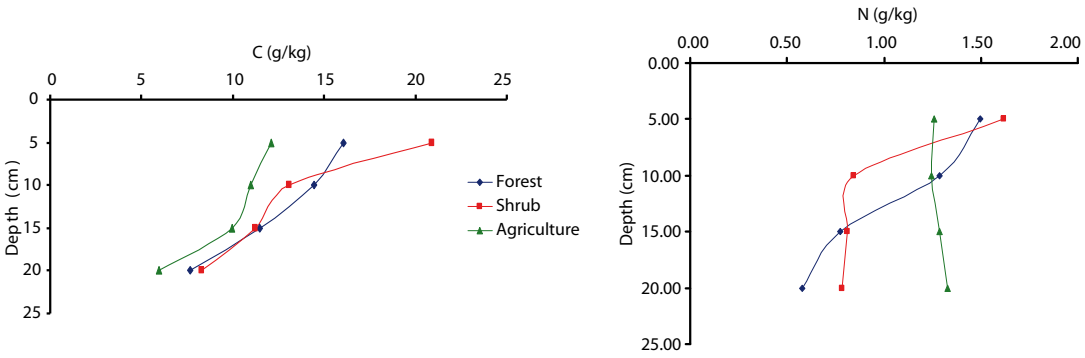


FIGURE 6. Mean depth profiles of C and N contents associated with forest, shrub and agricultural fields.

TABLE 2. Mean values for $\delta^{13}C$, $\delta^{15}N$, C, N and C:N ratios

Field	$\delta^{13}C$ (‰)	C (g/kg)	$\delta^{15}N$ (‰)	N (g/kg)	C:N
Forest (FR1)	–27.49 ± 0.18	12.99 ± 1.39	1.76 ± 0.75	1.04 ± 0.14	12.49± 1.94
Shrub (SR1)	–27.24 ± 0.23	13.05 ± 2.49	2.12 ± 1.00	1.02 ± 0.10	12.79± 2.74
Agriculture (AG1)	–27.33 ± 0.40	9.77 ± 3.96	1.59 ± 0.52	1.28 ± 0.04	7.63 ± 3.10

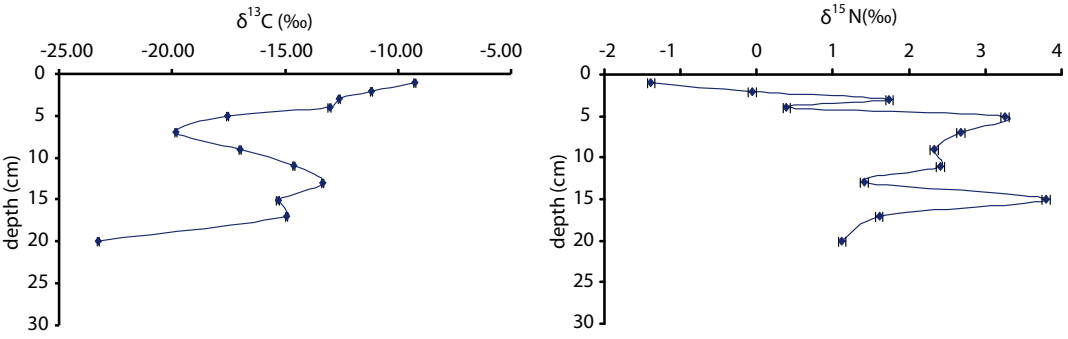


FIGURE 7. $\delta^{13}C$ and $\delta^{15}N$ depth profiles associated with a sediment core collected from the Talembout water reservoir.

the C content and C:N ratios which were higher for forest and shrub soils compared with agricultural soils.

The high correlation between $\delta^{13}C$ and C for forest (R^2 = 0.60) and shrub field (R^2 = 0.74) reflects a linear decrease in C content with an increase in $\delta^{13}C$. However, the correlation for the agricultural field was low (R^2 = 0.24). This can be explained by the high rates of organic matter decomposition arising from high erosion and periodic disturbance of the soil in the agricultural field, compared with the forest and shrub fields. Similar results were obtanied by Schaub and Alwell (2009).

On the other hand, depth profiles of $\delta^{13}C$, $\delta^{15}N$ and the C and N contents associated with one sediment core collected from Talembout water reservoir downstream of My Bouchta watershed (Figures 7 and 8) showed that $\delta^{13}C$ increased from the bottom (–23.25‰ at a depth between 17 and 20 cm) to the top (–9.26‰ at depth of between 0 and 2 cm). The mean value was about –15.15‰. Except for the value obtained at the lower depth which is characteristic of C_3 plants, all other values were higher than –20‰ indicating that the $\delta^{13}C$ signature values were derived from plants within the C_4 group.

These values differed according to the three types of fields (agriculture, forest and shrub) investigated in the uppper areas of the watershed which were derived from C_3 plants. Two interpretations can be provided at this stage:

- The main source of sediment deposits in the water reservoir was soil eroded from the upland areas cultivated by C_4 plants (e.g. maize which is cultivated alternatively with wheat).
- The historic cultivation of maize by farmers around the Talembout water reservoir.

Concerning the depth profile of $\delta^{15}N$, a rapid decline of $\delta^{15}N$ with depth was observed over the last 5 cm (Figure 7). This may be attributed to the increasing use of synthetic inorganic fertilizers on agricultural fields upstream of the reservoir during the last decade. Similar results were obtained by Oczkowski *et al*. (2011) for the sediment cores collected in Lake Manzala (Egypt) and Ghar El Melh Lagoon (Tunisia).

Carbon and N depth profiles are presented in Figure 8. They were maximum at a depth around 7 cm. The variations may reflect changed agricultural practices and fertilizer types.

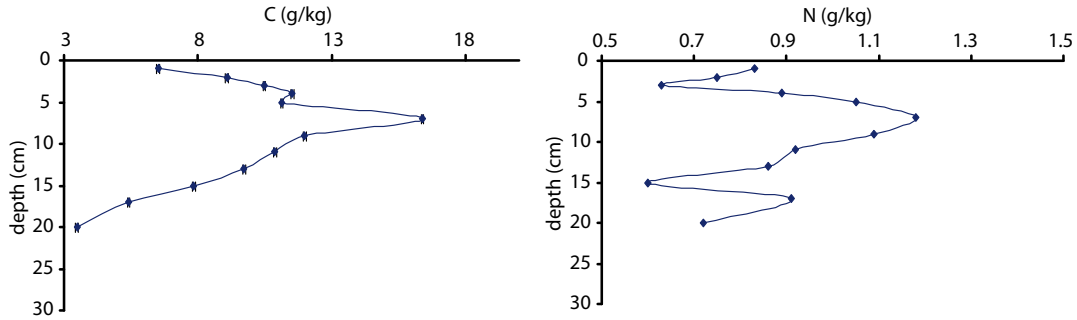


FIGURE 8. C and N depth profiles associated with a sediment core collected from the Talembout water reservoir.

CONCLUSIONS

These preliminary results show that the net soil erosion rate estimated for the My Bouchta watershed was about 22 $t \cdot ha^{-1} \cdot yr^{-1}$. Rates associated with agricultural fields were significantly higher than those for forest and shrub lands and indicate clearly the role of forest plantations and vegetation cover in protecting soils against erosion. A high sedimantation rate (about 0.50 g cm^{-2} yr^{-1}, (equivalent to 50 $t \cdot ha^{-1} \cdot yr^{-1}$) was obtained for the Talembout water reservoir where sediment deposits seemed to have originated mainly from fields cultivated with cereals, particularly maize (a C_4 plant).

There is an urgent need to develop new soil conservation strategies and new agricultural practices in the watersheds of northern Morocco to ensure sustainable crop production in agricultural landscapes and to protect water reservoirs against siltation. Results reported here from the use of stable N isotope indicate increasing use of synthetic inorganic fertilizers on upstream agricultural fields during the last decade. This work will be continued by using ^{7}Be as a tracer of short erosion events and compound-specific stable isotopes (CSSI) to identify and apportion with more precision, sediment source areas and erosion hotspots.

AKNOWLEDGMENT

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Use of Fallout Caesium-137 to Evaluate the Effectiveness of the FAO-LADA Approach for Assessing Soil Erosion-Induced Land Degradation in the Chinese Loess Plateau

Y. Li [1, 2*], H.Q. Yu[1], Y.Z. Zhang[1], B. Nyamdavaa[1] and G.Q. Liu[1]

ABSTRACT

The Land Degradation Assessment of Drylands methodology (LADA) has been reported to be a rapid and robust approach for assessing the state and nature of soil erosion at regional, national and global levels. However, it needs to be adapted for use under different environments. The studies reported here were to: (i) compare differences in soil redistribution rates determined by the LADA approach and by using the fallout caesium-137 (^{137}Cs) technique, and (ii) develop a validated LADA methodology to rapidly assess soil erosion-induced land degradation for use in the Chinese Loess Plateau. Forty two sites in three watersheds were selected (Liudaogou in Shenmu, Jiuyuangou in Suide, and Nianzhuang in Yan'an) in the Loess Plateau. Soil erosion rates were measured in grassland, shrubland, forestland and farmland by the LADA approach through measurements of plant/tree root exposure, a tree mound and build up against barriers, and rills and by the ^{137}Cs technique. Both approaches revealed similar spatial patterns for soil erosion rates, although the differences between rates determined by the two approaches were greater in grasslands than in forestland and shrubland, and rates derived from the LADA approach were lower in forest and grasslands but higher in farmland than those calculated by the ^{137}Cs technique. It is concluded that the LADA methodology is very useful for quickly assessing soil erosion under different hillslopes and land use types, although an area-wide evaluation of its suitability is still required.

Key words: *caesium-137 technique, LADA, soil erosion, Chinese Loess Plateau*

INTRODUCTION

The Loess Plateau located in west China is perhaps the area in the world most severely affected by soil erosion and deposition. It is estimated that about five billion tonnes of surface soil have been lost due to water erosion from the Loess Plateau, which accounts for 10 percent of total world soil loss. Inappropriate land use and intensive agricultural production with poor farming practices are responsible for this state of affairs. This accelerated soil erosion is a major force driving land and soil quality degradation, and is therefore a threat not only to China, but also to global food security and environmental sustainability (Li, 1995; Li and Lindstrom, 2001). However, the on-site and off-site impacts on productivity and agricultural sustainability of this well-documented acceleration of soil erosion and deposition remain debatable because of the compounding impact of changing climate and the compensating effects of high yields brought about by technological improvements (e.g. new varieties, fertilizer use, soil water conservation). New technologies are therefore urgently needed to develop decision support for sustainable agricultural production and agro-environmental sustainability (IAEA, 2008) based on a clearer understanding not only about the state and nature of soil erosion, but also the impacts of land management at regional, national and global levels.

Changes in soil quality degradation due to soil erosion are major factors to be considered in relation to food security (Lal, 1999). Soil erosion adversely impacts agricultural productivity and the environment through its effects on physical, chemical, and biological quality. Knowledge of these soil quality parameters is key to assessing the impacts of agricultural practices on land degradation in terms of productivity. Over the last 50 yr, soil erosion processes have been well documented in China through runoff plot observations and modelling, but their quantitative impacts on soil quality degradation are largely unknown, especially on an area-wide basis (Li and Lindstrom, 2001; Li *et al.*, 2004). So far no national map exists on soil erosion-induced land degradation, or on soils that have been restored through Sustainable Land Management (SLM) practices adopted over the last decades. In fact, simply by focusing solely on the negative trends is unlikely to provide the detailed understanding of the drivers and impacts of changes in land resources necessary to assess current and improved management and policy responses.

The recently developed Land Degradation Assessment (LADA) methodology by FAO and partners (LADA, 2009a and b), including its parameters for site selection and visual indicators for detailed assessment of soil erosion, is a rapid but robust assessment methodology. It offers the potential for a team of approximately five people with multi-sectoral expertise to implement land degradation assessment over a period of some two to three weeks, including time for analysis and report writing. Previous land degradation assessments have rarely moved much beyond descriptions and quantification of biophysical processes. The LADA methodology, on the other hand, aims to provide an understanding both of the state and nature of changes in land resources (soil, water, vegetation) and ecosystems, and also

1 Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing, 100081, P.R. China
2 College of Life Sciences, Hennan Normal University, Henan, Xinxiang 453000, P.R. China
* E-mail address of corresponding author: liyong@caas.cn

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of the drivers and impacts of land degradation LD/SLM. This methodology does not require substantial laboratory-based measurements but provides accuracy and validity by combining quantitative and semi-quantitative field measurements with qualitative information from local stakeholders. Its primary emphasis is on the assessment of the current status and dynamics of land resources — soil, water and vegetation in delivering the main productive services required by land-users from the land, i.e. the provisioning services. A second important consideration concerning the LADA approach is the need to identify and evaluate significant impacts of LD/SLM on other key ecosystem services, particularly on global-level systems and resources such as biodiversity etc. Nevertheless, the LADA methodology needs to be tested and compared with other approaches for determining soil erosion on detailed assessment sites so that it may be adapted for use under different environments.

Steep slopes and dense gullies with intensive crop production and over-grazing activities characterize the Chinese Loess Plateau. This emphasizes the existence of spatial variability of soil erosion-deposition and its impacts on soil quality in terms of productivity and environmental sustainability. Fallout radionuclides (FRNs) including ^{137}Cs, lead-210 (^{210}Pb) and berrylium-7 (^{7}Be) are useful for studying soil redistribution patterns within hillslope landscapes (Wallbrink *et al.*, 1999; Zapata, 2002; Wallbrink, 2004; Walling *et al.,* 2006; Zapata and Li, 2007; Ritchie and Ritchie, 2008) over the short (several months), medium (45-yr span), and long terms (100–150 yr span). Their use should therefore be helpful in developing a validated LADA methodology for assessing soil erosion-induced land degradation rapidly and that can be adapted for use across the main land use and ecosystems in the Chinese Loess Plateau.

The objectives of this study were: (i) to compare differences in soil redistribution rates determined by LADA approaches and ^{137}Cs techniques, and (ii) to develop a validated LADA methodology for assessing soil erosion-induced land degradation rapidly and adapting this for use across the main land use/ecosystems in the Chinese Loess Plateau. Some initial results are presented in this paper.

MATERIALS AND METHODS

Identification of study areas

To facilitate extrapolation of field-level results to the regional level of the entire Loess Plateau, the following three-tiered strategy of site selection was adopted for local assessment using the LADA approach:

(i) Geographic Assessment Area (GAA): Three areas were selected in the Loess Plateau to represent sand loess (Shenmu County), loam loess (Suide City) and loess areas (Yan'an City), respectively (see Figure 1).

(ii) Study Area (SA): One representative watershed was selected within each GAA for the field assessments, i.e. the Liudaogou watershed in Shemu County, the Jiuyuangou watershed in Suide City and the Nianzhuang watershed in Yan'an City.

(iii) Sites/full hillslopes for detailed assessments: 2–3 sites/full hillslopes (SITES) were chosen within each study area representing each land-use type (LUT) — cropland, grassland, forest /woodland — and changes in management practices and biophysical features (land units). The characteristics of each LUT were determined in the SA and are shown in Figure 2.

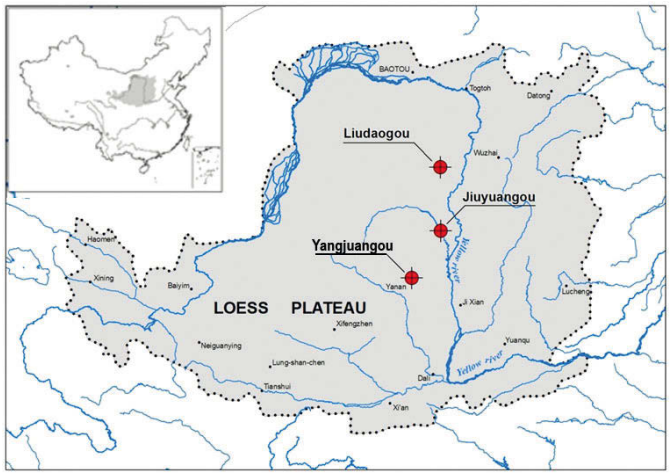


FIGURE 1. Map of the geographic assessment areas.

In total, 108 sites/full hillslopes within each study area (3GAA × 3SA × 3SITES × 4LUTs) were used for detailed assessment of soil erosion and field surveys of which 42 sites have been completed using the LADA approach and FRN techniques (Zapata, 2002).

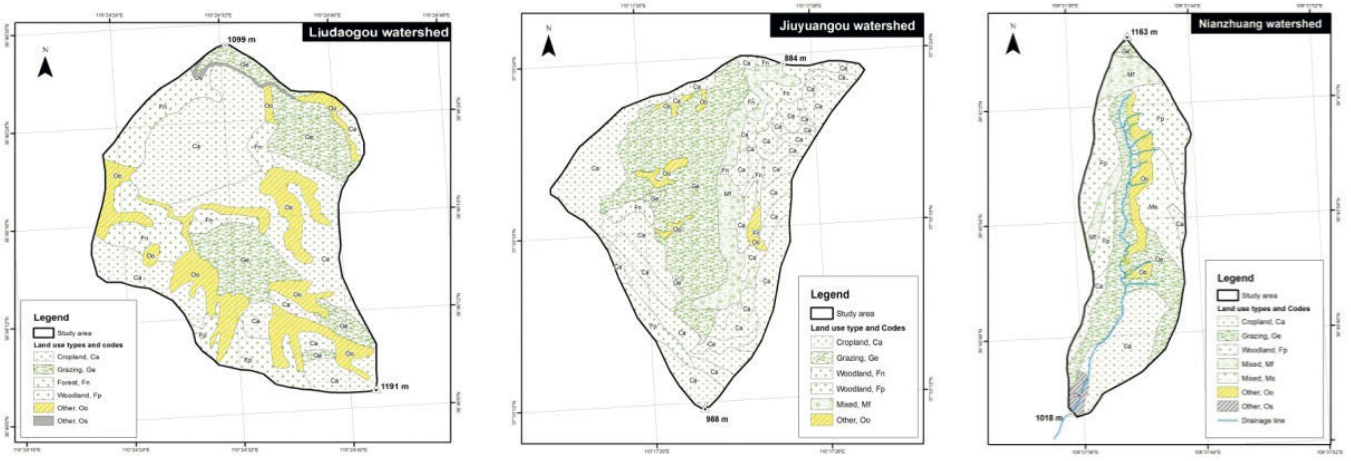


FIGURE 2. Study area (SA): Land use types in the Liudaogou (left), Jiuyuangou (middle) and Nianzhuang watersheds (right).

MEASUREMENT OF EROSION RATES BY LADA APPROACH

Soil erosion rates were measured in grassland, shrubland and forestland including through measurements of plant/tree root exposure, a tree mound and build-up against barriers through procedures and calculations described as follows:

Plant or tree root exposure

The distance from the soil surface to the points on the plant stem or tree trunk which could show the original ground level were measured using a ruler. This measurement gives an estimate of the soil lost since the plant or tree was planted. The annual soil loss ($t \cdot ha^{-1} \cdot yr^{-1}$) was then calculated by dividing the measured difference between the actual soil level and that which existed when the plant or tree started to grow, by the age of the tree. Annual soil loss was calculated using the following formula:

Annual soil loss ($t \cdot ha^{-1} \cdot yr^{-1}$) = Average soil level change (t/ha)/Average age of plants or tree (yr)

Where average soil level change (t/ha) = Average depth of soil loss (mm) × Soil bulk density (t/ha)

Build-up against tree trunk or plant stem

This measurement indicates the accumulation of soil on the upslope side of a tree trunk or plant stem in the target slopes. The volume of soil accumulated was calculated by measuring the depth of the accumulation (at the deepest point) and the distance it extends from the tree trunk or plant stem. For calculation it was assumed that the accumulation was a regular, half-cone shape. The amount of soil accumulated behind a barrier represents the build-up over time, and the eroded material lost in runoff occurring between the trees or plants and this eroded area between the trees or plants along the downslope will have been the contributing area. Detailed calculations were as follows:

(i) Total volume of saved soil

$$\sum \frac{1}{2}\left(\frac{1}{3}\pi r^2 d\right) = \textit{Total volume of soil saved}\ (m^3)$$

where r (m) — the depth of the accumulation (at the deepest point) and d (m) — the length of accumulation

(ii) The annual rate of soil accumulation (based on the age of trees or plants and land management practices):
Annual volume of soil accumulated (m^3/yr) = Total volume of soil saved (m^3)/Age of trees or plants *(yr)*

(iii) The total volume of soil lost from the estimated contributing area was expressed on the basis of square meters:
Total volume of soil accumulated (m^3/m^2) = Annual volume of soil accumulated (m^3/yr)/Contributing area (m^2), and

(iv) Soil loss rate was calculated using the volume of accumulated soil per square meter and the soil bulk density as below:
Soil lost ($t \cdot ha^{-1} \cdot yr^{-1}$) = Total volume of soil accumulated ($m^3\ m^{-2}$) × Bulk density (t/m^3) ×10 000

To estimate soil erosion rates from the farmland, visible rills induced by concentrated flow erosion events were measured in one year, with the below calculation:

Soil lost ($t \cdot ha^{-1} \cdot yr^{-1}$) = Total volume of rills (m^3) × Soil bulk density (t/m^3) ×10 000/Contributing area (m^2)

Soil erosion rates using ^{137}Cs technique

Soil samples for measuring ^{137}Cs inventories were collected from the same SAs from which the field assessments of soil erosion were carried out using the LADA approach. At the same time, samples were collected for determining reference ^{137}Cs inventories. Details concerning the samples and sites are given in Table 1.

Soil samples were air-dried, weighed, and divided into two parts, one passing through a 0.15-mm sieve for the measurement of soil organic carbon (SOC) concentrations and the other passing through a 2-mm sieve for the measurements of ^{137}Cs activities. Measurements of ^{137}Cs activities were conducted using a hyperpure coaxial Ge detector coupled to a multichannel analyzer (Li *et al.,* 2003). Caesium-137 activity was detected at the 662 keV peak using a counting time of over 80 000 sec, which provided an analytical precision of ± 5 percent (Li *et al.,* 2006).

Using ^{137}Cs to understand soil redistribution involves comparing the measured inventories (total activity in the soil profile per unit area) at all sample sites with an estimate of the total atmospheric input obtained from a "reference site". Also, one can determine whether erosion (less ^{137}Cs present than at the reference site) or deposition (more ^{137}Cs than at the reference site) has occurred. At each sampling point, calculation of soil erosion rates relied on the mass balance model developed by Walling *et al.* (2003).

RESULTS AND DISCUSSION

Soil erosion rates measured using the LADA approach

Table 1 summarizes the land use types and plant species for 42 sites in the SAs while Table 2 shows the results of the soil erosion rates in grassland, shrubland and forestland determined from the surveys carried out on these sites. It is believed that the data in Table 2 represent recent soil erosion rates in the SAs and arose from conversion of grass-shrub- and forestland to farmland since 1998. Noteworthy is that soil erosion rates increased according to the following order under different land uses in the Liudaogou and Jiuyuangou watersheds: grassland (average 0.89 and 0.84 $t \cdot ha^{-1} \cdot yr^{-1}$, respectively) < shrubland (average 5.5 and 4.5 $t \cdot ha^{-1} \cdot yr^{-1}$, respectively) < foreastland (average 6.0 and 6.1 $t \cdot ha^{-1} \cdot yr^{-1}$, respectively). For the Nianzhuang watershed, the rate of erosion was also higher under forestland than grassland. These results can be attributed to the easier identification of soil erosion in forestland than grassland, and the denser root distributions in grassland than in forestland which would enhance significantly the resistance of soil to runoff erosion (Li, 1995). Soil erosion rates on the farmland were estimated by measuring soil lost by rill erosion averaged 74.9 $t \cdot ha^{-1} \cdot yr^{-1}$. This value is 12 to 89 times higher than that measured in the forest and grasslands.

Soil erosion rates estimated using ^{137}Cs inventories

The rate of soil erosion derived from from ^{137}Cs averaged –7.0 $\cdot ha^{-1} \cdot yr^{-1}$ in forest hillslopes over the past 50 years (Figure 1). For the farmland, 18 soil samplings were conducted in the inter-rill and rill areas. From these, the soil erosion rate averaged 42.1 $t \cdot ha^{-1} \cdot yr^{-1}$ using the ^{137}Cs conversion model, much higher than forest and grasslands. This infers that reforestation on the cultivated hillslopes has significantly reduced soil erosion.

Comparing the LADA approach with FRN techniques

The spatial patterns of soil erosion were similar for the LADA approach and ^{137}Cs technique (Figure 1). The somewhat higher value

TABLE 1. Summarized land use types (LUTs) and plant species in the Chinese Loess Plateau

SA	LUTs	Plant species	Conversion of vegetation from farmland (yr)	Number of investigated sites
Liudaogou watershed in Shenmu county	Grassland1	Pennisetum flaccidum Griseb	10	3
	Grassland2	Tripolium vulgare Ness	5	3
	Forestland1	Populussimonii Carr	10	3
	Forestland2	Pinus tabulaeformis Carr	10	3
	Forestland3	Ulmus pumilaL.	10	3
	Shrubland1	Caragana intermedia	5	3
	Shrubland1	Caragana intermedia	10	3
Jiuyuangou watershed in Suide city	Grassland	*Medicago sativa Linn*		3
	Forestland	*Pinus tabulaeformis Carr*	7	3
	Shrubland	*Caragana intermedia*	7	3
	Farmland	Crop (Maize and Potato)	7	3
Nianzhuang watershed, Ya'an city	Grassland	*Pennisetum flaccidum Griseb*		3
	Forestland	*Robinia pseudoacaciaL.*	7	3
	Farmland	Crop (Maize and Potato)	7	3
Total				42

TABLE 2. Soil erosion rates with the LADA approach in the Chinese Loess Plateau

SA	LUTs	Soil erosion (cm)	Eroded period (yr)	Soil bulk density (g/cm)	Soil erosion rate $t \cdot ha^{-1} \cdot yr^{-1}$
Liudaogou watershed in Shenmu county	Grassland1	5	10	1.46	0.73
	Grassland2	3.8	5	1.37	1.04
	Forestland1	46	10	1.48	6.79
	Forestland2	40	10	1.31	5.24
	Forestland3	35	10	1.68	5.86
	Shrubland1	28	5	1.38	7.72
	Shrubland1	24	10	1.34	3.21
Jiuyuangou watershed in Suide city	Grassland	4.5	7	1.31	0.84
	Forestland	36	7	1.18	6.07
	Shrubland	25	7	1.27	4.52
Nianzhuang watershed in Ya'an city	Grassland	5	7	1.17	0.84
	Forestland	45	7	1.13	7.29

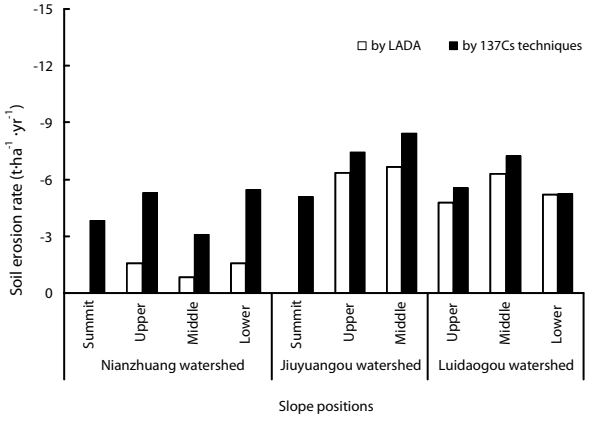


FIGURE 3. Comparison between spatial patterns of soil erosion rates by the LADA methodology and the ^{137}Cs technique in forest hillslopes.

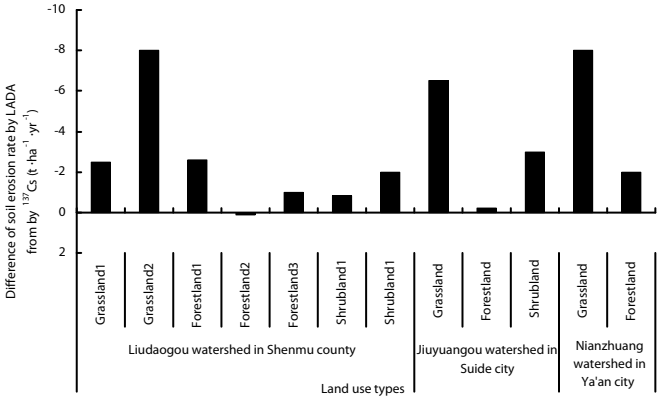


FIGURE 4. Difference in soil erosion rates determined by LADA and FRN techniques.

from the LADA approach is indicative of short-term soil erosion rates over 5–10 yr, while the ^{137}Cs technique estimates long-term average soil erosion over the past 50 yr. Overall, however, the results suggest that the LADA approach is very useful for quickly assessing soil erosion on different hillslope positions and land use types.

To compare the differences between the methods, soil redistribution rates determined by ^{137}Cs technique were subtracted from those obtained by the LADA methodology in the three SAs (Figure 2). For the different land use types, the differences between soil erosion rates determined by the LADA approach from those determined by ^{137}Cs were larger for grasslands (average 6.1 $t \cdot ha^{-1} \cdot yr^{-1}$, respectively) than for forestland (average 6.1 $t \cdot ha^{-1} \cdot yr^{-1}$) and shrubland (average 1.6 $t \cdot ha^{-1} \cdot yr^{-1}$). The LADA approach cannot measure soil lost by sheet erosion, and this may explain the larger difference in grassland than in forestland and shrubland. However, in farmland, the rill erosion rate was significantly higher (average 74.9 $t \cdot ha^{-1} \cdot yr^{-1}$) when measured by the LADA approach compared with using ^{137}Cs (average 42.1 $t \cdot ha^{-1} \cdot yr^{-1}$). Since little or no ^{137}Cs could be detected, it may be inferred that the ^{137}Cs method underestimated rill erosion.

CONCLUSIONS

The LADA approach is very useful for quickly assessing soil erosion on different hillslope positions and land use types, providing a spatial pattern similar to that obtained using the ^{137}Cs technique; soil erosion rates derived from the LADA approach are, nevertheless, lower than those calculated by the ^{137}Cs technique on forested hillslopes,

but higher on cultivated slopes in the Chinese Loess Plateau. Differences found between the methods in erosion rates may be explained by the LADA approach providing an estimation of visible soil loss, while figures obtained with the ^{137}Cs technique include both sheet erosion and inter-rill erosion. Also, the LADA approach yields information about short-term soil erosion rates (5–10 yr), while ^{137}Cs technique provides an estimate of long-term average soil erosion over the past 50 yr.

ACKNOWLEDGEMENTS

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Quantifying Agricultural Land Degradation Processes Related to Soil Carbon and Nitrogen Redistribution in Western China Using Fallout Radionuclide (FRN) and $\delta^{13}C$ Techniques

Y. Li[1,2,*], H.Q. Yu[1], Y.Z. Zhang[1], B. Nyamdavva[1] and Z.D. Zhang[3]

ABSTRACT

Over the last 50 years, soil erosion rates at field scale have been well documented in China, but knowledge of their quantitative impacts on soil quality is very limited, especially on an area-wide basis. The objective of this study was to quantify the agricultural land degradation processes related to soil carbon (C) and nitrogen (N) redistribution in cultivated slope land of the Chinese Loess Plateau. A cultivated sloping land (a slope gradient of 3.1°–16.8°) in Pucheng County on the Loess Plateau was selected and the history of upland degradation processes reflected by changes in water and tillage erosion and crop productivity was reconstructed through the dated chronology of soil accumulation at deposited sites using fallout radionuclides (FRNs) caesium-137 and excess lead-210 (^{137}Cs and $^{210}Pb_{ex}$), and sources of soil organic carbon (SOC) were identified using $\delta^{13}C$ tracer. The results showed that between 1954 and 2008, bulk soil translocation processes arising from intensive tillage activities were mainly responsible for land degradation in the cultivated slope catchment. Further, changes in crop productivity could be determined from records of C input by crop roots derived from the deposited profile. Models were established using FRN integrated with terrain attributes for slope-catchment evaluation of SOC and total nitrogen (TN) stocks covering a timeframe of 50–100 years. These models had a very high accuracy for quantifying changes in SOC and TN stocks in cultivated slope catchments. Using FRN profile dating in combination with natural $\delta^{13}C$ tracer, it was possible to explain the role of water erosion and intensive tillage processes in affecting upland degradation over the past 100 years.

Key words: *soil erosion, soil quality, fallout radionuclides, profile dating, organic carbon and nitrogen stocks.*

INTRODUCTION

Change in soil quality due to soil erosion is a major factor to be considered in relation to food security (Latta and Lal, 1999). Soil erosion adversely impacts agricultural productivity and the environment through its effects on physical, chemical, and biological quality (Li, Poesen and Valentin, 2004a; Nearing, Pruski and O'Neal, 2004; Lal, 2007). These soil quality parameters are key to assessing the impacts of agricultural practices on land degradation in terms of productivity (Li, 2004b; Wallbrink, 2004**)**. Over the last 50 years, soil erosion rates at field scale have been well documented in China, but understanding of their quantitative impacts on soil quality is very limited, especially on an area-wide basis (Li *et al.*, 2001 and 2004b). So far, no local or national map is available on soil quality degradation due to erosion. Such information is important to restore the quality of eroded soils because of the finite and easily degraded soil resources in China (Li and Lindstrom, 2001). There is increasing evidence that agricultural soil erosion perturbs the global carbon (C) cycle, but the changes in C dynamics induced by accelerated soil erosion and deposition in agricultural landscapes are poorly understood, especially in the steep cultivated hillslope landscapes of west China (Wallbrink, 2004; Li, *et al.,* 2007). The combined use of fallout radionuclide (FRNs) and stable isotopic techniques has the potential for developing quantitative relationships between soil redistribution and soil quality among soils and agricultural ecosystems so that cause-effect relationships can be established for introducing precision conservation measures.

Previous studies suggested that topography plays an important role in agricultural fields in shaping the spatial variability of soil quality parameters through soil redistribution processes (Li and Lindstrom, 2001). Water erosion was the primary cause of the overall decline in soil quality on a steep cultivated hillslope, while tillage erosion had a similar contribution to the overall level of soil quality on a terraced hillslope (Poesen, *et al.,* 1996; Li *et al*., 2006). Soil movement by tillage controlled the spatial patterns of organic matter (OM), N and phosphorus (P) on both terraced and steep cultivated hillslopes (Pennock, 2005). Selective removal of finer particles by water erosion caused a linear decrease in clay content of 0.02%/m, and a corresponding increase in silt content of 0.04%/m downslope of the steep cultivated hillslope (Li and Lindstrom, 2001). However, the relationship between soil redistribution and soil quality parameters needs to be up-scaled from a field scale to catchment and regional scales (IAEA, 2008 unpublished).

The objectives of the study reported here were to use fallout ^{137}Cs and $^{210}Pbex$ in combination with $\delta^{13}C$ to quantify land degradation processes: firstly, by understanding the magnitude and mechanisms of soil C and nutrient changes, and secondly, by reconstructing the evolution of soil organic carbon (SOC) sources in a cultivated slope.

1 Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing, 100081, P. R. China
2 College of Life Sciences, Hennan Normal University, Henan, Xinxiang 453000, P. R. China
3 Beijing Normal University, Beijing, 100875, P. R. China
* E-mail address of corresponding author: liyong@caas.cn

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MATERIALS AND METHODS

Study site

The study site was in Pucheng county (35°03′18.45″N, 109°38′25.34″E) of Shaanxi Province, which is in a clay loess area of the Chinese Loess Plateau. A typical sloping land with a slope gradient of 3.1°–16.8° and slope length 54 m at the site was selected for spatial analysis of changes in SOC and TN stocks induced by tillage and water erosion. The site has a westerly monsoon climate; long-term mean temperature is 13.2°C with a range of –16.7 to 42.8°C and precipitation is 540 mm, with 70 percent of annual rainfall occurring between July and September. Soil formed from loess parent materials has a uniform texture (25 percent clay, 70 percent silt, and 5 percent sand). Water and tillage erosion accelerated by poor soil management are responsible for the degradation of soil physical and chemical properties in cultivated slopes at the site. Soil organic C content in the loess soil is between 0.1 and 2.4 percent, but less than 1 percent in most cases. Wheat is the major crop in rotation with corn and sweet potato.

Soil sampling

The spatial patterns of SOC and TN stocks and of ^{137}Cs and $^{210}Pb_{ex}$ inventories were documented from soil cores taken on a grid of 5 m x 5 m from the cultivated slope catchment using an 8.0 cm diameter hand-operated core sampler. Reference sites for determining ^{137}Cs and $^{210}Pbex$ inventories were established on a level and undisturbed graveyard with a 100–yr history at the hilltop, less than 30 m from the study sloping land. Soil cores were taken at depths of 0–45 cm at upper and middle positions and at 0–60 cm at the lower positions to ensure that the core had penetrated to the full depth of the ^{137}Cs and $^{210}Pb_{ex}$ profile. Soil cores were also analysed for bulk densities (g/cm), calculated from the volume of soil cores and oven-dried soil mass. In total, 54 core profiles were collected.

A topographic survey was conducted using a 1-m grid and the coring locations from the slope catchment using a GPS-RTK (Ashtech Z-Xtreme, USA, resolution: 3 mm ± 1 ppm) for development of a digital elevation model (DEM).

Laboratory analyses

Soil samples were air-dried, weighed and divided into two parts, one passing through a 0.15 mm sieve for the measurement of SOC and TN concentrations and the other passing through a 2-mm sieve for measuring ^{137}Cs and $^{210}Pb_{ex}$ activities. Soil samples for measuring $^{210}Pb_{ex}$ were sealed in containers and stored for 28 d to ensure equilibrium between ^{226}Ra and its daughter ^{222}Rn (half-life 3.8 d). The amounts of $^{210}Pb_{ex}$ in the samples were calculated by subtracting ^{226}Ra-supported $^{210}Pb_{ex}$ concentrations from the total $^{210}Pb_{ex}$ concentrations. Measurements of ^{137}Cs and $^{210}Pb_{ex}$ activities were conducted using a hyper-pure coaxial Ge detector (BE5030, Canberra, USA) coupled to a multichannel analyzer. ^{137}Cs activity was detected at 662 keV while total ^{210}Pb concentration was determined at 46.5 keV, and the ^{226}Ra-supported ^{210}Pb was obtained at 609.3 keV using a counting time of over 80 000 seconds. This provided an analytical precision of ±5 percent for ^{137}Cs and ±8 percent for ^{210}Pb. Soil organic C was determined by dry combustion and using an Auto TOC Analyzer (Germany), and measurement of TN used the Kjeldahl digestion method.

Calculations of soil redistribution rates and soil C and TN inventories

At each sampling point, total soil redistribution (TSR) rates due to the combined action of tillage and water erosion were determined for the periods of 1954–2005 and 1857–2007 using ^{137}Cs and $^{210}Pb_{ex}$ inventories following the mass balance model developed by Walling, He and Appleby (1997) and Walling, Collins and Sichingabule (2003).

Soil organic C and TN stocks over the cultivated slope were calculated by multiplying the concentration of SOC and TN (g/kg) by

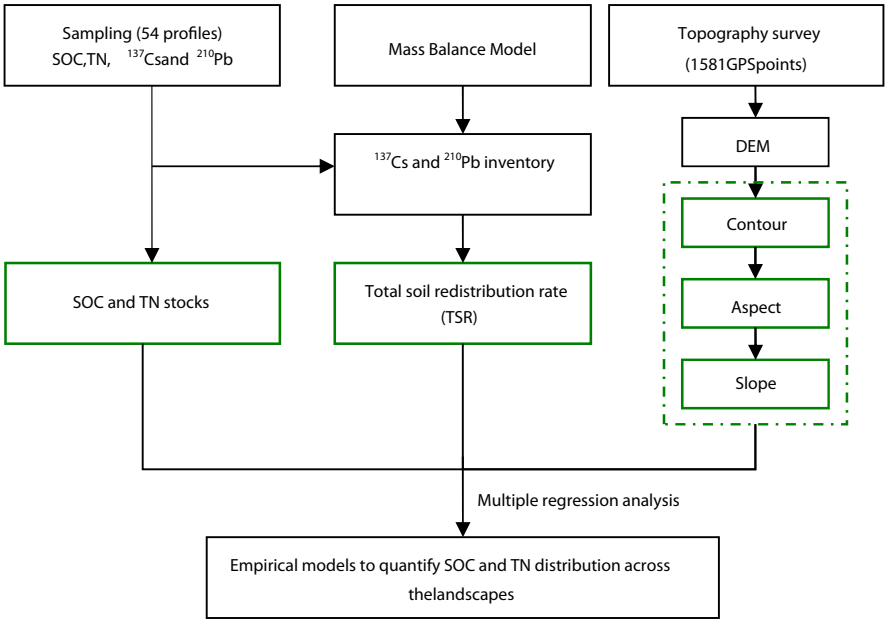


FIGURE 1. Procedure for developing empirical models to quantify SOC and TN distribution across landscapes using multiple regression analysis.

soil bulk density (g/cm) and soil depth (cm) of each sampled soil and expressed in mass per unit area (t·C/ha).

Development of empirical models to quantify soil C and N distribution across the landscapes

The procedure for model development is described in Figure 1. The input parameters include total soil redistribution rates ($t \cdot ha^{-1} \cdot yr^{-1}$) and selected terrain attributes such as relative elevation (E, m), slope gradients (S, in degrees) and slope aspect (A, in degrees). Outputs of the models include SOC and TN stocks (t/ha).

Reconstructing the evolution of land degradation by water and tillage erosion in cultivated slopes by identifying SOC sources at deposited sites

Three methods were adopted for determining the chronology of soil accumulation at deposited sites: using ^{137}Cs and $^{210}Pb_{ex}$ dating; identifying the sources of SOC in the profile of deposited sites using natural $\delta^{13}C$ tracer; and explaining the major factors driving land degradation on the cultivated slope through combined analysis of the first two. These approaches are described below.

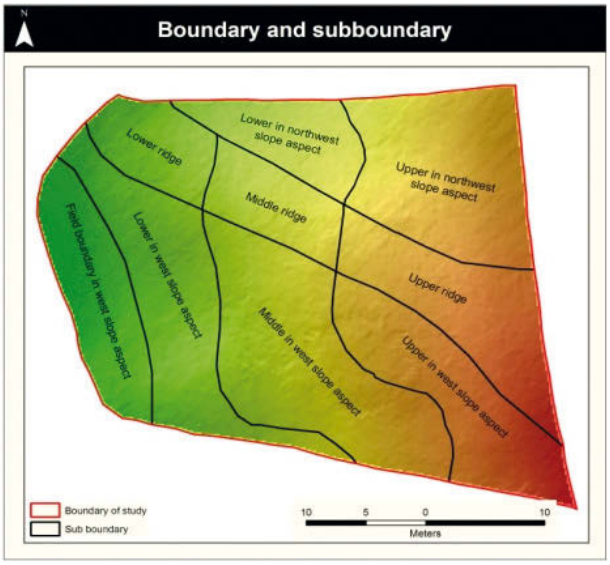


FIGURE 2. Sketch showing boundary and sub-boundary of the study slope catchment derived from the slope aspect.

RESULTS AND DISCUSSION

Spatial patterns of SOC, N, C:N ratios, ^{137}Cs and $^{210}Pb_{ex}$ in slope catchments

In order to analyse the spatial patterns in downslope direction, we divided the slope into three slope locations according to slope gradients: slope gradients ranged 3.1–13.7° for lower slope (slope length 13 m), 8.7–16.8° for middle slope (slope length 21 m), and 4.9–14.0° for upper slope (slope length 20 m). Soil organic C, total N (TN) and C:N ratios displayed similar spatial patterns to ^{137}Cs and $^{210}Pb_{ex}$ inventories in the slope catchment. In the ridge of the slope catchment (Figure 2), both ^{137}Cs and $^{210}Pb_{ex}$ inventories increased in the downslope and changed according to the slope location: lower slope > middle slope > upper slope. Reference values calculated for ^{137}Cs and $^{210}Pb_{ex}$ inventories were 1267 ± 132 Bq/m^2 and 10 675 ± 2599 Bq/m^2, respectively. Compared with the reference site, ^{137}Cs inventory losses were respectively 91.9 percent, 88.8 per cent and 70 percent at the upper, middle and lower slopes over the last 50 years. By contrast, compared with the reference site, $^{210}Pb_{ex}$ losses (which accounted for 50 percent of the total losses of ^{137}Cs inventories), were respectively 48.7 percent, 44.0 percent, and 7.7 percent at the upper, middle and lower slopes over the last 100 years.

Similarly, in the west slope aspect both ^{137}Cs and $^{210}Pb_{ex}$ inventories increased in the direction of the downslope and according to the following slope location: field boundary > lower slope > middle slope > upper slope. Compared with the reference site, ^{137}Cs inventory losses were respectively 89.9 percent, 75.4 percent and 54.8 percent at the upper, middle and lower slopes, while ^{137}Cs inventory gain was 17.6 percent at the field boundary. For $^{210}Pb_{ex}$, losses were respectively 43.8 percent, 38.7 percent, 23.1 percent and 5.2 percent at the upper, middle and lower slopes and at the field boundary. At the northwest slope aspect, ^{137}Cs and $^{210}Pb_{ex}$ inventory losses were respectively 83.2 percent and 54.5 percent.

Like ^{137}Cs and $^{210}Pb_{ex}$, SOC, TN and C:N ratios decreased in the order of slope location in both ridge direction and west slope aspects, but the highest values for SOC and TN were found at the lower field boundary of the slope catchment whereas much higher C:N ratios were found at the lower slope location than at the lower field boundary and other slope locations. For the northwest slope aspect, SOC, TN and C:N ratios were much higher at the lower slope than at the upper slope location. These spatial patterns of SOC, TN and C:N distribution suggest the existence of a mechanism associated with topographical attributes that controls soil redistribution arising from soil use and management practices.

Impacts of topography on distribution of SOC, N and C:N ratios

The influence of topography on SOC and TN was assessed by simple linear regression analysis of the relationship between SOC and TN stocks and topographical attributes (relative elevation, slope degree and slope aspect) (Table 1). The SOC and TN stocks were correlated negatively with both relative elevation ($p < 0.001$) and with slope ($p < 0.01$ and $p < 0.05$, respectively). However, while SOC stocks did not correlate significantly with slope degree, TN stocks did so ($p < 0.05$). These findings indicate that SOC and TN distribution were controlled by the relative elevation and slope aspect of the catchment.

Table 1. Relationships between slope relative elevation (E, m), slope degree (S), slope aspect (A) and SOC (t/ha) and TN (t/ha)

Linear regression	R^2	n	p
$SOC_{stock} = -1.7067E + 29.415$	0.6606	54	<0.001
$SOC_{stock} = -0.0685A + 42.018$	0.1354	54	<0.01
$SOC_{stock} = -0.2627S + 25.596$	0.0278	54	n.s.*
$TN_{stock} = -0.1088E + 2.7229$	0.4119	54	<0.001
$TN_{stock} = -0.0048A + 3.6425$	0.1012	54	<0.05
$TN_{stock} = -0.041S + 2.7364$	0.1036	54	<0.05

*Not significant

Slope catchment models to quantify SOC and TN stocks in cultivated landscapes

Table 2 shows the empirical models established for slope-catchment prediction of SOC and TN stocks in relation to soil redistribution and terrain attributes following the procedures listed in Figure 1.

Comparing the values measured with those estimated by the models from ^{137}Cs and $^{210}Pb_{ex}$, the relative errors for SOC stocks were less than 10 percent for 28 of the 54 observation points whereas those for TN were less than 10 per cent for 27 of 54 observations and 35 of 54 observations, respectively. For the 54 observation points, the average relative errors for SOC and TN were 9.5 percent and 8.3 percent respectively. This suggests that these models have a very high accuracy for quantifying changes in SOC and TN stocks in cultivated slope catchments.

Because the regression coefficients of terrain attributes in the models are relatively stable compared with soil redistribution rates, the models in Table 3 could be used to assess the effectiveness of soil use and management practices on SOC and TN over a time scale of 50–100 yr. For example, over the period 1954–2007, net SOC and TN losses from the slope due to soil erosion were calculated to be 0.36 $t \cdot ha^{-1} \cdot yr^{-1}$ and 0.14 $t \cdot ha^{-1} \cdot yr^{-1}$, respectively, indicating significant decreases in SOC and TN storage. In contrast, for the period 1907–1954, net SOC and TN gains due to soil erosion were respectively 0.204 and 0.027 $t \cdot ha^{-1} \cdot yr^{-1}$, indicative of noticeable increases in SOC and TN storage during this period.

However, much work remains to be done for an area-wide evaluation of agricultural land degradation induced by soil erosion. Future focus will therefore be directed at upscaling these slope catchment models from a sandy loess area to silt and loessial areas of the Chinese Loess Plateau.

Evolution of land degradation reconstructed by identifying SOC sources at deposited sites by application of FRNs and $\delta^{13}C$

Chronology of soil accumulation at deposited sites using ^{137}Cs and $^{210}Pb_{ex}$ dating

Two typical profiles were selected at both eroded and deposited sites of the cultivated slope catchment and ^{137}Cs and $^{210}Pb_{ex}$ were measured in both profiles (Figure 3). ^{137}Cs and $^{210}Pb_{ex}$ dating techniques were used to determine the chronology of soil accumulation

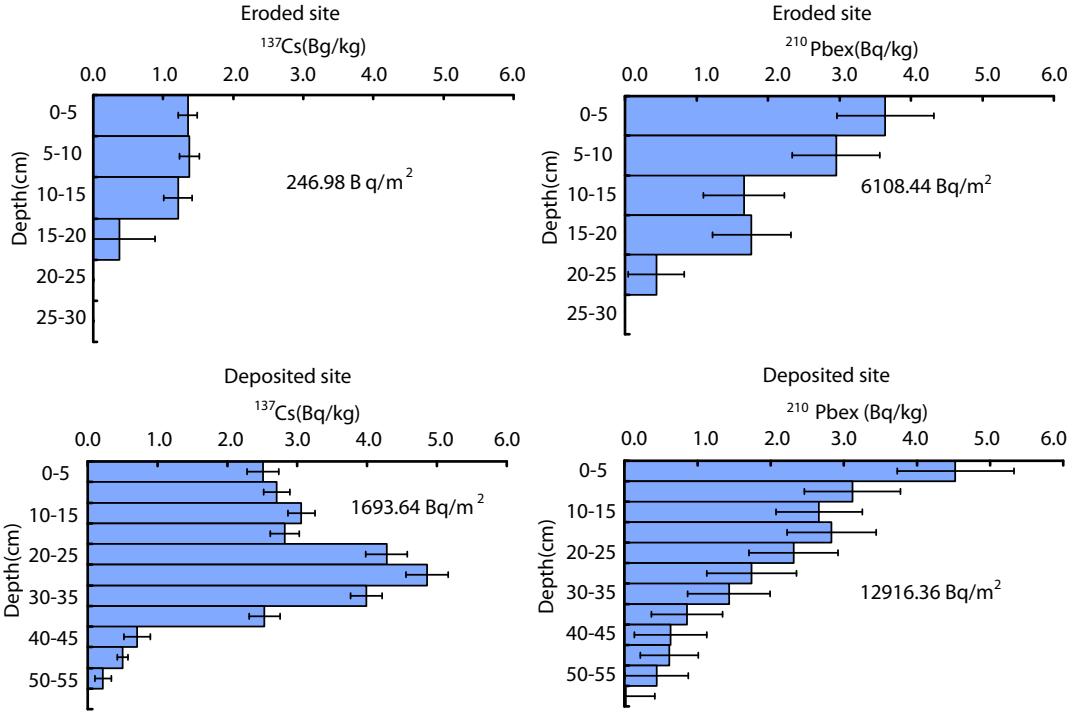


FIGURE 3. ^{137}Cs and $^{210}Pb_{ex}$ profiles at eroded and deposited sites of a cultivated slope catchment.

Table 2. Empirical models for predicting SOC and TN stocks at slope catchment scale using relative elevation (E), slope gradients (S), slope aspect (A) and total soil erosion (TSR)

Period	Multiple regression model	Correlation coefficient (R)	p value	Average % deviation from observed value
1954–2007	SOC_{stock}=44.34–1.49E–0.04A–0.38S+0.006TSR	0.8613	< 0.01	9.4
1907–2007	SOC_{stock}=45.14–1.53E–0.04A–0.39S+0.016TSR	0.8603	< 0.01	9.6
1954–2007	TN_{stock}=3.81–0.065E–0.002A–0.041S+0.002TSR	0.8135	< 0.01	8.6
1907–2007	TN_{stock}=3.75–0.071E–0.002A–0.036S+0.012TSR	0.8351	< 0.01	8.0

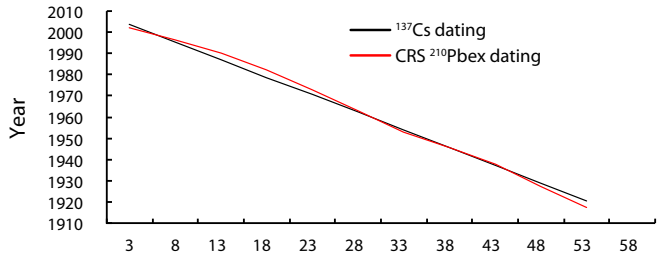


FIGURE 4. Radiometric chronology of soil accumulation profile at deposited site showing agreement between $^{210}Pb_{ex}$ CRS dates and ^{137}Cs dates.

at deposited sites of the slopes, while the $\delta^{13}C$ values of bulk soil and SOC were determined to identify the source of SOC in the profiles.

In the profile from the upland eroded site (Figure 3), ^{137}Cs was distributed uniformly within 0–15 cm of the plough layer whereas $^{210}Pb_{ex}$ decreased with soil depth, although this was also mainly distributed within 0–20 cm. By contrast, a peak of ^{137}Cs activity was found at depths between 25 and 30 cm (Figure 3) in the profile at the deposited site. This can be equated with the maximum atmospheric fallout in 1963. $^{210}Pb_{ex}$ activity decreased with soil depth and was still detected at a depth of 55 cm, i.e. much deeper than that at the eroded site. The temporal patterns of soil accumulation since 1927 at the deposited site of the cultivated slope were reconstructed using ^{137}Cs peak serigraphy in combination with CRS (constant rate of ^{210}Pb supply) $^{210}Pb_{ex}$ Dating Model (Figure 4). The identical chronology determined by these two independent methods suggests that it is possible to date recent soil accumulation with a high degree of accuracy, especially during the past 50 yr.

Profile distribution of SOC sources at deposited site of cultivated slope catchment using natural $\delta^{13}C$ tracer

Based on the data from Figure 5, SOC sources at deposited sites—were calculated using the following formulae:

$$Ce + Cr = Ct \quad (1)$$

$$\delta^{13}Ce \times Ce + \delta^{13}Cr \times Cr = \delta^{13}Ct \times Ct \quad (2)$$

where Ce (g/kg) — eroded soil C from upland; Cr (g/kg) — C input by crop roots; and Ct (g/kg) — total C in the deposited soil profile.

Higher values for Ce than for Cr were observed between 0 and 30 cm., indicating a major contribution to SOC sources by eroded SOC from upland (Figure 6). By contrast, at 30–60 cm depth, values for Ce were lower, indicating that crop inputs provided the major sources of SOC.

Dominant soil redistribution processes controlling upland degradation of slope catchment

To understand more fully the upland degradation processes induced by water erosion and intensive tillage activities, changes in the SOC content from eroded SOC and in inputs of crop roots were estimated by linking the chronology of soil accumulation (Figure 4) and SOC sources in deposited profiles at the lower field boundary of the slope (Figure 6).

It is clear that values for Ce and Cr showed distinct changes between the years 1927 and 1954 and between 1954 and 2008 (Figure 7). Values for Ce during the period 1927 to 1954 were much lower than between 1954 and 2008, ranging from 0.56 to 3.36 g/kg with a mean of 2.03 g/kg·compared with between 2.56 and 8.47 g/kg with a mean of 5.85 g/kg during the period 1954 and 2008, i.e. around a two-fold increase during the latter period.

Recent studies have indicated that over the last 50 yr soil movement from the cultivated slope catchment to the river system is mainly in the form of bulk soil. The present investigations also

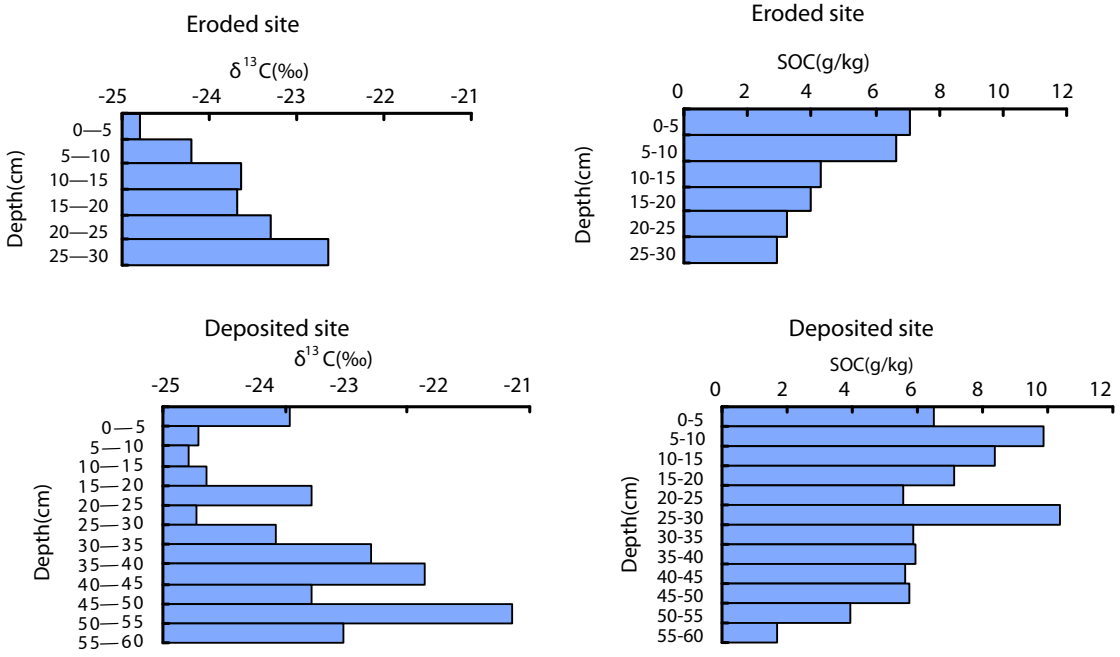


FIGURE 5. Profile distributions of SOC and $\delta^{13}C$ at eroded and deposited sites.

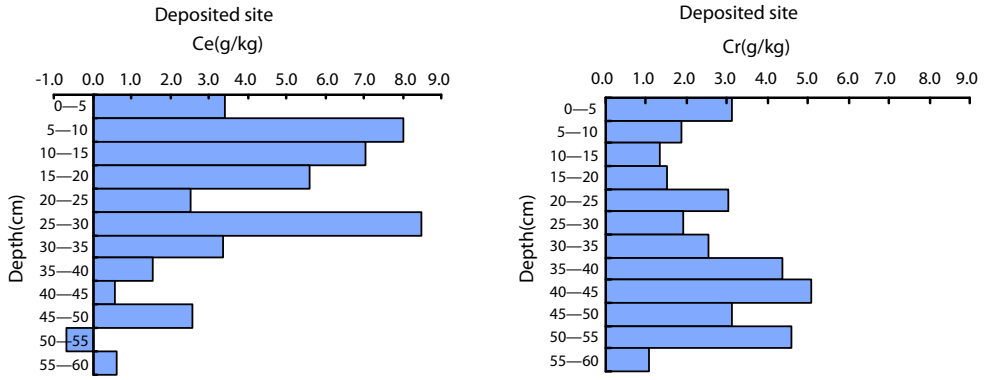


FIGURE 6. Profile distribution of SOC sources identified using $\delta^{13}C$.

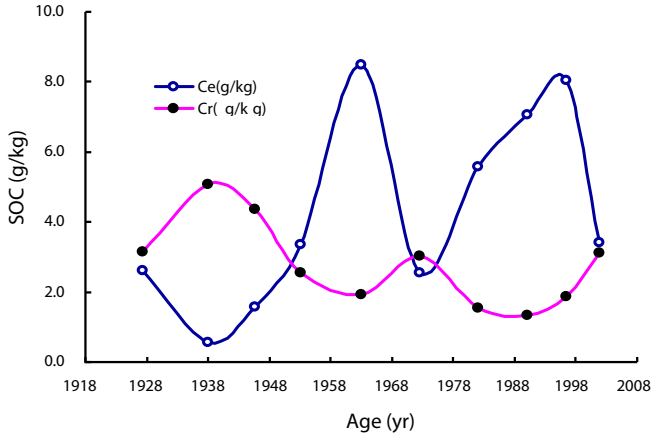


FIGURE 7. Reconstructed changes since 1918 in SOC sources in deposited profile at the lower field boundary of the cultivated slope. Ce — SOC eroded soil C from upland; Cr (g/kg); Cr — C input by crop roots (g/kg).

showed that soil accumulation at the lower field boundary was dominated by tillage erosion processes. It is therefore possible to use the reconstructed changes in SOC sources in the deposited profile (Figure 7) to explain the roles of water erosion and intensive tillage processes in bringing about upland degradation during the period 1927–2008. For example, in the period 1927–1954, the mean value of Ce in the deposited profile (2.03 g/kg) was lower than the values for SOC below the plough layer of 15 cm (these ranged from 2.92 to 3.97 g/kg with a mean of 3.37 g/kg). This suggests that sheet erosion was mainly responsible for land degradation of the cultivated slope catchment between 1927 and1965. By contrast, over the period 1954–2008, the mean value of Ce in the deposited profile (5.85 g/kg) was almost the same as the values for SOC within the plough layer of 0–15 cm (range 4.28–7.11 g/kg with a mean of 6.00 g/kg). This suggests that bulk soil translocation processes arising from intensive tillage were responsible for the land degradation of the cultivated slope catchment between 1954 and 2008.

Changes in upland crop productivity could be determined from the Cr values derived from the deposited profile (Figure 7). In the years before 1954 these were much higher than subsequently. Thus, during the period between 1927 and 1954, Cr values ranged from 2.54 to 5.07 g/kg with a mean of 3.78 g/kg, while for the period between 1954 and 2008, these ranged from 1.32 to 3.11 g/kg with a mean of 2.13 g/kg, i.e. a 44 percent decrease compared with the earlier period. This indicates that significant agricultural land degradation resulted from intensive tillage activities over the last 50 years.

CONCLUSIONS

By using ^{137}Cs and $^{210}Pb_{ex}$ integrated with terrain attributes, models were established for slope-catchment evaluation of SOC and TN stocks covering a time period of 50–100 yr. These models had a very high degree of accuracy for quantifying changes in SOC and TN stocks in a cultivated slope catchment.

By using FRN profile dating in combination with natural $\delta^{13}C$ tracer, the roles of water and intensive tillage erosion in bringing about upland degradation over the past 100 yr could be explained.

Future work will be directed towards validating and up-scaling the models for the entire Loess Plateau and for testing the suitability of combining FRN and $\delta^{13}C$ methodologies for understanding upland degradation induced by soil erosion on an area-wide scale.

ACKNOWLEDGEMENTS

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Determining Sources of Soil Erosion using Compound Specific Isotope Analysis: Application in a Rural Australian Catchment

G.J. Hancock[1,*] and A.T. Revill[2]

SUMMARY

Compound Specific Isotope Analysis (CSIA) was used to assess the ability of the delta carbon-13 ($\delta^{13}C$) signature of fatty acid compounds to discriminate erosion sources in the Logan-Albert catchment, a rural river catchment on the Australian east coast. The study augmented a previous sediment tracing study using fallout radionuclides and major/minor element geochemistry. Soil samples representing important erosion sources within the catchment were collected and the $\delta^{13}C$ values of bulk carbon and various fatty acids determined. It is found that surface soil from forest, pasture and cultivated land uses are well discriminated using CSIA. Furthermore, sub-surface soil sources associated with channel bank erosion and exposed subsoils (gullies and hillslope scalds) occurring specifically in the mid-western Logan catchment could also be discriminated. Sediment samples deposited during a high flow event in January 2008 were also collected and analysed, and the IsoSource mixing model was used to determine erosion sources contributing sediment. The results were compared with results obtained using other sediment tracers. For the lower Logan River, the CSIA tracing results are consistent with fallout radionuclide and element geochemistry tracing, with channel bank erosion being confirmed as the major sediment source. Moreover CSIA has quantified the significant contribution of exposed subsoils originating on hillslopes and drainage lines from the mid-western region of the Logan catchment. In the Albert River catchment about 40 percent of sediment comes from forest land use, although more than half of this may come from sub-surface sources. These results have demonstrated that CSIA has the potential to enhance significantly the ability of sediment tracing studies to determine the extent to which different land uses and erosion processes are contributing eroded soil to rivers, thus testing and validating model predictions and calibration of model parameters.

Key words: *compound specific isotope analysis (CSIA), $\delta^{13}C$, fatty acids, soil erosion, sediment tracing, land use.*

INTRODUCTION

Determining the proportions of eroded soils contributing sediment to rivers and streams using physical and chemical characteristics can be a direct and cost-effective way of directing erosion control actions. The method is particularly powerful when used in conjunction with catchment modelling and in-stream monitoring of suspended sediment loads to determine catchment sediment budgets (Hancock *et al.*, 2007; Rustomji *et al.*, 2008). The soil characteristics, or tracers, can take many forms: soil colour, geochemistry, stable isotope composition and mineralogy have been successfully employed to trace the spatial and/or geomorphological origin of sediment (e.g. Douglas *et al.*, 1995 and 2003; Walling 2005). Fallout radionuclides have also been found to be valuable tracers of surface soil erosion (Olley *et al.*, 1993; Wallbrink *et al.*, 1996 and 1998; Walling and Woodward, 2002; Walling 2005). In particular, anthropogenic caesium-137 (^{137}Cs) and naturally occurring fallout lead-210 (^{210}Pb) label the upper few cm of surface soil globally, providing an indication of whether erosion of surface or sub-surface soils are contributing to river sediment.

While these tracers, either separately or in combination, provide important erosion source information, catchment managers are often called to make soil conservation decisions on the basis of land use. Since different land uses can span geological boundaries and can lead to erosion of both surface and sub-surface soil there is no guarantee that traditional tracers can distinguish soils originating from (for example) forest, pasture or crop cultivation. Recent studies have utilized the stable isotope signature of a range of organic compounds in sediments to determine carbon (C) sources delivered to rivers and near-shore waters (e.g. Prahl *et al.*, 1994; Cook *et al.*, 2004; Seki *et al.*, 2010). Subsequently, the Compound Specific Isotope Analysis (CSIA) technique has been used to determine sediment sources using the delta carbon-13 ($\delta^{13}C$) signature of C compounds bound to the sediment particles as a tracer of their source. Compound Specific Isotope Analysis has successfully distinguished terrestrial and estuarine sediments (Hu *et al.*, 2006) and has been applied recently in New Zealand to distinguish catchment sources of eroded surface soil under different land uses (Gibbs, 2008), and in Britain to distinguish the effect of different crops on downstream stream sediment load (Blake *et al.*, 2012).

Compound specific $\delta^{13}C$ measurements as sediment tracers

The CSIA technique measures the $\delta^{13}C$ isotope signature of specific organic compounds associated with the organic matter bound to the soil/sediment. In this paper we investigate the use of fatty acids (FAs), organic compounds which are produced by plants and soil organisms. Due to their polar nature FAs are easily leached from the plant and can become tightly bound to soil particles (Thurman, 1985). It is proposed that different land uses will provide distinctive CSIA signatures due to the different types of vegetation and com-

1 CSIRO Land and Water, GPO Box 1666 Canberra, ACT 2601, Australia
2 CSIRO Marine and Atmospheric Research, GPO Box 1538, Hobart, Tasmania 7001, Australia
* E-mail address of corresponding author: Gary.Hancock@csiro.au

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munity structure which, on decomposition, make up the organic matter bound to soil particles (Chikaraishi and Naraoka, 2003; Gibbs, 2008). It is well known that various plants (e.g. grasses, shrubs, trees etc.) synthesize ^{13}C differently, leading to different $\delta^{13}C$ values for the vegetation of those plants (e.g. Peterson and Fry, 1987; Lamb *et al*., 2006). On decomposition of this vegetation the $\delta^{13}C$ values of the various C compounds are retained and transferred to the soil. Thus, different plants (e.g. "C3" and "C4" plants) associated with different land uses may allow the identification of erosion sources contributing sediment to rivers and estuaries. Despite continued degradation of FAs over time the isotopic signature is retained (Blessing, Jochmann and Schmidt, 2008).

While CSIA shows promise as a tracing technique of surface soils, it has not yet had widespread application, nor has it been applied to distinguish sub-surface erosion sources. In the Australian context the method has the potential to provide valuable information on erosion in rural areas such as those typified by coastal catchments along the eastern and southern coasts. The technique could be particularly relevant in the coastal catchments of central and northern Queensland where debate is occurring on the major erosion sources contributing sediment and nutrient to the Great Barrier Reef (GBR) lagoon. In particular, the relative contributions of human-induced erosion associated with various agricultural practices (cropping, irrigated and native pasture) are being compared with "natural" fluxes from native forest. While fallout tracers can identify surface soil erosion, they cannot directly determine the contributions of the different land uses, and estimates of erosion sources are often based on un-validated model estimates. Moreover, it is known that in many of these catchments erosion of soil from sub-surface sources dominates sediment fluxes. Fallout tracers have only a limited ability to distinguish these sources; for example both channel bank and gully erosion have little or no ^{137}Cs and excess ^{210}Pb. Neither, in many cases, does soil geochemistry provide discrimination, since soil geochemistry is largely controlled by the geology of the source rocks from which the soils are derived, and erosion processes are not always related to catchment geology. However, C sources of different sub-surface soils may differ, either as result of historical changes reflected by a contrast between B-horizon and A-horizon soils, or by the source of C being delivered to floodplains and channel banks. We therefore suggest that CSIA has the potential to distinguish different sources of sub-surface soils.

This paper describes work designed to assess the ability of the CSIA technique to discriminate eroded soil (and associated C) sources in a rural Australian catchment. We report the fatty acid CSIA characterization and discrimination of soil and sediment samples from the Logan and Albert River catchments. These two adjoining sub-tropical catchments are considered representative of many Australian rural eastern coastal catchments, both in regard to land use and the environmental concerns associated with eutrofication of estuarine waters. The results here may also have application to tropical catchments further north making up the GBR lagoon catchment. The Logan and Albert catchments were the subject of a study using traditional radionuclide and major/minor element tracers (Hancock and Caitcheon, 2010), the results of which provided new information on sediment sources and erosion processes. However these tracers could not discriminate between different land uses within the same geological province, in particular between the proportions of sediment originating from pasture, forest and cultivated regions. The different sub-surface soil sources were also not easily distinguished from each other. This study investigates the ability of CSIA to differentiate surface soils associated with specific land uses, particularly native forest and pasture. We also assess its ability to distinguish sub-surface soils, such as channel bank, and hillslope scalds and incised drainage lines. The proportioning of sources using CSIA is compared with previously published results using other tracers.

Study site

The catchment of the Logan and Albert Rivers comprises an area of 3 860 km^2 and is mainly rural. Three main water courses deliver water to the estuary: the Logan River with its tributary Teviot Brook, and the Albert River (Figure 1). Water flow is from the uplands in the south, to the estuary in the north. The dominant land uses are grazing (cattle), native forest, cropping and rural residential. The catchment headwaters are forested conservation zones (Lamington and Mt. Barney National Parks) and are in relatively pristine condition, but the catchment has been extensively cleared elsewhere for cropping, grazing and dairying. Grazing occurs in the middle and upper parts of the catchment and includes improved pasture and partially cleared grassed woodlands. Cultivation (cropping) occurs mainly along the river flats and floodplains of the major tributaries.

The Logan estuary is situated in the south of Moreton Bay and is characterized by high nutrient levels and turbidity. The ecological health of streams of the Logan catchment and the estuary is considered poor. The health of the Albert River is considered to be good, but its estuary is connected to the Logan estuary and it has a similarly poor ecological health ranking.

METHODS

Sample collection

Catchment soil samples and sediment samples for CSIA were collected from January 18–21, 2010. The sampling process was structured so that samples were collected representing the various potential sources of eroding soil contributing sediment transported from the catchment. Surface soil samples were obtained by collecting the top ~10 mm of soils from hillslopes and cultivated paddocks. Only the "mobile" or "potentially mobile" component of the surface soil was collected. These soils are defined as surface soil that had clearly been transported down-slope after recent rain, or was loose enough such that transport by overland flow was considered likely to occur during the next major rainfall event.

Soil samples representing distinct erosion sources were obtained by combining between three and five samples collected from hillslopes and paddocks with similar characteristics within a few km of each other. All combined samples represented the same major land use, and showed similar slope and vegetation cover. At each site 30–50 individual "grab" samples were taken over an area of at least 10 000 m^2. These were combined into a single sample. Thus each representative surface soil sample contained 100–200 individual grab samples collected along 10–20 km transects. Soil sampling locations are shown in Figure 1.

The surface soil samples were collected across the main tributaries of the Logan-Albert catchment, and included soils from the three main land uses:

- National Park and State Forest hillslopes.
- Permanent (uncultivated) pasture grazed by cattle for beef and dairy.
- Cultivated soils currently used for a range of crops planted and harvested throughout the year on a rotational basis (e.g. corn, sorghum, lucerne and various vegetables).

As noted above, pasture generally comprised gently sloping grassed hillslopes, either completely or partly cleared of woody vegetation. Protected forest hillslopes typically were steeper with extensive canopy cover, with little or no grass. Cultivated soils occur on or

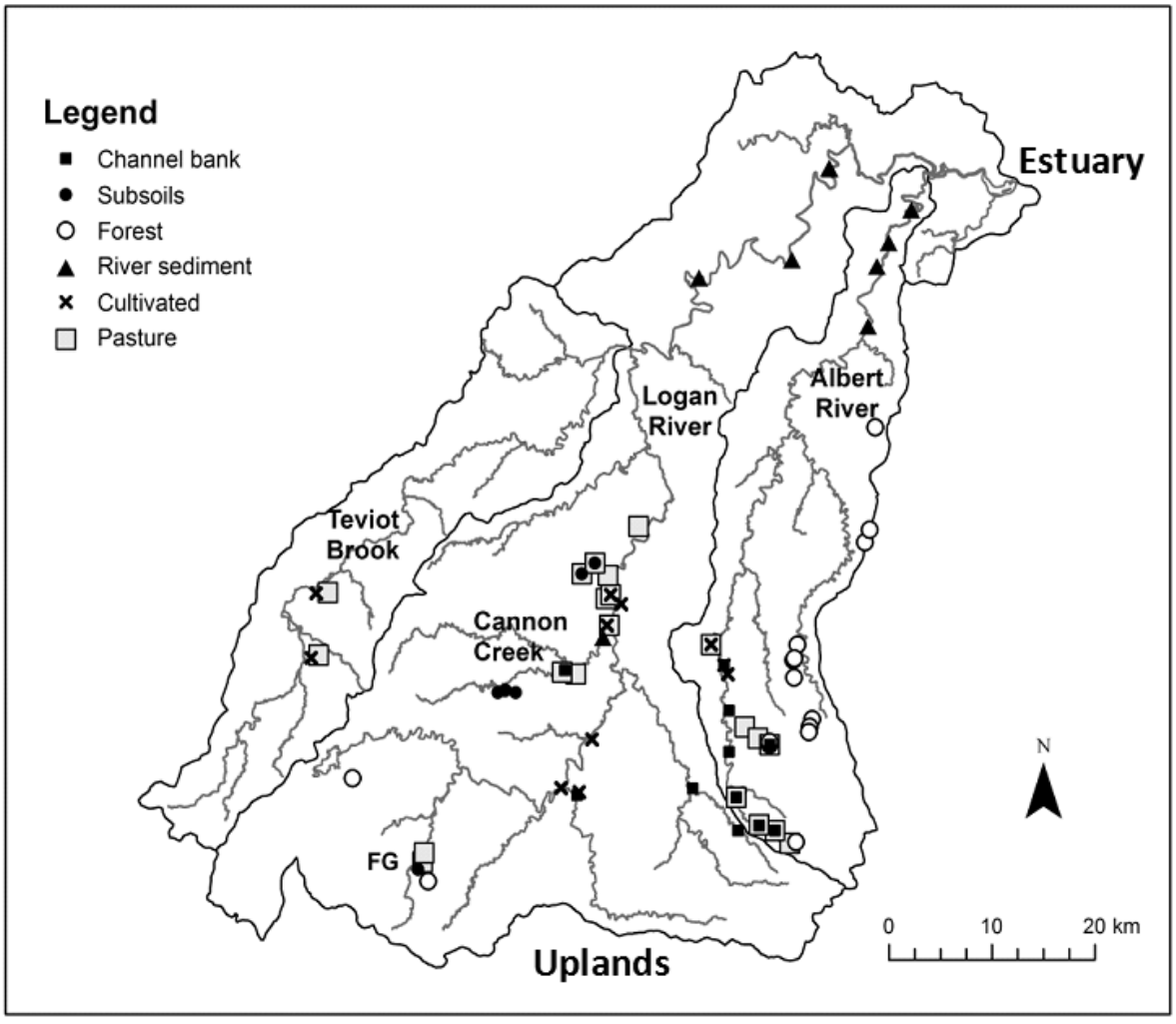


FIGURE 1. Map of the Logan-Albert catchment and locations of soil and sediment samples. Catchment boundaries are given by the dark solid line. Note that the sediment samples (closed triangles) for the lower Logan and Albert rivers were combined into a single sample for CSIA analysis. The forest gully sample is labelled as FG.

near river floodplains. Similarly, subsoil samples were collected from gully and scald sites that were clearly identifiable as being actively eroding. Again, only the mobile fraction was collected. Channel bank samples were collected from three sites along an approximately 5 km river reach and combined for analysis. Similar to hillslope sampling, multiple grab samples were collected at each site. Each eroding channel bank profile was sampled by scraping a thin layer (< 10 mm) of soil from the exposed vertical bank face. Because the whole vertical face was sampled a small component of surface soil or overbank sediment deposits from the top of the bank was included in the sample.

In addition to soil samples collected in 2010, a selection of river sediment samples collected in the January 2008 field trip immediately after the flood event were also analysed by CSIA (Figure 1, closed triangles). These samples represent deposits of fine sediment associated with the January 2008 flood event and were stored frozen. For the lower Logan and Albert rivers, sediment from four sample sites were combined into a single sample representative of the lower reaches of the River, and analysed by CSIA. A single sample from Cannon Creek, in the mid Logan catchment was also analysed.

Sample preparation and analysis

Representative soil samples were obtained by sub-sampling the bulk samples to obtain a single sample of ~100 g dry weight. Stored sediment samples were thawed and sieved in the same way. In the laboratory samples were extracted with n-hexane. The lipids were recovered and made up to a known volume with dichloromethane. Fatty acids were recovered from the aqueous fraction of the saponified mixture after the addition of 1 mL of HCl and converted to their methyl esters prior to analysis by treatment with acidified methanol (Christie, 1982).

Gas chromatography was performed using a Varian 3 800 equipped with a cross-linked 5 percent phenyl-methyl silicone (HP5) fused-silica capillary column using hydrogen as the carrier gas. Fatty acid fractions were analysed using a flame ionization detector, with the C_{23} fatty acid methyl ester as the injection standard. Compound specific isotope analysis was performed using a Thermo GC coupled to a Finnigan Mat Delta S isotope ratio mass spectrometer operating in continuous flow mode with helium as the carrier gas. Stable isotope values were corrected for the C added during methylation; the correction amounted to less than 1‰. Analytical precision is estimated at better than 0.5‰.

RESULTS AND DISCUSSION

Characterizing soil sources

Soil samples were grouped into five erosion sources: surface soils from hillslopes under the two major land uses (forest and pasture); the two major sub-surface erosion sources (in-stream channel banks and hillslope scalds) delivering soil derived from beneath the surficial soil layer (the A-horizon, or the upper ~10–15 cm). These two sub-surface soil sources are referred to separately as "channel bank" and "subsoils" the latter relating to hillslope scalds and incised drainage lines clearly seen in the mid-western region of the Logan catchment. The fifth erosion source is cultivated paddocks, which, due to the tilling process will contain both surface and sub-surface soils.

Mean concentrations for each fatty acid (FA, units of µg/g) and bulk C (units of % dry weight) for the five soil sources are shown in Figure 2. The FA concentrations range from about 10 µg/g to less than 0.1 µg/g with the highest concentrations shown by the 16 and 18 C-chain derivatives (C16:1w7, C16:0, C18:1w, C18:0). The highest soil source FA concentrations are shown by forest soils and the lowest by hillslope subsoils, the latter being derived primarily from B-horizon soils. This trend closely matches the bulk C concentrations, with forest soils having an average 7.7 percent C (dry weight), and subsoils 0.6 percent C. The FA concentration trend closely matches the bulk C trend for all soil sources.

The $\delta^{13}C$ FA data are summarized in Table 1, which gives mean $\delta^{13}C$ values for all FAs and bulk C for each soil source. Also shown is the standard error (±1 sigma) on the mean. Not all soils yielded meaningful $\delta^{13}C$ values for all the FAs, especially the higher chain FAs derived from soils with low C content. Overall, there is good separation between the FA $\delta^{13}C$ values for pasture and forest soils, with pasture generally showing the highest $\delta^{13}C$ (mean value range –13 to –26‰) and the forest soil showing the lowest (mean value range –22 to –36‰). These trends are also shown by the bulk C $\delta^{13}C$ measurements, with mean bulk C $\delta^{13}C$ values for pasture soils (–15.3‰) and forest soils (–24.4‰) showing the greatest difference. These results are consistent with previous work on bulk soil C, where bulk $\delta^{13}C$ values for warm-climate grasses (C4 plants) were found to be less negative and well separated from those of shrubs and trees (C3 plants) (Fry and Sherr, 1984; Peterson and Fry, 1987). The FA and bulk $\delta^{13}C$ values for the other source soils lie between the pasture and forest values.

The CSIA discrimination between soil sources was quantified using the statistical t-test to compare the significance of differences between pairs of soil sources for each fatty acid. The results of the test are expressed as the parameter T_{ab}, given by the difference between the FA mean $\delta^{13}C$ values of source soils *a* and *b*, divided by the standard error of that difference; i.e.

$$T_{ab} = \frac{\left|\overline{X}_a - \overline{X}_b\right|}{\sqrt{(\sigma_a^2 + \sigma_b^2)}} \quad (1)$$

where $\overline{X}_a$ and $\overline{X}_b$ represent the mean $\delta^{13}C$ values of each FA and σ_a, σ_b represent the standard errors on the mean.

T_{ab} values for the 10 soil source pairs are shown in Table 2. Some fatty acids (C20:0 to C30:0) are not included because subsoil data are missing. Values of T_{ab} can range from 0 (no discrimination) to >5 (excellent) and soil sources with T_{ab} values of 5 and above (equivalent to a statistical t-test with $p < 0.01$) were selected for the mixing model. These values are summarized in Table 2. Excellent pasture:forest discrimination is seen and both pasture and forest are well discriminated from the other sources. The two subsoil sources (channel bank and subsoils) and cultivated soils show a lesser degree

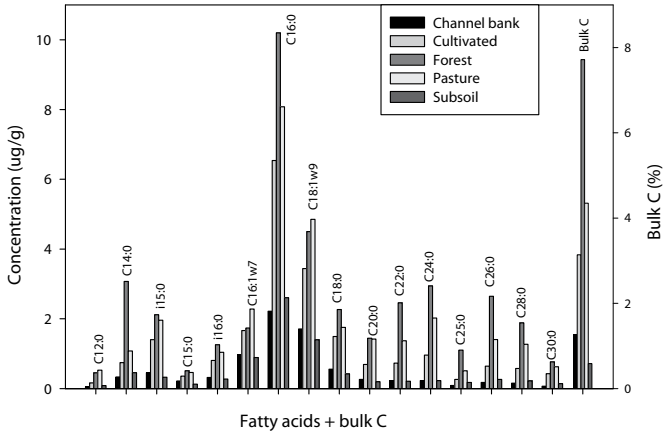


FIGURE 2. Fatty acid concentrations for the five soil sources. Also shown is % bulk C (dry weight). Note separate y-axis for % bulk C.

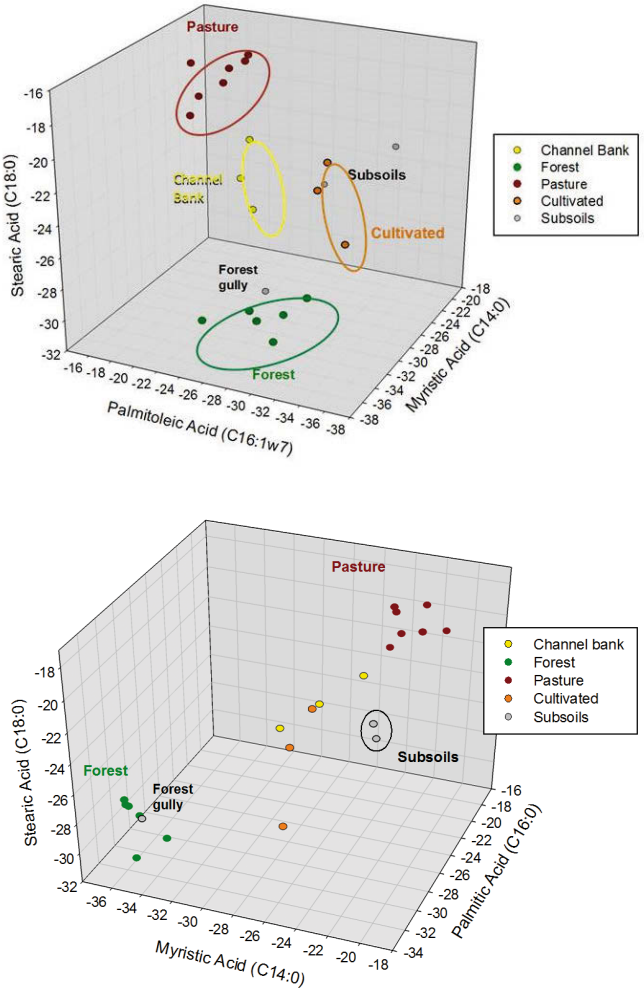


FIGURE 3. A (Top): 3-D plot of soil sources and three FAs showing the separation between sources. B (Bottom): The same plot rotated to the left showing the separation of the two west-Logan subsoil sources (circled). The third subsoil sample, the forest gully sample from Mt Barney NP, plots with the forest samples.

TABLE 1. Mean % C and $\delta^{13}C$ (‰) for FAs and bulk C for each soil source. Note the forest gully sample is excluded from the subsoil mean value. Standard error (SE) is given (±1σ), with the number of measurements given in brackets. Note that the number of measurements varies for each source due to the low concentrations of some FAs not allowing a meaningful measure of $\delta^{13}C$.

	Channel Bank		Cultivated		Forest		Pasture		Subsoils	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
C (%)	1.26	0.24 (3)	3.14	0.34 (3)	7.72	1.23 (6)	4.35	0.49 (7)	0.59	0.10 (2)
Bulk $d^{13}C$	–21.10	0.33(3)	–18.85	0.69 (3)	–24.42	0.79 (6)	–15.30	0.56 (7)	–20.64	0.70 (2)
C12:0	–22.02	1.26 (3)	–22.59	0.49 (4)	–31.44	1.52 (6)	–21.10	0.54 (7)	–20.66	0.72 (2)
C14:0	–26.86	1.21 (3)	–26.24	0.23 (4)	–35.25	0.51 (6)	–22.46	0.43 (7)	–22.51	0.47 (2)
i15:0	–20.64	1.79 (3)	–19.87	0.57 (4)	–26.34	0.47 (6)	–15.40	0.44 (7)	–19.60	0.06 (2)
a15:0	–18.71	1.09 (3)	–20.59	0.90 (4)	–22.10	0.77 (6)	–13.20	0.49 (7)	–19.28	0.49 (2)
C15:0	–24.01	2.32 (3)	–22.03	0.69 (4)	–32.82	1.72 (6)	–19.86	1.46 (7)	nm	
i16:0	–24.92	1.90 (3)	–20.87	0.45 (4)	–30.16	0.71 (6)	–17.64	0.25 (7)	–19.71	1.00 (2)
C16:1w7	–23.23	0.83 (3)	–30.05	0.62 (4)	–29.33	1.09 (6)	–17.96	0.85 (7)	–30.64	2.80 (2)
C16:0	–23.76	0.82 (3)	–29.01	1.30 (4)	–31.78	0.46 (6)	–20.70	0.78 (7)	–26.05	1.56 (2)
18 comb	–23.78	0.79 (3)	–24.19	1.23 (4)	–26.89	0.13 (6)	–18.12	0.56 (7)	–22.96	0.95 (2)
C18:0	–23.41	0.80 (3)	–23.95	1.10 (4)	–28.47	0.49 (6)	–19.11	0.55 (7)	–23.24	1.42 (2)
C20:0	–23.77	3.40 (2)	–23.97	1.01 (4)	–30.73	0.94 (6)	–20.79	0.36 (7)	–18.54	(1)
C22:0	–29.22	1.99 (2)	–27.17	0.73 (4)	–32.92	0.28 (6)	–23.46	0.55 (6)	nm	
C24:0	–31.65	(1)	–26.93	1.24 (4)	–32.29	0.40 (6)	–23.05	0.66 (6)	nm	

nm — not able to measure

TABLE 2. Discrimination between soil sources by fatty acid CSIA and bulk $\delta^{13}C$ as measured by T_{ab} (see text for details). Values of T_{ab} greater than 5 are shaded. Fatty acids with missing subsoil data (C20:0 to C30:0) have not been included. The FAs selected for mixing model analysis are also indicated by shading of the column headings.

Soil source pairs	Bulk $\delta^{13}C$	C12:0	C14:0	i15:0	a15:0	i16:0	C16:1w7	C16:0	18 comb	C18:0
Pasture:Forest	9.4	6.4	19.1	17.0	9.8	16.6	8.2	12.2	15.2	12.6
Pasture:Cultivated	4.0	2.0	7.7	6.2	7.2	6.3	11.5	5.5	4.5	3.9
Pasture:Channel Bank	8.9	0.7	3.4	2.8	4.6	3.8	4.5	2.7	5.8	4.4
Pasture:Subsoils	5.9	0.5	0.1	9.5	8.7	2.0	4.3	3.1	4.4	2.7
Forest:Cultivated	5.3	5.5	16.1	8.7	1.3	11.0	0.6	2.0	2.2	3.8
Forest:Channel Bank	3.9	4.8	6.4	3.1	2.5	2.6	4.5	8.5	3.9	5.4
Forest:Subsoils	3.6	6.4	18.3	14.1	3.1	8.5	0.4	3.5	4.1	3.5
Channel Bank:Cultivated	2.9	0.4	0.5	0.4	1.3	2.1	6.6	3.4	0.3	0.4
Channel Bank:Subsoils	0.6	0.9	3.4	0.6	0.5	2.4	2.5	1.3	0.7	0.1
Cultivated:Subsoils	1.8	2.2	7.1	0.5	1.3	1.1	0.2	1.5	0.8	0.4

of discrimination, although at least one FA shows good discrimination for each source pair. Based on this analysis the following FAs were selected to provide the best overall discrimination for all soil sources: myristic acid (C14:0), palmitoleic acid (C16:1w7), branched-chain $isoC_{15:0}$ (i15:0), and stearic acid (C18:0). Palmitic acid (C16:0) was substituted for (C16:1w7) for some IsoSource runs. Following the suggestion of Gibbs (personal communication), the fifth input term (tracer) used in IsoSource was $\delta^{13}C$ of the bulk (whole) sample as it makes up the largest mass component of the soil (1 000-fold greater than the FA signatures).

The discrimination of sources by isotopic composition of three members of the FA suite can be assessed visually using a 3-D plot (Figure 3). Good separation can be seen between the three land uses (pasture, forest and cultivation), as well as river channel banks. Rotation of the plot and substituting palmitic acid (C16:0) for palmitoleic acid (C16:1w7) (Figure 3B) allows separation of the two subsoil samples from the western mid-Logan catchment. It is interesting to note that the subsoil sample collected from a gullied drainage line in National Park forest plots close to surface soil samples from forested areas.

Determining sediment sources

Mixing models are primarily used to apportion sources to sediment comprising a mixture of soils. As suggested by Gibbs (2008) we have used the isotopic mixing model IsoSource (Phillips and Gregg, 2003) to estimate soil sources for sediment samples collected at various locations in the river network. The model calculates the feasible solutions within a given tolerance (e.g. ± 1‰) of the tracer concentrations of the target mixture. Results are displayed as a frequency

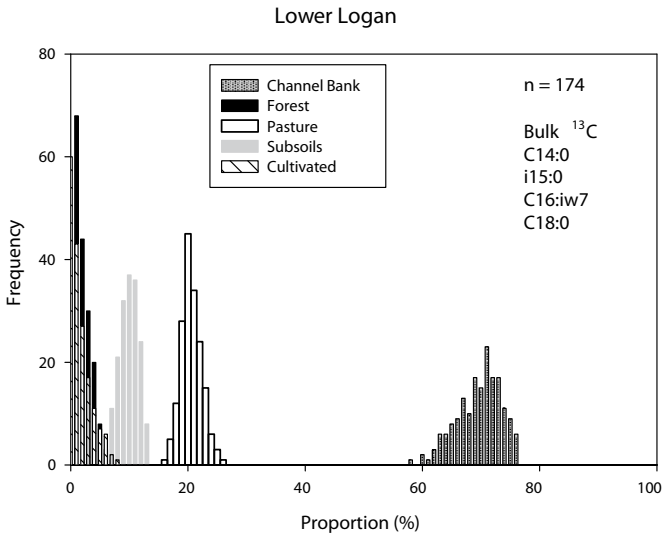


FIGURE 4. IsoSource frequency plot for the proportions of soil sources contributing to sediment from lower Logan River. The five tracers used are listed; the number of feasible solutions, n, is 174.

distribution of all feasible solutions. Whereas each feasible solution is equally possible, the most frequently occurring solution (statistical mean) provides a best estimate and the range of feasible solutions represents the level of uncertainty.

Because the CSIA method traces C rather than the soil itself, the results given by the mixing model need to be adjusted to account for the different amounts of each FA in each of the soil sources. This is done by using the % C content of each source, as described by Gibbs (2008), i.e.

$$\%Soilsource_n = \frac{(P_n / C_n)}{\sum_n (P_n / C_n)} \times 100 \qquad (2)$$

where P_n (%) is IsoSource mean solution for source soil *n;* and C_n is % C of the soil.

An example of the output of soil source apportioning by IsoSource is shown in Figure 4. Five soil sources are considered. The target sediment mixture is a combined sediment sample collected from the lower Logan River in January 2008, representing Logan River sediment downstream from its junction with Teviot Brook to the beginning of the Logan estuary, a distance of ~40 km (Figure 1). Four sediment samples collected along this reach were combined into a single sample and analysed by CSIA. The frequency distribution plot in Figure 4 indicates that the FA and C contents of lower Logan sediment are sourced mainly from channel bank soil (68 ± 4 percent), with lesser amounts from pasture soil (20 ± 2 percent) and subsoils (9 ± 2 percent). Only minor amounts of cultivated and forest soil are indicated, with no valid solutions being seen above 8 percent for either, and most of the solutions being less than 3 percent. All soil sources are well constrained.

Table 3 summarizes the IsoSource mean proportions and uncertainties for each soil source contributing C to the lower Logan sediment. Also shown are the proportions of each soil source after application of Equation 2 to correct for the percent C content of each source. The relatively high C content of the pasture soil and the relatively low content of subsoils has seen their proportions decreased and increased respectively (pasture = 6 ± 1 percent; subsoils 20 ± 4 percent). The channel bank proportion is almost unchanged.

TABLE 3. Apportionment of FA (C) sources as determined from $\delta^{13}C$ values using IsoSource (column 2). Soil sources in column 3 are then determined using bulk C values and Equation 2

	Bulk C (%)	Nominal C sources (%)	Soil sources (%)
Lower Logan			
Channel bank	1.26	68 ± 4	72 ± 4
Cultivated	3.14	2 ± 2	1 ± 1
Forest	7.72	1 ± 1	< 1
Pasture	4.35	20 ± 2	6 ± 2
Subsoils	0.59	9 ± 2	20 ± 4
Cannon Creek			
Channel bank	1.26	22 ± 12	17 ± 10
Forest	7.72	11 ± 5	1 ± 1
Pasture	4.35	20 ± 6	5 ± 1
Subsoils	0.59	47 ± 3	77 ± 6
Lower Albert			
Channel bank	1.26	14 ± 2	48 ± 8
Cultivated	3.14	7 ± 2	9 ± 3
Forest	7.72	78 ± 1	42 ± 1
Pasture	4.35	1 ± 1	< 2

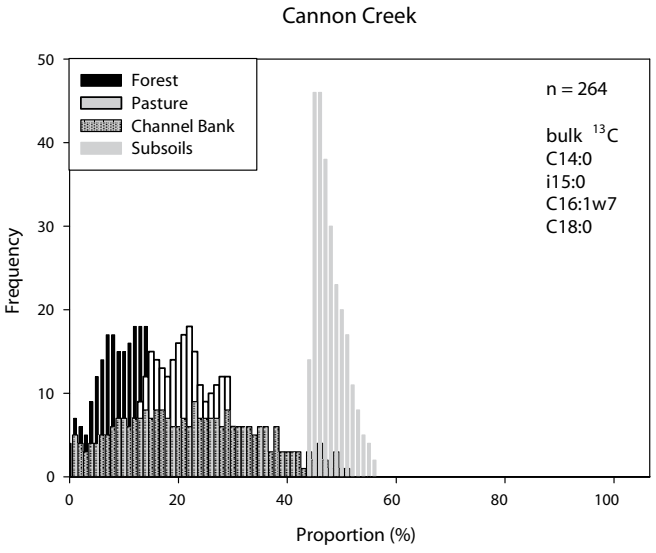


FIGURE 5. IsoSource frequency plot for the proportions of soil sources contributing to sediment to Cannon Creek. Only four soil sources are considered (cultivated soils are excluded); n = 264.

It is interesting to consider the role of the mid-western region of the mid-Logan catchment in delivering subsoils to the lower Logan. Geochemical tracing by Hancock and Caitcheon (2010) indicated that a significant proportion (up to 25 percent) of sediment delivered to the Logan River in the middle catchment region is eroded from soils associated with the Marburg geological formation. Surface tracers indicate that subsoil erosion is significant in this region. These eroded subsoils are almost certainly coming from creeks draining the mid-western region of the Logan catchment, all of which drain soils from the Marburg geological province. Figure 5 shows the IsoSource output for 2008 flood sediment collected from one of these

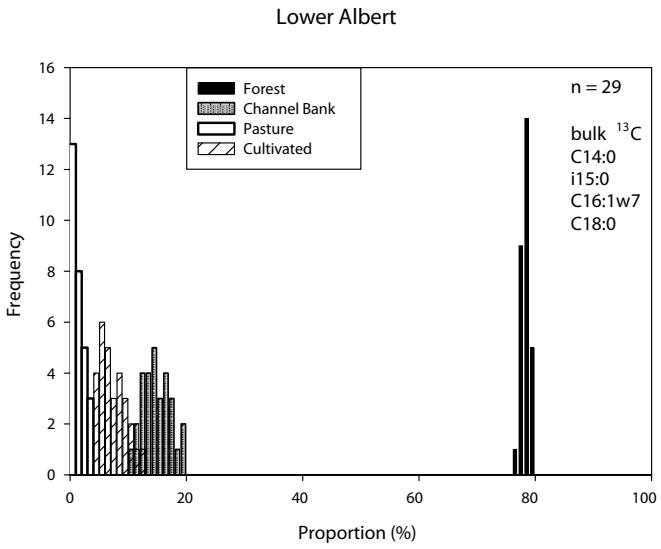


FIGURE 6. IsoSource frequency plot for the proportions of soil sources contributing to sediment from lower Albert River. Only four soil sources are considered (subsoils are excluded); (n = 29).

creeks, Cannon Creek. Crop areas are minimal in this catchment and cultivated soils were not considered as a source term.

While the resolution of minor sources is poor with the reliability of the minor source predictions being reflected by their uncertainties, IsoSource predicts a high likelihood of major input from hillslope subsoil C (range 42 to 62 percent). This is despite the low C and FA content of this source. When corrected for soil C content the model predicts that 77 ± 6 percent of Cannon Creek sediment is sourced from eroding subsoils (Table 3).

Table 4 compares the results of all tracer techniques (surface tracers, geochemistry and CSIA) for the lower Logan and Cannon C sediment samples. Fallout radionuclides are assumed to have been mostly derived from surface forest and pasture soils since they are the dominant land uses on an areal basis. The remaining sediment is assumed to come from the sub-surface sources of channel bank and subsoils (gullies) as indicated by catchment modelling results (Caitcheon *et al*., 2001). Cultivated soils could not be distinguished by fallout tracers. The results of all three tracer techniques are consistent, with the CSIA technique confirming the dominant role of channel banks as a sediment source to the lower Logan (50–70 percent) and the relatively minor surface soil inputs from forest and pasture. Cultivated soils are also a minor source. The CSIA results corroborate the indication given by fallout tracers that a smaller but significant input (20–30 percent) comes from hillslope subsoils in the western creeks catchments.

Another example of soil source estimation by CSIA is illustrated by the IsoSource results for the lower Albert River. In this analysis only four soil sources are considered due to the fact that hillslope subsoils were not sampled for this catchment. Figure 6 shows the IsoSource frequency graph of valid solutions. The low number of solutions (n = 29) indicates high reliability. It is concluded that forest soils dominate C inputs to the lower Albert (78 ± 1 percent). After a soil C content correction is applied soils sourced from the forest regions are still seen to be major (42 ± 1 percent, Table 3) with channel bank being identified as the other potentially significant source. A minor component of cultivated soil is indicated.

When compared with other tracer results (Table 4) the CSIA analysis confirmed channel bank as the major erosion source (~50 percent). Importantly though, CSIA in conjunction with fallout tracers indicated that not all soil from the forested region is surface soil, since the fallout radionuclide results show that no more than 20 percent can be sourced by sheet erosion of surface soils, compared with more than 40 percent predicted by CSIA. Fallout tracers indicate that subsoil sources are contributing in the Albert catchment (30 ± 10 percent, Hancock and Caitcheon, 2010). This information in combination with the observation that the single forest subsoil (gully) noted above matched the forest surface soil signal suggests that the high forest soil component estimated by CSIA is due in part to sub-surface

TABLE 4. Comparison of the results of sediment source tracing in the Logan and Albert River catchments using CSIA, fallout radionuclides and element geochemistry. Values given are proportions (%) of soil sources. Note fallout radionuclides are assumed to have been mostly derived from surface forest and pasture soils.

	Forest	Pasture	Cultivated	Channel Bank	Subsoils
Lower Logan					
Geochemistry	Lower Logan sediment contains a mixture of eastern catchment soils from the upper and mid-catchments (Lamington soils, 70%) and mid-western catchment soils (Marburg soils, 15–25%)				
CSIA	< 1	6 ± 1	<2	72 ± 4	20 ± 4
Fallout tracers	10 ± 5*		nc	50 ± 10	40 ± 10
Cannon Ck					
Geochemistry	Creek sediment is at least 90% mid-western catchment soils (Marburg)				
CSIA	1 ± 1	5 ± 1	nc	17 ± 10	77 ± 6
Fallout tracers	10 ± 5*		nc	90 ± 5	
Lower Albert					
Geochemistry	Lower Albert sediment is made up of at least 90% Lamington soils sourced from the forested part (the southern half) of the catchment.				
CSIA	42 ± 1	< 2	9 ± 3	48 ± 8	nc
Fallout tracers	15 ± 5*		nc	55 ± 10	30 ± 10

nc — source not considered in the model

*Note the surface soil proportion predicted by surface tracers is assumed to come from the combined sum of forest and pasture land uses

soil sources within the forest. The erosion processes responsible are likely to include hillslope slumping and deep rilling.

CONCLUSIONS

In this study the $\delta^{13}C$ values of at least eight FAs gave sufficient discrimination to distinguish surface soil erosion from pasture and forest land uses. Bulk $\delta^{13}C$ also provided good discrimination. Although surface soil erosion was found to be a minor contributor to sediment in the Logan-Albert catchment, it is thought to be significant in tropical regions, contributing more than 50 percent of sediment to some northern rivers (e.g. Bartley, Olley and Henderson, 2004). Consequently there has been much debate whether grazed pastures or conservation forests are the source of this surface soil. The CSIA technique has the potential to enhance significantly the ability of sediment tracing studies to investigate this important issue.

Other sub-surface soil sources were also distinguished, although to a lesser extent. Nevertheless, for sediment transported in the Logan-Albert River system the CSIA technique was able to provide realistic estimates of the proportions of sub-surface soil sources such as channel banks and hillslope B-horizon soils, a result surface tracers and elemental geochemistry often fail to achieve. In combination, the three tracing techniques – surface tracers, element geochemistry and CSIA – offer the opportunity to discriminate five potentially important erosion sources, providing spatial, land-use and erosion process source information.

While cultivated soil in the Logan catchment could be discriminated from other sources, the signature associated with specific crops was not investigated. However, we suggest that where a single crop such as sugar cane has been harvested on a long-term (decadal) time scale, discrimination between soils eroded from cane fields, pasture and forest soils may prove viable. Thus CSIA may be an important technique to identify soil sources contributing sediment along the tropical and sub-tropical east Australian coast to near-shore regions and the Great Barrier Reef Lagoon.

Finally, since CSIA traces organic C attached to sediment the technique has the potential to provide improved estimates of the sustainability of surface (A-horizon) soils, an important issue in some agricultural regions (Bui, Hancock and Wilkinson, 2011). Since organic C is essential for a productive A-horizon, rapidly depleting surface soil stocks are likely to result directly in a loss of agricultural productivity, especially on hillslopes where the A-horizon is thin.

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Tracing Crop-Specific Sediment Sources in Agricultural Catchments with Compound-Specific Stable Isotope (CSSI) and Geochemical Markers

W.H. Blake[1,*], K.J. Ficken[2], P. Taylor[1], M.A. Russell[3] and D.E. Walling[4]

ABSTRACT

Compound specific stable isotopes (CSSI) in soils have the potential to trace suspended sediment in river channels back to source areas determined by specific crop type. As such they offer to provide support for soil resource management policies and also to inform sediment risk assessment for the protection of aquatic habitats and water resources. This manuscript summarises a study which aimed to assess the potential for CSSI tracers to provide information on sediment sources in mixed land use agricultural catchments in the United Kingdom (UK). Source discrimination was undertaken in two ways using: (i) CSSI properties and (ii) more conventional geochemical markers. The properties of the source areas were compared with those of suspended sediment collected during a major storm event using established unmixing models. Results from both methods suggested that grassland was an important source of sediment (ca. 0.13 ± 0.02 t/ha) but the geochemical fingerprinting method appeared to be slightly biased to cultivated sources owing to the influence of prior ploughing on the geochemical signals in soil of temporary pasture fields (ley pasture) that were in rotation. With prior knowledge of ley and permanent pasture management practice, analysis of the discrepancies between the two sets of tracers highlights the importance of damaged permanent pasture as an erosion hotspot. Compound specific stable isotopes offer a powerful means of addressing the impacts of land management on soil erosion and downstream sediment problems, but further research is required into the influence of crop rotations on CSSI signature development and implications for downstream interpretation.

Key words: *compound specific stable isotopes, geochemical markers, sediment sources, erosion hotspots.*

INTRODUCTION

Compound specific stable isotope (CSSI) sediment tracing technology offers a new and exciting tool to inform soil and sediment management (Gibbs, 2008, Blake *et al.*, 2012, Hancock and Revill, 2013). The basic principal of the approach is akin to more established sediment fingerprinting and tracing methodologies but offers an additional dimension in terms of tracing material to specific crop type as opposed to generic source end members e.g. surface or subsurface material.

Sediment tracing methods, in general, rely on the ability to characterise and discriminate material from different sediment source areas using a suite of natural tracer properties (e.g. geochemical, mineral magnetic, radiochemical or biomarkers). If this can be achieved, then the properties of material from downslope or downstream locations can be compared with the source areas and relative contributions determined, resulting from effective precipitation (PPT) events (see Figure 1; Collins and Walling, 2002), subject to some important assumptions. The most important assumption made is that sediment fingerprint properties are not transformed during transportation or storage in the river or stream system.

Gibbs (2008) first demonstrated the potential use of CSSIs to trace estuarine sediment back to specific catchment sources, in this case to demonstrate the importance of forestry practice in delivering silt to an estuary. The study showed that CSSI signatures of particle-associated plant fatty acids (FAs) under exotic pine plantation were statistically different from these of soils under native forest cover and agricultural land uses. This results from the fact that FAs readily leach from plant leaves and roots and bind strongly to surface mineral particles in the upper soil profile. Of importance to tracer applications, sediment-associated FAs carry a consistent carbon-13 isotopic signature (delta C-13, $\delta^{13}C$) that reflects the C isotope fractionation processes within the source soil plant cover. Different plant types produce the same particle-reactive compounds (e.g. FAs) but with different CSSI signatures (Chikaraishi and Naraoka, 2003). In terms of satisfying the key assumption that tracer properties are not transformed between the source and receptor landscape units, it has been suggested that the $\delta^{13}C$ signature of the organic compounds does not change or degrade over time since volatilisation, dilution, dispersion and sorption do not cause isotopic fractionation (Blessing, Jochmann and Schmidt, 2008). Evidence has, however, been presented that shorter chain lipids can be degraded preferentially by soil microorganisms (Matsumoto *et al.*, 2007) which has implications for the way CSSI signatures are used and interpreted. Perhaps of greater concern is the influence of legacy effects of prior land use where fields are in a crop rotation system. These factors all require further investigation. Regarding physical factors that might influence properties, it is generally believed that CSSI properties are not modified by transport processes or fluvial sorting. In the case of geochemical tracer properties, the effect of fluvial sorting and particle size effects

1 School of Geography, Earth and Environmental Sciences, Plymouth University, Plymouth, Devon, PL4 8AA, United Kingdom
2 Department of Geography, College of Science, Swansea University, Singleton Park, Swansea, SA2 8PP, United Kingdom
3 Environment Agency, Exminster House, Exminster, Devon, EX6 8AS, United Kingdom
4 College of Life and Environmental Sciences, University of Exeter, Exeter, EX4 4RJ, United Kingdom
* E-mail address of corresponding author: william.blake@plymouth.ac.uk

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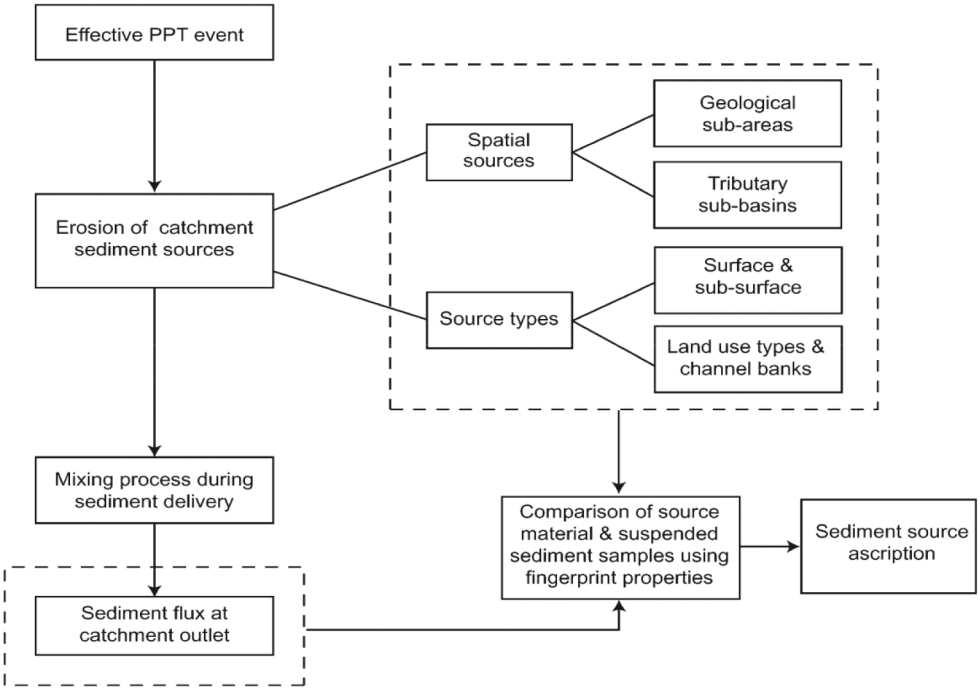


FIGURE 1. Schematic overview of the sediment fingerprinting concept (reproduced from Collins and Walling, 2002).

on sediment properties has been shown to influence fingerprint properties in some systems leading to particle size correction factors begin applied in many studies (Walling, 2005). As CSSI properties are not particle size specific, no such correction is required.

Against this background, this study aimed to explore the potential for using CSSIs as tracers in a United Kingdom (UK) agricultural context and further, to compare the results from CSSI tracing with those from more conventional geochemical tracers.

MATERIALS AND METHODS

The study was undertaken in a small (145 ha) catchment in south-west UK (Figure 2). The land cover at the time of sampling was dominated by pasture (86 ha) cover.

Land use at the time of study comprised a mix of 69 ha ley pasture (temporary grassland as part of a crop rotation system) and 17 ha permanent pasture (i.e. not previously cultivated). Cultivated land was maize (24 ha) and winter wheat (16 ha). The upper catchment was wooded (ca 5 ha) and riparian zones in places were fringed by deciduous trees. In order to characterise the material from the source areas, spatially-integrated samples of soil were collected from fields with maize, winter wheat, permanent pasture and ley pasture cover. For each sample, random transects were laid out in targeted fields and soil material collected at 1 m intervals was bulked into one bag. Integrated samples from deciduous woodland and channel banks were also taken. In total, 62 samples of soil and seven channel bank samples were collected. Samples were dried at <45°C prior to being gently disaggregated and sieved. The <63 micron fractions of the source materials were analysed for CSSI properties and also a suite of major and minor element geochemical properties. Suspended sediment samples were collected during a significant rainstorm event (24.4 mm over 5 hours). Bulk samples of sediment and water were collected at regular intervals during the storm hydrograph and returned to the lab for centrifugation, drying, sieving and CSSI and geochemical analysis as above. Flow and suspended sediment concentration were monitored at the catchment outlet at 15-minute intervals.

To comply with Gibbs (2008), bulk $\delta^{13}C$ ratios for specific extracted fatty acids were determined. Geochemical properties were determined by standard ICP-MS analysis and particle size properties by laser granulometry. Full analytical details are provided in Blake *et al.* (2012).

CSSI and geochemical data were used to discriminate slightly different, but related, source end members in accord with the theoretical basis of each approach (Figure 3). Whereas the CSSI end members are in theory defined exclusively by crop and plant cover, the geochemical signatures are controlled by vertical and horizontal variability in the geochemical properties of the substrate. In the case of cultivated *versus* uncultivated land, this is largely due to mixing of the weathering profile. In this specific catchment, the main area of woodland in the upper catchment had a different geological substrate. When comparing results from the different methods, it is essential to have a good theoretical underpinning of signature development.

Geochemical data were processed and unmixed following standard approaches based on Collins and Walling, (2002). CSSI data were processed and unmixed following the approaches outline by Gibbs (2008) using the Isosource model (Phillips and Gregg, 2003).

RESULTS AND DISCUSSION

Source discrimination

Bulk $\delta^{13}C$ of soil from under the different crop types showed some discrimination between the identified end members (Figure 4), but the extent of discrimination expected from the literature was not present. This is especially notable when comparing maize, a C4 plant, to winter wheat, a C3 plant, since the maize values would be expected to be less negative. This implies that the bulk carbon in the soil comprises material derived from previous crop rotations and also potentially weeds and cover crops.

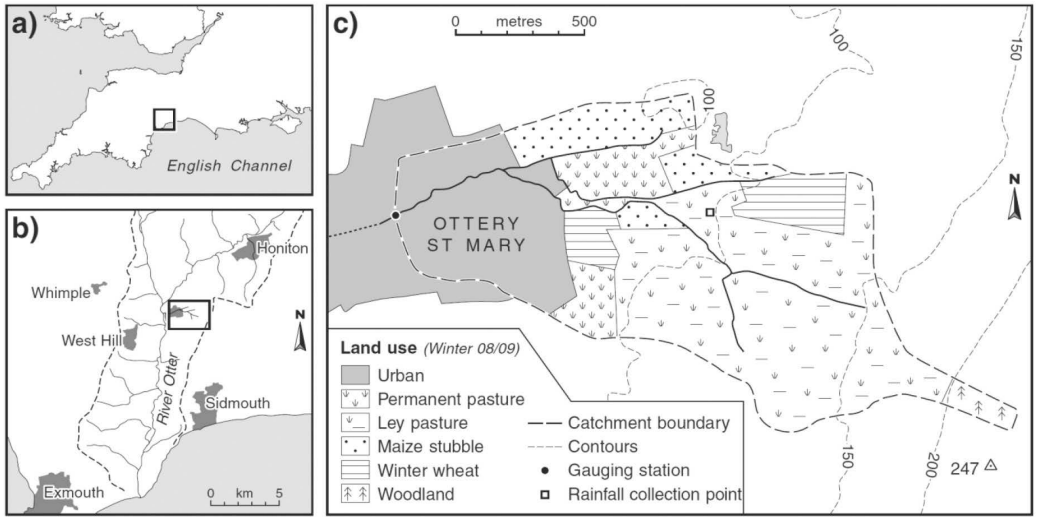


FIGURE 2. Study catchment location (a and b) and land cover (c) at the time of sampling (reproduced from Blake *et al.*, 2012).

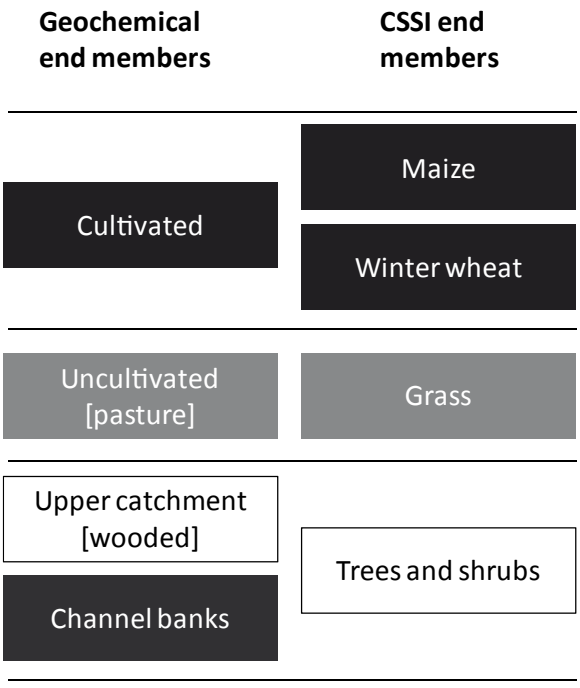


FIGURE 3. Relationship between the different source end members as defined by geochemical and CSSI approaches.

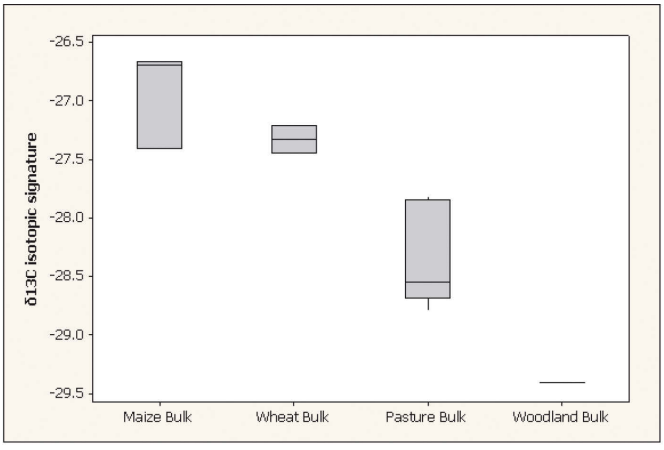


FIGURE 4. Range of $\delta^{13}C$ signatures for bulk C from different source end members.

The CSSI signatures of the soil, however, showed more promising discrimination between the land cover types in shorter chain length (C-16 and C-18) fatty acids. The range of $\delta^{13}C$ values for soil beneath maize was notably different than that for winter wheat in the C-18 length fatty acids (Figure 5). While winter wheat and grassland showed overlap, grassland had a greater range of values with many sites showing more negative $\delta^{13}C$ values than winter wheat. Woodland samples were limited in number, but showed better discrimination in C-16 chain length data (not shown). The longer chain fatty acids did not show the same degree of discrimination as seen in C-16 and C-18 groups. This was slightly surprising as the same signature was expected in all compounds. If microbial action (Matsumoto *et al.*, 2007) is a factor it could be that legacy short-chained fatty acids from previous crops were broken down, whereas the longer-chain fatty acids from previous crops remained in the soil, in line with the bulk $\delta^{13}C$ data. Alternatively, the observations could relate to other ecological activities in the soil specific to the cultivation practice. Further work is required to explore the internal discrepancies between the CSSI signatures of different fatty acids, but in the context of the study aims, the discrimination seen allowed the study to move to the next stage, i.e. apportionment of sediment.

The geochemical properties offered discrimination based on major and trace element components of the mineral fraction (see Blake *et al.*, 2012 for full details). Generally, cultivated soils were discriminated by lower acid-soluble concentrations of metals linked to a greater proportion of primary minerals in the cultivated profile. Channel banks were notably labelled by palladium (Pd) derived from road runoff which is channelled into the stream network. Importantly, the basis for discrimination between the cultivated and uncultivated soil indicates that ley pasture signatures are likely to reflect past cultivation, i.e. the approach will potentially overestimate contributions from cultivated land at the time of sampling.

Source apportionment

Comparison of the CSSI properties of suspended sediment samples (Figure 6) with source materials enabled the temporal dynamics

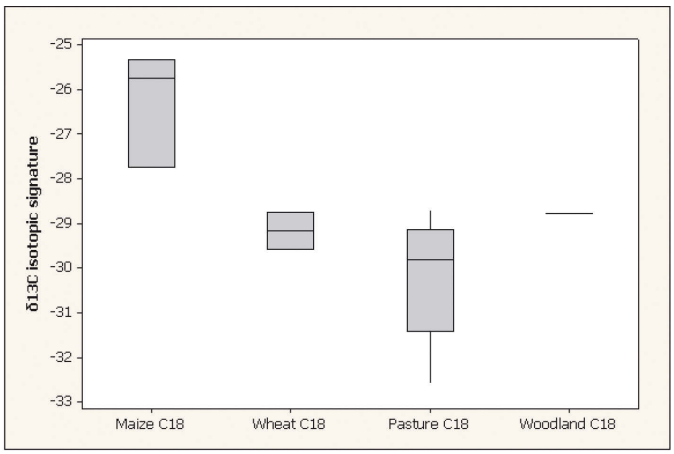


FIGURE 5. Range of CSSI signatures ($\delta^{13}C$) for C-18 chain length fatty acids from different source end members.

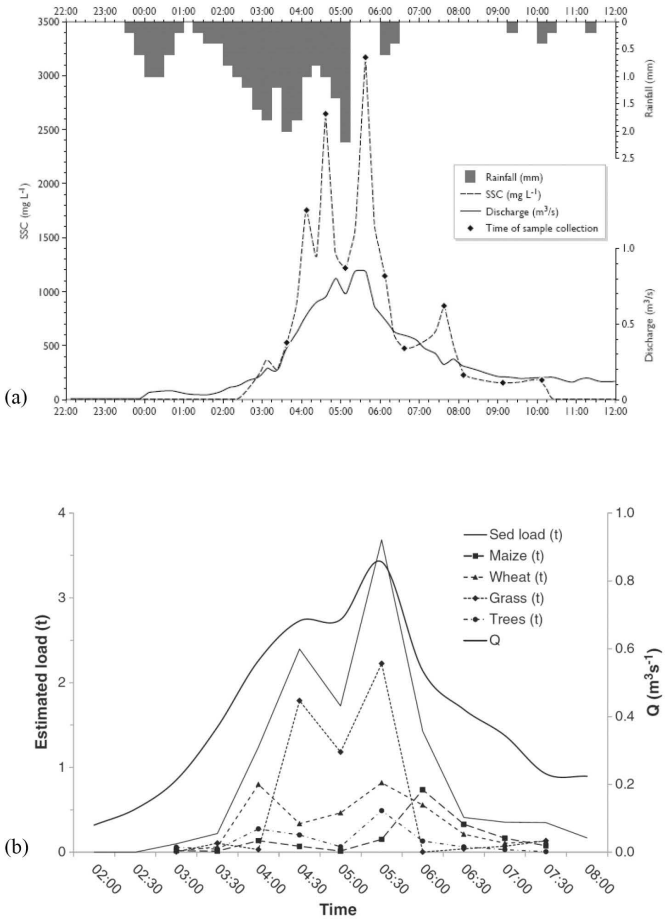


FIGURE 6. (a) Hydrograph and suspended sediment sampling points for the studied storm event and (b) sediment load apportioned to specific crops according to CSSI data (reproduced from Blake *et al.*, 2012).

of sediment delivery from different crop types to be explored. Soil from grassland sources dominated the sediment load with a near synchronous pattern in discharge and sediment. This implies a rapid response from the grassland which is corroborated by a parallel study involving rainfall simulation experiments in the catchment (Hogan, Deeks and Read, 2009). That work showed that pasture slopes were highly compacted with a rapid response to rainfall. Maize and wheat inputs were fairly continuous, in line with compaction due to farm machinery (on tractor tram lines), with a late peak in the maize input suggesting saturation driven run-off from the main field areas. This is again corroborated by rainfall simulation data (Hogan, Deaks and Read, 2009).

In terms of total sediment yield during the event, results from the CSSI data (Table 1) indicated that pasture was a dominant source of suspended sediment in the catchment. This important contribution to total sediment load was a function of the large area of grassland (65 percent) within the catchment at the time of sampling, within which compaction and poaching of the soil surface by livestock are likely to have enhanced the potential for sediment mobilization (Russell, Walling and Hodgkinson, 2001; Bilotta, Brazier and Haygarth, 2007).

The areal yield from this land cover (0.13 ± 0.02 t/ha) appeared quite high in the context of literature values for gross pasture erosion rates in southwest UK, e.g. 0.1. – 0.4 t·ha^{-1}·yr (Walling *et al.*, 2006), which implies high connectivity in this system. Grassland erosion was lower than that of winter wheat at 0.44 ± 0.15 t/ha, but similar to that from maize (0.14 ± 0.02 t/ha). According to CSSI data alone, winter wheat displayed the greatest erosion rate and sediment delivery at the time of the study. Consideration of the geochemical data results, however, offers additional insights into the sediment delivery patterns during the storm event.

Geochemistry-based source specific sediment yield data (Table 2) did not fully cohere with CSSI data. The sum of maize and winter wheat yields from the CSSI results (10.4 ± 3.0 t), is ca. 3 t lower than the yield from cultivated areas indicated by the geochemical data. Likewise, the CSSI derived grassland yield was 11.0 ± 1.4 t, some 2 t greater than the geochemistry derived yield. This can be linked to the influence of prior cultivation of the ley pasture, i.e. the geochemistry-based approach identified some ley pasture as cultivated. Given that the area of permanent pasture was known, i.e. 17 ha of the total 86 ha of grassland, it can be inferred that ley pasture contributed, as a minimum, the 3 t discrepancy noted above. If this is the case, then the remainder of the grassland derived material (a maximum of ca 9 t) must have been derived from the 17 ha of permanent pasture, which implies a sediment yield of up to 0.5 t/ha. This has important implications for grassland management in the catchment. Eighty five percent of the sites examined by Hogan, Deaks and Read (2009) had "very slow" (< 1 mm/h) to "slow" (< 5 mm/h) infiltration rates, based

TABLE 1. Storm sediment yield from different crops types based on CSSI tracers

	ha	t	t/ha	kg/m^2
Maize stubble	24	3.4 ± 0.5	0.14 ± 0.02	0.01
Winter wheat	16	7.0 ± 2.5	0.44 ± 0.15	0.04
Grassland	85	11.0 ± 1.4	0.13 ± 0.02	0.01
Trees/shrubs	5	2.7 ± 1.5		

TABLE 2. Sediment yield from different crops types based on geochemical tracers

	ha	t	t/ha	kg/m^2
Cultivated	40	13.3 ± 0.6	0.34 ± 0.02	0.03
Pasture	85	8.9 ± 1.2	0.10 ± 0.01	0.01
Woodland	5	0.5 ± 0.2	0.10 ± 0.03	0.01
Channel banks	n/a	1.3 ± 0.8		

on the categories given by Landon (1991), leading to high surface run-off rates. The combined results of the two tracing approaches suggest that permanent pasture fields where the surface is damaged can act as an erosion hotspot.

CONCLUSIONS

The $\delta^{13}C$ of particle-reactive fatty acids extracted from soil enabled sediment in streams to be linked back to fields under specific crop cover. Furthermore, the combined CSSI and geochemical tracing approach used in this study provided unique insights into sediment source and delivery dynamics that could not have been derived from each tracer alone. Damaged permanent pasture was identified as an erosion hotspot with sediment yields equivalent to those from a juvenile winter wheat crop. The evidence presented by this and other studies within FAO/IAEA Coordinated Research Project D1.20.11 suggests that CSSIs in combination with fallout radionuclide and other tracers have great potential to identify erosion hotspots in agricultural river basins. This study has also raised some important methodological considerations and research questions about the development and application of CSSI biomarkers. In particular, the effect of crop rotation and legacy signals from prior cultivation practices needs attention. Linked to this, future work needs to explore how soil microbial action and the biodegradation of fatty acids might affect soil and sediment signatures.

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Compound-Specific Stable Isotope Techniques for Improving Soil Conservation Strategies: An Overview of the Lessons Learned from an FAO/IAEA Coordinated Research Project

G. Dercon[1,*], M. Gibbs[2], F. Rasche[3], C. Brandt[3], G. Cadisch[3], P. Boeckx[4], S. Bodé[4], G. Hancock[5], W. Blake[6], D.E. Walling[7], L. Mabit[1], M. Benmansour[8], N. Lam[9], D. Lobb[10], P. Schuller[11], V. Golosov[12], X. Zhang[13], Y. Li[14], W. Froehlich[15], A. Asfary[16] and M. L. Nguyen[1]

ABSTRACT

This paper summarises key findings from a five-year (2009–2013) coordinated research project (CRP) on "Integrated Isotopic Approaches for Area-wide Precision Conservation to Control the Impacts of Agricultural Practices on Land Degradation and Soil Erosion", organized and funded by the International Atomic Energy Agency through the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. The project brought together fifteen participants, from Australia, Belgium, Canada, Chile, China, Germany, Morocco, New Zealand, Poland, the Russian Federation, the Syrian Arab Republic, the United Kingdom and Vietnam. The project involved the use of isotopic and nuclear techniques to assess soil erosion and develop soil conservation strategies at a landscape level. The overall objective of this CRP was to develop integrated isotopic approaches using not only fallout radionuclides (FRN) but also compound-specific stable isotope (CSSI) techniques to identify critical areas of land degradation in agricultural catchments so that effective soil conservation measures can be implemented. Compound-specific stable isotope (CSSI) techniques are based on the measurement of carbon-13 (^{13}C) natural abundance signatures of specific organic compounds (e.g. fatty acids of plant and animal origins) in the soil profile. A harmonized protocol for the application of CSSI techniques measuring ^{13}C of fatty acids extracted from soils was developed to identify critical sediment source areas and erosion hotspots at the catchment scale in a range of environments and land-use systems. The results obtained show that FRN and CSSI based techniques are complementary as fingerprints and tracers of sediment redistribution within agricultural catchments. The CCSI technique provides information on sources while FRN techniques can provide information on the extent of soil losses so that effective soil conservation measures can be targeted to criticallly degraded areas in agricultural landscape.

Key Words: *compound-specific stable isotopes, fallout radionuclides, soil erosion, precision soil conservation, agricultural landscapes.*

INTRODUCTION

Current concerns to enhance food security for an ever-growing population and to arrest widespread land degradation have highlighted the important influence of agricultural land use and management on soil erosion losses and related impacts on farmers' environments. New technologies will need to be developed and applied to better understand and manage natural and agricultural resources in agroecosystems to meet the dual goal of enhancing agricultural productivity and environmental sustainability.

Precision soil conservation is a rapidly developing key science integrating geospatial techniques, models and other tools to better understand the spatial variability of erosion across a landscape by connecting farms to natural surrounding areas and identifing erosion hot spots within agricultural lands. This integrated approach allows informed decisions on the management of critically-degraded areas and assists land managers to target soil conservation measures and appropriate land-uses to these hot spots. This will ultimately result in

1 Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria
2 National Institute of Water and Atmospheric Research, Hamilton, New Zealand
3 Institute of Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim, Stuttgart, Germany
4 Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
5 Division of Land and Water, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Canberra, Australia
6 School of Geography, Earth and Environmental Sciences, Plymouth University, Plymouth, Devon, UK
7 College of Life and Environmental Sciences, University of Exeter, Exeter, Devon, UK
8 Centre National de l'Energie, des Sciences et des Techniques Nucléaires, Rabat, Morocco;
9 Hanoi University of Agriculture, Hanoi, Vietnam
10 Faculty of Agricultural and Food Sciences, University of Manitoba, Winnipeg, Canada
11 Instituto de Ciencias Químicas, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile
12 Faculty of Geography, Lomonosov Moscow State University, Moscow, Russia
13 Institute of Mountain Hazards and Environment, Chengdu, Sichuan, P. R. China
14 Institute of Environment and Sustainable Development in Agriculture, Beijing, P. R. China
15 Institute of Geography and Spatial Organization, Polish Academy of Sciences, Poland
16 Department of Soil Science and Reclamation, Faculty of Agriculture, University of Aleppo, Aleppo, Syria
* E-mail address of corresponding author: g.dercon@iaea.org

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improved efficiency of resource use, economic returns and environmental sustainability.

OVERVIEW ON COMPOUND-SPECIFIC STABLE ISOTOPES TECHNIQUES FOR PRECISION SOIL CONSERVATION

Through the IAEA funded coordinated research project (CRP) entitled "Integrated Isotopic Approaches for Area-wide Precision Conservation to Control the Impacts of Agricultural Practices on Land Degradation and Soil Erosion (2009–2013)", an approach based on compound-specific stable isotope (CSSI) techniques was identified for further development to support the implementation of precision conservation. These techniques are based on the measurement of carbon-13 (^{13}C) natural abundance signatures of specific organic compounds (e.g. fatty acids of plant and animal origins) in the soil profile. By linking fingerprints of land use to the sediment in deposition zones, CSSI techniques have been shown to be useful tools for identifying the source of eroded soil or transported sediment and thereby identifying areas sensitive to land degradation/erosion (Gibbs, 2010; Blake *et al*., 2012).

Significant progress in the development of these techniques was made in this CRP, as indicated below:

- A harmonized protocol was developed for the application of CSSI techniques using ^{13}C signatures in soil fatty acids to identify critical sediment source areas and erosion hotspots at the catchment scale in a range of environments and land-use systems (Gibbs, 2010). This protocol was tested successfully in Australia, Austria, Belgium, Canada, Ethiopia, Germany, New Zealand, United Kingdom and Vietnam, and was further tested by seven other countries across the world in 2013
- A summary was produced of the types, advantages and limitations of organic compounds that can be used as fingerprints to identify sediment sources and critical areas of soil loss (Table 1).
- Bulk ^{13}C signatures in soils (deposited sediment) can provide general information about sources of soil losses, but ^{13}C signatures in soil fatty acids offer important opportunities for obtaining more precise and detailed information. Bulk nitrogen-15 (^{15}N) might also help with broad-brush identification.
- With regards to CSSI techniques, the analysis requires specific intruments and skilled operators (linking gas-chromatography via an on-line combustion interface to isotope ratio mass spectrometry or GC-c-IRMS), and therefore an analytical service provider is needed. While current costs of analysis are high, these are expected to drop dramatically in the coming years, reducing to a as little as US$65 per sample.

OVERVIEW ON THE INTEGRATION OF COMPOUND-SPECIFIC STABLE ISOTOPES AND FALLOUT RADIONUCLIDES TECHNIQUES FOR PRECISION SOIL CONSERVATION

The CRP team also assessed the advantages and the way forward for the integration of CSSI with fallout radionuclide (FRN) based techniques. As FRNs have been proven to be powerful tools for assessing landscape-wide soil redistribution and identifying erosion processes, their integration with CSSI analysis will open new opportunities for improving area-wide soil conservation strategies (Gibbs, 2010; Dercon *et al*., 2012). For instance, Gibbs (2010) reported that the use of beryllium-7 (^{7}Be), an FRN with a short half-life of 53 d, allows identification of recent sediment deposits. Information obtained can then be used to collect sediment samples for CSSI analysis so that hotspots of recent land degradation (sources and intensity of soil loss) in the New Zealand study site can be identified. In addition, the linking of FRNs such as caesium-137 (^{137}Cs) and lead-210 (^{210}Pb) (half-lives of 30 and 22 years, respectively) with CSSI analysis showed how past land degradation and its link with land-use history over the last hundred years can be reconstructed (Gibbs, 2010). Combined use of FRNs and CSSI has also been used successfully in Australia to identify hot spots and assess changes in soil erosion–deposition (sediment dynamics) across a landscape (e.g. relative importance of channel bank erosion and sources).

TABLE 1. Summary of suitable specific organic compounds to identify and apportion areas sensitive to erosion through CSSI analysis.

Type	Origin	Applications	Advantages	Limitations
Fatty acids (^{13}C)	Root exudates; plant materials; animals	Land-use soil source identification to the root depth	Isotopic signature is conservative. Fatty acids are polar, move deep into the soil with water and are tightly bound to clays.	Concentrations may be low in older or sandy soils requiring larger sample analysis. Mixed land-use history (frequent crop rotations) may be difficult to resolve.
Alkanes (^{13}C)	Leaf waxes	Surface soil discrimination by land-use. Top layer only.	Isotopic signature is conservative. Non-polar and hence do not move by water. Waxes are adsorbed onto surface soil layers only.	Surface layer will be eroded first so signature rapidly removed from source. Need for different GC column to separate.
Resin acids (^{13}C)	Pine trees	Identifying pine harvest as soil source.	Specific to pine trees. Specific resin acid half-life gives time since deposition.	Rapid decay of abietic acid in sunlight within a month
Lignin (^{13}C)	Plants	Terrestrial vs aquatic plant sources.	Discriminates terrigenous soil source proportions	—
Fatty acids (Deuterium)	Root exudates; plant materials; animals	Altitude in a single land use.	Adds new dimension. The D isotopic signatures may separate similar land uses from different altitudes with > 500 m differences (rainfall influence).	Extra cost for analysis

Overall, the results obtained from the CRP demonstrated that the combination of FRNs and CSSI provides integrated information on erosion processes, spatial distribution of eroded soils and erosion sources. The CSSI-based techniques provide information on sources and, in the context of monitoring programmes, can provide quantitative information. They are complementary to the FRN techniques as fingerprints and tracers of soil redistribution within a landscape. Thus the CCSI-based techniques provide an additional dimension to generic "soil redistribution" information that is more relevant to land-use management decision-making.

CHALLENGES IN THE USE OF COMPOUND-SPECIFIC STABLE ISOTOPIC TECHNIQUES

The need for uncertainty analysis and error propagation when combining methodologies has been considered in this CRP. Sample numbers are a key consideration and should be scaled to the size and complexity of the study area and the number of land uses. Sufficient samples (minimum five per source), are required for a robust estimate of appropriate descriptive statistics. The nature of uncertainty is likely to be different for different approaches and this needs to be considered on a case-by-case basis. In this context, a detailed knowledge of land-use history within the catchment can be useful in some situations.

For those countries without easy access to CSSI analysis, intermediate approaches should be identified. In this case, bulk isotopic signatures can be an intermediate approach for identifying hot-spots. Bulk ^{13}C signatures can provide general information about soil sources (e.g. agricultural soils are different from karst soils; C_3 versus C_4 plants), but they will not provide the more precise and detailed information provided by CSSIs. Bulk ^{15}N might also help with broad-brush identification over short time-scales (d). However, when using ^{15}N, account should be taken of the N dynamics in the agroecosystem, and advantage taken of the many factors influencing ^{15}N signatures from different land uses. Bulk ^{15}N should only be used as a tracer in flowing water in wet systems, where the ^{15}N signature may not have changed since it entered in the geochemical cycle.

CONCLUSIONS

CSSI and its integration with FRNs show potential for developing soil conservation strategies for agricultural landscapes. Combined tracer approaches will help overcome the complexities for assessing land degradation and soil distribution at a landscape level. Interdisciplinary approaches including soil science, geomorphology, hydrology and biogeochemistry potentially assist in identifying key processes of land degradation and soil distribution. Information obtained can then be used to identify the most appropriate tools and sampling strategies.

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SESSION 3

SOIL AND WATER CONSERVATION ZONES FOR POLLUTION CONTROL

Water Conservation Zones in Agricultural Catchments for Biomass Production, Food Security and Environmental Protection

K. Sakadevan[1,*], L. Heng[1] and L. Nguyen[1]

ABSTRACT

This paper reports the preliminary results obtained from an FAO/IAEA coordinated research project (CRP) involving eight countries. Results obtained are discussed with respect to sources and sinks of water and nutrients in water conservation zones that help farmers to use water when it is required. Three types of water conservation zones, namely farm ponds, wetlands and riparian buffer zones were studied using stable isotopes of oxygen-18 ($\delta^{18}O$) and hydrogen-2 ($\delta^{2}H$) to trace the movement of water, and nitrogen-15 (^{15}N) to trace nitrogen (N) and determine N use efficiency. Preliminary results showed that $\delta^{18}O$ and $\delta^{2}H$ effectively identified sources of water in water conservation zones and the interactions between water conservation zones and water from the catchment. Approximately 90 percent of water in water conservation zones was provided by runoff from the catchment while the remainder came from sub-surface flows and/or direct rainfall inputs. Water balance estimations showed that these water conservation zones with a surface area of less than three percent of the catchment area were able to capture more than 90 percent of water generated as runoff. Besides water retention, one water conservation zone in Estonia trapped N in the runoff water (up to 60 percent) effectively and converted nitrate into N_2 gas (170 to 350 kg·ha/yr) through denitrification, thus potentially reducing nitrate inputs into downstream receiving waters. The nitrate and ammonium trapped in water conservation zones could also be used to provide N for crops through irrigation.

Key words: *water conservation zones, stable isotopes, wetlands, ponds, riparian buffer zones.*

INTRODUCTION

Global land and water resources are under threat as land use transforms from natural to urban and agricultural uses, and through population growth, increased demands for food security and socio-economic well-being and the contamination of the environment (UNESCO, 2006). Poor crop yields as a result of water stress are one of the main reasons for prevailing hunger and rural poverty. The Green Revolution of the sixties and seventies in many parts of the world, particularly in Latin America and Asia depended partly on water management (Das, 2002). However, in the foreseeable future the majority of food supply will still need to come from rain-fed agriculture. Water conservation zones in agricultural catchments play an important role in the capture and storage of water and nutrients from farmlands and providing these for crop and biomass production in times of need in rain-fed areas.

In many regions, water conservation zones are considered as an important part of water resource management strategies that have been developed to prevent reservoir siltation, reduce water quality degradation, mitigate flooding, enhance groundwater recharge and provide water for farming (Gonfiantini, 1986). In addition to making crop production possible in dry areas, water conservation zones minimize soil erosion, improve soil moisture status through capillary rise and enhance soil fertility and quality (SIWI, 2001). These water conservation zones include (i) natural and constructed wetlands (including riparian wetlands), (ii) farm ponds, and (iii) riparian buffer zones. They provide a wide range of socio-economic and environmental services including food, fibre, water supply and purification, carbon storage and biodiversity protection (Fasina, 2005).

The management of water conservation zones has been a challenge due to the poor understanding of the relationship between upstream land use and the functions of these zones and their internal dynamics. Activities and hydrological processes occurring in the upper catchment often affect water and nutrient capture and storage in these zones (Bramley and Roth, 2002). Knowledge of the sources and sinks of water such as the magnitude, frequency, duration, timing, rate of the water flow regimes and nutrient cycling into and out of the system as influenced by upland activities is therefore needed to identify management practices to optimize their performance for conservation and re-use of water and nutrients (Akhter *et al.,* 2005). Redefining water and nutrient budgets for water conservation zones is important for optimizing the capture, storage and use of water and nutrients in agricultural landscapes.

This paper presents and discusses preliminary results obtained from a coordinated research project (CRP) on "Strategic Placement and Area-Wide Evaluation of Water Conservation Zones in Agricultural Catchments for Biomass Production, Water Quality and Food Security" initiated in 2008 by the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture. The overall objective of this CRP is to assess and enhance ecosystem services provided by wetlands, ponds and riparian buffer zones for improving water storage and nutrient use within agricultural catchments. The specific objectives are to (i) determine the capacity of water conservation zones for water storage, (ii) assess nutrient attenuation capacities, (iii) assess the link between water and nutrient dynamics, and (iv) optimize water conservation zones for improving water storage and quality.

1 Soil and Water Management & Crop Nutrition Section, FAO/IAEA Programme, International Atomic Energy Agency, Vienna, Austria
* E-mail address of corresponding author: k.sakadevan@iaea.org

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MATERIALS AND METHODS

The research network operated within the framework of the CRP included eight research contract holders (China, Estonia, Iran, Lesotho, Nigeria, Romania, Tunisia and Uganda), supported by two technical contract holders (France and United States) and two research agreement holders (France and United States). Three types of water conservation zones were used in all participating countries based upon their use in agricultural catchments, namely: (i) water and nutrient storage for downstream irrigation use (farm ponds), (ii) *in situ* crop and biomass production (wetlands including riparian wetlands), and (iii) downstream water quality (riparian buffer zones). Piezometers were installed in and around most of the zones at various depths to more than four m to record groundwater depth fluctuations and to collect water samples. Oxygen-18, hydrogen-2, and nitrogen-15 stable isotopic signatures were used to identify sources and factors influencing water and nutrient capture, dynamics and storage in these water conservation zones. For each country, one catchment was selected for field studies based on the importance of agricultural production and water management practices. Preliminary land-use surveys were carried out for experimental catchments in all eight countries and the results are provided in Table 1.

Based on the CRP objectives, the water conservation zones in Tunisia (farm pond) and Iran (Ab-bandans, i.e. man-made water storage ponds) were grouped under water conservation zones that gather catchment runoff for improving food production through irrigation of crops downstream. China, Lesotho, Nigeria and Uganda (wetlands) were grouped under water conservation zones that are used for improving food production through *in situ* crop production. Finally, Estonia and Romania (riparian zones) were grouped under water conservation zones that regulate nutrient cycling, protect downstream water quality and generate biomass within the system.

A brief description of the studies carried out in each of the zones is provided below:

- The sources of water to the farm pond in the Kamech catchment in Tunisia were investigated using $\delta^{18}O$, $\delta^{2}H$ and hydrological processes.
- Isotopic and water mass balance studies were carried out in 30 Ab-bandans in northern Iran that capture water from the surrounding catchments to irrigate downstream rice fields.
- Field investigations using isotopic and conventional techniques were carried out in northern China to assess water conservation zones for sustainable agricultural production.
- The *in situ* rice production and N use were investigated for rice wetlands in eastern Uganda using ^{15}N isotopic techniques.
- In Nigeria, the environmental issues related to integrated management and characterisation of water conservation zone were studied.
- The hydrological and management constraints in two water conservation zones were examined in Lesotho.
- The N pathways in a riparian buffer zone were investigated in the Arges river catchment in Romania.
- Two riparian buffer zones in Estonia were studied for optimizing N removal through dinitrification.

Data on rainfall and other weather related information were collected for all catchments to establish water balance for the catchment and sources of water inflow to water conservation zones.

Oxygen-18, hydrogen-2 and water chemistry measurements were carried out for water samples collected from water conservation zones, runoff water from the catchment, rainfall, stream water and from the piezometers (four m below the ground). In addition, biomass yield and N uptake in the water conservation zones were measured in all countries.

RESULTS AND DISCUSSION

As the overall objective of the CRP was to optimize the capture and storage of water and nutrients, the preliminary results focussed only on water conservation zones in China, Estonia, Iran, Tunisia and Uganda with emphasis being given to the capture and storage of water and nutrients.

TABLE 1. Land use of experimental catchments.

Country	Catchment	Major land-use	Area (ha)
China	Sanjiang Plains	Rice	92 000
Estonia	Porijogi	Livestock	12 600
Iran	Ab-Bandons	Rice	11 700
Lesotho	Ha-Matela	Maize, sorghum and livestock	300
Nigeria	Ekiti Valley	Rice, maize, yam	2 500
Romania	Galvacioc	Wheat and maize	3 200
Tunisia	Kamech	Wheat, barley, oat, etc.	260
Uganda	Manafwa	Rice	300

TABLE 2. Monthly water budget for the Kamech Lake (m^3)

Month	Sept-09	Oct-09	Nov-09	Dec-09	Jan-10	Feb-10
Net Exchanges	–7871	5511	–3776	–9256	–13 129	–11 352
Month	**Mar-10**	**Apr-10**	**May-10**	**Jun-10**	**Jul-10**	**Aug-10**
Net Exchanges	–16 603	–11 944	–3 047	1119	–2413	–5985

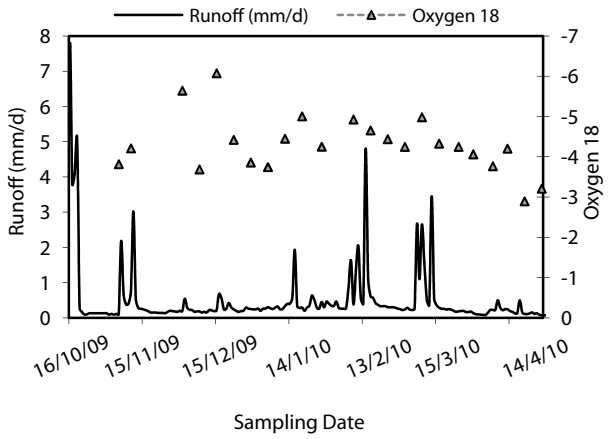


FIGURE 1. Isotopic signature ($\delta^{18}O$) and runoff volume for Kamech streamflow.

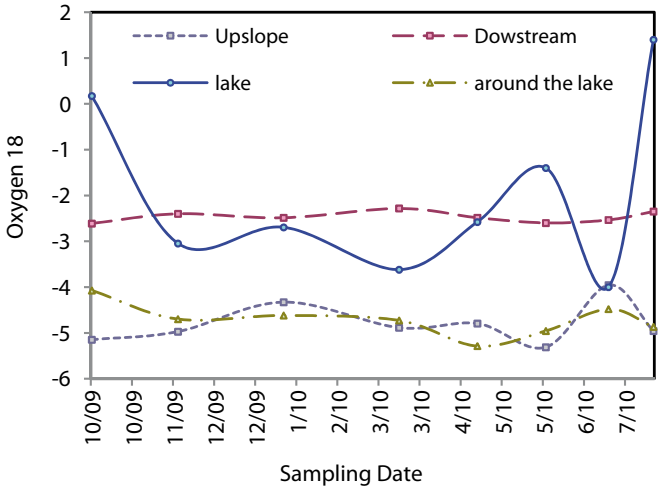


FIGURE 2. Stable isotopes variation of Kamech groundwater.

TABLE 3. Isotopic signatures of various source waters in the Kamech catchment

Isotope	Rainfall	Stream flow	Farm Pond	Run–off
$\delta^{18}O$	–14 to 0.1	–7 to +3	–4 to +5	–6 to –3
$\delta^{2}H$	–89 to +5	–37 to +3	–199 to +219	

Water conservation zones for downstream irrigation

The water budgets established monthly during 2009–2010 for the farm pond in Kamech catchment in Tunisia revealed that for most of the time the farm pond was recharging the unsaturated zone (Table 2). Runoff in the catchment is mainly produced in autumn and winter (between October and March each year) during which annual rainfall occurs. The water balance data showed that October and June were the only months during which water exchange was dominated by unsaturated zone contribution to the farm pond (positive sign, Table 2).

Stream flow and the corresponding $\delta^{18}O$ values for various time periods are provided in Figure 1. Seasonal variations in isotopic signatures clearly showed that it was influenced by runoff. A more detailed study of this data coupled with flood events will permit understanding of flood processes of the catchment.

Delta oxygen-18 ($\delta^{18}O$) and $\delta^{2}H$ values of various water sources in the Kamech catchment are provided in Table 3. The signatures of steam flow have the same range as those of rainfall indicating that direct runoff from rainfall is the major contributor to stream flow. However, these are seasonally variable depending on rainfall and temperature. Isotopic signatures along with water balance calculations indicate that runoff from the catchment is the major source of water to the farm pond.

Different isotopic signatures were found between water in the upslope, around the farm pond and downstream of the farm pond (Figure 2). The water downstream of the farm pond was less enriched, indicating that the downstream recharge was a mixture of overflow, releases and infiltration. Isotopic signatures of water samples collected from the farm pond showed clear seasonal trends closely related to the variations in volume. During the rainy season, isotopic signatures were similar, being influenced by precipitation, stream flow and the volume of water discharged from the water conservation zone. However, during the non-rainfall season, the water was continuously enriched due to evaporation that became the most important component of the water balance during this period (Figure 2).

The water balance calculations (from Table 1) and the closeness of the isotopic signatures of various source waters (Table 3) showed that more than 90 percent of water to the farm pond in the Kamech catchment is contributed by runoff water generated from rainfall.

TABLE 4. Seasonal changes in water quality parameters

Parameters	Spring			Summer			Winter		
	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.
EC (dS/m)	2.8	0.1	0.9	3.3	0.2	1.1	1.0	0.1	0.4
pH	8.4	6.7	7.8	7.8	5.9	7.2	8.9	6.9	8.0
SO_4-S (mg/L)	5.9	0.7	2.5	108	1.3	21.8	5.3	0.9	2.1
P (mg/L)	9.4	0.0	1.3	5.3	1.5	3.2	18.1	0.0	1.4
NO_3-N (mg/L)	13.7	0.2	2.2	33.8	1.1	8.9	30.0	1.0	15.4
NH_4-N(mg/L)	7.4	0.0	2.2	25.6	0.0	2.1	1.4	0.0	0.1
$\delta^{18}O$ (‰)	3	–7	–4	5	–7	–1			
$\delta^{2}H$ (‰)	0.2	–43	–28	19	–38	–12			

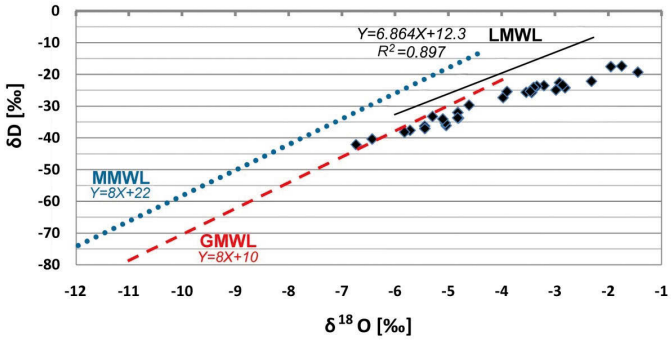


FIGURE 3. Plot of $\delta^{18}O$ versus $\delta^{2}H$ values from 30 Ab-bandans in the north of Iran shown in relation to the global meteoric water line (GMWL), Mediterranean meteoric water line (MMWL) and local meteoric water line (LMWL) (Vreča *et al.*, 2006; Ogrinc *et al.*, 2008; Wassenaar, Athanasopoulos and Hendry, 2011).

Water quality and isotopic signatures for water samples collected from 30 Ab-bandans during winter, spring and summer are shown in Table 4. The analysis of variance between water quality and isotopic signatures showed that a number of factors including land use, fertilizer application and the location of these Ab-bandans influenced the amount of water captured and the chemical characteristics and isotopic signatures of the water (Table 4).

Figure 3 shows the plot of $\delta^{18}O$ versus $\delta^{2}H$ of selected Ab-bandans in relation to the global meteoric water line (GMWL) and the Mediterranean meteoric water line (MMWL). The local meteoric water line (LMWL) was determined from the linear regression of precipitation data collected during the water sampling period. Data showed that the isotopic signatures for almost all Ab-bandans lay below the LMWL and GMWL, indicating that water in these Ab-bandans was affected by evaporation. The results showed that most Ab-bandans in the north of Iran do not receive sufficient inputs of water to minimize the effects of summer evaporation on isotopic signatures (with average $\delta^{18}O$ and $\delta^{2}H$ values of –4‰ and –29‰, respectively). Runoff from precipitation (rainfall and snow melt) during autumn and winter (September to March) was the main contributor to the Ab-bandan water.

Water balance calculations showed that on average 7.6 million m^3 of water with 86 and 17 t of N and P, respectively were captured annually by 30 Ab-bandans mainly through runoff and were available for irrigation. Flood irrigation using this water at a rate of 10 000 m^3/ha over the growing season (April to September) was able to produce rice in an area of 730 ha with a yield of 3.5 t/ha. However, changing the irrigation method from flood to an eight-d irrigation interval was able to cultivate 1500 ha with a similar yield and a significantly increased water use efficiency and reduced energy use (Figure 4).

Water conservation zones for *in situ* biomass production

Isotopic signatures for surface water and unsaturated zone water (water depth at four m below ground) in three typical rice wetlands in Honghe (HH), Qianfeng (QF) and Qianshao (QS) farms in China, showed that different processes were occurring in these three rice wetlands during the period 2005–2009. At inter-annual scale (between years), water levels tended to decrease, with that in the HH wetland decreasing the most, followed by QF and QS farms. At intra-annual scale (within a year especially from April to the middle of May), water levels decreased by up to four m, being controlled by unsaturated zone water extraction, i.e. for rice-farming. This demand decreases when rain appears in May each year. As a result, water levels rise back to levels before irrigation.

In July, average values of $\delta^{2}H$ and $\delta^{18}O$ in rain water were -81.5‰ and -11.2‰, respectively. The $\delta^{2}H$ and $\delta^{18}O$ for unsaturated zone water (four m below ground) in Honghe farm ranged from –98.6‰ to -68.3‰, and from –13.5‰ to –8.8‰ respectively, with mean values of –92.6‰, and –12.1‰. Similarly, the $\delta^{2}H$ and $\delta^{18}O$ of unsaturated zone water in Qianfeng farm ranged from –86.1‰ to –102.4‰, and from –13.8‰ to –10.8‰, respectively with mean values of –97.0‰ and –12.8‰. Isotopic signatures of irrigation water from paddy fields entering drainage channel ranged from –9.0‰ to –12.6‰ for $\delta^{18}O$ with a mean value of –10.4‰. These values are comparable with those in rain water. This closeness between the isotopic signatures of rain water and unsaturated zone water suggest that the unsaturated zone water in this area is recharged during the rainy period. As the unsaturated zone water level decreased before the rainy season (April and May each year), it is evident that the contribution of unsaturated zone water to the wetland is minimum.

The performance of wetlands for *in situ* rice production and nutrient use in the Manafwa catchment, Uganda (Doho rice scheme) showed that these rice wetlands remove 64 t of N in a single growing season from the incoming river water through biomass production. The three water management practices in these wetlands, namely (i) irregular and low (poor), (ii) regular and low (moderate) and (iii)

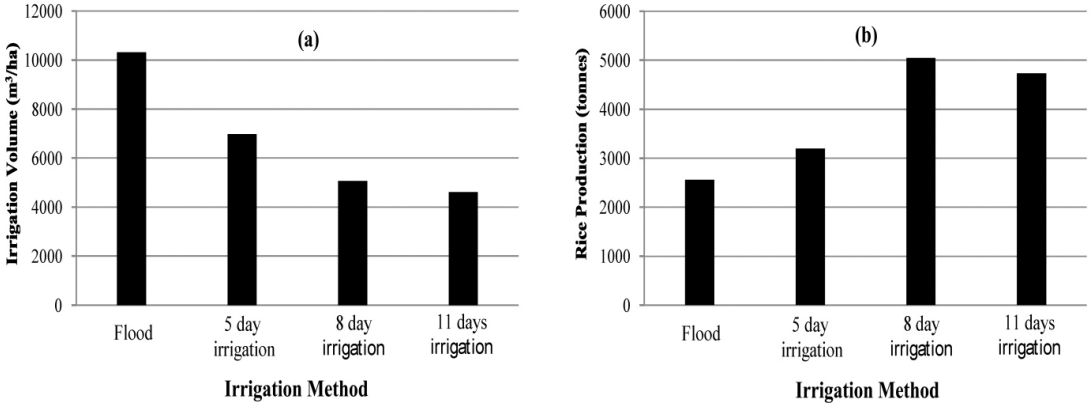


FIGURE 4. Influence of irrigation methods on (a) amount of water used and (b) total rice production of Ab-Bandans.

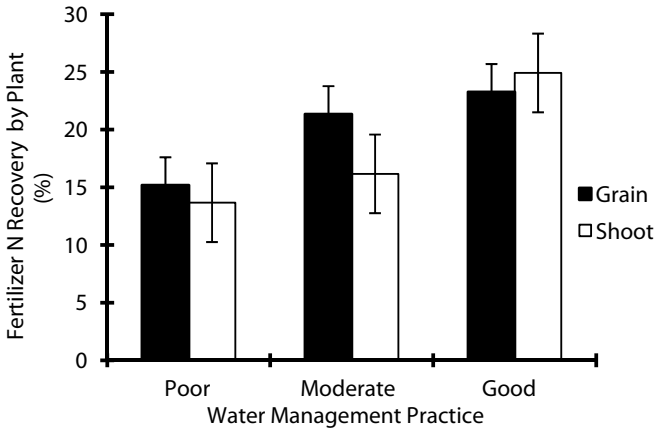


FIGURE 5. Fertilizer N recovery by rice crop as influenced by water management practice in Doho rice wetlands.

TABLE 5. Grain yield, biomass production and N uptake by rice in Doho wetlands

Treatments	Yield (t/ha)		N uptake (kg/ha)	
	Grain	Biomass	Grain	Biomass
Fertilized	7.7	24.7	96	184
Unfertilized	6.2	19.3	70	144

regular, high and continuous (good) demonstrate that good water management practice played an important role in biomass production and nutrient use efficiency of rice (Figure 5). However, fertilizer application made a significant difference to biomass productivity in wetlands, increasing grain yield by 25 percent and biomass production by 27 percent, while N uptake increased by 36 percent and 28 percent, respectively in grain and biomass (Table 5).

^{15}N isotopic signatures of rice grain and shoot showed that water management practices affected the amount of fertilizer N taken up by the rice crop, ranging from 34.6 kg N/ha under poor water availability to 57.7 kg N/ha under permanent water availability (Figure 5).

At the end of rice growing season, less than two percent of fertilizer N was present in the top 30 cm soil depth suggesting possible N losses through leaching, denitrification and volatilization. The rice wetland removed more than 70 percent of the applied fertilizer. The wetland rice production provided a minimum net economic return of US$1300 per ha per cropping season.

Regulating water and nutrient cycling in water conservation zones

Studies on nitrous oxide (N_2O) emissions from two differently loaded riparian grey alder (*Alnus incana)* dominated forests in agricultural landscapes of southern Estonia showed a negative correlation between the concentration of N_2O-N and the rate of conversion of nitrate to N_2O in groundwater in both Porijõgi and Viiratsi catchments (Figure 6A). The negative correlation suggests that the majority of nitrate denitrified is converted to N_2 gas. The isotopic signatures of $\delta^{18}O$ exhibited a relatively large variability and site preference (SP), typically ranging between 10‰ and 50‰ and with a close correlation between $\delta^{18}O$ and ^{15}N (Figure 6B) (Well, Weymann and Flessa, 2005). Results obtained are in agreement with those reported by Mander *et al* (2003) and Well, Weymann and Flessa (2005), showing that N_2O emissions at both sites were significantly lower (0.5 and 0.6 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ in Porijõgi and Viiratsi, respectively) than emissions of N_2 (51.2 and 47.4 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$, respectively).

These results show that in riparian alder forests, denitrification leads mainly to the release of N_2 rather than N_2O, which is a significant boost to reducing N_2O emission to the atmosphere (Well, Weymann and Flessa, 2005). These riparian buffer zones can remove between 170 and 350 kg·N/ha from the incoming water, mainly as N_2 gas.

CONCLUSIONS

Water conservation zones are major sources of water for groundwater recharge as shown by similar δ^2H (–102‰ to –68‰) and $\delta^{18}O$ (–14‰ to –9‰) signatures. Isotopic signatures of water in runoff, rainwater, stream water and water conservation zones along with water balance calculations showed that more than 90 percent of the water captured by water conservation zones is by surface runoff during rainy periods. Nitrogen captured in water conservation zones is a major source of N for biomass production (215 $kg{\cdot}N{\cdot}ha{\cdot}yr^{-1}$)

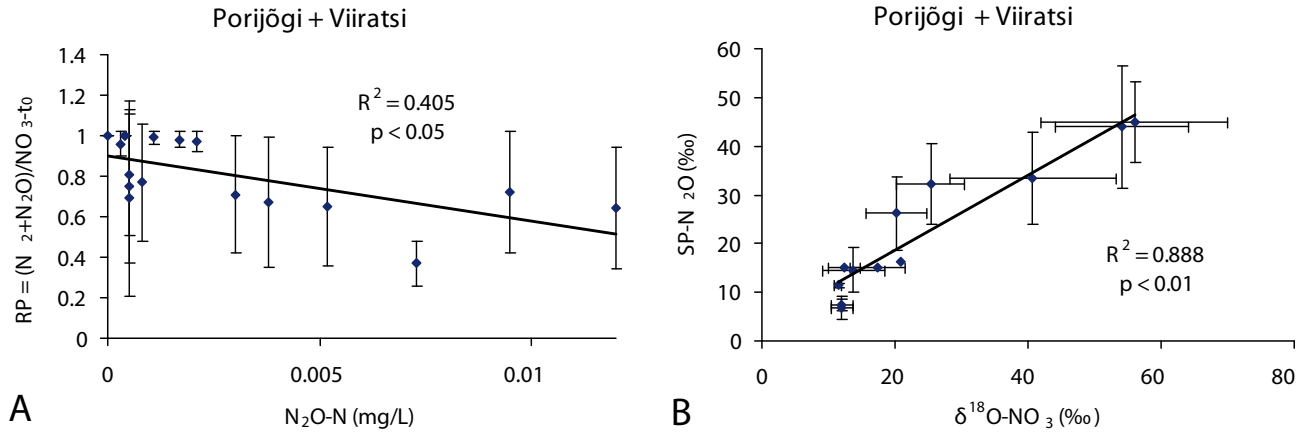


FIGURE 6. Groundwater N_2O vs reaction progress (RP) (A) and $\delta^{18}O$ vs SP (B) in riparian grey alder forests of Porijõgi and Viiratsi, Estonia.

within these zones and for irrigating adjacent farmlands and potentially reducing N input to downstream water. More than 60 percent of N removed by water conservation zones in agricultural catchments occurred through denitrification in Estonia as shown by N-15 techniques. Nitrogen-15 labelled nitrate and urea were useful for quantifying denitrification and biomass N use efficiency in wetlands, farm ponds and riparian buffer zones. These preliminary results showed that in wetlands and riparian buffer zones, denitrification is a major process leading to N removal from water (>60 percent of N removal), and that most of this denitrification leads to the formation of N_2 gas rather than N_2O thus reducing greenhouse gas emissions to the atmosphere. Information collected from this research is useful for preparing guidelines and management practices that help farmers to optimize the capture and storage of water and nutrients in water conservation zones and their subsequent use for agricultural production, as well as to improve downstream water quality and quantity.

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Nutrient (Nitrogen and Phosphorus) Management in Agricultural Catchments for Improving Crop Productivity and Water Quality

K.R. Reddy[1,*] and G.J. Hochmuth[1]

ABSTRACT

Managing land and water resources is increasingly challenging as a result of increasing competition for natural resources because of population and economic growth, climate change and other drivers. Nutrient management in agriculture plays an important role in crop production intensification and improving the quality of land and water resources in agricultural catchments. In this paper, a brief overview is presented on critical issues related to nutrient management in agricultural catchments for improving crop productivity, nutrient use efficiency and water quality. Nitrogen (N) and phosphorus (P) are critical nutrients for global agricultural productivity and food security. Increased demand for food production has resulted in extensive use of these nutrients resulting in impacts on surface and groundwater quality. Optimal nutrient management can be established with knowledge of nutrient budgets for site-specific conditions, nutrient use efficiency by crops and availability of legacy nutrients. Long-term goals of nutrient management in agricultural watersheds should include conservation and enhancement of soil quality. Future soil and nutrient management practices should consider nutrient imbalances resulting from surplus and deficits in fertilizer use in the catchment.

Key words: *soil quality; nutrient use efficiency; nutrient budgets; nitrogen isotopes; watersheds.*

INTRODUCTION

Globally, land and water resources are threatened by land-use shifts from natural to urban or agricultural environments and increased population. This change in land use has altered the demography as a large number of people moved from rural to urban environments, resulting in increased demand for food, water security and socio-economic well-being, and in environmental impacts from industrial, municipal and agricultural pollution (UNESCO, 2006). Agricultural, forested, rangeland and urban land management play an integral part in influencing sustainable land and water resource use for crop production and natural resource protection and conservation. The objective this paper is to provide a brief overview of nutrient management in agricultural catechements for sustainable agricultural productivity by improving nitrogen (N) and phosphorus (P) use efficiency by crops/grasslands and reducing non-point source pollution.

1 Soil and Water Science Department, University of Florida, Gainesville, Fl 32611 USA

* E-mail address of corresponding author: krr@ufl.edu

Agricultural ecosystems include: crops, livestock and horticultural production systems, rangelands, aquaculture and animal agriculture. Non-point source pollution of water resources such as streams, rivers, groundwater, lakes, wetlands and estuaries is linked to the agricultural water and nutrient management practices used in the watershed. The questions of immediate concerns are:

- Are current agricultural practices compatible with sustaining economic crop productivity and preserving the quality of our natural resources?
- Are current agricultural practices adequate to meet current demands and future needs to sustain economic crop productivity and protect the quality of natural resources?

Many current agricultural management practices may be compatible, but not adequate to sustain natural resource quality. Society demands that the quality of natural resources be protected, placing a greater demand on producers to deliver environmentally sound goods. The major challenges for sustainable global agriculture are: (i) meeting the food and fibre needs of a world population projected to exceed nine billion by the year 2050, (ii) decreasing the rate of soil degradation and ameliorating degraded soils, and (iii) protecting the quality of natural resources. During the past decade, implementation of best management practices (BMPs), mostly in developed countries, has helped to improve soil and water quality. However, many watershed management practices currently used around the world are clearly insufficient to deal effectively with stresses placed on land and water resources from burgeoning populations. For sustainable land use and management, integration of the information from diverse domains (e.g. physical, biogeochemical, economic, social, cultural and demographic) at spatial and temporal scales is required to develop predictive tools across environmental, hydrologic, economic and social gradients. The US National Research Council Committee (NRC, 1993) on long-range soil and water conservation defined four broad issues that show promise for maintaining sustainable agriculture, while protecting water quality. These include: (i) conserve and enhance soil quality as a fundamental step to environmental improvement, (ii) increase nutrient, pesticide, and irrigation use efficiencies in farming systems, (iii) increase the resistance of farming systems to erosion and run-off, and (iv) make greater use of field and landscape buffer zones. Although these issues were identified almost two decades ago, many are not adequately addressed in both developed and developing regions of the world.

Understanding the nature of soil quality, which is defined as "The ability of the soil resource to produce and maintain ecosystem production of plant, animal, and microbial biomass and to buffer or improve water quality", is fundamental to meeting the challenges identified above (Figure 1).

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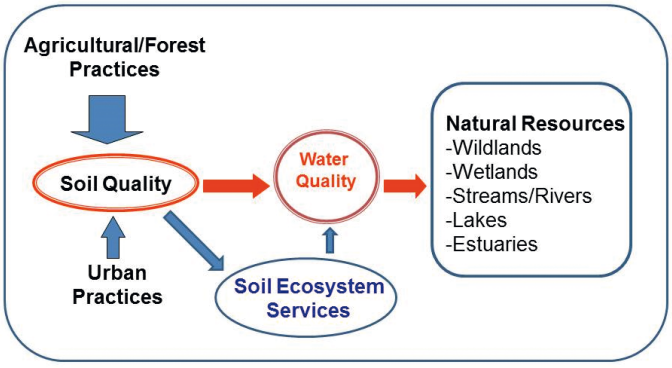


FIGURE 1. The linkage between soil and water quality.

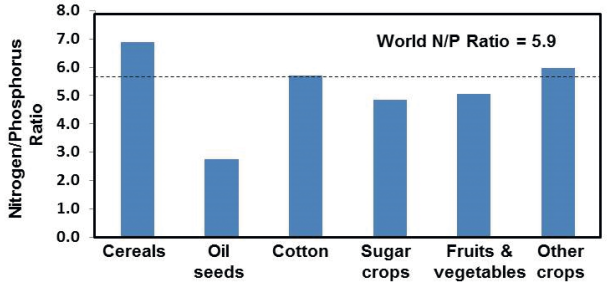


FIGURE 2. Nitrogen and phosphorus ratio of fertilizers used by major crops in the world during the year 2008 (Heffer, 2009).

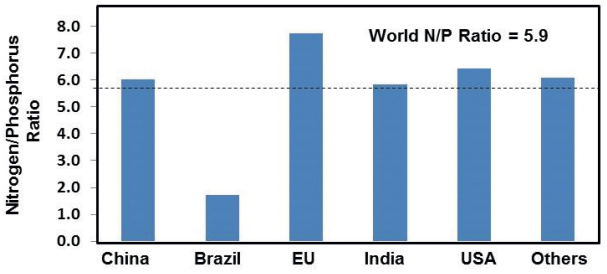


FIGURE 3. Nitrogen and phosphorus ratio of fertilizers used by selected counties in the world during the year 2008 (IFA, 2009).

Land-use changes and alterations in management practices are having significant impacts on the quality of groundwater, adjacent streams, wetlands, lakes and estuaries. For example, approximately 218 million Americans in USA live within 10.6 km of an impaired water body. In the United States, agricultural and forest management alone contribute 70 percent of the pollution in rivers and 60 percent of the pollution in lakes (Carey, 1991). Excess nutrients in aquatic systems have detrimental effects on designated or existing uses, including drinking water supply, recreational use, aquatic life and fisheries (USEPA, 2000). In the southeastern United States, approximately four million ha of lakes, and 560 000 km of rivers are impacted by pollutants, with sediment and nutrients as major types. New regulations place a burden and demand on farmers to change current practices or develop new practices in order to protect water quality while maintaining a profitable production system. To meet this demand, it is critical to review and analyse the available databases and develop new data at both site-specific and watershed levels, with a focus on developing tools that will help farmers and managers to assess soil quality and its linkage to water quality and sustainable productivity.

A holistic, integrated approach to research and education is required to develop alternate practices that will maintain environmentally sound management of these land and water resources. Since the soil is the primary driver regulating ecosystem processes and functions, its quality has a direct influence on water quality and ecosystem productivity. Soils are integral players in biogeochemical cycles that regulate ecosystem functions. Thus, it is appropriate to define soil quality in terms of functions that soil plays in these ecosystems. Policies that protect soil resources should sustain the soil's capacity to serve several functions simultaneously, including the production of food, fibre and fuel, nutrient storage, carbon sequestration, waste storage and the maintenance of ecosystem stability and resilience.

GLOBAL NITROGEN AND PHOSPHORUS FERTILIZERS USE

Nitrogen and P are critical nutrients for global food security. However, the use of these nutrients is not only linked to food production but also the potential environmental impacts on surface and groundwater quality (Heathwaite, Sharpley and Gburek, 2000; McDowell, Sharpley, and Kleinman, 2002; Reddy and Jawitz, 2010). Global N fertlizer consumption stayed on a par with increasing population and demand for food and fibre. It is estimated that approximately 100 million tonnes (t) of N fertilizer was consumed during 2008, representing 55.3 percent of world fertilizer N applied to cereal crops (corn, rice, wheat and other cereals), with oil seed crops accounting for 6.3 percent, cotton and sugarcane for 7.1 percent, fruits and vegetables for 15.3 percent and other crops 15.9 percent, respectively (IFA, 2009). It is estimated that approximately 17 million t of P fertilizer was consumed during 2008, representing 46.6 percent of world fertilizer P applied to cereal crops (corn, rice, wheat and other cereals), with oil seed crops accounting for 12.3 percent, cotton and sugar crops for 8.0 percent, fruits and vegetables for 17.8 percent and other crops 15.3 percent, respectively (IFA, 2009).

Global distribution of N fertilizer consumption in 2008 (100.5 million t), with China (32 percent) and India (14.5 percent) accounted for more than 35 percent, countries in the European Union (EU; 11.5 percent), United States (11.5 percent), Brazil (2.7 percent), and all other countries (27.9 percent), respectively (IFA, 2009). Global distribution of P fertilizer consumption in 2008 (17.1 million t), was led by China (31 percent), followed by India (14.7 percent), United States (10.5 percent), Brazil (9.4 percent), EU (8.8 percent), and all other countries (26.9 percent), respectively (Heffer, 2009).

The global N and P fertilizer consumption ratio is estimated to be 5.9 (IFA, 2009), while the ratio is 6.1 for North America (Mullins, Joern and Moore,, 2005), and 6.8 for the State of Florida (Reddy, Lowe and Fontaine, 1999 and Reddy et al, 2011), respectively. Fertilizer N and P consumption ratios for crops ranged from 2.7–6.9 (Figure 2), with low ratios observed for oil seed crops and high ratios for cereal crops. Geographically, N and P consumption ratios ranged from 1.7–7.7 with low ratios observed for crops grown in Brazil and high ratios for crops grown in the EU countries. The low fertilizer N and P consumption ratios in Brazil are probably due to the high P fixation capacity of soils and low plant available P. This results in high rates of P application in relation to N. High ratios for crops in EU countries are probably the result of reductions in P application rates. Similarly, higher ratios were noted for North America and the State of Florida, reflecting reductions in P fertilization (Figure 3).

The demand for N fertlizers will contiue to increase as the world population is projected to exceed nine billion by 2050. A significant correlation was noted between global population increase and N fertilizer use for agriculture (Raun and Schepers, 2008). It is estimated that world-wide demand for N fertilizers may increase to 186 million t by 2050, placing a major demand on energy to produce this fertilizer (Raun and Schepers, 2008). Similarly,, the 2008 estimate for world P fertilizer consumption was 17.1 million t. Rock phosphate is a non-renewable resource and currently is the major source of inorganic P, with current annual production estimated to be about 20 million t (Cordell *et al.*, 2011). It is estimated that peak P production will occur in 2035, and after that period demand would outweigh supply, suggesting P fertilizer scarcity (Cordell, Drangert and White, 2009; Carpenter and Bennett, 2011; Childers *et al.*, 2011; Neset and Cordell, 2012). Global distribution of soil P content is uneven. In some areas of the world the soils are saturated with P as a result of long-term application of fertilizers and other sources including manures and other organic wastes. Soils in other areas may be P deficient as a result of low P applications and highly reactive soils (dominant in iron and aluminum). Recommended P application rates to crops grown in these highly reactive soils are relatively high to maintain adequate plant available P. In some areas, inorganic N and P fertilizers are supplemented with organic wastes and manures. However, application of manures and organic wastes based on needed N results in excessive P load, and when based on P needs results in N limitation. In many cases, applications of organic solids based on crop N needs may result in application of P in excess of ecosystem requirements, resulting in adverse impacts on surface and groundwater quality. For example, the average N content of biosolids is about three percent of the dry weight and the average P content is about 1.8 percent (Reddy, Lowe and Fontaine, 1999). The N:P ratio of the biosolids is 1.7, which means that when land application rates are calculated based on the N content, there is a potential to apply high levels of P which can potentially create water quality problems. However, if land application rates are determined based on P content, rates would be much lower and supplemental inorganic N fertilizer would be required to meet the N requirement of the crop. Organic waste loading rates should be based on site soil characteristics, the bioavailability of N and P and the hydrologic characteristics of the site. In order to protect the aquatic ecosystems of watersheds, organic waste loading rates should also be based on the P content and the soil's capacity to retain P. Considering potential future deficits in inorganic P supply, manures and other organic wastes offer potential sources of P to crops.

NUTRIENT BUDGETS

Accounting of various inputs and outputs of N and P at multiple scales (field plot to watershed scales) in agricultural ecosystems will aid in improving nutrient use efficiency by crops and in developing strategies to reduce the environmental impact of non-harvestable nutrients on adjacent water bodies. Nutrient budgets typically identify major flow paths of a nutrient in question and associated stores in various pools in the soil–plant system and biogeochemical transformations within and between pools. For example, N budgets in various agricultural ecosystems have been used for more than 100 years to determine sources and sinks for N (Meisinger *et al.*, 2008). The basic principle in developing nutrient budgets is simple: nutrient inputs minus nutrient outputs equals change in stores in the system. These stores can be positive or negative depending on inputs and outputs and the nutrient use efficiency of the cropping system used. Nutrient budgets are useful for guiding land managers in determining where to apply the most effort for increasing crop nutrient use efficiency, reducing environmental pollution, or for reducing the waste of a nutrient that would decrease agricultural profitability. Budgets depend on an understanding of the sources, flows and fates of a nutrient. Budgets help determine the balance of nutrients in the agricultural system, a negative balance resulting from more outputs relative to inputs and a positive balance from more inputs relative to outputs. Negative balances could mean there are losses of nutrients to the environment. Positive balances do not always demonstrate good nutrient management because the buildup of nutrients in the system could lead to large nutrient release should a production practice change or a sudden wet period occur leading to erosion. Knowing the particular nutrient balance and understanding the pools and fates of the nutrients are key to adjusting the balance.

Major nutrient pools

In an agricultural setting there are four major nutrient pools. These are inputs, those nutrients being stored and cycled, exports and outputs (losses) to the environment. Note that in a farm budget, nutrient outputs can be separated into loss to the environment and exportation via the produce or animal meat transported to market. Nutrient inputs and outputs for an agricultural setting are shown schematically in Figures 4 and 5. Nutrient budgets are created from numerous sources of information. Some pools are easy to quantify from farm records, such as fertilizer brought onto the farm and used for crop production. Some farms are equipped to collect and weigh livestock manure, especially farms that operate under a USDA Natural Resource Conservation Service comprehensive nutrient management plan. The amounts of crops harvested, packaged, and sold off the farm can be obtained from farm records. Some researchers quantify certain nutrient pools from published information in the scientific literature pertaining to similar farm operations. Some nutrient pools must be measured for the best accuracy. For example, crop uptake can be measured by analysing above-ground and below-ground plant parts for nutrient content. Nutrients in the irrigation water can be determined by laboratory analyses. Nitrogen and P have distinct transformations and flow paths that regulate fate and transport of these nutrients (Figures 4 and 5). A major portion of soil N is in organic form (95 percent of total soil N) and it is tightly coupled to soil organic carbon. Thus, breakdown of organic matter through decomposition processes can result in mineralization of organic N and release of inorganic N.

Some examples of different types of nutrient budgets can be found in the literature and this has become a popular area of research, probably due to the strong interest in nutrient losses from farms. Davis *et al.* (2003) took advantage of the Magruder Plots at Oklahoma State University in Stillwater, Oklahoma that have been under continuous tillage since 1892. These researchers calculated an average N use efficiency of 33 percent for wheat. Gentry *et al.* (2009), used direct measurements of several N pools in a watershed in Illinois, finding that fertilizer N and soybean N2 fixation dominated the N inputs and grain export dominated the outputs. In a dry year, inputs were greater than outputs and in a wet year, outputs were greater than inputs.

Scale of nutrient budgets

It is important to determine the specific goal for developing the nutrient budget to understand the effects of changes in fertilization rates on nutrient pools. A nutrient budget can be determined for different scales depending on the needs of the farm operator. Budgets can describe the nutrient pools at the field, farm or watershed level. A budget can be developed for a specific farm process, for example cattle feeding operations, urban areas (N in a residential watershed)

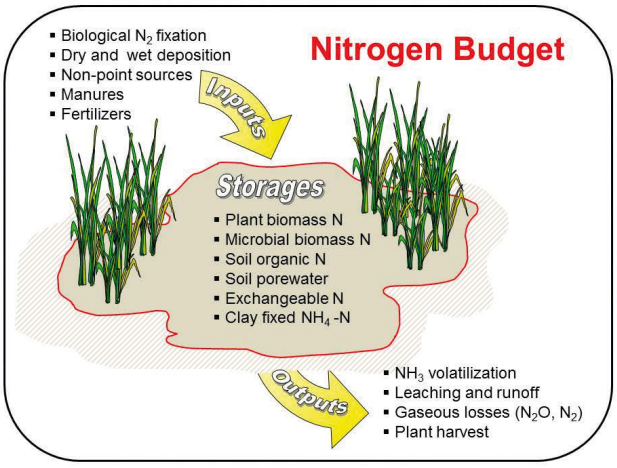


FIGURE 4. Schematic showing nitrogen budget for a typical cropping system.

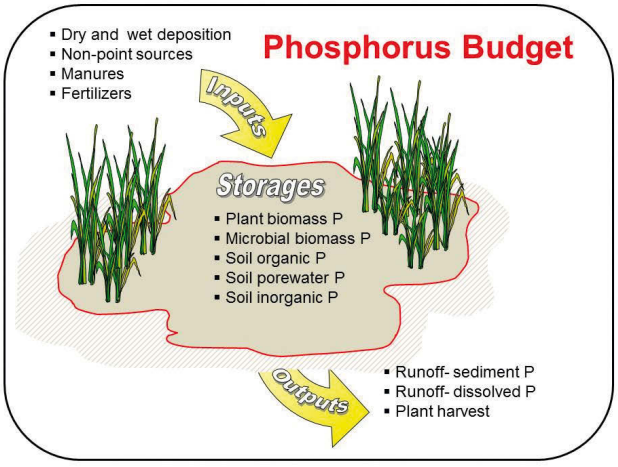


FIGURE 5. Schematic showing phosphorus budget for a typical cropping system.

and natural areas such as forests. Many times the nutrient budget for agriculture is a component of a larger watershed nutrient budget. Therefore, one objective of a nutrient budget should be to determine the specific scale of the budget to be developed. Several reviews of N budget research in agriculture have been made (Allison, 1955 and 1966; Legg and Meisinger, 1982; Meisinger *et al.*, 2008).

Nutrient budgets and balances have been determined for watersheds that include agricultural and urban areas. For example, McMahon and Woodside (1997) studied eight sub-basins in the Albemarle-Pamlico drainage basin in the United States. They used published information about agriculture in the basins and directly measured values to construct a N budget for the watershed. Greatest stream N concentrations were found in areas dominated by agriculture, intermediate loads were from mixed agricultural/urban areas, and lowest outputs were from agricultural/forested areas. Atmospheric N comprised a significant source of N and pointed to challenges in managing this source in the budget. One-half of the nutrient inputs could not be accounted for by stream loads or crop removal. Using a similar approach, Harned, Brian Atkins and Harvill (2004) found that high N concentrations in streams were associated with high urban development, and high P concentrations were associated with agriculture. Improvements in waste water treatment probably were associated with decreasing trends in stream N levels after 1987. Annual variations in animal populations and fertilizer consumption were associated with changes in stream N and P concentrations.

Challenges for nutrient budgets

Nutrient budgets are still difficult to calculate, even by today's scientists. Nitrogen, for example, exists in various oxidation states in the environment, so different analytical methods are required to quantify all the N forms and processes in the soil. Nitrogen undergoes numerous transformations in the environment and can move between pools in the budget. Nitrogen uptake by crops can be determined by the "difference" method where N accumulation by plants growing without N fertilization are compared with N accumulation by fertilized crops. Phosphorus can precipitate with calcium and can be adsorbed onto soil particles so that it becomes difficult to measure the P pool that is currently important for plant uptake in a nutrient budget. Quantifying the pools of stored N and P (Figures 4 and 5) and tracking mineralization and immobilization in these pools are particularly challenging aspects of nutrient budget research. Some scientists have used controlled-environment greenhouse-scale and micro-plot studies to calculate nutrient budgets, often as a prelude to larger-scale field studies.

Pools of nutrients lost from the farm are a challenge to quantify because of the need for special equipment and the associated operational costs. If there are streams entering or leaving the farm, stream flow and nutrient content can be measured. Leaching loads can be measured by drainage lysimeters. Run-off and leaching loads are sometimes measured directly in a nutrient budget calculation, but more often are included in the "unaccounted-for" nutrient pool. In addition to leaching and runoff, gaseous losses (volatilization and denitrification) are often not measured directly and included in the unaccounted-for pool.

Animal production in agriculture has been at the forefront in understanding nutrient budgets and balances on the farm, especially dairy farms around the world (Castillo *et al.*, 2000; Spears, Young and Kohn, 2003; Cabrera *et al.*, 2006; Wang *et al.*, 2010). Cornell University (2013) developed a nutrient balance calculator in Excel format for any type of farm (livestock, crops, etc.) to help farmers understand more about the quantities of inputs and outputs on their own farms.

Information on nutrient budgets for horticultural crops is limited. In a study on N budgets for butternut squash in Canada, Van Eerd (2010), found that apparent N losses increased significantly when fertilizer N rates increased above the recommended rate and that squash did not respond to fertilization on some soils. The author pointed out that farmers should identify those soils that would likely be non-responsive to N fertilizer before they apply fertilizer. Jackson (2000) followed nitrogen-15 (^{15}N) in a lettuce and cover crop system in California, found that of the 40 percent of N mineralized from a cover crop, 50 percent was taken up by the lettuce, 25 percent was lost in gaseous forms, and the remainder ascribed to potential leaching loss and inorganic and microbial N in the soil. Hochmuth and Bennett (2011) used published information from numerous sources to calculate a P budget for Florida watermelons. Over the last 20 yr, the Florida watermelon crop has transitioned from using seeded to seedless cultivars. The seeds contain most of the P in the fruits and therefore a significant amount of P left the State when seeded cultivars were shipped. When farmers switched to seedless cultivars (very little P in the fruits) the P fertilizer inputs were not reduced resulting in considerable P accumulation in the Statewide budget today com-

pared with 20 yr ago. Nearly 55 times as much P was exported in the 1985 watermelon crop compared with 2008.

Importance of nutrient budgets

Nutrient budgets can provide information on surplus or deficit of nutrients with respect to crop needs. In many areas of the world, both N and P fertilizers are applied in excess of crop needs. Application rates are usually determined based on nutrient use efficiency of crops and yields. Long histories of fertilizer and other P-rich material applications have built up soil P levels in many watersheds, and the residual P may be sufficient to meet some or all the requirements of the crops grown on these soils. Agricultural scientists and some farmers use plant and soil analyses to determine the P status and requirements of crops. The most common approach is to use soil test procedures, which involve extraction of soils with selected chemicals. The amount of P extracted is related to crop yields to determine the P fertility of soils. These relationships have been developed for various crops and soil types. Calculating agricultural nutrient budgets and balances like those illustrated above will be increasingly important for determining and refining nutrient best management practices on farms. This process will take considerable and continued investment in research and education. Unless the inputs and outputs in agricultural watersheds are understood and can be demonstrated to farmers it will be difficult to make progress in reducing nutrient losses from farms (Vitousek *et al.*, 2009).

ROLE OF ISOTOPIC AND NUCLEAR TECHNIQUES IN NUTRIENT MANAGEMENT

The ^{15}N stable isotope can be used to determine N uptake from labelled N fertilizers by plants and to trace the flows of applied N as well as developing more accurate nutrient budgets. With labelled N, the researcher must know the goal of the research and select the proper labelled N form, for example N-15 nitrate ($^{15}NO_3$-N) or N-15 ammonium ($^{15}NH_4$-N) (Jankowski, Schindler and Holtgrieve, 2012). More information on the benefits and challenges with using labelled N in crop- and watershed-scale N budgets is provided by Meisinger, Calderon and Jenkinson (2008). Methodology based on the use of a novel isotope tracer, oxygen stable isotope in phosphate (delta oxygen-18 [$\delta^{18}O$-P]), to investigate P transport in soil–plant systems is currently being evaluated to bring new insights into the understanding of the processes driving P cycling in the soil and environment (Tamburini *et al.*, 2010).

CONCLUSIONS

Long-term goals of nutrient management in agricultural watersheds should include conservation and enhancement of soil fertility and soil quality. Environmental regulations and related policies to reduce nutrient loads from ecosystems should seek to improve soil quality as a first step for improving nutrient use efficiency by crops. Future soil and nutrient management practices must be compatible with extreme climatic change events. Economic values of soil ecosystem services and tradeoffs associated with changes in soil and nutrient management practices should be considered in crop production. Nutrient budgets should be determined for site-specific conditions and used in developing recommendations for sustainable production of crops while reducing nutrient loads to adjacent water bodies.

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Assessment of the Impact of Reclaimed Lands for Rice Fields on Water Budget and Quality for Sustainable Agriculture in Northeast China: Using Isotopic Tracers

Z. Pang[1,*], L. Yuan[1], T. Huang[1], Y. Kong[1], J. Li[1] and L. Luo[1]

ABSTRACT

The Sanjiang Plain in Northeast China is one of the main grain production areas in the country and is supporting a rich biological diversity. However, the wetlands and forest lands have shrunk to one fifth of their original size in the last five decades because of increasing population and land reclamation for agriculture. A major part of the reclaimed land has been used for rice production (rice wetlands). Sustainable management of these rice wetlands is important to protect water resources. Isotopic signatures of oxygen-18 (delta O-18, $\delta^{18}O$) and hydrogen-2 ($\delta^{2}H$), water chemistry and depth of groundwater were monitored on three farms, namely, Honghe (HH), Qianfeng (QF) and Qianshao (QS). Results showed that the $\delta^{18}O$ for groundwaters in all three farms varied from –8.8 per 1000 (expressed as mil, ‰) to –13.8‰ with an average of –12.4‰. However, $\delta^{18}O$ for groundwater from a single farm showed large variations, suggesting a complex source and mixing in the groundwater. Groundwater nitrate (NO_3^-) levels underneath these rice wetlands were less than baseline values (6 mg NO_3^-/L) suggesting that NO_3^- contamination of groundwater under the Sanjiang Plain wetlands is not a major environmental issue. The groundwater $\delta^{18}O$ in HH (–12.9‰) and QF (–13.0‰) farms showed that lateral groundwater flow probably dominates the groundwater recharge. However, the groundwater on the QS farm is uniformly enriched with an average $\delta^{18}O$ of –12.2‰. This suggests that the aquifer on the QS farm is probably influenced by the vertical infiltration, and there exists a strong groundwater–surface water interaction. The results have important implications for wetland reclamation and agricultural production on the Sanjiang Plain.

Key words: *isotopic signatures, groundwater, nitrate, wetlands, Sanjiang, water management.*

INTRODUCTION

Sustainable agricultural development requires optimized water management at the watershed or river basin scale in order to achieve efficient water use and sustainable agricultural productivity. Therefore, it is necessary to understand the storage and nutrient attenuation capacities of water systems and the interactions of wetlands rice. Northeast China, especially in the so-called Songhuajiang and Sanjiang catchments where there are many wetlands, is probably one of the most suitable regions for agriculture on the North China Plain (Liu *et al.*, 2005). The region is a major source of food supply for the population. The 'black soil' prevalent in the area is very rich in nutrients, and the annual precipitation is high in most part of the region. Other conditions are also favourable for agriculture. In order to ensure food security for the country, the local governments have launched plans to increase crop production in the region by an estimated five million tonnes (t) per year through improved water conservation measures. To achieve this ambitious goal means that more water will be diverted from surface and groundwater sources to meet irrigation needs in the crop areas and reclamation of more wasted land will have to be carried out in the west of Jilin Province and the low land of Heilongjiang Province. The excess water from these irrigated rice wetlands will recharge groundwater leading to a groundwater table rise and salinization risks. It will also impose an additional risk on the quality of surface water. Therefore, it is of paramount importance to assess these impacts in order to sustain the agricultural production in this area.

The Sanjiang Plain in the northeast of China, with an area of about 1.2 million ha, is the largest food supply base in China. It is famous for large-scale land reclamation and 35 percent of the wetlands have been reclaimed for rice paddies since the 1950s. In order to meet the crop water requirement for paddy cultivation, groundwater exploitation is at about 2.2 million mega litres (L/yr). This will lead to a steady decline of groundwater level and deterioration of water quality.

In short, issues of water and nutrient budgets of agricultural wetlands, as well as hydrological and biogeochemical processes within and around a wetland system require thorough investigation and understanding in order to increase the efficiency and reduce the risks of agricultural activities on surface and groundwater resources. There is a major water conservation and quality issue in the reclaimed rice lands in the Northeast China region. The question is whether or not irrigation using groundwater can be sustainable in view of the water table decline and surface water quality deterioration. The overall objective of this project is therefore to study the hydrological and biogeochemical processes in rice wetlands systems and to develop and optimize water conservation measures for sustainable agriculture in Northeast China.

1 Key Laboratory of Engineering Geomechanics, Institute of Geology and Geophysics, Chinese Academy of Sciences, Beijing 100029, China
* E-mail address of corresponding author: z.pang@mail.iggcas.ac.cn

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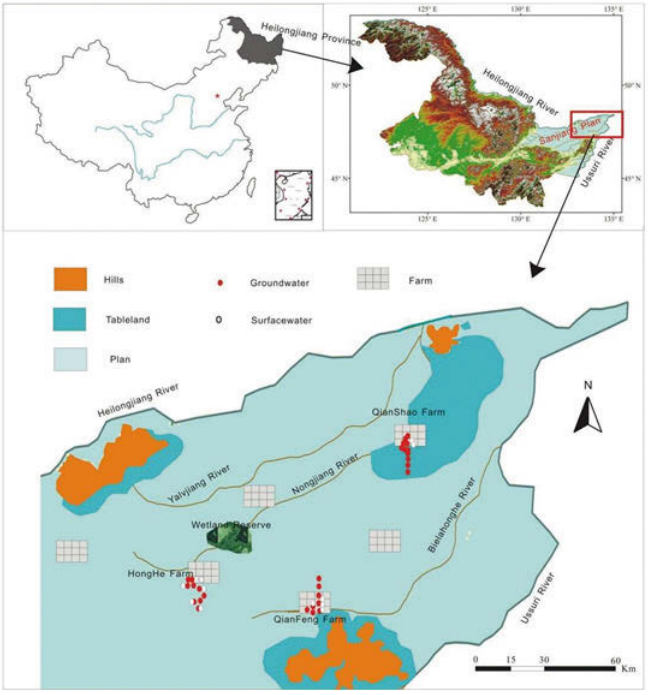


FIGURE 1. The Sanjiang Plain with the Nongjiang and Bielahonghe rivers and the three farms, namely Honghe (HH), Qianfeng (QF) and Qianshao (QS).

MATERIALS AND METHODS

Study area

The Sanjiang Plain (43°49′–48°27′N, 129°11′–135°05′E) is located in the northeastern part of Heilongjiang Province, Northeast China (Figure 1). It encompasses a total area of 6.2 million ha. The Sanjiang Plain is bounded by mountains to the west and south, and the Heilongjiang river and Wusuli river to the north and east, respectively (Figure 1). Two sites namely Heilongjiang and Jilin Provinces were selected for this study.

The elevation of the low plain ranges from 50 to 60 m, while the elevation of the highest mountain is 1 429 m. The mean annual temperature increases from 1°C in the southern mountain region to 3°C in the northern plain (Liu, 2007). The mean annual precipitation in the plain is around 600 mm, most of which falls between June and September, accounting for about 70 percent of annual rainfall. Except for the Songhua River which is a perennial river, there are many ephemeral rivers, e.g. the Nongjiang and Bielahonghe rivers running through the Sanjiang Plain (Figure 1).

The Sanjiang Plain contains a historically famous marsh, named Bei Da Huang (Huang *et al.*, 2009). In the 1940s, more than 5 million ha of marshes and wet meadows existed (Liu and Ma, 2002). However, in order to meet the food demand for the increasing population, part of the plain was reclaimed for agriculture. Thereafter, the cultivated land area increased from about 0.79 million ha in 1949 to 5.24 million ha in 2000. As a result, the wetland area has decreased from 5.35 million ha in 1949 to 0.84 million ha in 2000 (Li, Zhang and Zhang, 2002). Paddy cultivation dominates the agricultural sector, leading to considerable groundwater exploitation (6.65 mega $L{\cdot}ha^{-1}{\cdot}yr^{-1}$, corresponding to 665 mm) and fertilizer application dominates rice production with rice crop receiving 170 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ (mainly urea, diammonium and ammonium bicarbonate).

Sampling and analyses

Groundwater samples, as well as surface water in paddy fields, drainage channels, and the rivers, were taken for isotopic (^{2}H and ^{18}O) and chemical analyses from three farms (HH, QF and QS, respectively) at the northeastern part of the Sanjiang Plain in July 2009. Following preliminary interpretation of the data, a further sampling campaign was conducted in August of 2011 along a transect throughout the plain extending 250 km in an east–west direction for measuring ^{2}H and ^{18}O isotopic signatures. Two typical hydrogeological conditions, namely unconfined aquifer to the west and confined aquifer to the east are present on the Plain. Precipitation samples from the Sanjiang station (China precipitation isotope network, CPIN) were collected every month from August 2010 to July 2011. Groundwater monitoring wells were established on each farm (10, 9 and 11 wells in HH, QF and QS, respectively), and groundwater depth information was collected every year for all monitoring wells. Isotopic signatures for oxygen-18 ($\delta^{18}O$) and hydrogen-2 ($\delta^{2}H$) along with water chemistry were measured. Locations of all samples and monitoring wells are shown in Figure 1a.

Water chemistry was measured using ion chromatography (Dionex-500™) at the Beijing Research Institute of Uranium Geology. The cation measurements were based on National Analysis Standard DZ/T0064.28-93 while anion determinations were based on DZ/T0064.51-93. Alkalinity was measured on an automatic titrator (785 DMP™). Analytical precision was 3 percent based on the reproducibility of samples and standards, and the detection limit was 0.1 mg/L. The charge balance error for all samples was within ± 4 percent. Stable isotope signatures were analysed using a Picarro L1102-i laser absorption water isotope spectrometer in the Water Isotope Lab of Institute of Geology and Geophysics, Chinese Academy of

TABLE 1. Initial isotopic signatures ($\delta^{18}O$ and $\delta^{2}H$) and total dissolved solids for groundwater on three farms

Farm	Groundwater depth (m)	$\delta^{18}O$ (‰)	$\delta^{2}H$ (‰)	TDS (mg/L)
HH	15	–12.5	–93	229
QF	18	–12.8	–97	231
QS	20	–12.2	–93	136
HH-Paddy Field		–11.1	–80	103
QF Paddy Field		–10.6	–79	89
HH Channel		–9	–73	92
QS Channel		–9	–77	93

The concentrations of major cations and anions, such as Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, HCO_3^{-}, CO_3^{2-}, and SO_4^{2-} in groundwater are similar to the baseline of natural groundwater as shown in Figure 2 (Zhang, 2009)

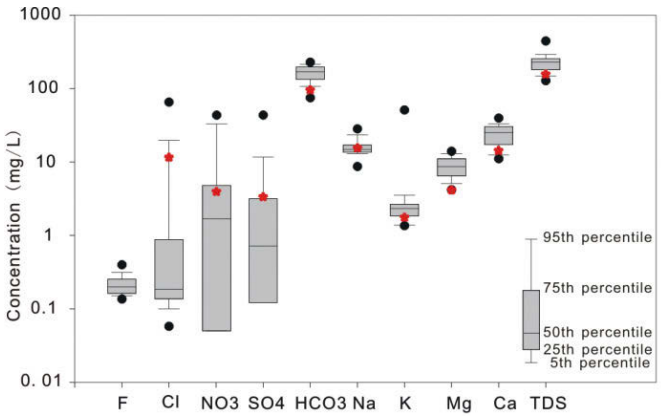


FIGURE 2. Box plots of major anions, cations and total dissolved solids (TDS) of groundwater samples. The red pentagon represents the baseline of the groundwater.

Sciences. Tritium was determined on electrolytically enriched water samples by low-level proportional counting and the results reported as tritium unit (TU) with a typical error of 1 TU. The measurement was performed at the Open Laboratory of Environmental Geology and the Central Laboratory of Hydrogeology, the Ministry of Land Resources, China.

RESULTS AND DISCUSSION

Groundwater quality

The initial groundwater data are presented in Table 1. The total amount of dissolved anions and cations (total dissolved solids, TDS) in groundwater varied widely between the three farms compared with the differences in $\delta^{18}O$ and $\delta^{2}H$ in groundwater between these farms. The channels and the paddy field also had similar $\delta^{18}O$ and $\delta^{2}H$ values as those presented in groundwater.

Concentrations of nitrate (NO_3^-) in groundwater beneath agricultural land concern people most, which is considered to be one

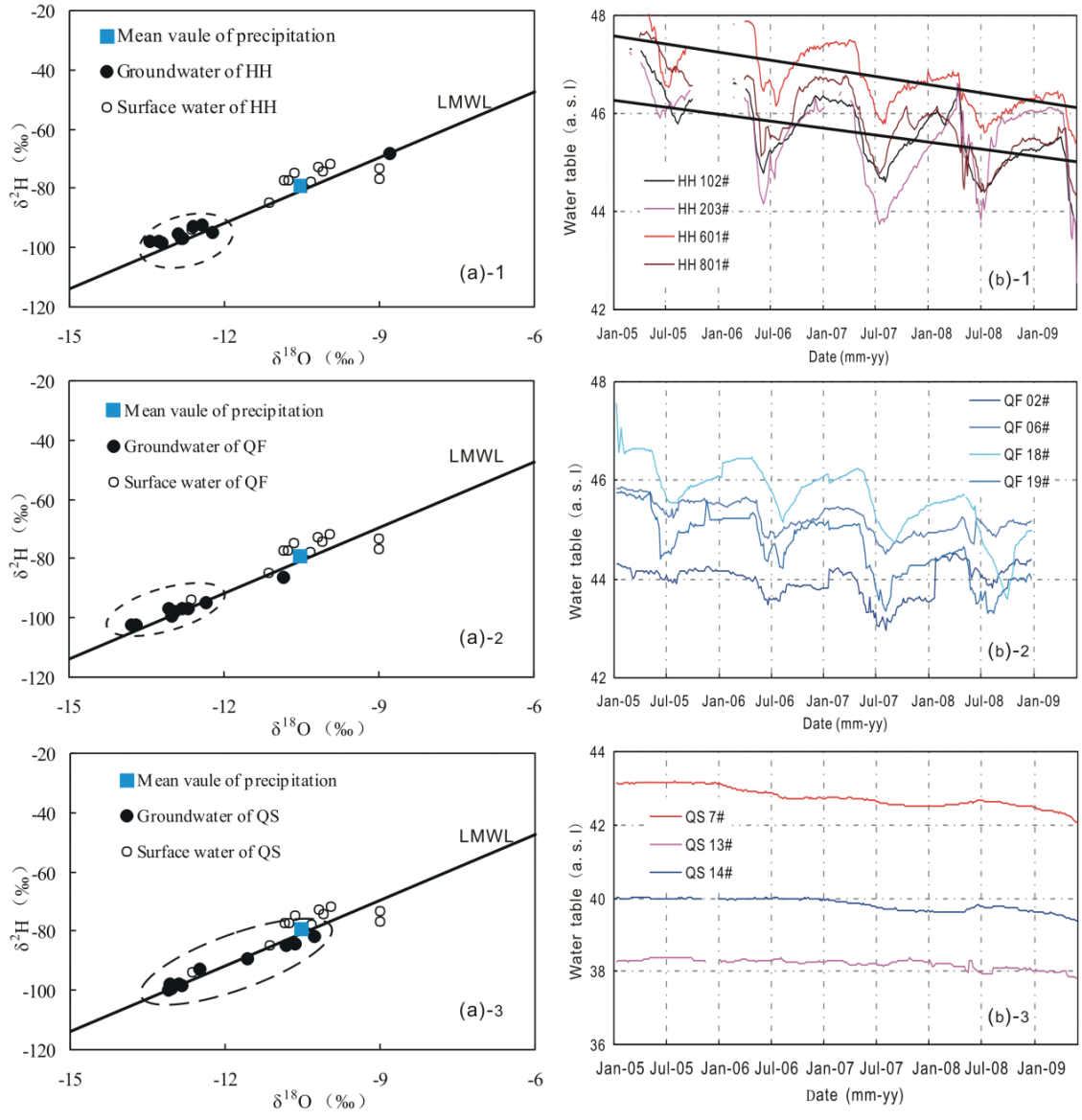


FIGURE 3. Isotopic signatures of precipitation and groundwater on Honghe (HH), Qianfeng (QF) and Qianshao (QS) farms and groundwater depth regimes (LMWL) regression of $\delta^{2}H = 7.4\ \delta^{18}O–3.1$ with a correlation coefficient of 0.8 (n = 12).

of the parameters for evaluating the sustainability of an agricultural system. About 75 percent of the groundwater samples collected from beneath the rice wetlands had NO_3^- concentrations lower than the baseline (6.6 mg NO_3^- /L). Groundwater NO_3^- ranged from values lower than the detection limit to 17.9 mg/L. The HH9 and QF7 were exceptions, having relatively higher NNO_3^- concentrations (32.9 and 43.2 mg/L, respectively during one sampling event), indicating that elevated NO_3^- input may occur locally. Possible reasons include sampling time relative to the time of fertilizer application, land management practices and probably high spatial variability of NO_3^- in the groundwater. This needs to be further investigated for the respective locations.

Groundwater recharge and residence time

Groundwater recharge

Four categories of recharge to the groundwater are possible on Sanjiang Plain including local rainfall, river recharge, irrigation returns and lateral groundwater flow (flow from the mountain areas with high altitude). According to the rainfall samples collected from Sanjiang station, modern rainfall can be characterized by the $\delta^{18}O$ and $\delta^{2}H$ values. The local meteoric water line (LMWL) is provided by the relationship $\delta^{2}H = 7.4\ \delta^{18}O - 3$ (Figure 3(a)-3).

The annual weighted rainfall $\delta^{18}O$ and $\delta^{2}H$ values in 2005 at Sanjiang station were –10.7‰ and -79.0‰, respectively. The $\delta^{18}O$ and $\delta^{2}H$ values of water for the Nongjiang River were –10.1‰ and –74.4‰, respectively and the line laid on the LMWL. The $\delta^{18}O$ values of irrigation water from rice wetlands entering the drainage channel ranged from –9.0‰ to –12.6‰, with an average of –10.4‰. The $\delta^{18}O$ for all groundwaters collected from the three farms ranged from –8.8‰ to –13.8‰ with an average of –12.4‰.

The groundwaters were therefore significantly lighter (in ^{18}O) than precipitation, river water and rice wetland and probably reflected high altitude precipitation from the higher mountain range at the west and south (data not shown).

On HH and QF farms, the $\delta^{18}O$ of groundwater ranged respectively from –13.5‰ to –12.2‰ and –13.8‰ to –12.3‰, with averages of –12.9‰ and –13.0‰, respectively. The depleted composition indicates that lateral groundwater probably dominates the groundwater recharge process on these two farms. However, there were exceptions to this observation. Water samples collected from wells HH9 and QF7 had elevated NO_3^- levels and were enriched in $\delta^{18}O$ (–8.8‰ and –10.8‰, respectively). These data suggest that the high levels of NO_3^- are probably accompanied by vertical infiltration (leaching), e.g. rainfall, irrigation, or some combination, which are characterized by enriched $\delta^{18}O$.

Oxygen isotopic composition of groundwater on the QS farm was evenly distributed and ranged from –13.1‰ to –10.3‰ with an average of –12.2‰ (Figure 3(a)–3). The groundwater on the QS farm was enriched with $\delta^{18}O$ compared with that on HH and QF farms. This enrichment suggests that the aquifer on the QS farm is probably influenced by the vertical infiltration of water (irrigation and rainfall water moving through the soil profile) with a relatively strong connection between groundwater and the surface water.

Residence time of groundwater

Tritium is one of the most important transient and ideal tracers used in hydrological research. It is produced naturally in the stratosphere by cosmic radiation on ^{14}N leading to a level of about 15 tritium units (TU) in precipitation (Brown, 1961). The substantial input during late 1950s and early 1960s created a tritium reservoir in the stratosphere, which has contaminated global precipitation systems for over four decades and provides a useful tracer for water originating from this period. A recharge date can be estimated from a decay line, which is constructed using the input function and decay equation with a half-life of 12.32 years (Unterweger *et al.*, 1980). However, there are strong variations in the global distribution of tritium. Circulation in the stratosphere is constrained with respect to latitude, resulting in a latitudinal banding of tritium in rainfall (Eriksson, 1965).

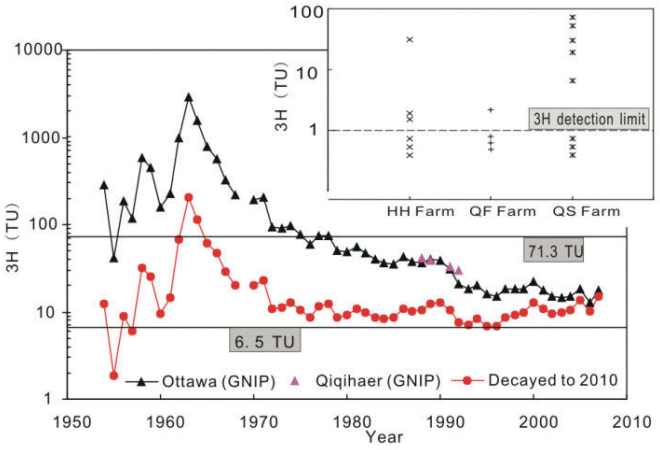


FIGURE 4. Tritium in groundwater, precipitation from 1954 to 2007 and that decayed to 2010.

Figure 4 shows that groundwater recharged before the "bomb test" has tritium levels lower than 6.5 TU. Groundwater with high levels of tritium indicates the influence of the "bomb test" (Figure 4). Tritium levels in the groundwater from HH and QF farms were low, with a narrow range of < 1–1.9 and < 1–2.2 TU respectively, indicating that groundwater on HH and QF farms is older than 50 years.

Groundwater on the QS farm showed a wide range of tritium (< 1–71.3 TU), but levels of tritium are related to the sampling locations. Samples with high levels of tritium (6.5–71.3 TU) were collected near the river, and those collected away from the river showed low levels (< 1 TU). This suggest that groundwater near the river has a relatively short residence time (recharge occurring).

Figure 3 shows the fluctuations in groundwater levels recorded in the period from 2005 to 2009 in the 11 monitoring wells. The measurements highlight the existence of two different groundwater regimes on the three farms. As shown in Figures 3b1 and 2, groundwater levels on HH and QF farms showed similar dynamics. Groundwater levels in these two farms changed significantly and had an intra-annual scale that fluctuated with the groundwater exploitation. On the other hand, groundwater levels in the QS farm declined moderately and the intra-annual fluctuation could not be found. Different regimes suggest that groundwater is more easily recharged at the QS farm, following a short groundwater residence time.

The one exception (HH8) at the HH farm with a tritium level of 31.3 TU further proves the hypothesis that NO_3^- is brought by vertical infiltration. However, groundwater samples with elevated tritium collected at the QS farm were consistently enriched in ^{18}O, but had low levels of NO_3^- (< 17.9 mg/L). A probable explanation is that the source of recharge (precipitation and surface waters) had low levels of NO_3^- (< 6.0 mg/L).

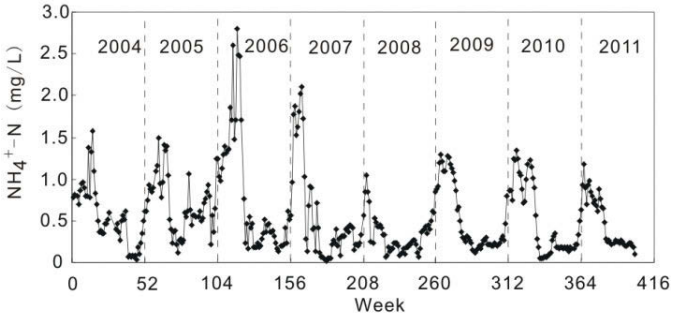


FIGURE 5. Ammonium in the Songhua river (Tongjiang transect) as measured in weekly samples. (Data obtained from the Ministry of Environmental Protection of the People's Republic of China).

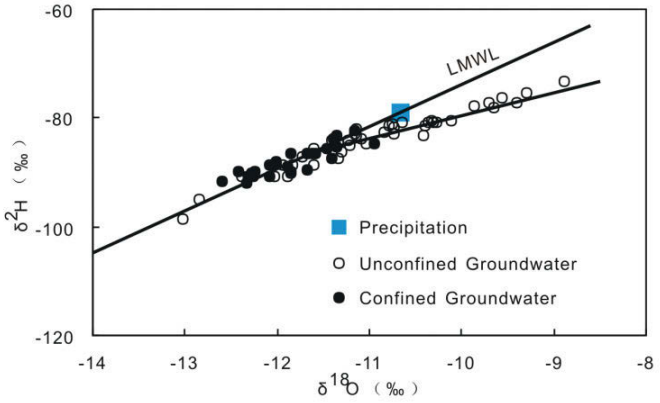


FIGURE 6. Plot of $\delta^{18}O$ and δ^2H of groundwaters from the Sanjiang Plain.

Implications for agriculture

Nutrient retention capacity of wetland

In April, with N applications amounting to 170 kg/ha (urea or ammonium), the concentrations of ammonium (NH_4^+) and NO_3^- in paddy fields can reach up to 24.2 mg N/L and 3.4 mg·N/L, respectively. However, $NH_4^+$was found in the Songhua River only during the first few weeks of the year (Figure 5). The average concentration of NH_4^+in river water from June to December was 0.3 mg·N/L, and the drinking water standard for NH_4^+ is 0.5 mg N/L. The time of first occurrence of elevated NH_4^+ in water could be due to the leaching of the applied urea fertilizer.

During the growing period, groundwater is pumped to the paddy fields, after which it flows into a small drainage channel and to the river. Compared with the elevated NH_4^+ in paddy fields during the growing season (from May to September), the river NH_4^+ levels were low suggesting riverine wetland might have taken most NH_4^+ (Zedler, 2003; Mander, Hayakawa and Kuusemets, 2005). One of the functions of wetland is to remove nutrients (Maltby, Digby and Baker, 2009). Greater nutrient retention of the catchment would be attributable to the high coverage of natural wetland on the Sanjiang Plain. Natural wetland covers about 13 percent of the catchment area. Studies by Mitsch, Day and Gilliam (2001) and Arheimer *et al.* (2005) showed significant increases in water quality at the catchment scale when wetland accounts for about 2–7 percent of the total catchment.

Spatial arrangement of wetland reclamation

Stable isotope signatures of groundwaters collected from unconfined and confined areas displayed significant differences. In the confined area, $\delta^{18}O$ values of groundwaters were depleted (average of –11.8‰) compared with the local weighted mean value for the rainfall (–10.7‰). On the other hand, in the unconfined area, the $\delta^{18}O$ for groundwaters varied widely (–8.9‰ to –13.0‰), and a plot along a line with a slope of 5.3 had an intercept on the local meteoric water line at –11.8‰ (Figure 6), which is identical to the mean value of groundwaters sampled in the confined area. These data suggest that the Sanjiang Plain aquifer is significantly recharged by the high altitude precipitation from the surrounding mountain which is characterized by depleted isotopic signatures. However, in the unconfined area, extra recharge exists and the groundwater system is recharged from the land surface by rainfall or by infiltration from rivers, irrigation or a combination of each.

CONCLUSIONS

The stable isotopic signatures, tritium and water chemistry data demonstrate that the use of reclaimed land for rice production on the Sanjiang Plain is sustainable as far as water pollution is concerned as it has not affected the quality of groundwaters even though 170 kg $N \cdot ha^{-1} \cdot yr^{-1}$ as composite fertilizer is being applied. Limited vertical infiltration (leaching) of water from rice wetlands reduced the influence of agricultural activities on the NO_3^- contamination of groundwaters. The low levels of NH_4^+ in surface waters (wetlands and channels) and of NO_3^- in groundwaters near the river are probably attributable to NO_3^- and NH_4^+ retention in wetlands. With certain wetland coverage, groundwater quality is safe on the Sanjiang Plain. However, with limited recharge, groundwater levels will continue to decline, imposing a risk on sustainable agriculture. Therefore integrated use of surface water and groundwater is recommended.

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Gaseous Nitrogen Fluxes and Nitrous Oxide Isotopic Signatures in Riparian Grey Alder Forests

Ü. Mander[1, 2,*], R. Well[3], D. Weymann[4], K. Soosaar[1], M. Maddison[1], A. Kanal[1], A. Kull[1], K. Lõhmus[5], J. Truu[1] and J. Augustin[6]

ABSTRACT

Nitrous oxide (N_2O) and nitrogen (N_2) gas emissions and isotopic signatures of N_2O and nitrate (NO_3^-) in groundwater of two differently loaded riparian grey alder stands in southern Estonia were investigated over a period of nine months. One area was a 38-year-old stand in Porijõgi (PJ), where uphill agricultural activities had been abandoned since the middle of 1990s, and the second area was a 55-year-old alder stand in Viiratsi (Vi), which still receives polluted lateral flow from uphill fields applied with pig slurry. Gas fluxes were measured in six sampling sessions, and water samples were analysed for nitrate (NO_3^-), N_2, N_2O, and isotopic signatures of oxygen-18 (delta ^{18}O, $\delta^{18}O$) and nitrogen-15 (delta ^{15}N, $\delta^{15}N$) in N_2O and NO_3^- in four of the six sessions. The N_2O and N_2 fluxes from both riparian zones did not differ significantly, being 9.6 ± 4.7 and 14.5 ± 3.9 μg N_2O–N $m^{-2}{\cdot}h^{-1}$, and 2 466 ± 275 and 3 083 ± 371 μg N_2–N $m^{-2}{\cdot}h^{-1}$ in PJ and Vi sites respectively, suggesting that gaseous N_2 is the dominant gas emission from these alder stands. The isotopic signatures of N_2O and NO_3^- were not significantly different between PJ and Vi study sites suggesting possible conversion of NO_3^- to N_2O in both areas. The greater prevalence of N_2 emissions over N_2O in both areas, and the strong relationship between NO_3^- and N_2O concentrations ($r = 0.92$, with $p < 0.01$) further suggested that denitrification is the main source of N_2O and N_2 fluxes in these grey alder stands. The dominant emission of N_2 over N_2O showed that these riparian zones play an important role in reducing the emissions of N_2O while removing NO_3^- from water.

1 Institute of Ecology and Earth Sciences, University of Tartu, 51014 Tartu, Estonia
2 Hydrosystems and Bioprocesses Research Unit, National Research Institute of Science and Technology for Environment and Agriculture (Irstea), 1 rue Pierre-Gilles de Gennes CS 10030, F92761 Antony cedex, France
3 Institut für Agrarrelevante Klimaforschung, Johann Heinrich von Thünen -Institut, 38116 Braunschweig, Germany
4 Forschungszentrum Jülich IBG-3, Wilhelm-Johnen-Straße, 52428 Jülich, Germany
5 Institute of Ecology and Earth Sciences, University of Tartu, 51005 Tartu, Estonia
6 Institute of Landscape Matter Dynamics, Leibniz-Centre for Agricultural Landscape and Land Use Research (ZALF), D-15374 Müncheberg, Germany
* E-mail address of corresponding author: ulo.mander@ut.ee

Key words: *denitrification, nitrogen, isotopic signatures, nitrate, nitrification, nitrous oxide, site preference.*

INTRODUCTION

Riparian ecosystems are important landscape elements that control water quality in rivers and other water bodies but they are also potential hot-spots of nitrous oxide (N_2O) emission to the atmosphere (Villain *et al.*, 2012). Nitrous oxide plays an important role in altering stratospheric chemistry, including depletion of the ozone layer. The radiative forcing of N_2O is 296 times higher than that of the same amount of carbon dioxide (CO_2), and is therefore a potent greenhouse gas (GHG). Despite its relatively minor contribution to global warming (6 percent), a small increase in emissions can lead to a large accumulation of N_2O in the troposphere, a phenomenon resulting from the long residence time of N_2O, approximately 120 years (Forster *et al.*, 2007). Nitrous oxide is produced by (i) reduction of nitrate (NO_3^-) to nitrogen gas (N_2), and (ii) oxidation of ammonium hydroxylamine (NH_2OH) to nitrite (NO_2^-), and the reduction of NO_2^- to N_2O and N_2 under aerobic conditions. Apportioning N_2O to these source oxidation-reduction processes is a challenging task. A better understanding of the N_2O processes is, however, required in order to improve mitigation strategies (Well *et al.*, 2012).

Considerable NO_3^- reduction is possible, especially in agricultural areas with high N fertilizer inputs. Dinitrogen (N_2), the main gaseous component of Earth's atmosphere, is the final product of this process, and thus the quantification of groundwater N_2 arising from denitrification (excess N_2) can facilitate the reconstruction of historical N inputs, because NO_3^- loss is derivable from the sum of denitrification products (Weymann *et al.,* 2008). The concentration of excess N_2 produced by denitrification in groundwater is estimated by comparing the measured concentrations of argon (Ar) and N_2 with those expected from atmospheric equilibrium, assuming that the noble gas argon (Ar) is a stable component (Weymann *et al.,* 2008). It is also very important to consider the excess N_2 value when calculating indirect N_2O emission from the aquifer resulting from NO_3^- leaching (Weymann *et al.*, 2008).

It has been suggested that the information obtained from measuring the intra-molecular distribution of ^{15}N on the central (α) and the end (β) position of the linear N_2O molecule is crucial for a better understanding of the apportioning of N_2O between nitrification and denitrification, but also source and sink processes (Toyoda *et al.*, 2011). The N_2O site-specific ^{15}N signatures from denitrification and the NH_2OH to N_2O pathway of nitrification have been shown to be clearly different, making this signature a potential tool for N_2O

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source identification. Most published studies have been dedicated to the analysis of ^{15}N and ^{18}O isotope and isotopic signature ($\delta^{15}N_{\alpha}$ and $\delta^{15}N_{\beta}$) of emitted N_2O (Toyoda *et al.,* 2011), while there are only a limited number of studies dedicated to the analysis of dissolved N_2O in groundwater (Well *et al*., 2012). The main objective of this study was therefore to compare gaseous N_2O and N_2 fluxes with the isotopic signatures of N_2O and NO_3^- in the groundwater of two differently loaded riparian alder stands in southern Estonia.

MATERIALS AND METHODS

Study sites

The study areas are (i) a 38-year-old stand in Porijõgi (58°12′41″N, 26°46′55′E), in which uphill agricultural activities had been abandoned since the middle of the 1990s, and (ii) a 55-year-old stand in Viiratsi (58°20′N, 25°39′20″E), which still receives polluted lateral flow from uphill fields fertilized with pig slurry (Figure 1). The estimated lateral N inflow in Viiratsi is twice as high as in Porijõgi (Soosaar *et al.,* 2011).

In the Poriõgi area, a 20-m-wide grey alder stand grows on a Thapto-Mollic (Endogleyic) Gleysol with groundwater table depths of 0–0.8 m and 0–0.1 m in the upper and lower sites, respectively. In Viiratsi, a 12-m-wide wet patch (*A. incana — Filipendula ulmaria*) on Mollic Gleysol (considered as 'upper' site with groundwater table depth 0–0.05 m) is followed by a 28-m-wide grey alder forest on Thapto-Mollic Endogleyic Umbrisol (considered to be a 'lower' site with groundwater depth of 0–0.5 m; Figure 1). At each study site in both areas, 50 mm water sampling wells and collars for gas sampling chambers were installed.

Gas sampling and analyses

The closed-chamber method was used for the measurement of N_2O fluxes, and the helium–oxygen (He–O) method (Teiter and Mander, 2005) was used for the measurement of N_2 emissions. Gas samplers were installed in five replicates at upper and lower sites in both the Porijõgi and Viiratsi study areas (Figure 1). During each gas sampling session at each microsite, the depth of the groundwater table (cm) in observation wells (using 50 mm internal diameter, 1.5 m deep PVC pipes perforated and sealed in a lower 0.5 m part), and soil temperature were measured at three depths (0–10, 20–30 and 30–40 cm).

Gas sampling was carried out once a month in April, May, July, August, October, November and December 2008 using standard gas collection procedures (Mander *et al.,* 2003). The soil temperature, redox potential and water depth in the sampling wells were measured simultaneously, and the NH_4^+–N and NO_3^- –N concentrations in the soil samples were analysed using standard methods (APHA, 1989). The gas concentration in the collected air was determined using the Shimadzu 2014 gas chromatographic system.

Intact soil cores (0–10 cm) were taken from the sites in which the gas samplers were installed. Soil samples were collected immediately after gas sampling. Soil samples were weighed, kept at low temperature (4°C) and transported to the laboratory for N_2 and N_2O measurements by the He–O method (Soosaar *et al.,* 2011).

Water sampling and analyses

For the analysis of N_2O, N_2, NO_3^- and their isotopic signatures, water samples were taken in 4–9 replicates from water sampling wells using a peristaltic pump. The samples were stabilized with the addition of 0.1 ml saturated mercuric chloride ($HgCl_2$) solution. Nitrous oxide, N_2 and ammonium (NH_4^+) were measured using standard procedures. The N_2 produced from denitrification was calculated using the method of Weymann *et al.* (2008).

Initial water nitrogen concentration as nitrate (NO_3^--N) at a given location ($cNO_3^-{}_{t0}$) on the aquifer surface is defined by the NO_3^--N concentration of the recharging water before alteration by denitrification in the groundwater (Weymann *et al.,* 2008). It is assumed that NO_3^- consumption on the groundwater flow path between the aquifer surface and a given sampling spot originates from denitrification. The process results in the accumulation of gaseous denitrification products (N_2O and N_2) in groundwater. The concentration of N_2 in groundwater is referred to as Excess N_2. From this, $cNO_3^-{}_{t0}$ can be calculated as the sum of the residual substrate and accumulated products (Weymann *et al.,* 2008). Thus $cNO_3^-{}_{t0}$ is given by the following equation:

$$cNO_3^-{}_{t0} = Excess\ N_2 + cNO_3^- + cN2 \quad (1)$$

where cNO_3^- is residual NO_3-N; and cN_2O is N_2O-N concentration (i.e. N concentration as N_2O) in the groundwater.

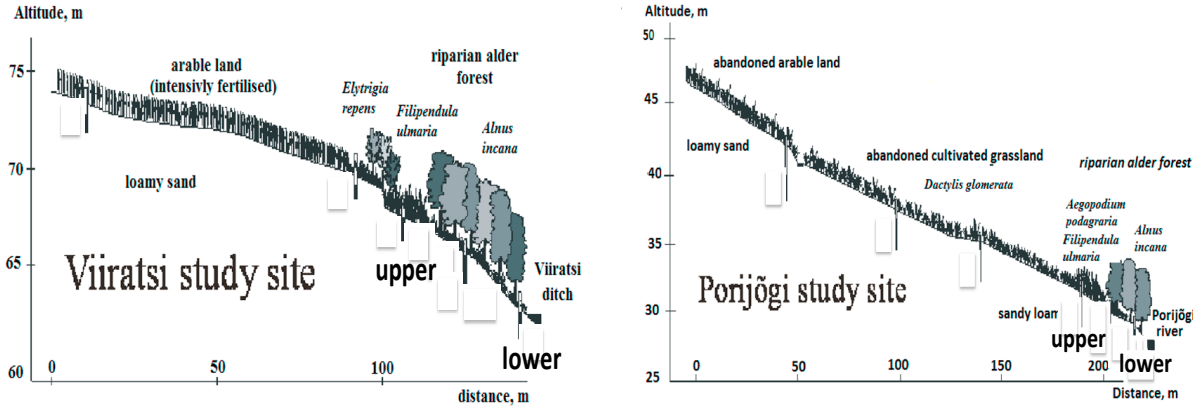


FIGURE 1. Illustrations of riparian study sites in Estonia.

Reaction progress (RP), the ratio between the products and the starting material of a process can be used to characterize the extent of NO_3^- elimination by denitrification. It is calculated as follows:

$$RP = \frac{Excess\ N_2 + cN_2O}{cNO^-_{3\,to}} \quad (2)$$

In this study, all of the concentration values are calculated to mg/L.

Isotope analyses

The isotopic signatures ($\delta^{18}O$ and of $\delta^{15}N$) in N_2O, the bulk ^{15}N (the total ^{15}N signature in N_2O, $^{15}N^{bulk}$-N_2O) and ^{15}N from the central N position ($^{15}N^{\alpha}$), were measured using isotope ratio mass spectrometry (Well *et al.*, 2012).

The site preference (SP‰) ^{15}N (i.e. the ^{15}N in the N^{β} position of N_2O) was obtained using the following equation:

$$SP = 2(^{15}N^{\alpha} - {}^{15}N^{bulk}N_2O) \quad (3)$$

The isotopic ratios of a sample (R_{sample}) were expressed as the deviation from the $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios of the reference standard materials (R_{std}), atmospheric N_2 and standard mean ocean water (SMOW) respectively as

$$\delta X = \left(\frac{R_{sample}}{R_{std}} - 1\right)1000 \quad (4)$$

where X = $^{15}N^{bulk}$-N_2O, $^{15}N^{\alpha}$, $^{15}N^{\beta}$, or ^{18}O.

Statistical analysis of data

Linear correlation analysis and a *t*-test were used to compare the relationship between the variables. For all cases, the significance value of $p < 0.05$ was accepted.

RESULTS

Gaseous nitrogen fluxes

The average values of N_2O and N_2 fluxes from both riparian zones did not differ significantly throughout the whole study period (9.6 ± 4.7 and 14.5 ± 3.9 μg N_2O–N $m^{-2}{\cdot}h^{-1}$ and 2 466 ± 275 and 3 083 ± 371 μg N_2–N $m^{-2}{\cdot}h^{-1}$ in Porijõgi and Viiratsi, respectively). The N_2:N_2O ratio in Viiratsi (278 ± 60) was significantly lower than in Porijõgi (995 ± 360) (Figure 2).

Different nitrogen forms in water samples

The average N concentrations as NH_4^+(NH_4^+–N) in groundwater for the whole study period in Viiratsi were significantly lower than those in Porijõgi (Figure 3a). The measured groundwater NO_3^--N and N_2O-N concentrations were significantly lower in Porijõgi than in Viiratsi (Figure 3a).

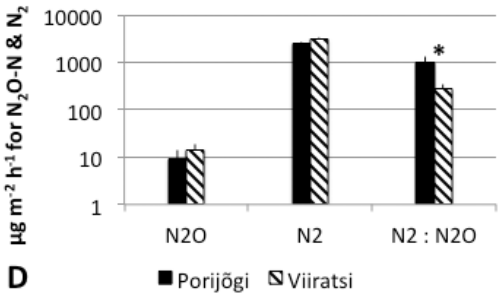


FIGURE 2. N_2O and N_2 emissions and N_2:N_2O ratios averaged over the whole study period (* indicates significant at $p < 0.05$).

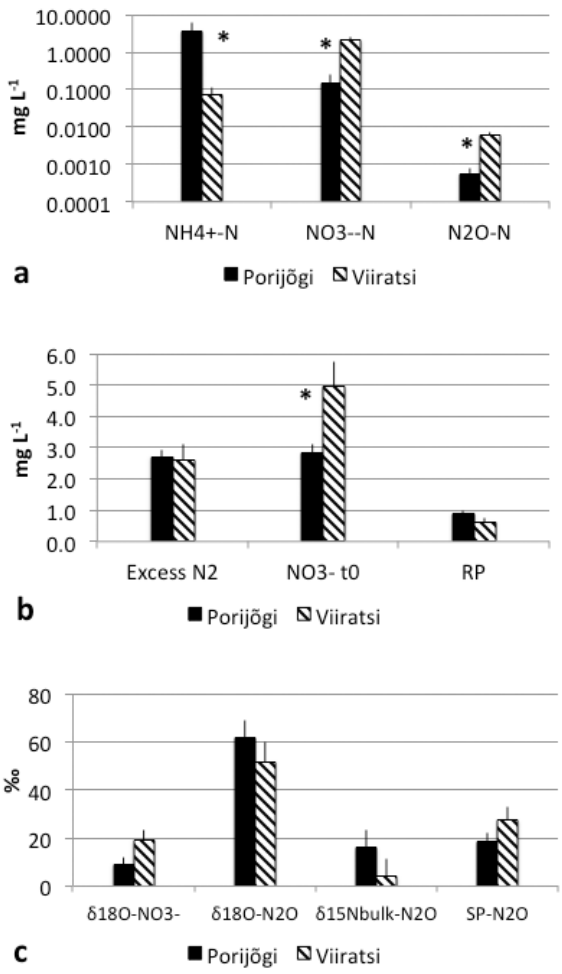


FIGURE 3. NH_4^+-N, NO_3^--N, N_2O-N (a); excess N_2, initial NO_3^--N ($NO_3^-t_0$), reaction progress (RP) (b); $\delta^{18}O$-NO_3^-, $\delta^{18}O$-N_2O, $\delta^{15}N^{bulk}$-N_2O, and site preference of the N_2O molecule (SP-N_2O) (c) in Porijõgi and Viiratsi (* indicates significant at $p < 0.05$).

The excess N_2 concentration in both Porijõgi and Viiratsi was quite similar (Figure 3b). The average value of $NO_3^-{}_{t0}$ was significantly higher in Viiratsi than in Porijõgi (Figure 3b). The RP value was also not significantly different between Porijõgi and Viiratsi (Figure 3c)

Isotopic signature ($\delta^{18}O$ in NO_3^-, $\delta^{18}O$ in N_2O, and $^{15}N^{bulk}$ in N_2O) values were not significantly different between Porijõgi and Viiratsi (Figure 3c). Also there was no significant difference in isotopic signature of SP-N_2O between Porijõgi and Viiratsi (Figure 3c).

Relationship between excess N_2, different nitrogen forms in groundwater and N_2O isotopic signatures

Groundwater N_2O-N correlated negatively to reaction progress (RP) (Figure 4A). The excess N_2 related positively to N_2O (Figure 4B), while the site preference (SP) N_2O was correlated negatively to the bulk ^{15}N in N_2O (Figure 4C) and positively to the $\delta^{18}O$ of NO_3^- (Figure 4D). The bulk ^{15}N in N_2O also correlated negatively to $\delta^{18}O$ of NO_3^- values (Figure 4E).

N_2O emissions correlated positively with NO_3^--N, N_2O-N and $NO_3^-{}_{t0}$ concentrations in groundwater (Table 2). Similarly, a signifi-

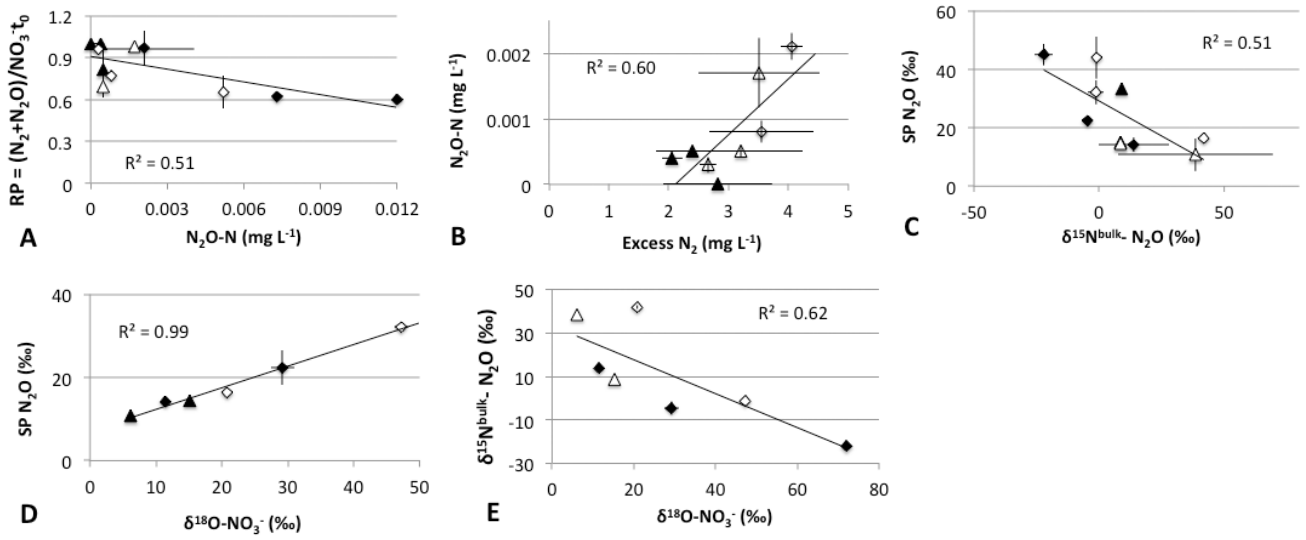


FIGURE 4. Correlation between N_2O, reaction progress (RP), excess N_2, bulk ^{15}N in N_2O, site specific (SP) N_2O, $\delta^{18}O$ in NO_3^- in Porijõgi (triangles) and Viiratsi (diamonds) in Estonia (The open and closed symbols refer to upper and lower sites at each location).

TABLE 2. Correlation coefficients between N_2O and N_2 emission, water characteristics (* = p < 0.05; ** = p < 0.01)

	NO_3^--N	N_2O-N	NO_3^--t_0
N_2O emissions	0.53*	0.66*	0.92**
N_2 emissions	0.66*	0.86**	0.48

cant positive correlation was also found between N_2 emissions and NO_3^--N and N_2O-N concentrations (Table 2).

DISCUSSION

Gaseous nitrogen fluxes

Due to intensive N cycling in alder stands, gaseous N fluxes in these ecosystems are also intensive. In a long-term study (2001–2009) on gaseous N fluxes from the Porijõgi and Viiratsi areas, the range of N_2O was from -0.6 to 87 μg N_2O-N $m^{-2}{\cdot}h^{-1}$ in Porijõgi and 0.5–38 μg N_2O-N $m^{-2}{\cdot}h^{-1}$ in Viiratsi, showing somewhat higher to medium values in Viiratsi (1.7 and 2.1 μg N_2O-N $m^{-2}{\cdot}h^{-1}$ for Porijõgi and Viiratsi respectively). The N_2:N_2O ratio, however, was significantly higher in Porijõgi, ranging between 10–7600 and 40–1200 in Porijõgi and Viiratsi correspondingly (Soosaar *et al.,* 2011). These results are consistent with earlier studies carried out in Porijõgi and Viiratsi (Teiter and Mander 2005). On the other hand, N_2:N_2O ratio in riparian alder forests was up to two magnitudes higher than that reported for fertilized fields (Bol *et al.*, 2003). These high ratios can probably be attributed to relatively long residence time of N_2O due to low diffusivity of N_2O in wet soils and/or further conversion of N_2O to N_2.

Different N forms in water samples

In Viiratsi, NH_4^+-N concentrations were always very low (< 0.5 mg/L), whereas in Porijõgi, high concentrations were recorded in the upper site in May (3.5 ± 2.7 mg/L) and October (22.3 ± 5.7 mg/L). This might be related to the lower groundwater NH_4^+-N level during these sampling sessions. Elevated NO_3^--N (> 1 mg/L) has mostly been found in Viiratsi, but not in Porijõgi. Possible reasons for the relatively low NO_3^- levels in Porijõgi are inhibited nitrification under saturated conditions, NO_3^- leaching and intense denitrification. The excess N_2 ranged from 1.5 to 4.5 mg/L at most sites. The highest values were observed in Viiratsi, which coincided with higher NH_4^+-N and NO_3^--N levels. This can be considered as evidence of intense denitrification with associated N_2O formation (Well, Weymann and Flessa, 2005) and is supported by the higher N_2O-N levels in Viiratsi, with concentrations mostly >10 μg/L and up to 100 μg/L compared with Porijõgi.

Isotopic signatures of NH_4, NO_3^- and N_2O

High values of $\delta^{18}O$ in N_2O (> 40‰) measured in the field are typical for N_2O production by denitrification in aquifers (Well *et al.,* 2012). Such values were also found in Viiratsi at times of elevated NO_3^- levels suggesting that N_2O emission processes are similar and are related to NO_3^- levels. The $\delta^{18}O$ in NO_3^- (varying from 6 to 72‰) reported in this study are comparable with those published in earlier studies on the $\delta^{18}O$ of NO_3^- in groundwater under agriculture (Well *et al.*, 2012)

The comparison between $\delta^{15}N$ in NO_3^- and the bulk ^{15}N in N_2O ($\delta^{15}N^{bulk}N_2O$) shows that the difference between these values is approximately 20 to 30‰, which is in line with the isotopic signatures recorded during NO_3^- reduction to N_2O via denitrification, suggesting that denitrification may be the main process responsible for N_2O emissions in alder stands Data from this study showed that $\delta^{18}O$ in N_2O was greater than 35‰ and SP was greater than 10‰. This is indicative of the production of N_2O by denitrification and the partial reduction of N_2O to N_2 (Well *et al.,* 2012).

The relationship between emitted N_2, different N forms in groundwater and N_2O isotopic signature

A significant negative correlation was found between N_2O-N concentrations in gas samples collected from chambers and RP (Figure 4A), which is typical evidence for the domination of denitrification processes in aquifers (Well *et al.*, 2012). Another indicative characteristic of denitrification was the strong positive correlation (R^2 = 0.99) between $\delta^{18}O$ in NO_3^- and SP values (Figure 4D). However, Porijõgi, with its high $\delta^{18}O$ and low SP values, seems to be different. The low

SP could be explained by nitrification–denitrification with little reduction to N_2. Therefore this might be related to the changing water table in the upper site that temporarily allows sufficient aeration of soil and consequently enhances nitrification.

Perspectives for further studies

The increased N_2 and N_2O emissions in th 1e Viiratsi study area as shown in this study may be a result of the age (> 50 years) of the grey alder stand compared with that at Porijõgi (38 years old), but may also be caused by the long-term nutrient load of this riparian alder stand (Soosaar *et al.*, 2011). However, over time the buffering capacity of continuously loaded riparian buffers will decrease, requiring careful management of these riparian forests (e.g. selective cutting of older trees). Further studies are recommended to clarify the impact of age and environmental stress factors on denitrification of the riparian buffer ecosystems.

In general, for a better understanding of the relationship between nitrification–denitrification processes and in order to distinguish between N_2O sources in riparian zones and wetlands in general, a more detailed and long-term comparison is needed of potential lateral N_2O fluxes (groundwater discharge) using isotopic signatures of both water and gaseous emissions. The latest development in research technology allows the use of novel laser spectroscopic techniques for the continuous analysis of N_2O isotopic signatures *in situ* (Köster *et al.,* 2013). This would open up new horizons in isotope studies in all ecosystems.

CONCLUSIONS

The main gaseous flux from both riparian alder stands was in the form of N_2, which was 278 (Viiratsi) to 995 times (Porijõgi) higher than the amount of N_2O emitted. Nitrous oxide accumulation in the groundwater was moderate, i.e. not higher than typical values in NO_3^-contaminated denitrifying aquifers. Therefore the fluxes of N_2O along with water from both study areas were small in comparison with surface fluxes. The dynamics of N_2O turnover are similar to denitrifying aquifers, with the lowest N_2O accumulation at the start and end of the reaction progress. Site preference signatures are higher than those of N_2O from unsaturated soils, confirming that denitrification in the saturated zone exhibits a broad range of SP with most values > 30‰ (Well *et al*., 2012). Both N_2:N_2O ratios and isotope data suggest that the main source of N_2O in both areas is denitrification. Due to the more fluctuating groundwater depth in Porijõgi, a significant part of N_2O may be produced by nitrification, at least temporarily. This study also confirms that isotopic signatures of N_2O may be used to distinguish N_2O fluxes from ecosystems with unsaturated and saturated groundwater situations. Further, the study showed that in riparian alder stands saturated with water, N_2 is the predominant denitrification product compared with N_2O which is a significant boost to N_2O emissions to the atmosphere.

ACKNOWLEDGEMENTS

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Importance of Near and In-stream Zones in Small Agricultural Catchments to Buffer Diffuse Nitrogen Pollution

G. Pinay[1,*], E. Hamilton[2], M. Sébilo[3], J. Pritchard[2], A. Bale[2] and M. Furey[2]

ABSTRACT

Riparian ecosystems play an important role in removing nitrogen (N) from water and improving water quality downstream. However, factors influencing N retention in these water conservation areas are not fully understood, hindering effective management and their potential use for N removal. The objective of this paper is to evaluate the N buffering capacity of landscape features, including riparian zones, using isotopic signatures of nitrogen-15 (delta ^{15}N, $\delta^{15}N$) of aquatic and riparian vegetation as an indicator for determining sites of biogeochemical N transformation. Studies in fourteen countries across Europe with a range of climatic conditions showed that nitrate (NO_3^-) removal by riparian buffer zones (plant uptake and denitrification) varies from 0 to 30 percent (%), depending on NO_3^- loading to these zones and the local hydraulic gradient rather than climatic conditions. The negative relationship between in-stream NO_3^- concentration and the $\delta^{15}N$ of diatoms in water showed that the ^{15}N natural abundance in diatoms can be used as a proxy for in-stream denitrification and point source pollution of N. In flood plains, the percentage reduction in NO_3^- along the flow path and an increased $\delta^{15}N$ in the remaining NO_3^- suggest NO_3^- is lost through denitrification. However, the increased ^{15}N in the vegetation showed that plants compete with denitrification for NO_3^- . The isotopic signature of ^{15}N is an important tool to assess in-stream, riparian zone and flood plain denitrification thus allowing us to improve management practices for reducing the fluxes from croplands to streams.

Key words: *wetland, riparian zone, denitrification, natural isotopic abundance.*

INTRODUCTION

River ecosystems control the transport of nutrients and organic matter from terrestrial sources (Townsend-Small, McClain and Brandes, 2005), produce organic material within aquatic environments, degrade organic matter while transporting it downstream (Hedges *et al*., 2000) and carry the fingerprint of human activities (Rosenberg, McCully and Pringle, 2000). Floodplains and in-stream zones are the key components of river ecosystems controlling these functions (Fischer *et al*., 2005). These riverine landscape features act as biogeochemical hot spots, in particular for nitrogen (N) cycling (Forshay and Stanley, 2005). They also represent functional retention areas (Carling, 1992) which control and maintain river water quality (Pinay and Décamps, 1988). At the landscape scale, three fundamental interrelated principles regulate the cycling and transfer of carbon and nutrients in rivers ecosystems. The first principle, i.e. connectivity, is related to the delivery patterns of carbon and nutrient inputs controlled by the flow regime along river ecosystems. River systems and their retention zones can be viewed as open ecosystems dynamically linked longitudinally, laterally and vertically by hydrologic and geomorphologic processes (Ward, 1989). The second basic principle is that the area of water-substrate interface (i.e. water-sediment or wetland-upland length of contact) is correlated positively with the efficiency of nutrient retention and use in river ecosystems. These positive relationships occur both in the main channel itself and in the riparian and floodplain zones (Jones and Holmes, 1996). The third principle is related to the role of water levels, especially flow and flood pulses affect N cycling in alluvial soils by controlling the duration of oxic and anoxic phases.

THE ROLE OF RIPARIAN ZONES AS NITROGEN BUFFERS

Numerous studies have demonstrated that groundwater NO_3^- concentrations may decrease substantially as water moves through riparian ecosystems before being discharged into streams (Burt, Pinay and Sabater, 2010). As a result, there is considerable interest in exploiting the N 'filtration' capacity of riparian ecosystems in order to improve surface water quality (Figure 1). However, there is still much uncertainty about the mechanisms and controls of N retention in riparian ecosystems, thus hindering their potential use and effective management.

Yet, the evaluation of the N buffering capacity of riparian zones is not a trivial task. For example, in a pan European study, Sabater *et al*. (2003) evaluated N removal efficiency by riparian buffers at 14 sites scattered throughout seven European countries subject to a wide range of climatic conditions. The sites also had a wide range of NO_3^- inputs, soil characteristics and vegetation types. Dissolved forms of N in groundwater and associated hydrological parameters were measured at all sites; these data were used to calculate NO_3^- removal by the riparian buffers (Figure 2).

Nitrate removal rates (expressed as the difference between the input and output NO_3^- concentrations in relation to the width of the riparian zone) were mainly positive, ranging from 5 percent per

1 OSUR –ECOBIO University of Rennes 1, campus de Beaulieu, Avenue du général Leclerc, 35042 Rennes cedex, France
2 School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston B15 2TT, United Kingdom
3 BIOEMCO, University Paris VI, Pierre & Marie Curie, Jussieu, 75005 Paris, France
* E-mail address of corresponding: gilles.pinay@univ-rennes1.fr

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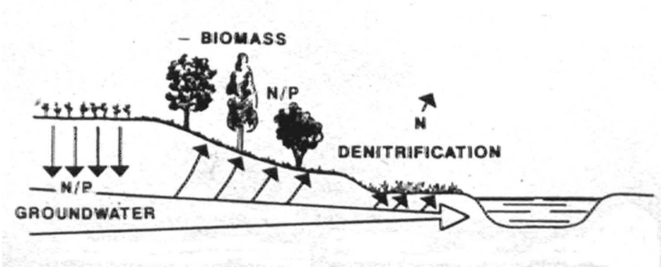


FIGURE 1. Schematic view of the nutrient fluxes under riparian zones.

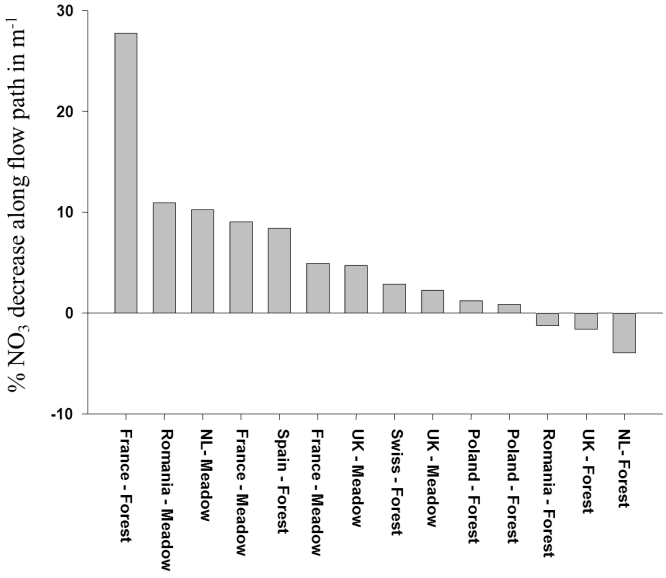


FIGURE 2. Percentage of decrease in NO_3^- in riparian zones (Sabater *et al.*, 2003).

meter (5%/m) to 30%/m, except for a few sites where the values were close to zero (Figure 2). On the basis of this inter-sites comparison, it was concluded that the removal of NO_3^- by biological mechanisms (e.g. denitrification, plant uptake) in the riparian areas is related more closely to NO_3^- load and local hydraulic gradient than to climatic parameters.

Quantifying nitrogen buffering capacity

In order to evaluate the importance of local hydraulic gradient on diffuse N buffering capacity of riparian zones, Ocampo, Oldham and Sivapalan (2006) used the Damköhler ratio to quantify the relative importance of transport *versus* reaction in the attenuation of NO_3^- concentrations within the riparian zones. The Damköhler ratio can be defined as the ratio between a transport timescale ($\tau_{transport}$ = L/v) and a reaction timescale ($\tau_{reaction}$ = $1/K_1$) where L is the travel length; v is the velocity; and K_1 is the rate of NO_3^- consumption.

This dimensionless number is therefore the ratio between the rate of transport (rate of NO_3^- input to the site) and the rate of reaction (denitrification in the site). Thus, it is a measure of the competition between transport and reaction processes. The higher the ratio is, the more efficient to remove NO_3^- is a given site. The Ocampo, Oldham and Sivapalan (2006) study based on results published in several sites all over the world predicted the N removal capacity of riparian zones with a very good accuracy (Figure 3).

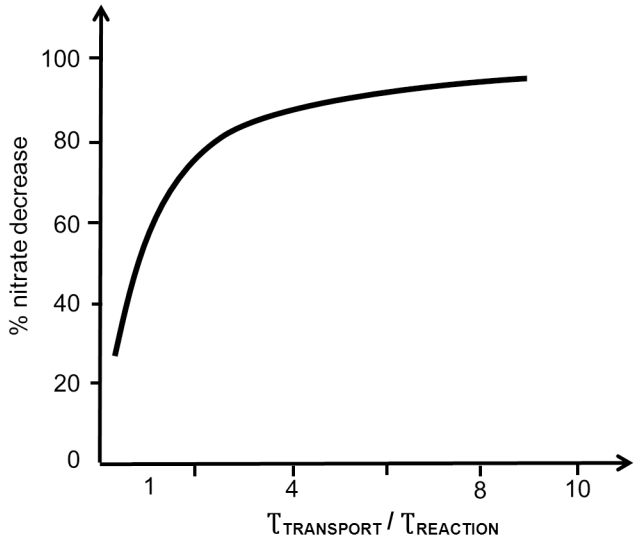


FIGURE 3. Percentage of NO_3^- removal in a riparian zone as a function of the Damkölher ratio (Ocampo, Oldham and Sivapalan, 2006).

Natural isotopic abundance of N as proxy for denitrification

If the Damköhler ratio can provide a good framework to evaluate N buffering capacities at the riparian scale, it does not inform on the processes at stake, i.e. uptake or denitrification. Natural abundance N isotopic signature ($\delta^{15}N$) can provide such insights into the NO_3^- removal processes occurring within riparian zones (Mengis *et al.*, 1999). There are two stable isotopes of N, namely ^{14}N and ^{15}N. The most common isotope, ^{14}N, accounts for approximately 99 percent of atmospheric N. Although the N isotopic composition of the standard (atmospheric N_2) is constant, other materials have variable isotopic compositions because some biological processes discriminate (i.e. fractionate) between N isotopes. The lighter isotope (^{14}N) often reacts more rapidly in biogeochemical cycles than the heavy one (^{15}N); therefore processes involved in the N cycle can also affect the ratio between the ^{14}N and ^{15}N isotopes in environmental N pools. Among these processes, microbial denitrification significantly alters the N isotope ratio, resulting in the progressive enrichment of the remaining NO_3^- pool with ^{15}N (Mengis *et al.*, 1999). In contrast to denitrification, NO_3^- uptake by terrestrial vegetation appears to fractionate minimally or not at all.

An example of the use of $\delta^{15}N$ to decipher the respective role of denitrification and plant uptake in N buffering capacity of riparian zones has been provided by Clément *et al.* (2003). They used the natural abundance distribution of N isotope in both the groundwater NO_3^- and riparian plant tissues along transects to determine the extent of groundwater NO_3^- decline that resulted from denitrification and/or plant uptake. They found that the decline in groundwater NO_3^- concentration along flow paths under the riparian zone was correlated to an increase of $\delta^{15}N$ in the remaining NO_3^- in groundwater, indicating that denitrification was the main process responsible of NO_3^- decline. However, analysis of $\delta^{15}N$ in plant growing along the transect showed a good correspondence with groundwater NO_3^- $\delta^{15}N$ (1:1 line, Figure 4).

This implies that plant uptake was also contributing to NO_3^- removal along the flow paths. Yet, the seasonal analysis of the $\delta^{15}N$ showed that during the low water period (Figure 4, triangle symbols), groundwater contribution from below the root zone to plant

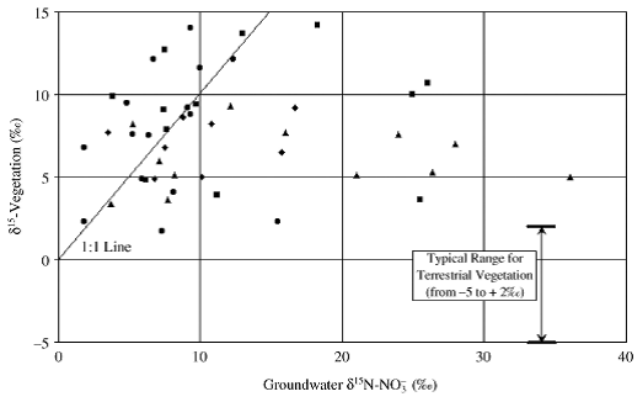


FIGURE 4. Relationship between δ^{15}N-NO_3^- in riparian groundwater and the δ^{15}N isotopic composition of overlying vegetation in April (diamonds), June (squares), August (triangles) and February (circles) (Clément *et al.*, 2003).

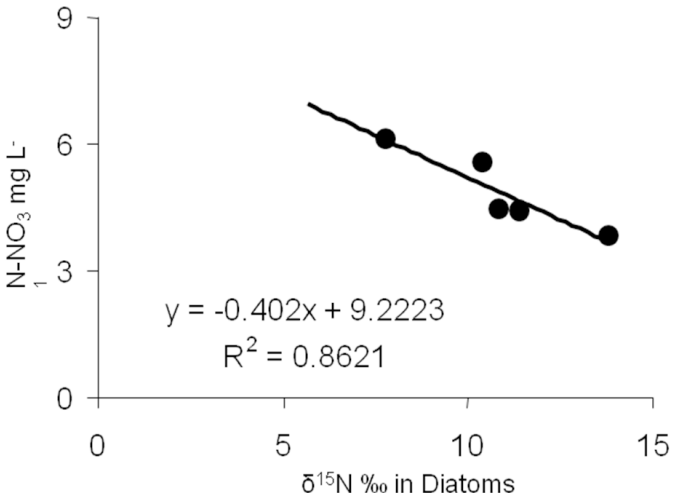


FIGURE 5. Relationship between stream NO_3^- concentration and δ^{15}N in diatoms (Bale, 2010).

NO_3^- uptake was minimal and ^{14}N-NO_3^- resulting from nitrification of ammonium (NH_4^+) in upper soil horizons contributes to plant uptake.

Natural isotopic abundance as a marker of in-stream denitrification

Nitrogen natural isotopic abundance in diatoms was used by Bale (2010) to evaluate potential in-stream denitrification along the Rolleston Brook, a 13 km-long stream situated on the Staffordshire/Derbyshire county boundary, ca. 50 km North West of Birmingham, UK. The results show a negative relationship between stream NO_3^- concentration and δ^{15}N in diatoms present on the stream sediment (Figure 5).

This negative trend was noticeable along the river flow path with high NO_3^- concentration and low δ^{15}N in diatoms in the upstream part of the stream, low NO_3^- concentration and high δ^{15}N in diatoms in the downstream part. This result suggests that in-stream denitrification occurs to reduce NO_3^- concentration, resulting in an increase of δ^{15}N of the remaining NO_3^- available for diatoms. Therefore, diatoms could be used as proxy to in-stream denitrification activity, a self-purification process.

Natural isotopic abundance as a marker of floodplain denitrification

At a larger scale, i.e. the floodplain, Pritchard (2009) used the variation in δ^{15}N measurements of aquatic and riparian vegetation within a small English catchment to identify areas of denitrification. The method used involved sampling river water and plant matter during low flow period (0.6 m^3/sec) along a 23 km stretch of the River Tern (drainage basin 92 km^2), located in the vicinity of Market Drayton in Shropshire, approximately 80 km from the University of Birmingham (UK). The main transect used in the study aimed to capture the N transfer through a small catchment. Eleven study sites were located on the River Tern with three situated on the longest tributaries. A short transect was also chosen to study N transfer at a smaller spatial scale in order to identify local factors which may influence biogeochemical processes. Two different types of plants were selected to determine their ^{15}N signatures. *Salix fragilis* (Crack willow), a medium-large deciduous tree which grows rapidly to between 10 and 20 m, usually found beside rivers on deep, damp soil. *Veronica anagallis-aquatica* (Blue water speedwell), an herbaceous perennial found in or beside streams, marshes and wetlands. *Salix fragilis* and *Veronica anagallis-aquatica* presented a significant relationship in their δ^{15}N content and the percentage of change of NO_3^- load per km of stream flow (Figure 6).

In the Tern catchment context most of the NO_3^- was related to diffuse pollution, with NO_3^- load increasing with distance from the source. A reduction of the slope of change, i.e. a decrease of the percentage of change of NO_3^- load per km, revealed that some NO_3^- was removed from the catchment in these particular areas. The significant increase of δ^{15}N in *Salix fragilis*, a typical tree species of the riparian zones, could be attributed to competition with denitrification for NO_3^- uptake in the riparian sites located in the NO_3^- load reduction (Figure 6A). Similarly, the increase of δ^{15}N in *Veronica anagallis-aquatica* with the decrease in percentage of change of NO_3^- load reveals that similar competition with denitrification of NO_3^- uptake occurred also in-stream (Figure 6B).

Natural isotopic abundance as marker of point source pollution

Natural isotopic abundance of N can also be used to obtain information on the source of N pollution in streams. In this context, Furey (2010) measured NO_3^- concentrations and δ^{15}N in diatoms collected in the riverbed of the Arrow stream, near the city of Reddich, West Midlands, UK. Similar to what was found along the Rolleston Brook (Figure 5), he found a negative relationship between stream NO_3^- concentration and δ^{15}N in diatoms (Figure 7). Yet, two sampling points (open circles) did not fit with this negative trend and presented significantly higher NO_3^- concentration and δ^{15}N in diatoms.

These two data points corresponded to sites downstream from urban sewage treatment plants. Indeed, wastewater N presents a high δ^{15}N because animal and human metabolic wastes lead to enrichment in the ^{15}N. Volatilization of ammonia during waste water treatment also increases the percentage of ^{15}N as well.

CONCLUSIONS

A large range of human activities have increased the fluxes of NO_3^- and $NH_4^+$to such an extent that it has reached the planet's carrying capacity. This increase has affected not only the N cycle but also those of carbon and phosphorus, both on land and in the ocean. Modern agriculture is considered to be the most prominent human activity which increased N fluxes in the last 50 years. As early as the 1980s

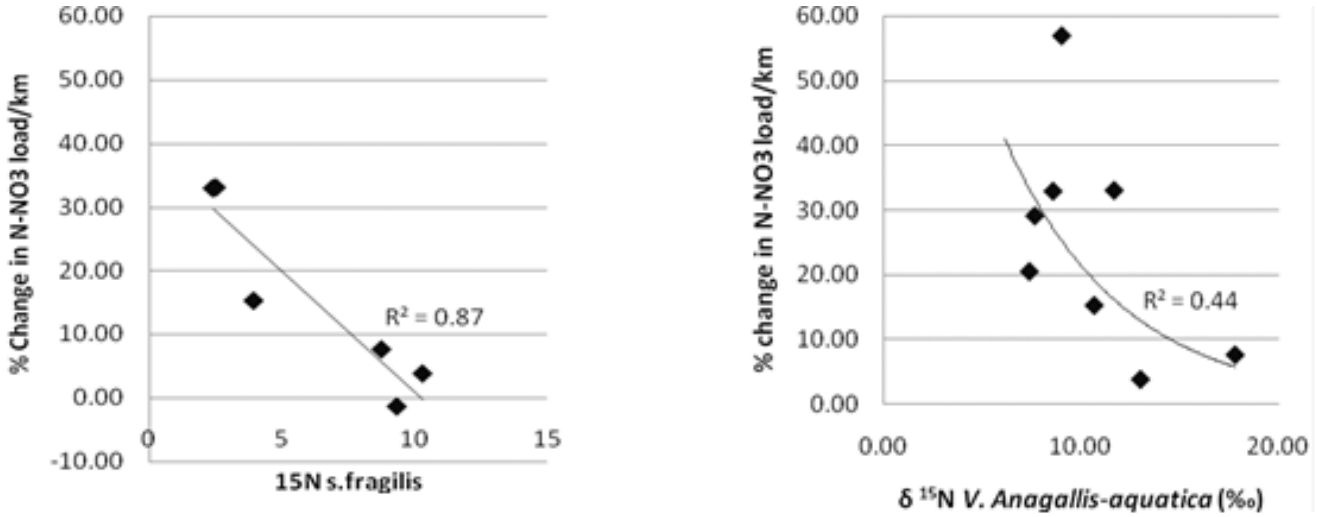


FIGURE 6. Relationship between $\delta^{15}N$ in *Salix fragilis* (A) and in *Veronica anagallis-aquatica* (B) and percentage of NO_3^- load change per km of stream.

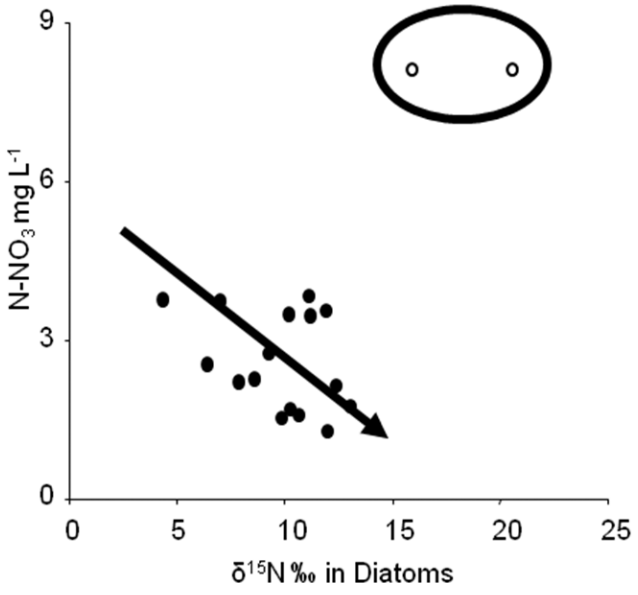


FIGURE 7. Relationship between stream NO_3^- concentration in the Arrow stream network and $\delta^{15}N$ in diatoms. The two open circles correspond to sampling sited downstream from urban wastewater treatment plants (Furey, 2010).

the positive relationship was demonstrated between the percentage of agricultural land cover in catchments and NO_3^- fluxes at their outlets. At the same time, it was demonstrated that riparian zones along streams could buffer diffuse N fluxes. Yet, their evaluation at the drainage basin scale is still a challenge due to a substantial local heterogeneity of farming activities. Isotopic signatures of ^{15}N ($\delta^{15}N$) in aquatic and riparian vegetation were found to be valuable indicators for determining sites of biogeochemical N transformation. These results confirm also the importance of in- and near-stream N buffering capacity along small agricultural catchments using $\delta^{15}N$ as a proxy for denitrification activity.

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Denitrifying Bioreactors: Opportunities and Challenges for Managing Offsite Nitrogen Losses

A.J. Gold[1,*], L.A. Schipper[2], K. Addy[1] and B.A. Needelman[3]

ABSTRACT

In watersheds that deliver elevated levels of agricultural nitrogen (N), denitrifying bioreactors — often simple trenches or denitrification walls filled with a solid carbon (C) substrate — hold great promise for treating non-point discharges of nitrate-rich water. These systems have been developed largely in the temperate zone for high-input, high-production croplands and nitrified waste streams. A wide range of nitrate removal rates (0.014–22 g $N \cdot m^{-3} \cdot d^{-1}$) have been reported in field-based bioreactor studies generally reflecting differences in C substrates, hydrologic setting, temperature, seasonal/site variation in N loading and hydraulic residence time. The use of stable N isotopes has been critical for determining that denitrification is the process most responsible for observed declines in nitrate loads. Further, investigations with N isotopes provide insights into factors that can inform placement and design to minimize the extent of nitrous oxide (N_2O) and methane (CH_4) emissions from this type of management practice.

Key words: *nitrogen, nitrate, bioreactor, denitrification, denitrification wall.*

INTRODUCTION

To address the food security needs of a growing global population in a changing climate, agricultural lands in many areas may receive more intensive management, including additional nitrogen (N) fertilization, irrigation and artificial drainage. Each of these practices can exacerbate offsite losses of N, which can lead to surface water degradation. In addition, reactive N can undergo transformations that generate nitrous oxide (N_2O), a potent greenhouse gas.

Denitrifying bioreactors (Figure 1) hold great promise for reducing edge-of-field N losses through denitrification (Schipper *et al*., 2010). Denitrifying bioreactors are filled with a solid carbon (C) source, such as wood chips or corn cobs. Under saturated conditions, such as found in groundwater or from pipe flow and channelized discharges, this plant material degrades slowly, creating anaerobic conditions and labile C that can foster microbial denitrification. These bioreactors are being incorporated into many different types of settings and designs, but optimizing their value requires site-specific information on hydrology, temperature and nitrate (NO_3^-) loading rates. In addition, there are a number of important research questions that warrant further testing and mechanistic study, many of which would benefit from the use of isotopic techniques.

DENITRIFYING BIOREACTORS

The simplest denitrifying bioreactors are denitrification beds (Figure 1). These beds are often a lined container filled with particulate C. Nitrogen-bearing water is fed in at one end and discharged at the other. In general, these beds are used to treat NO_3^- rich discharges from conveyance systems, e.g. tile drainage or effluents (Figure 2a; e.g. Schipper *et al*., 2010; Woli *et al*., 2010). Denitrification beds have been adapted to other environments, such as in a stream bed (Figure 2b; Robertson and Merkely, 2009) to treat drainage once it enters the stream.

Denitrification walls (Figure 3) are an adaption to treat non-point discharges of NO_3^- rich ground water before it reaches surface water or tile drains. A trench of soil is excavated perpendicular to ground water flow and back-filled with a solid C source.

Rates of removal and controlling factors

Schipper *et al.* (2010) calculated a geometric mean of N removal across a range of denitrifying bioreactors as 3.4 $g \cdot N \cdot m^{-3} \cdot d^{-1}$ (where m^{-3} refers to a volume of the bioreactor). An informal review of published papers since 2010, indicates that the average N removal in denitrifying bioreactors may be closer to 5–7.5 $g \cdot N \cdot m^{-3} \cdot d^{-1}$ (Schipper, 2012) when focusing on systems that were not limited by NO_3^- concentrations.

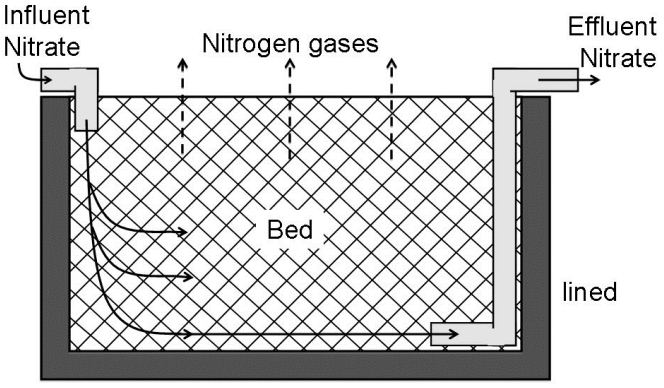


FIGURE 1. Denitrification beds are lined denitrifying bioreactors filled with a solid carbon source receiving pipe flow of N-enriched water.

1 University of Rhode Island, One Greenhouse Road, Kingston, RI 02881 USA
2 University of Waikato, Private Bag 3105, Hamilton 3240, New Zealand
3 University of Maryland, 204 H. J. Patterson Hall, College Park, MD 20903 USA
* E-mail address of corresponding author: agold@uri.edu

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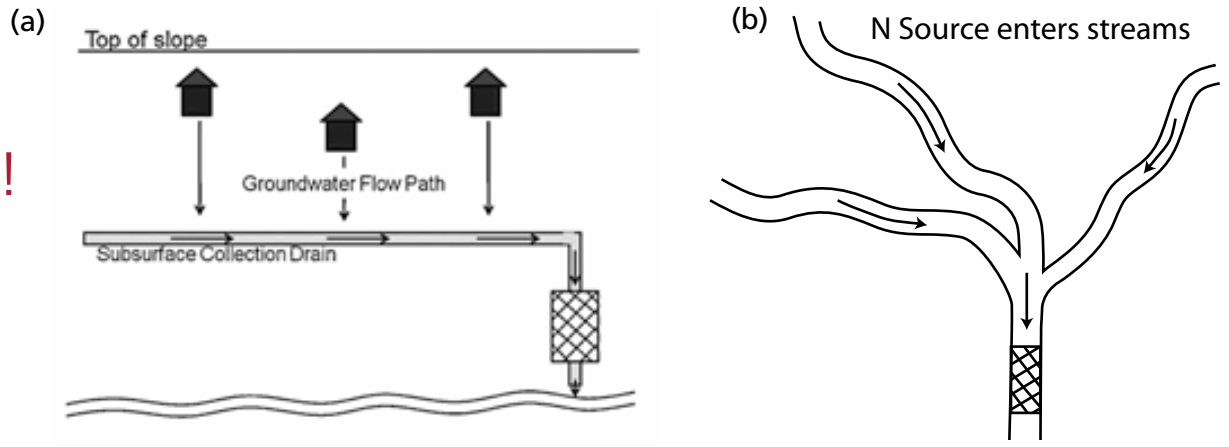


FIGURE 2. Denitrification beds can be placed in sequence to treat tile drainage (a), within a stream to treat stream water (b), or in a trench in the ground to intercept and treat ground water. (From Schipper *et al.*, 2010).

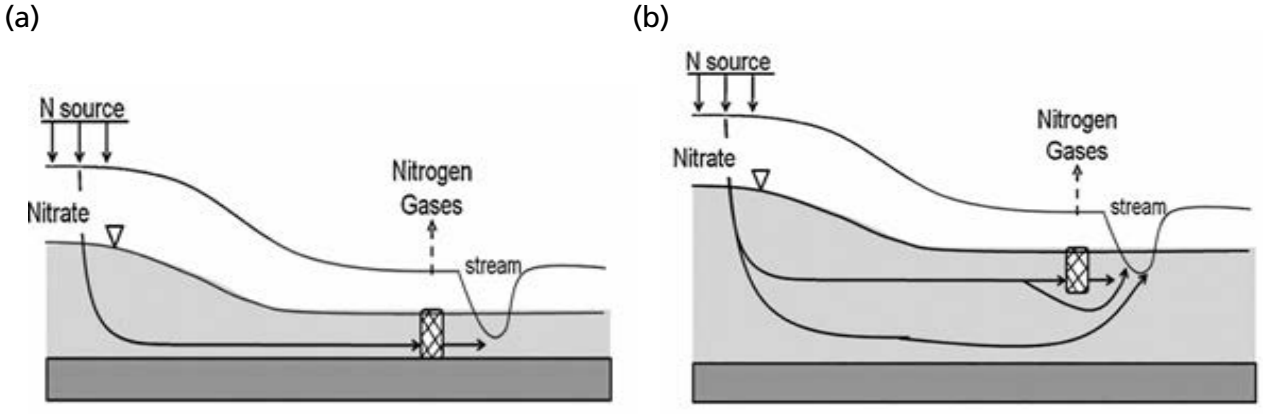


FIGURE 3. Denitrification walls are passive hydrologic systems that intercept NO_3^- enriched ground water. Inception and treatment of groundwater is enhanced when walls are placed close to the source or where a shallow confining layer (a) forces ground water through the wall. In deep aquifers (b), substantial flux of NO_3^- enriched ground water can by-pass the wall minimizing its effectiveness (From Schipper *et al.*, 2010).

The factors that control NO_3^- removal rates and denitrification in these systems are temperature, C source, and absence of oxygen. Stable isotope studies with ^{15}N-labelled NO_3^- point to denitrification as the dominant NO_3^- removal process within denitrifying bioreactors (Greenan *et al.*, 2009; Warneke *et al.*, 2011a). Greenan *et al.* (2009) indicated that in column studies of wood chip material, minimal N_2O was produced. This suggested that N_2 gas production may be a predominant product of denitrification instead of nitrous oxide (N_2O), a potent greenhouse gas. Further studies using N-15 techniques (either natural abundance or enriched) are needed to refine estimates of N_2O production from bioreactors.

Warneke *et al.* (2011a) compared four approaches to measure denitrification rates in a denitrification bed treating hydroponic glasshouse effluent (Figure 4a). The *in vitro* acetylene inhibition method yielded highly variable rates and overestimated the NO_3^- removal rate. Denitrification rates were also obtained from two groundwater push-pull tests based on the production of ^{15}N enriched N gases from ^{15}N-labelled NO_3^- amendments. The denitrification rates from the natural abundance stable isotopic method, when coupled with estimates of retention time, corresponded well with the decline in $NO_3^-$and with the changes in dissolved N_2 concentration along the length of the bed (Figure 4b).

Seasonal and annual temperature differences between regions are likely to account for some of the variability observed in bioreactor performance. In general, biological reaction rates positively correlate with temperature. In the Schipper *et al.* (2010) review of non-NO_3^- limiting bioreactors, a general trend of increasing denitrification rates with increasing average annual temperature was noted. However, some studies suggest that long-term N removal may be lowered in warmer climates since the C substrate may decompose more rapidly (Cameron and Schipper, 2010). In general, as temperature increases by 10°C, the denitrification rates in bioreactors increase two-fold (Figure 5). Cameron and Schipper (2011) found that it was possible to increase pilot-scale bioreactor's temperature by 3–4°C using passive solar heating.

Denitrifying bioreactors will only continue denitrification as long as there is available labile C. Until recently, it was not clear how long denitrifying bioreactors would continue to remove NO_3^- but three studies have now demonstrated that bioreactors constructed with woodchips or sawdust will remove NO_3^- for nine yr or more (Moorman *et al.*, 2010; Roberston *et al.*, 2010; Long, Schipper and Bruesewitz, 2011). These studies also estimated the future performance of denitrifying bioreactors by examining decay rates of wood material in denitrification walls and estimated bioreactor material half-life as between 4.6 and 37 yr in Iowa depending on sample depth (Moor-

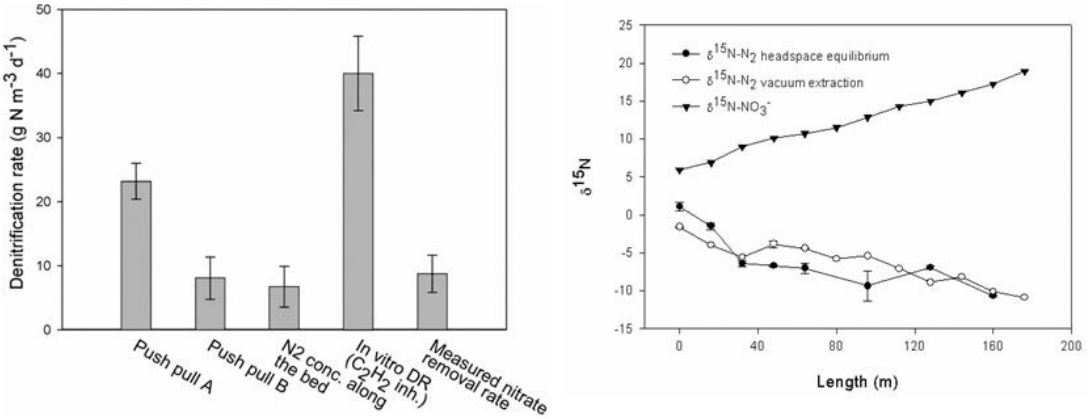


FIGURE 4. (a) Denitrification (estimated by a variety of methods; push-pull using ^{15}N- NO_3^- additions and N_2 concentrations along bed using ^{15}N natural abundance) and NO_3^- removal rates within a denitrification bed indicating the importance of denitrification to NO_3^- removal. (b) Using the natural abundance technique, N_2 was found to decline along the length of the denitrification bed. From Warneke *et al.* (2011a).

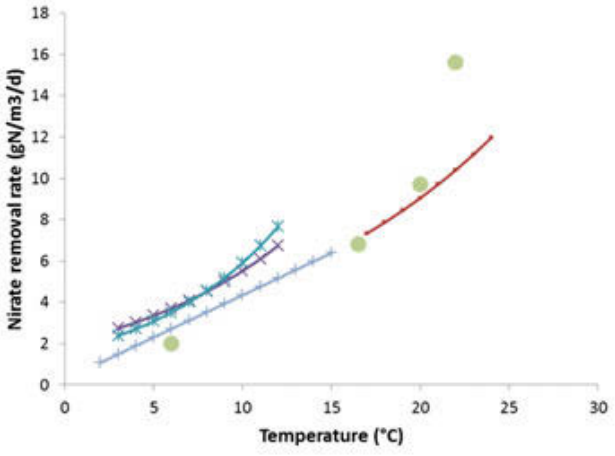


FIGURE 5. Response of nitrate-N removal rates in denitrifying bioreactors to temperature. Adapted from Schipper *et al.* (2010).

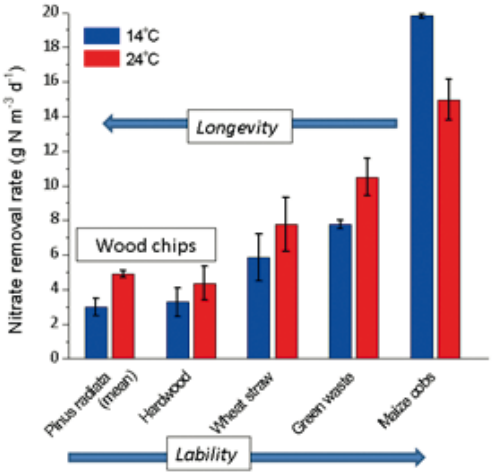


FIGURE 6. Nitrate-N removal rates in denitrifying bioreactors with different carbon sources. Adapted from Cameron and Schipper (2010).

man *et al.*, 2010) and as 11 yr in New Zealand (Long, Schipper and Bruesewitz, 2011). Warneke *et al*. (2011b) measured total losses of C from a large denitrification bed and estimated a life time of up to 39 years. Use of material with higher lability such as maize cobs, may compromise the longevity of the denitrifying bioreactor (Figure 6; Cameron and Schipper, 2010). These studies suggest that NO_3^- removal will be sustained for decades once the bioreactors are constructed, although with a likely declining efficiency through time.

Hydrologic considerations and isotopic approaches for siting denitrifying bioreactor

Hydrologic site conditions are critical in designing a bioreactor. Denitrification walls (Figure 3) are passive systems, restricted by construction practicalities to the upper 1–2 m of groundwater. They have the greatest potential to intercept NO_3^- enriched groundwater in shallow aquifers, where the confining layer is within several meters of the surface. In deep aquifers, where the denitrification wall is not installed to the confining layer depth, flow paths may go below the wall restricting the extent of treatment. Because groundwater flow paths are often relatively shallow within 15–50 m of the site of infiltration, placing walls relatively close to a "hotspot" or elevated source of NO_3^- inputs can enhance the likelihood of intercepting and treating groundwater that is contaminated with NO_3^- . Assessing groundwater flowpaths and aquifer characteristics can be a costly and time-consuming enterprise. Isotopic methods can assist in the selection and strategic placement of denitrification walls. The abundance of stable isotopic signatures of oxygen-18 and hydrogen-2 ($\delta^{18}O$ and $\delta^{2}H$) in ground water provides a finger print of the source water (Craig, 1961; Ingraham and Taylor, 1991). Local, shallow groundwater tends to be isotopically heavier (^{18}O enriched). Enrichment of ^{18}O in base flows of streams may serve as a positive indicator for the use of denitrification walls where they will intercept shallow groundwater flow.

Because bioreactor beds are positioned to intercept channelized flow or tile drainage, the extent of removal within these designs can be limited by hydraulic residence time and N loading. Sizing bioreactor beds warrants careful understanding of void space volume as well as the temporal variations in flow and spatial variation of inputs. Design criteria for beds need to optimize costs vs. performance when considering seasonal variation and storm generated pulses of hydrologic input.

Environmental trade-offs

Uncertainty surrounds the extent of unintended environmental trade-offs. Initial leaching of dissolved labile C from the solid carbon source within the bioreactor can temporarily affect dissolved oxygen concentrations of receiving waters. However, careful start-up procedures may be able to minimize losses of dissolved C, such as pre-leaching of wood chips.

Bioreactors may produce other unwanted pollutants, such as N_2O and methane (CH_4), both potent greenhouse gases, from either incomplete denitrification or prolonged retention times that promote highly reducing conditions within the bioreactor. When NO_3^- loading is high and retention times are limited, there is the potential for more N_2O to be produced since denitrifiers reduce nitrate to N_2O and not completely to N_2 (Elgood *et al.,* 2010). On the other hand, under conditions of low NO_3^- loading and extended retention periods (as can occur during low flow seasons), highly reduced conditions can be generated within the C bioreactors. Controlling NO_3^- losses without generating substantial greenhouse gas emissions will likely depend on scaling bioreactors to ensure that the N concentrations within the bioreactor are poised to ensure complete denitrification while preventing CH_4 production from methanogenesis (Warneke *et al*., 2011b).

A further reason for optimizing the size of denitrification bioreactors is management of the production of methyl mercury which can be released by sulphate under reducing conditions (Shih *et al*., 2011). When NO_3^- concentrations are high, sulphate reduction is suppressed and toxic methyl mercury is not formed (Shih *et al*., 2011). Again, proper design will require consideration of NO_3^- loading and retention to promote complete denitrification, minimize methanogenesis and suppress methyl mercury production.

Costs

Based on a functional life time of 20 yr, Schipper *et al*. (2010) estimated a removal cost of between US$2.39 and US$15.17 per kg N, which compares well with other agricultural management techniques such as controlled drainage, soil testing, wetlands and autumn cover crops. Estimates from later studies have suggested more constrained and lower costs of between US$3.67 and $4.72 (Schmidt and Clark, 2012). The lower costs were achieved when local resources, including the wood chips, lining and flow structures and equipment to dig were available.

Strategic placement

Geospatial analysis has been used to integrate data layers at various scales to allow for the spatial targeting and interpretation of BMP implementation. The efficacy of bioreactors is dependent on spatially and temporally variable factors such as physiography, seasonality of drainage, aquifer properties, soils, precipitation and temperature. At regional scales, geospatial analysis can therefore provide broad guidelines for bioreactor BMP implementation, based on variations due to climatic and generalized physiographic characteristics. This type of regional analysis has been applied to demonstrate differences in riparian buffer efficacy for pollution abatement across nine physiographic provinces in the Chesapeake Bay watershed (Lowrance *et al*., 1997). In that analysis, the effectiveness, optimum design and management of buffer systems were linked to differences in hydrologic connections associated with different physiographic provinces. Similarly, there would be benefits for regional geospatial analysis that provide broad interpretations for bioreactor BMP implementation based on variations due to temperature, seasonal runoff patterns and farming practices. Physiographic provinces are also important considerations for these artificial sinks; for example, agricultural regions dominated by artificially drained soils are likely locations for denitrification beds rather than walls.

Geospatial data are also useful for design and siting at more localized scales. County-level soil survey data and associated interpretations have a long history of guiding conservation practices. Design and placement of denitrifying bioreactors would benefit from soil survey data on depth to restrictive layers, seasonal water table elevations and hydraulic conductivity. Stream gauging networks can afford extensive, spatially explicit datasets on area-normalized flow that provide insight into expected magnitudes and seasonality of flow (Armstrong, Parker and Richards, 2004).

Given our understanding of bioreactors, to optimize NO_3^- removal they should be located in areas with the greatest NO_3^- loads to surface waters (Crumpton *et al.,* 2008). David, Drinkwater and McIsaac (2010) showed locations within the Mississippi River basin where artificial sinks such as denitrifying bioreactors would be strongly beneficial, based on the co-location of tile drainage combined with intensive agriculture in producing high winter and spring nitrate loads.

Denitrifying bioreactors hold considerable promise for reducing nitrate export from agricultural catchment. These systems have been developed largely in the temperate zone for high-input, high-production croplands and nitrified waste streams. As agricultural practices continue to intensify in developing countries with different climatic regimes and different cropping systems, there is a need for denitrifying bioreactors to be tailored to these conditions. Future designs may include incorporation into constructed wetlands where food and fibre can be generated.

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Large Scale Evaluation of Water Conservation Zones for Water Quality Improvements and Biomass Production in Northern Iran

M.A.M. Shalmani[1,*], K. Sakadevan[2], A. Khorasani[1], N. Piervali[1], V. Feiziasl[3], M.K Rudsari[4]

ABSTRACT

Water scarcity and uneven distribution of rainfall are the most important limiting factors for the development of agriculture in Iran. In order to assess water quality, quantity and characterize seasonal variation in isotopic signatures of oxyge-18 ($\delta^{18}O$) and hydrogen–2 (δD), a study was conducted during 2010 to 2011 in 30 different ponds in the north of Iran (Ab–bandans). Water samples were collected in winter, spring and summer of 2010 and 2011 and analysed for chemical and isotopic compositions. Data showed that highest $\delta^{18}O$ and δD were recorded in summer (–1.15‰ and –12.11‰ for $\delta^{18}O$ and δD) and the lowest $\delta^{18}O$ and δD were recorded in winter (–7.50‰ and –47.32‰ for $\delta^{18}O$ and δD), respectively. The $\delta^{18}O$ and δD signatures showed that the water at the Ab–bandons were enriched from spring (–3.57 and –27.72‰) to summer (–1.15 and –12.12‰), respectively. The relationship between $\delta^{18}O$ and δD for pond water and local/global precipitation showed that rainfall and snowmelt can be a major source of water for these Ab-bandans. Water and nutrient balance based on input, output and storage showed that on average 7.6 million cubic meters of water along with 86 tonnes of nitrogen (N) and 17 tonnes of phosphorus (P) were captured and stored by these ponds and are available for irrigating downstream rice crops. Flood irrigation of this water at a rate of 10 000 m^3/ha over the growing season (April to September) was able to produce rice in an area of 730 ha with a yield of 3.5 t/ha. However, changing the irrigation method from flood to an eight-day irrigation interval was able to cultivate 1500 ha with similar yield and significantly increased water use efficiency by 53%. The results of the study are useful to identify the sources of water in the pond and to improve land and water management practices to optimize the capture and storage of water and nutrients for downstream irrigation.

Key words: *Ab-bandans, ponds, $\delta^{18}O$, δD, northern Iran, water quality, water reservoir*

INTRODUCTION

In Iran, water scarcity is one of the main limiting factors for low agricultural productivity. Although irrigation agriculture generally provides 2 to 3 times more crop yield than rainfed agriculture, crop yields are still on the lower end (2 tonnes/ha) by international standards (Smedema, 2003). Although arid and semi-arid climate with low rainfall (annual average of <250 mm) covers over two third of Iran, a narrow part between the Caspian Sea and the Alborz mountains could provide humid climate with average annual rainfall between 1 200 to 1 800 mm (varies along the coastline). In this respect, constructed or man-made wetlands and ponds can be suitable options to capture, store and use water and nutrients in these areas to improve agricultural productivity and environmental quality.

In the southern Caspian lowlands, one of the most important types of wetlands is the "Ab-bandans", a number of small, manufactured ponds. These shallow water ponds (sometimes also called as wetlands), varying in size from 3 ha to 1 000 ha, most were originally built as temporary water storage providing water for irrigation of rice fields during summer growing seasons (Teif Saz Sabz, 2004). Recent surveys by Department of the Environment, Islamic Republic of Iran have showed that there are still about 2160 ponds in the Guilan Province, Northern Iran covering an area of 8 353 ha. These ponds are one of the major sources of water for agriculture in Caspian low lands.

Scientific knowledge of all available water resources, including sources and fluxes is vital for the optimal utilization of scarce water resources in arid and semi-arid areas (Leontiadis, Vergis and Christodoulou, 1996). The use of oxygen-18 and hydrogen-2 stable isotopes as naturally occurring tracers is a valuable tool to identify water sources and fluxes to and from rivers and lakes in catchments (Bowen, 2010). Studies have shown that the evaporation of surface water increases the isotopic signatures of oxygen-18 ($\delta^{18}O$) and hydrogen-2 (δD) in the remaining water and deviate linearly from the local meteoritic water line (LMWL). However, such studies are often restricted to local scale water dynamics (Vandenschricka *et al.*, 2002). Wassenaar *et al.* (2011) used isotopic signatures of oxygen-18 and deuterium ($\delta^{18}O$ and δD) to assess water sources of different lakes in western Canada. They showed that the $\delta^{18}O$ and δD of surface waters were more positive than mean annual precipitation, indicating the basin-scale evaporation of surface waters. With isotopic mass-balance modelling, they also found that about 35% of inflow to the lake watershed was lost to evaporation. Jonsson *et al.* (2009) compared isotope signature of the sub-Arctic lakes in northern Sweden and showed that lake waters showed a range of isotopic

1 Agricultural, Medical and Industrial Research School, Nuclear Science and Technology Research Institute, P.O. Box 31485- 498, Karaj, Iran
2 Soil and Water Management & Crop Nutrition Section, IAEA, VIC, P.O. Box 100, 1400 Vienna, Austria
3 Iranian Rainfed Agricultural Research Institute, P.O. Box 119, Maraghe-Iran
4 Post Graduated Student, Department of plant pathology, Tehran University
* E-mail address of the corresponding author: amoosavi@nrcam.or

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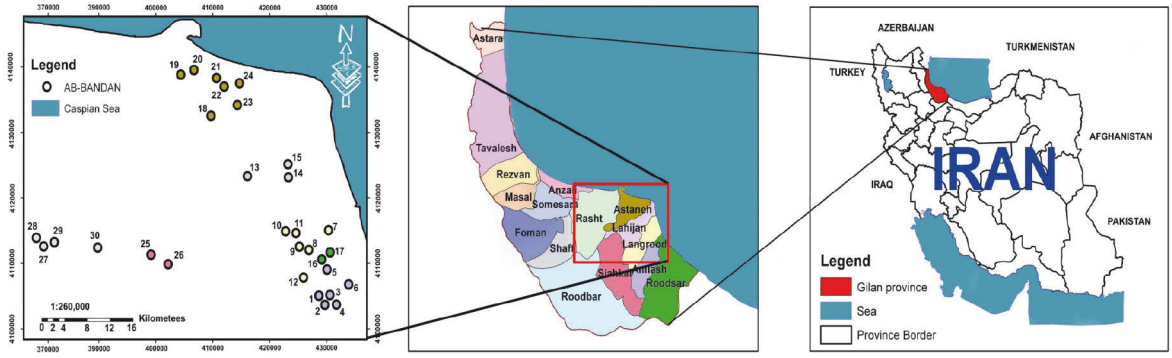


FIGURE 1. Locations of 30 Ab-bandans in northern Iran.

TABLE 1. Geographical and climate characteristics of pond catchments

No	Catchment area (ha)	Maximum elevation of catchment (m a.s.l)*	Minimum elevation of catchment (m a.s.l)	Average annual rainfall (mm)	Average ET (mm/year)
1	831	261	11	1218	763
2	831	261	11	1218	763
3	831	261	11	1218	763
4	831	261	11	1218	763
5	172	5	0	1218	763
6	112	6	0	1218	763
7	140	–17	–22	1431	792
8	370	243	–7	1431	792
9	370	243	–9	1431	792
10	315	430	–5	1431	792
11	190	383	–13	1431	792
12	247	100	25	1431	792
13	2542	–3	–16	1431	792
14	1332	–10	–19	1431	792
15	1332	–10	–19	1431	792
16	106	6	–4	1218	763
17	72	–1	–8	1218	763
18	440	–6	–20	1359	831
19	171	–16	–23	1359	831
20	231	–17	–24	1359	831
21	418	–15	–20	1359	831
22	1704	–8	–20	1359	831
23	304	–7	–23	1359	831
24	153	–20	–20	1359	831
25	105	122	46.1	1146	788
26	59	114	53.2	1146	788
27	120	177	41	1369	831
28	105	169	41	1369	831
29	34	138	70.2	1369	831
30	115	103	41.5	1369	831

*meters above sea level (m.a.s.l)

signatures between different sites as effected by catchment elevation and timing of snow-melt. Until now no comprehensive assessment of water fluxes in ponds was undertaken. In this respect, an assessment of water quality, quantity and water and nutrient balance in relation to upland activities and land use practices is important for developing strategies for effective management of water for agricultural use.

The objective of this paper was to assess the dynamics of water using isotopic signatures of oxygen-18, hydrogen-2, and nutrients of water ponds in Northern Iran in order to optimize the capture and storage of water and nutrients in these ponds.

MATERIALS AND METHODS

The study was carried out during 2010–2011 in Guilan province, close to the Caspian Sea, Northern Iran bounded by geographical co-ordination of X: 368376 to 43358 and Y: 4104370 to 4139522 (Figure 1). The hydrological regime for the area is a seasonal water cycle with maximum input to the ponds in autumn and winter due to precipitation (rainfall and snow) and minimum input in summer. The water data used in the study were collected from 30 man-made ponds. These ponds have a range of surface area, volume and depth. Surface area of ponds and their usage were taken in to consideration when selecting these 30 ponds. The major losses of water from the pond are evapotranspiration and discharge for irrigation during the crop growing season. The elevation ranges for the ponds were classified by Digital Elevation Model (DEM) of watersheds. Catchment area, elevation, and long term average rainfall and evapotranspiration are provided in Table 1. Land use, soil types, topography and climate for the catchment were characterized and details were provided elsewhere (Teif Saz Sabz, 2004). As shown in Table 1, the ponds were grouped based on drainage area, and elevation.

Measurements

Climate, hydrology and physiographical data were collected from the selected weather stations covering all 30 ponds. Water samples were collected during three different seasons, (i) at the beginning of the irrigation period (June 2010), (ii) at the end of the irrigation period (August 2010), and (iii) at the end of precipitation season (March 2011). Water samples were analyzed for chemical compositions (pH, CO_3^{2-}, Cl^-, SO_4^{2-} TDS, Na^-, Ca^{2+}, Mg^{2+}, NO_3^-, NH_4^+ and P), $\delta^{18}O$ and δD during spring and summer 2010 and winter 2011. The amount of water, nitrogen (NH_4-N +NO_3-N) and phosphorus (P) captured in the ponds, the change in $\delta^{18}O$ and δD of water and other hydro chemical properties of water were measured during this period. Nitrogen and P budget for all ponds were estimated based on the amount of water in the ponds and the concentration of N and P in the water. Statistical analyses for the chemical compositions were carried out using multivariate analysis (Johnson and Wichern, 2002).

The stable isotopic signatures of O-18 and D are expressed in δ-values representing deviations in per mil (‰) from the standard for O-18 and D such as

$$\delta^2{}_{sample}(‰) = \left(\frac{R_{sample} - R_{standard}}{R_{standard}} - 1\right) \times 1000$$

where R is the $^{18}O/^{16}O$ and $^2H/^1H$ ration in sample and standard. The $\delta^{18}O$ and δD values for water samples were plotted by linear regressions to obtain its relationship with Global Meteoric Water Lines (GNWL). From this relationship, the deuterium excess (d-excess) which provides information about changes in seasonal precipitation or moisture sources in the region (Saulnier-Talbot *et al*., 2007).

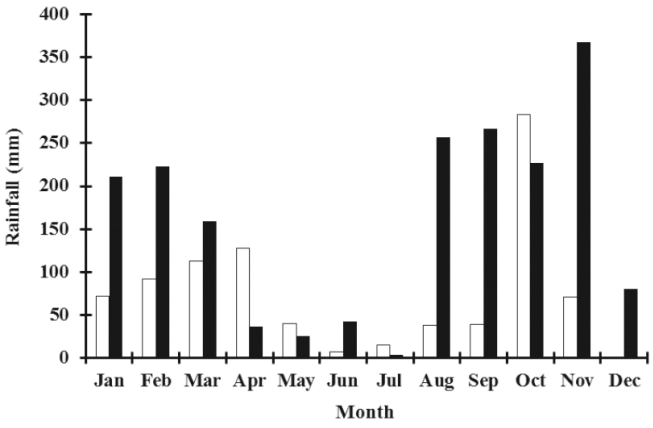


FIGURE 2. Rainfall in the catchment in 2010 (□) and 2011 (■).

RESULTS AND DISCUSSION

Annual rainfall

The 2010 and 2011 annual rainfall for the catchment is provided in Figure 2. The total rainfall for 2010 (900 mm) is lower than the long-term average (1330 mm). However, total rainfall in 2011 (1 900 mm) is much higher than both the long-term and the year 2010. Eventually there was more water available in pond in 2011 than 2010. As characterised by the hydrological cycle, most rainfall occurred in autumn and winter of both years with less rainfall during spring and summer. As a result of higher rainfall during 2011, the amount of water in these ponds increased by more than 30%.

Pond characteristics

The highest water depths were recorded in the winter (average of 1.89 m) and the lowest were in the summer (average 0.38 m). The volume of water decreased from winter to summer (0.27 to 0.018 million cubic meters). On average, the reduction in water column depth and water volume from winter to spring were 26.9% and 5.3% and from spring to summer were 23.7%, and 3.1%, respectively. The main reason for this phenomenon is the demand of water for land preparation at the end of winter and irrigation during summer. All 30 ponds were being recharged during winter through rainfall, runoff and snow melt which increased the volume of water. With minimum rainfall and higher evapotranspiration losses and water discharged for irrigation, the amount of water available in these ponds decreased during summer.

Data obtained showed that water depth, pond area, and pH, CO_3^{2-}, Cl^-, SO_4^{2-}, Na^-, Ca^{2+}, Mg^{2+}, NO_3-N NH_4-N and P of pond water vary significantly ($p<0.01$ Duncan's test) during spring, summer and winter (not shown). The concentrations of anions and cations increased from spring to summer and then decreased over the period from summer to winter.

Nitrogen

The range and average concentrations of NO_3-N, NH_4-N and dissolved P of pond waters during winter, spring and summer are shown in Table 2. As seen, they are highly variable during the monitoring period which was also observed for other inland water bodies (Quirós, 2003). The average nitrate (NO_3-N) concentration was recorded during the winter (15.4 mg/L) and was greater than that

TABLE 2. Nitrate, ammonium and dissolved P concentrations (mg N or P/L) in different seasons

Variable		Winter	Spring	Summer
Nitrate-N	Range	1.0–30.0	0.2–13.7	1.2–33.8
	Average	15.4	2.2	8.8
Ammonium-N	Range	<0.1–1.38	0–7.4	0–25.6
	Average	0.14	2.2	2.0
Dissolved P	Range	0–18.1	1.5–4.5	1.5–5.3
	Average	1.4	3.2	3.2

recorded in summer (8.8 mg/L) and spring (2.18 mg/L). This may be due to greater NO_3-N input by runoff from the catchment to the pond during winter. In addition, reduced losses of NO_3-N from the pond during winter through plant uptake and microbial activity caused by low temperatures may also lead to the presence of high NO_3-N in the water during this period.

Average ammonium-N (NH_4-N) concentrations were lower than NO3-N concentrations for all three period (Table 2). Unlike NO_3-N, the NH_4-N concentrations of pond water were lower during winter than in spring and summer. Reasons for low NH_4-N concentrations during winter are not clear.

The average concentration of dissolved P was lower in winter compared to spring and summer seasons (Table 2). In this respect water samples during spring and summer were not significantly different (Table 2).

Water, nitrogen and phosphorus budget for the ponds

Water, N and P balance in the ponds over winter, spring and summer showed that on average of 7.6 million cubic meters of water is collected annually. However, in 2011 the volume of water collected

TABLE 3. Isotopic signatures of oxygen-18 and hydrogen-2 in all thirty ponds during winter, spring and summer

Pond No.	$\delta^{18}O$‰ (vs SMOW)				δD‰ (vs SMOW)				d excess
	Max	Min	Mean	SD	Max	Min	Mean	SD	
1	0.45	–7.47	–3.21	4.0	–1.44	–45.96	–23.53	22.3	2.13
2	2.54	–7.26	–1.95	4.9	0.18	–46.57	–17.54	23.6	–1.93
3	–0.90	–7.11	–3.54	3.1	–8.97	–45.70	–25.66	18.4	2.69
4	–0.41	–7.78	–3.34	3.7	–4.57	–48.12	–23.71	21.8	3.02
5	–0.17	–7.11	–2.91	3.5	–3.53	–46.22	–22.58	21.4	0.73
6	–0.20	–7.71	–4.61	3.8	–3.97	–46.43	–29.70	21.4	7.17
7	–1.61	–7.62	–4.81	3.0	–17.25	–48.24	–33.78	15.5	4.68
8	5.26	–8.51	–1.44	6.9	18.57	–60.09	–19.27	39.3	–7.73
9	3.41	–6.52	–1.75	5.0	11.67	–42.96	–17.35	27.3	–3.39
10	–2.85	–7.75	–5.30	2.4	–17.26	–48.40	–33.26	15.6	9.14
11	–0.32	–5.57	–2.81	2.6	–12.26	–37.82	–24.23	12.8	–1.71
12	–1.24	–6.89	–4.82	2.9	–16.33	–43.55	–31.99	13.7	6.58
13	–0.01	–8.05	–2.98	4.1	–8.81	–49.65	–24.91	20.6	–1.06
14	1.02	–7.85	–2.31	4.5	–6.05	–48.50	–22.18	21.4	–3.68
15	–1.04	–8.54	–5.05	3.8	–16.09	–54.10	–36.10	19.0	4.26
16	1.26	–7.58	–3.44	4.4	1.71	–49.07	–25.69	25.4	1.81
17	1.35	–6.42	–3.40	3.9	2.37	–41.68	–24.08	22.2	3.15
18	–1.46	–7.42	–5.06	3.0	–17.51	–46.33	–35.22	14.5	5.26
19	–2.41	–8.08	–5.44	2.8	–20.47	–49.13	–36.32	14.4	7.21
20	–4.16	–6.93	–5.72	1.4	–29.44	–42.46	–37.62	6.6	8.12
21	–3.53	–7.36	–5.83	1.9	–26.72	–44.85	–38.19	9.2	8.42
22	–3.45	–8.15	–5.44	2.4	–26.38	–50.69	–37.04	12.2	6.51
23	–1.77	–7.25	–4.83	2.7	–18.68	–44.73	–33.70	13.1	4.91
24	–3.82	–6.03	–5.11	1.1	–27.24	–37.63	–34.05	5.3	6.79
25	–4.66	–8.14	–6.43	1.7	–33.76	–49.42	–40.33	7.9	11.12
26	–5.27	–8.12	–6.73	1.4	–32.41	–50.83	–42.16	9.2	11.71
27	–1.35	–6.42	–3.98	2.5	–14.87	–38.58	–27.35	11.9	4.45
28	–1.04	–7.67	–3.90	3.3	–5.58	–47.37	–25.39	20.9	5.82
29	1.83	–8.85	–3.46	5.3	2.02	–54.08	–25.34	28.1	2.37
30	1.69	–9.54	–2.86	5.6	5.82	–60.92	–23.34	33.5	–0.48

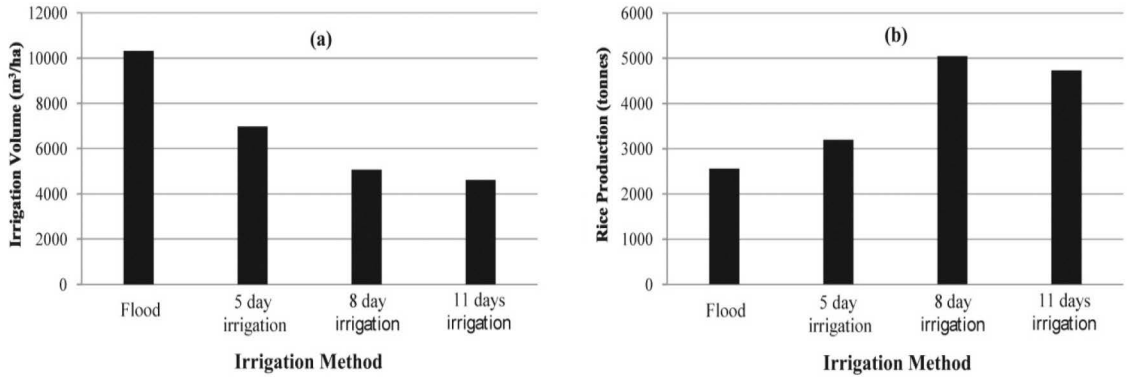


FIGURE 3. Influence of irrigation methods on amount of water used (a) and total rice production (b) of ponds.

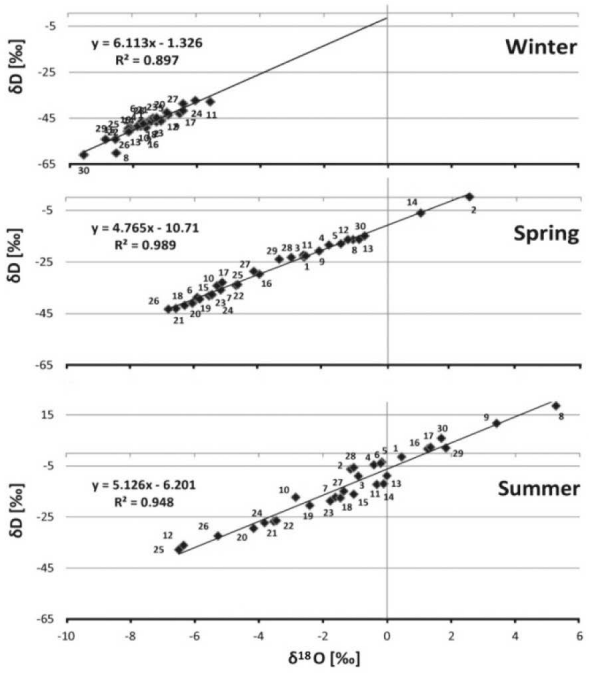


FIGURE 4. Relationship between $\delta^{18}O$ and δD signatures in pond water during winter, spring and summer seasons.

through runoff was about 40% more than the volume of water collected on an average year. Based on N and P concentrations of water about 86 tonnes of N and 17 tonnes of P were collected by these ponds and were available for rice crops.

Rice production related to the irrigation management

The water available in the pond can be used to irrigate an area up of 1500 ha producing 5000 tonnes of rice. Irrigation management practice had a major influence on the use of pond water for irrigation and rice yield (Figure 3). Flood irrigation at 10 000 m^3/ha of water over the growing season was able to irrigate an area of 730 ha and produced a total of 2 561 tonnes of rice. However, by changing the irrigation method from flood to 8 days irrigation (4 500 m^3/ha), an area of 1 500 ha was able to be used for rice cultivation with a total production of 5 050 tonnes of rice. This showed that cultivation area and rice production can be increased by more than 50% and 94%, respectively by changing irrigation practices. In addition, water use efficiency of rice production can be increased by more than 50%.

Isotope signatures of $\delta^{18}O$ and $\delta^{2}D$ and their seasonal variations

The maximum, minimum and the mean isotopic signatures of pond water measured during winter, spring and summer are shown in Table 3. These values are within the broad range of signatures observed for lakes in other agro-climatic conditions (Jasechko *et al.*, 2013).

Data showed that ponds have a range of $\delta^{18}O$ (2.54‰ to –9.54‰) and δD (5.82‰ to –69.9‰) values and these isotopic signatures are influenced by local hydrology and different seasonal parameters (including rainfall, temperature and land use in the catchment). For any given pond, the $\delta^{18}O$ and δD signatures were dependent on water inputs (direct precipitation, groundwater, surface and stream inflows) and outputs (evaporation, groundwater loss, surface and stream outflows).

The relationship between isotopic signatures of $\delta^{18}O$ versus δ*D* in water during winter, spring and summer are shown in Figure 4. Isotopic signatures of pond waters during winter, spring and summer showed that most ponds in winter have light isotopic signature compared with other seasons (Figure 4).

The relationship between $\delta^{18}O$ and δD in rainfall is well understood on a global scale (Craig, 1961), known as the Global Meteoric Water Line (GMWL), and is given by the equation

$$\delta D = 8\delta^{18}O + 10‰ \quad (1)$$

For the local meteoric water line (LMWL), Vreča *et al.* (2006) obtained correlation for the relationship between $\delta^{18}O$ and δD in precipitation water and is provided by the equation

$$\delta D = 8\delta^{18}O + 10‰ \quad (2)$$

The slopes of the relationship between $\delta^{18}O$ and δD in water during winter (6.113), spring (4.765) and summer (5.126) are lower than that of both global and local meteoric water lines (Equations 1 and 2). Also the mean deuterium excess (d–excess) for water during winter (–1.326), spring (–10.71) and summer (–6.201) are lower than that of both global and local meteoric water lines. The value of d becomes higher as evaporation rates are high due to high temperature and low relative humidity in the atmosphere during the formation of water vapour. However, the significantly lower d-excess values (< 10‰) of water sample from ponds may be indicative of water in the ponds was sourced from rainfall/runoff that are subjected to evaporation in a warm and dry atmosphere in the region.

CONCLUSION

The "Ab-bandans" in Northern Iran are an important source of water and nutrients for rice crop by capturing and storing rainfall and snowmelt during winter and spring. This water can be used to irrigate up to 1500 ha of cropland providing more than 5 000 t of rice. The $\delta^{18}O$ and δD signatures showed that the water in the Ab-bandons was enriched from spring (–3.57 and –27.72‰) to summer (–1.15 and –12.12‰), respectively. The seasonal variation of $\delta^{18}O$ and δD in pond water depended on the input of winter snowmelt (with light isotopes) during the early spring period and the enriched summer rainfall and evaporation in late summer. The linear relationship between $\delta^{18}O$ and δD, their slopes and d-excess showed that the pond water is mainly derived from rainfall/runoff and snow melt. The results also showed that most ponds in the north of Iran do not receive sufficient inputs (groundwater input or rainfall) of water during summer.

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SESSION 4

MANAGING SOILS FOR CLIMATE CHANGE ADAPTATION AND MITIGATION

Carbon Sequestration in Agricultural Soils: Separating the Muck from the Magic

E.T. Craswell[1,2*], H.P. King[1] and Z. Read[1]

ABSTRACT

Intense interest in soil organic matter (SOM) over its role in the storage of terrestrial carbon (C) and in the release of greenhouse gases to the atmosphere manifests itself in a renewal of research efforts to assess the impacts of different soil management systems on C dynamics. This paper assesses the future role of selected systems for managing agricultural and pastoral land; addressing the global land area, potential to sequester carbon, side effects on other sources of greenhouse gases, and other limitations. Some systems such as organic farming and the use of biochar may have positive impacts over limited areas, but agroforestry, grazing lands and conservation agriculture appear to have the greatest potential to sequester carbon by covering vast areas on which C loss is reduced or positive C sequestration occurs. An over-riding problem is the difficulty of measuring changes in soil C. Isotopic techniques applied in comparative studies of different soil management systems offer new insights that will guide land managers and policy makers.

Key words: *soil carbon, decomposition, agroforestry, conservation tillage, holistic rangeland management, permanent pasture.*

INTRODUCTION

Soil organic matter (SOM) plays a key role in soil productivity: as a nutrient reserve; in the formation of stable aggregates and protecting the soil surface; in the maintenance of the vast array of biological functions, including the immobilization and release of nutrients; in the provision of ion exchange capacity; and in the storage of terrestrial carbon (C) (Craswell and Lefroy, 2001). The importance of SOM and its challenging chemical complexity has spawned a vast published literature, e.g. Waksman (1936) cited 1 311 references in his book on humus. Since Waksman's time, land transformation for agriculture through clearing of native vegetation and cultivation has led to accelerated decomposition rates over large areas of land. This led to major declines in soil organic carbon (SOC) levels estimated to have released 55 to 90 Pg (1×10^{15} g = 1 billion tonnes (t)) to the atmosphere as carbon dioxide or methane (CH_4; Lal, 2006). A concomitant decline in soil nutrient content led to the widespread adoption of fertilizer inputs in many countries, whereas many farmers in regions such as sub-Saharan Africa have not adopted fertilizers (Craswell and Vlek, 2013). Soil erosion by wind and water has also displaced soil nutrients and organic C to a level estimated at 4.0–6.0 Pg/yr (Lal, 2003). The greenhouse gases released from decomposition and from the use of fertilizers also continue to contribute to global warming. Consequently the research emphasis has changed from studying the role of SOM in productivity to understanding its function in climate change mitigation by reducing or reversing greenhouse gas emissions. Given the current unprecedented need to expand food production to meet population growth, improving land management to achieve both productivity and sequestration objectives provides an opportunity for a grand win-win success.

This paper draws on several recent reviews (e.g. Trumbore, 2009; Sanderman, Farquharson and Baldock, 2010; Powlson, Whitmore and Goulding, 2011; The World Bank, 2012) to consider how selected agricultural systems may contribute to greenhouse gas mitigation. It then considers how isotope techniques can be applied to increase our understanding of the processes of C accumulation and decomposition.

Land management effects on carbon sequestration

The management systems selected for this paper are a mixture of traditional and novel systems that may have, or have been advocated to have significant impacts on C sequestration in agricultural soils. As discussed below, some of the claims made regarding the potential for C sequestration do not meet a reality check based on valid measurements or potential rates of adoption by farmers. Hence our concern to separate the muck from the magic. The systems chosen are not mutually exclusive, e.g. combinations of biochar production with agroforestry or minimum tillage. The greenhouse gas issues associated with paddy rice in flooded rice systems have been considered in detail by various authors, so we limit our consideration of rice soils to the newly advocated system for rice intensification.

Table 1 summarizes and generalizes a huge amount of published information. Some more detailed comments on the individual systems are given below, focusing particularly on estimates of the current and potential land area in relation to C sequestration:

Agroforestry

Different definitions of agroforestry exist, but broadly agroforestry involves the integrative growing of trees in harmony with other agricultural activities including cropping and livestock enterprises. According to Nair *et al.* (2010) forms of agroforestry have existed for centuries, reportedly going back as far as 13 000–9 000 BC in Southeast Asian fishing communities. Today, agroforestry has a diversity of forms depending on the climatic zone, environmental need and socio-economic factors. Agroforestry can include alley cropping, silvopastoral systems such as grazing or "cut and carry" practices, windbreaks and shelterbelt systems. When compared with other

1 Fenner School of Environment and Society, College of Medicine, Biology and Environment, Australian National University, 0200, Australian Capital Territory, Canberra, Australia
2 Crawford Fund, PO Box 4477, Kingston, ACT 2603, Australia
* E-mail address of corresponding author: eric.craswell@anu.edu.au

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TABLE 1. List of selected land management systems indicating some key limitations to effects on net greenhouse gas production

Management system	Comments	Limitations
Agroforestry	Adds standing biomass plus soil C; currently 1 023 million ha with potential to expand on degraded tropical lands	1
Conservation tillage	Extensive areas globally, but crop residues decompose and C may accumulate only in the surface soil	1,2
Holistic rangeland management	Potentially large areas, but may be hard to implement; residues and roots incorporated by trampling	1,3
Permanent pasture	Well documented way to increase stable SOC	3
Organic farming	External organic inputs, but low yield over limited area	1,3
Biochar application	Inert C inputs have long half life in soil, but economics may limit the potential area	1
System for rice intensification	External C inputs; drying and re-flooding may increase decomposition rates	1,3

Limitations: 1 — few reliable soil C data; 2 — associated N_2O emissions due to increased N fertilizer use; 3 — increased CH_4 emissions from animals or soil

agricultural activities, agroforestry has the benefit of utilizing and rehabilitating degraded soil (Albrecht and Kandji, 2003), providing windbreaks, food, fodder, habitat, wood products, reducing erosion and improving crop productivity through shelter and cooling (Nair, Kumar and Nair, 2009) and contributing to the terrestrial pool of C by adding stable forms of SOC.

Agroforestry sequesters C in both the above ground biomass and in the soil, but the actual quantity sequestered depends on a large number of variables including site location, species mix, stand age, soil type, soil depth, management and climate. Nair *et al.* (2009) has estimated that globally, 1 023 million hectares (ha) of agricultural land is used for agroforestry production with a further 630 million ha of unproductive agricultural land having the potential to be converted to agroforestry. Estimates of above and below ground C sequestration attributable to agroforestry are difficult to derive due to site variability. Nair *et al.* (2009), in summarizing the literature has found above ground C sequestration rates ranged between 0.29 and 15.21 $Mg{\cdot}ha^{-1}{\cdot}yr^{-1}$ and in soil between 1.25 and 173 $Mg{\cdot}\ ha^{-1}{\cdot}yr^{-1}$. The variability in reported C sequestration rates indicates the difficulty in predicting a global average rate of sequestration.

Limitations/constraints include:

- risk of loss due to natural or anthropogenic causes;
- protocol and method variability of soil sampling depths and increments;
- allometric equations for estimating above ground biomass may be applied inappropriately;
- problems in estimating area under agroforestry because of variation in distance between rows;
- tree species composition influences quality and quantity of SOC;
- decomposition rates vary depending on climatic zone and soil type.

Conservation tillage

Conventional tillage (CT) generally disturbs the soil significantly because the implements used are designed to invert the surface soil. Conventional tillage methods aim to prepare a clean seed bed for cropping activities by controlling weeds and pests (Sanderman, Farquharson and Baldock, 2010). Lal (1997) estimated that conventional mechanized farming methods typically leave less than 15 percent of crop residues on the soil surface following tillage. Conventional tillage disrupts stable soil aggregates, and its repeated use may reduce aggregation significantly, leading to increased susceptibility to wind and water erosion. Conventional tillage also exposes formerly protected C to mineralization thereby increasing emissions of CO_2 to the atmosphere.

Contemporary methods of CT refer to agricultural practices that include minimum tillage, direct drill and zero-till operations. These practices are adopted to reduce the risk of soil erosion, improve soil aggregation, increase stubble retention and have the potential to enhance the sequestration of C in soil (Sanderman, Farquharson and Baldock, 2010). Derpsch *et al.* (2010) estimated CT was practised on 111×10^6 ha of agricultural land globally in 2009, with an estimated annual increased area of adoption totalling 6×10^6 ha. Lal (1997) estimated that $1\ 352 \times 10^6$ ha of global arable land would be under some form of CT by 2020. Further, the Intergovernmental Panel on Climate Change (IPCC, 2000) suggested that 60 percent of arable land could potentially be farmed by using CT methods. Annual rates of C sequestration associated with CT have been estimated by Lal (1997) to be 0.002 percent to a depth of 1m. This is extended to a global increase in SOC of 0.125 $Pg{\cdot}C{\cdot}yr^{-1}$.

Carbon sequestration rates vary widely between sites and climatic zones. Reasons for this include: the natural spatial and temporal heterogeneity of SOC across the landscape; the SOC concentration at time of conversion to CT; a disproportionate increase in the labile C fraction contributing to higher decomposition rates; and responses of different soil types to C (Blanco-Canqui and Lal, 2004). Conservation tillage contributes an increase in C particularly near the surface, but is reported to make little increase and can even decrease SOC content at depth (Lal, 1997). Carbon to nitrogen (N) ratios are an additional factor contributing to the complexity of C sequestration. Since organic matter decomposition requires and immobilizes N, phosphorus (P) and sulphur (S) (Kirkby *et al.*, 2011), the sequestration of C under CT systems may require additional fertilizer inputs which may be counter-productive if increased nitrous oxide (N_2O) is lost from the N fertilizer to the atmosphere.

Limitations/constraints include:

- landscape heterogeneity;
- stability and residence times of C additions;
- can decrease C at depth;
- other greenhouse gas emissions.

Holistic rangeland management

Grazing is an important land use especially in drylands and on degraded lands that are not suitable for cultivation. It is also a major livelihood in developing countries; for example 40 percent of land in Africa is dedicated to pastoralism. However, grazing is also a cause of land degradation with an estimated 100 Tg CO_2-e (27 million Tg C) emitted annually from grazing-induced desertification (FAO, 2009). Improved grazing management has significant soil C sequestration potential, with wide-reaching co-benefits from restoring productivity to degraded lands. The wide range of conventional grazing practices can be categorised broadly into continuous grazing (CG) and rotational grazing (RG). Conant, Paustian and Elliot (2001) found that improved grazing management increased soil C

by 0.35 $Mg{\cdot}C{\cdot}ha^{-1}{\cdot}yr^{-1}$ on average but results were variable between studies. Pastures with a long history of grazing and low productivity showed a mean C increase of 7.7 percent whereas pastures with high productivity and a short grazing history showed a mean soil C loss of 1.8 percent, — likely a reflection of greater capacity to store C in more degraded soils. Studies comparing different grazing intensities showed high intensity grazing increased soil C by 0.19 $Mg{\cdot}C{\cdot}ha^{-1}{\cdot}yr^{-1}$ relative to moderate intensity.

There is strong anecdotal evidence that holistic rangeland management (also known as planned grazing, high intensity short duration grazing, cell grazing, time controlled grazing) regenerates degraded land and enhances soil C sequestration. Although it is a type of RG, it is fundamentally different from conventional practices which are rotations determined by animal production and time period, in that timing and duration of grazing and rest periods are determined primarily by plant physiological needs. Properly implemented, stocking rate is very high in order to break up and incorporate litter into the soil, grazing periods are very short to avoid plants being re-grazed when they start to re-shoot, and rest periods are sufficiently long for plants to recover but not so long that they senesce. By stimulating plant growth, net primary productivity is increased, and by avoiding selective grazing, desirable plants are able to compete leading to a shift to a higher proportion of more productive perennials in the pasture.

Although holistic rangeland management was developed over 40 years ago (Savory and Parsons, 1980), it has been the subject of little research. Most studies of improved grazing management have focused on stocking rate or CG/RG comparisons, rather than fundamentally different methods. Nevertheless, Kahn, Earl and Nicholls, (2010) recently found that high intensity short duration grazing resulted in a productive and stable grassland with greater perennial cover, less bare ground and a 78 percent higher stocking rate. While these factors could be expected to enhance soil C sequestration, increased livestock would also lead to high CH_4 emissions from enteric fermentation. However, these emissions may be reduced by improved nutrition and pasture management (Beauchemin *et al.*, 2008) and the CH_4 sink provided by grasslands soil (Dalal *et al.*, 2008). Research is needed to estimate the methane balance from grazing and pasture.

Limitations/constraints include:

- low adoption rate;
- higher CH_4 emissions from enteric fermentation if livestock numbers are increased — although healthy grasslands may absorb CH_4.

Permanent pasture

Permanent pastures (used here to include modified pastures and rangelands) account for 69 percent or 3.4 billion ha of global agricultural land and are estimated to hold 30 percent of global soil C stock (FAO, 2009). They cover a wide range of agro-ecological zones with different inherent C carrying capacities and potential sequestration rates based on edaphic and climatic factors. Of the 3.4 billion ha of permanent pasture, 73 percent are affected by soil degradation (FAO, 2009a) resulting in reduced soil C and loss of productive capacity; but also providing significant soil C sequestration potential due to the large area covered. In a review of 115 papers, Conant, Paustian and Elliot (2001) found that mean soil C increased by 0.54 $Mg{\cdot}C{\cdot}ha^{-1}{\cdot}yr^{-1}$ (0.11 to 3.04 $Mg{\cdot}C{\cdot}ha^{-1}{\cdot}yr^{-1}$) with improved management and concluded that grasslands could become a significant C sink. Results were influenced by biome and climate with grassland and woodland increasing most. As most inputs of soil C are through root turnover and root exudates, management practices that increase below ground net primary productivity and improve aggregation and other soil properties that stabilize C would be expected to raise soil C levels over time. Providing irrigation water where water is a limiting factor should increase productivity. However, excess water can lead to water logging and result in emissions of other greenhouse gases, e.g. N_2O and CH_4. Effective rainfall can be increased by reducing run-off or evaporation, or by increasing water infiltration and soil water holding capacity, e.g. by maintaining ground cover or improving soil structure. Tongway and Ludwig (2010) estimated that 4 $Mg{\cdot}C{\cdot}ha^{-1}$ was sequestered in the top 10 cm of soil over 15 yr at a "water ponding" site in the semi-arid zone of central-western New South Wales, Australia.

Fertilization that provides a supply of limiting nutrients can be expected to increase productivity subject to other potentially limiting factors being available, e.g. water, temperature, sunlight. However, fertilization can result in increased emissions of other greenhouse gases, e.g. N_2O; and there are significant greenhouse gas emissions from the production and transport of synthetic fertilizers. Biological nitrogen fixation by legumes was found to have a greater influence on soil C sequestration than fertilization (Conant, Paustian and Elliot, 2001). However in pasture ley systems, increasing the time under pasture and reducing cropping time may lead to increased CH_4 production that counteracts the benefits of C sequestration in the soil. Sowing more productive species of grasses showed the greatest increase in soil C sequestration of the management improvements studied (Conant, Paustian and Elliot, 2001). Legumes also improve fertility and result in higher productivity. Changes in grazing management can also shift pasture composition with a greater proportion of perennials and higher net pasture productivity expected under rotational than under continuous grazing. Wilson and Edwards (2008) have proposed that enteric CH_4 from ruminants, which make up 11 percent of total greenhouse gas emissions in Australia, could be significantly reduced by the greater adoption and production of low-emission kangaroo meat. This may be a peculiarly Australian solution.

Limitations/constraints include:

- other greenhouse gas emissions such as CH_4 from ruminants;
- land clearing for pasture establishment reducing above ground C stocks;
- loss of old (stable) C with input of new (labile) C — the "priming effect" (Fontaine *et al.*, 2011).

Organic farming

Organic farming has been advocated for more than a century as a movement opposing the widespread use of artificial fertilizers and other agrochemicals in food production. In 2009 the total area (certified and uncertified) was estimated by FAOSTAT to be 29.8 million ha or 0.6 percent of the total agricultural area. The areas in 2007 and 2008 were respectively 28.9 and 29.5 million ha indicating a levelling out of the rate of increase in land utilized for organic farming, which serves what is essentially a niche market. This is in line with calculations by Connor (2008) indicating that organic farming cannot feed the world because of the high cost in terms of land area planted to legumes to meet the demand for N rather than using fertilizers for N inputs.

The rate of C sequestration in organically farmed soil would be expected to be high and FAO (2009b) estimated a potential of 0.9–2.4 Pg CO_2e per annum globally. A key unanswered question is the extent to which organic matter turns over in such soils, the C dynamics of which have not been studied in depth.

Limitations/constraints include:

- few controlled carefully conducted studies (Scialabba and Muller-Lindenlauf, 2010);

- reduced use of N fertilizers may reduce N_2O emissions;
- increased tillage to control weeds may increase fossil fuel use and hasten organic matter decomposition.

Biochar application

The production of biochar from crop residues and other feedstocks has been advocated as a major innovative approach to C sequestration for climate change mitigation (Lehmann, Gaunt and Rondon, 2006; Sohi *et al*., 2010). Biochar is produced by pyrolysis of biomass at low oxygen levels and temperatures less than 700°C. When applied to soils, biochar is inert and has a long residence time, hence Roberts *et al.* (2010) calculated that the annual rate of C sequestration by utilising all of the "unused" crop residues produced globally would be 0.65 Pg CO_2e. The idea that so much of the residues of crops are unused may not reflect the real alternative needs of farmers for these residues for animal feed and bedding, for fuel and for protection of the soil surface against erosive forces of water and wind. The value of biochar as a soil amendment derives from the historical practice of using biochar amendments to create the productive Terra Preta soils from acid, infertile soils in the Amazon region. Biochar can increase the nutrient- and water-holding capacity of soils and provides a better substrate for microbes including beneficial mycorrhizae. More research is needed to gain a better understanding of which soil types benefit most from the biochar applications. For example, the C sequestration achieved in studies reviewed by The World Bank (2012) ranged from 2.3 Mg to 3.8 $Mg{\cdot}ha^{-1}{\cdot}yr^{-1}$, yet actual levels achievable will depend on the availability of feedstock, and the economics of the capital and operating costs of a pyrolysis unit. Transport will also add to the costs. The importance of these variables underlines the need for a life cycle analysis that takes account of the biochar production issues as well as the direct and indirect impacts on greenhouse gases (Roberts *et al.,* 2010).

Limitations/constraints include:

- inconsistency in product quality which may also contain contaminants;
- availability of feedstock and transport may be major constraints;
- markets for trading C do not exist and institutional basis for certification lacking;
- threshold price of C of US$37 per tonne to make biochar economic;
- needs precautionary research in relation to large scale adoption effects on biodiversity.

System for rice intensification (SRI)

The system of rice intensification was developed in Madagascar and is based on the use of young seedlings planted at wide spacings. Another key element is the management of water to allow wetting and drying cycles during crop growth. Soil management is based on the use of compost rather than chemical fertilizers, and major increases in yield due to the adoption of the SRI have been claimed. A concerted effort, largely by non-government organizations in 40 countries, has led to two million rice farmers adopting the scheme (Kassam, Stoop and Uphoff, 2011). The use of compost and the reduction in fertilizer use would be expected to increase C sequestration and reduce greenhouse gases such as N_2O, and the aeration of the soil during wetting and drying cycles would reduce CH_4 production. However, Dumas-Johansen (2009) found little evidence of C sequestration under SRI in Cambodia, and found out that Cambodian farmers do not have sufficient water control to implement SRI. Thus SRI remains a controversial system which requires more research to pinpoint where it can be effective.

Limitations/constraints include:

- wetting and drying soil may increase organic matter decomposition rates (Patrick and Wyatt 1964);
- use of compost may increase sequestration but the restricted use of NPK fertilizers will limit C sequestration.

Overall assessment of different systems

Several overarching features are noteworthy. Firstly, increases in soil C sequestration may be offset by increased emissions of other more potent greenhouse gases (Table 2). Secondly, the productivity and C sequestration potentials of different systems depends on adequate supplies of stabilizing nutrients such as N, P and S. Ultimately the total impact of land management depends on the actual and potential areas of land under the particular system. On this basis, the most important systems are agroforestry, conservation tillage, holistic rangeland management and permanent pasture.

Measurements of soil carbon

Many published reports are not based on rigorous measurements of changes in SOC. The main problem with monitoring changes in SOC using conventional methods centres on the need to measure, over a limited number of experimental years, small changes in the relatively large pool of organic C, which has high spatial variability. The long term experiments that help resolve this problem are expensive and relatively rare. Furthermore, since effective sequestration depends on the long-term sustainability of observed changes, so the dynamics as well as the quantity of SOM need to be better understood. In particular, differentiating the labile fraction of SOC from the more passive long-term C can provide such information to be fed into simulation models for use in predictions and economic policy analysis.

TABLE 2. Current and potential areas, and greenhouse gas impacts of selected land management systems; areas based on FAOSTAT (* information not available, ** numbers are approximate)

Management system	Current area 10^6 ha (% ∑ Ag area)	Potential area Increase (10^6 ha)	Issues
Agroforestry	1023 (21%)	630	Above ground C↑
Conservation tillage	100 (2%)	1252	N_2O↑
Holistic rangeland management	*	*	CH_4↑
Permanent pasture	3356 (69%)	*	CH_4↑
Organic farming	30 (0.6%)	*	N_2O↓
Biochar	*	*	*
System rice intensification**	2 (0.02%)	4	N_2O↑ CH_4↑

Conventional soil carbon studies

When monitoring changes in SOC following changes to land use and management there are a number of uncertainties associated with methodological strategies and measurement techniques that can be used. Differences in SOC concentration can vary from micro-site scale, to field, farm, landscape, catchment and national scales. A number of heterogeneous characteristics including topography, relief, bulk density, gravel content, nutrient availability, mineralogy, soil texture and structure, previous land use, vegetation composition, spatial variability and seasonal variation can influence the SOC concentration. Therefore, within economic constraints, it is valuable to replicate sampling and analysis as much as possible.

The World Bank (2012) has classified soil C assessment into two broad methods: direct and indirect methods. Direct methods are referred to as methods which measure SOC empirically based on field sampling and laboratory analysis. However, this is costly and time consuming. Indirect methods are those that are based on C simulation models. The advantage of using indirect methods is that they can provide a cost effective means of estimating spatial and temporal changes to SOC at a range of scales, and can also be used for C accounting. In summary the following issues need attention:

- high variability requires longer time frames between measurements;
- dearth of long-term studies;
- bulk density a key parameter;
- landscape dimension not considered sufficiently; lack of awareness and rigour in many studies.

Role for isotope studies

Isotope techniques provide a unique means for studying decomposition processes and retention of soil C, as well as its interactions with other soil processes and especially nutrients such as N (Celano *et al.,* 2012). Some examples are the following:

- C turnover studies using ^{13}C natural abundance to track C4 versus C3 plant residues and organic matter. This technique can be used to trace corn crop residues (C4) in soils previously dominated by C3 crops or native vegetation;
- associated studies of real time ^{13}C dynamics in gases respired in soils with different crop residues. This new methodology provides data on the dynamics of ^{13}C in systems in which soil moisture and temperature can be varied; useful for studies of the effects of global warming on decomposition and isotope fractionation processes;
- profile studies of ^{13}C and ^{15}N abundances to analyse decomposition processes;
- radiocarbon dating of soil C and respired C to distinguish soil organic fractions with short and long mean residence times;
- ^{15}N abundance and C:N ratios in SOM turnover to differentiate biologically fixed N and its contribution to C dynamics and storage; ^{14}C pulse and continuous labelling for tracing organic C dynamics and particularly the residence time of different C pools.

The objectives of these studies should be clearly defined, particularly the search for the "holy grail" — the labile C fraction that is differentiated from the passive C that has a long residence time. The time is ripe for a coordinated global effort to apply such techniques (especially 1. and 4.) in studies of soil C dynamics at diverse sites where different high priority management systems are compared.

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A Review of Carbon Sequestration through Conservation Agriculture

S. Corsi[1,2,*], T. Friedrich[1], A. Kassam[1] and M. Pisante[2]

ABSTRACT

This review aims at developing a clear understanding of the impacts and benefits of the prevalent types of agriculture with respect to soil organic carbon (SOC) sequestration and carbon (C) pools, and examining if there are any misleading findings at present in the scientific literature. Most of the world's agricultural soils have been depleted of organic matter and soil health over the years under tillage-based agriculture (TA), compared with their state under natural vegetation. This degradation process can be reversed and the review identifies conditions that can increase soil organic matter (SOM) content and improve soil health under conservation agriculture (CA) practices which involve minimum soil disturbance, maintenance of soil cover and crop diversity. The review also discusses the need to refer to specific C pools when addressing C sequestration, as each C category has a different turnover rate. With respect to greenhouse gas emissions (GHG), sustainable agricultural systems based on CA principles are described which result in lower emissions from farm operations as well as from machinery manufacturing processes, and that also help to reduce fertilizer use. The review concludes that terrestrial sequestration of carbon can be achieved efficiently by changing the management of agricultural lands from high soil disturbance TA practices to low disturbance CA practices and by adopting effective nitrogen (N) management practices to provide a positive nitrogen balance for C sequestration. However, full advantages of CA in terms of C sequestration can usually be observed only in the medium- to longer-terms when CA practices and associated C sequestration processes in the soil are well established.

Key words: *conservation agriculture, soil organic carbon sequestration, environmental conditions; crop rotation, organic matter returned, soil disturbance.*

INTRODUCTION

Concerns about rising atmospheric carbon dioxide (CO_2) levels and climate change mitigation efforts have stimulated an interest in using the world's soils for carbon (C) sequestration due to their large sink potential. Soils are important for C management due to their large C content, and also because soil organic carbon (SOC) is particularly responsive to modification through agricultural land use.

Usually, conventional agriculture is tillage-based (TA) in industrialized as well as developing countries and relies as a key operation on mechanical soil tillage with no organic mulch cover for seed bed preparation. This kind of agriculture is generally considered to speed up the loss of soil organic matter (SOM), by increasing its mineralization and through soil loss by erosion. In addition, tillage is a high energy-consuming operation that uses large amounts of fossil fuel per ha in mechanized systems.

In contrast to tillage-based systems, conservation agriculture (CA) is an agro-ecological approach to resource-conserving agricultural production that requires compliance with three linked practical principles, namely: (i) minimum mechanical soil disturbance (with no-till and direct seeding); (ii) maintenance of permanent organic soil cover (with crops, cover crops and/or crop residues); and (iii) species diversification through crop rotations and associations (involving annual and/or perennial crops including tree and pasture crops) (FAO, 2012).

This review aims at developing a clear understanding of the impacts and benefits of the two aforementioned types of agriculture, TA and CA, with respect to the SOC sequestration and C pools, and examining if there are any misleading findings at present in the scientific literature and highlighting the evidence that exposes their flaws.

METHODOLOGY

The review is based on a desk study of the research literature on soil C sequestration and soil organic matter (SOM) stabilization published in leading peer-reviewed journals.

The relevant literature was reviewed to identify the variables that intervene in CA and TA management systems and produce verifiable estimates of their effect on C accumulation in agricultural soils. The variables analysed were: (i) environmental conditions, (ii) the pattern of the crop rotation, (iii) the quantity and the type of the organic matter returned to the soil, (iv) the management of crop residues, and (v) soil disturbance. This review aims at developing a clear understanding of the impacts and benefits of CA and TA on SOC sequestration, soil C pools, and the potential GHG emissions.

The C footprint of other variables in addition to the above that constitute the CA and TA production cycles was also analyzed because many agronomic practices and methods often recommended to increase C accumulation in soils contain hidden C costs in terms of ancillary greenhouse gas (GHG) emissions. The following variables were included in the review: (i) the C costs attributable to the use of machinery, (ii) GHG effluxes from the soil induced by different treatments, (iii) the use of fertilizers, and (iv) strategies to reduce nutrient leaching, methane and nitrous oxide emissions.

1 FAO, Plant Production and Protection Division, Viale delle Terme di Caracalla, 00100 Rome, Italy
2 University of Teramo, Department of Food Science, Viale C. Lerici - Teramo - Italy
* E-mail address of corresponding author: Sandra.Corsi@gmail.com

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RESULTS

There are different C pools in the soil as a result of transformations from the undecomposed form to the decomposed stable form. The C sequestration potential of any soil, for the C pool considered, depends on the vegetation it supports (which influences the amount and chemical composition of organic matter being added), soil moisture availability, soil mineralogical composition and texture, depth, porosity and temperature. Therefore, when addressing C sequestration, rates should always be referred to specific C pools, as each C category has a different turnover rate.

In most soils no measurable C is sequestered when rotations do not return enough biomass to the soil, when crop residues are removed for other concurrent uses and under repeated mono-cropping in no-till systems (Angers *et al.*, 1997; Wanniarachchi *et al.*, 1999, VandenBygaart, Gregorich and Angers, 2003). Several studies showed that changing from mono-crop to multi-crop rotation results in higher SOC concentration due to a more balanced "diet" the latter provides to soil organisms, although different rotations have different potential to promote and support C sequestration. In general terms, C accumulates in the soil when the nitrogen (N) balance of the crop rotation is positive, i.e. when the input from N fixation or fertilizer is higher than the N exported with harvested produce plus the amount lost by leaching or in gaseous forms (Sidiras and Pavan, 1985; Boddey *et al.*, 1997; Alves *et al.*, 2006).

With respect to SOC accumulation in deeper soil layers, it is quite often reported that when soil sampling is extended deeper than 30 cm, the SOC concentration in deep soil layers is usually higher under TA vis-à-vis undisturbed soil (Centurion and Demattê, 1985; Corazza *et al.*, 1999; Baker *et al.,* 2007). The reason for this is that the top layer, which is C-enriched through fertilization, is turned upside down. However, in this way the recalcitrant C from deeper layers becomes exposed to rapid oxidation and mineralization at the soil surface. Further, SOC accumulation achieved with deeper fertilizations regresses as soon as the external C input is interrupted.

Soil disturbance is another very important determinant for soil C accumulation, as many of the factors determining the soil C budget are influenced by land management practices. Most of the world's agricultural soils have been depleted of organic matter and soil health over the years under TA systems compared with their state under natural vegetation. This degradation process has been shown to be reversible and the main ways to increase SOM content and improve soil health seem to be: (i) keeping the contact and interactions between mechanical implements and soil to an absolute minimum, (ii) using effective crop rotations and associations, and (iii) returning crop residues as C source to the soil.

The implementation of these practices can help restore a degraded soil to a sustainable and productive state. However, SOC sequestration is generally non-linear over time (Freibauer *et al.*, 2004), and the effectiveness of conversion of TA to CA depends on many variables: for example, soil C sink strength increases most rapidly soon after a C-enhancing change in land management has been implemented, and reduces with time and the stable SOC stock approaches a new equilibrium (Smith, 2004). Although some authors report significant increases in microbial activity soon after transition to CA, full advantages of CA in terms of soil health can usually be seen only in the medium- to longer-terms when CA practices and soil biological processes become well established within a farming system. To provide an idea of the time scale, Smith (2004) reported that the period for European agricultural soils to reach a new steady state level after a C-enhancing land-use change was introduced is approximately 100 years.

Other aspects relevant for C budgets and GHG emissions are the power and energy requirements. Not tilling the soil results in less fuel consumption, lower working time and slower depreciation rates of equipment per unit area per unit of output, all leading to emission reductions in the farm operations as well as in the machinery manufacturing processes (FAO, 2001). In addition, the crop residues left in CA fields return the C fixed in the crops by photosynthesis to the soil C, resulting in improvement in soil health and fertility leading to reduced fertilizer use and CO_2 emissions. Other GHG emissions from agriculture, namely methane and nitrous oxide, can also be reduced within a CA environment with some complementary practices.

DISCUSSION

Conservation agriculture allows agro-ecosystems to store more and emit less CO_2 and overall, improves ecosystem functioning and services, such as the control of run-off and soil erosion, C sequestration also below the plough layer and, when a mulch cover is adopted, increase infiltration (Pisante *et al.*, 2010). Despite the beneficial environmental impacts of CA, the main incentives for farmers to shift to it are related to productivity and economics rather than environmental sustainability, i.e. improving farm competitiveness and cutting some of the most relevant production costs, thereby increasing profit margins (Hengxin *et al.*, 2008).

Nevertheless, where TA is deeply rooted in the cultural background, lack of knowledge about CA systems and their management make it particularly difficult for farmers to produce crops without ploughing. Additionally, tillage is efficient in the short term (the season) and it is relatively easy to control since it relies on external inputs and actions that the farmer can control and that give the feeling of delivering fast results (such as fertilizer applications). Most farmers would be able to incorporate chemical nutrients mechanically into the soil, bury weed seeds, and recreate a temporary soil structure on a seasonal basis as a precarious environment favourable for crop growth. Conservation agriculture, on the other hand, is a different approach to farming that requires many technical skills and knowledge to be implemented correctly.

Fewer farmers would know how to set up a crop rotation aimed at producing adequate biomass, providing soil nutrients, reducing weed growth in time, diminishing pest incidence and producing competitive yields. For it to work, CA needs to start a virtuous process in the soil, so that in time soil life and a more balanced ecosystem can reduce labour, the need for external inputs and increase the resilience of the ecosystem. Lack of knowledge about CA systems and their management is why technical extension is crucial in the transition phase. The shift to CA has indeed been achieved where: (i) farmers have been informed of the system and convinced of its benefits by experience, (ii) training and technical support to pioneers have been provided, and (iii) adequate support policies (e.g. funding through C sequestration contracts with farmers) have been implemented.

The important lessons learned from around the world regarding the high potential for C sequestration with CA systems (Lal *et al.*, 1998) and the associated opportunity for C trading and reduction in greenhouse gas emissions should be taken into consideration in any future climate change mitigation and sustainable crop production strategies.

CONCLUSIONS

The relevant literature was reviewed to identify the most frequent situations in which no C is sequestered under non-traditional agricultural systems. This is most frequently associated with any one or a combination of the following reasons: (i) soil disturbance, (ii)

monocropping, (iii) specific crop rotations, and (iv) poor management of crop residues.

Terrestrial sequestration of C can be achieved efficiently by changing the management of agricultural lands from high soil disturbance practices to low disturbance and by adopting effective N management practices as described by CA.

With CA fewer or smaller tractors can be used and fewer passes over the field done, which also result in lower fuel and repair costs. However, full advantages of CA can usually be obtained only in the medium- to longer-terms when CA practices and soil biological processes are well established.

The combined environmental benefits of CA at the farm and landscape levels can contribute to global environmental conservation and also provide a low-cost option to help offset emissions of the main greenhouse gases.

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The Use of ^{13}C and ^{15}N Based Isotopic Techniques for Assessing Plant C and N Changes under Conservation Agriculture System

K. Ismaili[1,*], M. Ismaili[1] and J. Ibijbijen[1]

SUMMARY

A long-term field experiment was conducted to investigate the effect of tillage and the addition of residues in a wheat–faba bean rotation. The soil was fertilized with a total of 150 kg nitrogen (N)/ha enriched with 9.96 percent nitrogen-15 (^{15}N) atom excess, in four applications. The first crop was corn, a C_4 plant cropped under till (T) and no-till (NT) conditions. Wheat delta carbon-13 ($\delta^{13}C$) values changed significantly with the addition of corn residues (R^2 = 0.53–0.64) and with time. The wheat residues had the lowest $\delta^{13}C$ (–30.5‰) and the seeds the highest (–28.5‰). Residue and till treatment (RT) had the highest wheat percent ^{15}N values (1.5 and 1.9 percent, respectively). Residues and no-till (-) had the lower percent ^{15}N values (1.1 and 1.1 percent, respectively). Residues increased mineralization by 50 percent as the quantity of ^{15}N taken up by wheat increased with tillage. Tillage and residues treatments did not affect biological nitrogen fixation (BNF). Following wheat and faba bean, crops derived less N from fertilizer (10 to 2 percent). Residues and tillage increased significantly the percent N derived from fertilizer (Ndff). The wheat crop recovered 6.45 kg·N·ha^{-1} (4.3 percent), 13 kg·N·ha^{-1} (8.7 percent) and 11.1 kg·N·ha^{-1} (7.4 percent) for no residues no-till (NRNT), no-residues and till (NRT), residue and no till (RNT) and residue and till (RT) treatments, respectively. Faba bean recovered less N in all treatments (0.66–1.74 kg·N·ha^{-1} or 0.4 to 1.2 percent). The fourth wheat crop recovered between 1.5 (1 percent) and 5.63 kg·N·ha^{-1} (3.7 percent). Cumulative N recovery during the four growing seasons was 122 kg·N·ha^{-1} (81.5 percent) for RT and 116 kg·N·ha^{-1} (77.1 percent) for RNT. Less N was recovered in the NRNT treatments in the four cropping seasons: 104 kg·N·ha^{-1} (69.3 percent) and 106 kg·N·ha^{-1} (71 percent) respectively. Nitrogen not recovered by the crops amounted to 18.5 percent for RT, 22.9 percent for RNT, 31 percent for NRNT and 29 percent for NRT. Most of the N not recovered was in the soil organic matter (SOM).

Key words: *conservation agriculture, ^{13}C, ^{15}N, soil organic matter, tillage.*

INTRODUCTION

The effect of drought and changing climate continue to reduce cereal production in Morocco. Conservation agriculture (CA) based on management of crop residues reduces soil degradation, increases water and nitrogen (N) fertilizer use efficiency, sustains yields and improves soil fertility and structure. Long-term sustainability of crop production is the agricultural strategy adopted worldwide and this can only be reached through good management of water resources and soil fertility and giving particular attention to soil N (mineralization and immobilization) (Galloway *et al.*, 2008). Zeller and Dambrine (2011) traced the fate of litter-derived N in beech forest soils and showed that after 4–5 years on litter decomposition, most of the nitrogen-15 (^{15}N) input was present as particulate organic matter and that litter N became rapidly re-available for trees. Natural abundance stable isotope investigations in plant ecology have focused largely on variations in bulk delta carbon-13 ($\delta^{13}C$) (Bowling, Pataki and Randerson, 2008). Recently-formed soil organic matter (SOM) may follow different pathways of mineralization and/or stabilization depending on soil properties and climate conditions (von Lutzow *et al.*, 2006). Smith *et al.* (2000) showed that $\delta^{13}C$ values of plants can be used to predict irrigation needs and optimize production. Crop residues reduce the effect of drought and improve water and N storage in soil and protect soil from the impacts of high temperatures (Cassman *et al.*, 2003). The wide diversity of $\delta^{13}C$ values of grassland plants was found to be due to environmental conditions (Dungate *et al.*, 2010). Previous studies have reported differences in leaf bulk $\delta^{13}C$ values between life forms (Zheng and Shangguan, 2007). Only between 30 percent and 50 percent of fertilizer N is taken up by crops (Ladha *et al.*, 2005), the remainder becoming part of the SOM or being lost (Tillman, 1999). Nitrogen that remains immobilized will lead to reduced N losses via leaching and/or denitrification (Scow, 1997). Soil temperature and humidity affect the activity of soil micro-organisms and increasing soil temperature induces rapid SOM degradation and increased emission of carbon dioxide (CO_2) (Grall *et al.*, 2006).

The ^{15}N isotope dilution technique is used to investigate N recovery by crops, available soil N and SOM-N pools (IAEA, 2003). Soil organic matter has the ability to store nutrients and improve soil structure, and has long been used as a key indicator of the sustainability of cropping systems (El Alami and Ismaili, 2007a). The form of N application, i.e. organic versus inorganic or in combination, may affect the amount of C that becomes sequestered (Moran *et al.*, 2005). Carbon isotope discrimination ($\delta^{13}C$) was correlated with grain yield under water stress conditions (Monneveux *et al.*, 2005). The $\delta^{13}C$ values decreased with increased water stress and increased with increasing N stress (Stewart *et al.*, 1995). These authors also reported a negative correlation between $\delta^{13}C$ values of the plant and water use efficiency (WUE), with water stress and increasing N availability improving WUE. The use of minimum tillage and mulching limits the potential for water erosion (Pansak *et al.*, 2007), the relationship and slope between available NO_3-N and $\delta^{13}C$ values suggesting that

1 Université Moulay Ismail, Faculté des Sciences BP 11201 Zitoune, Meknès 50000, Morocco
* E-mail address of corresponding author: Ismailih2000@yahoo.fr

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differences in ^{13}C isotopic discrimination were more related to availability of N than to differences in water availability. In a thorough literature review, Giller *et al.* (2009) showed positive effects of CA on water availability (reduced soil evaporation, reduced water runoff, increased water infiltration and reduced soil temperature).

Cerri *et al*. (1985) used differences in $\delta^{13}C$ values of C_3 plants (varying from –24 to –34‰) and C_4 plants (range –6 to –19‰) to evaluate WUE in arid environments. The ^{13}C abundance of incoming SOM is preserved during mineralization and humus formation and the $\delta^{13}C$ content of SOM reflects the ^{13}C content of the vegetation. A significant water and N effect on maize $\delta^{13}C$ values was shown by Dercon *et al*. (2006). If a change occurs from C_3 to C_4 plants at a known time, it is possible to infer the turnover time of the SOM. The ^{13}C natural abundance technique provides useful data to test SOM models in complex cropping systems in which both C_3 and C_4 plants are intercropped or rotated (Diels *et al*., 2004). The ^{13}C natural labelling in combination with modelling was used by Diels *et al*. (2000) to study long-term SOM changes in agro-forestry systems through investigation of yearly changes of $\delta^{13}C$ in plant materials. Important and consistent within-year variations were observed for legume tree pruning (up to 2.4‰ $\delta^{13}C$) and weeds (up to 7‰ $\delta^{13}C$) pointing to the need for repeated sampling within a single year.

The objectives of this study were to: (i) evaluate the beneficial effects of a CA system (no-till and residues left on the soil) on a wheat–faba bean rotation, (ii) estimate the extent of biodegradation of corn crop residues through time and quantify the contribution of residues to wheat and faba bean N nutrition and BNF, and (iii) determine the effect of corn residues on the $\delta^{13}C$ values of follow-on crops (wheat and faba bean).

MATERIALS AND METHODS

A field experiment was conducted to investigate a CA system based on wheat (*Triticum durum* Oum Rbia G3 2004) — faba bean (*Vicia faba* L.) — wheat rotation. Average rainfall was 500 mm/yr and the experiment lasted four years (four cropping seasons). The soil was a well-drained sandy loam of poor fertility and low water holding capacity. The first crop was corn (*Zea mays* var. rugosa), a C_4 plant seeded on 16 plots (16 m^2) fertilized with 150 kg·N·ha^{-1} in four applications: 25 kg·N·ha^{-1} at seeding, 25 kg·N·ha^{-1} two weeks after seeding, 50 kg·N·ha^{-1} one month after seeding and 50 kg·N·ha^{-1} two months after seeding. Nitrogen was added as ammonium sulphate enriched with 9.96 percent atom ^{15}N excess at all applications. The corn crop (a C_4 plant) was used to enrich the soil with C_4 plant residues (23 825 kg/ha of residues or 9616 kg/ha total C, with a $\delta^{13}C$ = –12.4‰, and 340 kg/ha total N labelled with 4.28 percent atom excess ^{15}N (Vanlauwe *et al.*, 2001).

Soil was amended with phosphate (100 kg P_2O_5/ha) and potassium (40 kg K_2O/ha) before the corn crop was established. The corn crop was irrigated as needed, but the following wheat (second crop) and faba bean (third crop) crops were only rain-fed (El Alami and Ismaili, 2007b). The second crop was wheat (a C_3 plant) planted after the corn under no-till and till and with or without corn residues left on the soil. The first wheat crop of the wheat-faba bean system was sampled three times during the first growing season: at one and two months after seeding, and at the end of the season. After the first wheat crop, subsequent crops (faba bean as a third crop and wheat as fourth crop in the wheat-alfalfa system after the first crop of corn to provide C_4 plant residues) were sampled only at harvest time. The $\delta^{13}C$ and percent ^{15}N values were determined at each sampling time and at harvest.

After the first corn crop, the treatments were NRNT — no residues, no-tillage, NRT — no residues with tillage, RT — with residues and tillage, RNT — with residues and no tillage. The percent C and ^{13}C natural abundance, percent N and percent ^{15}N excess were determined simultaneously with a Europa Scientific ANCA-SL stable isotope mass spectrometer. Carbon-13 natural abundance was expressed in $\delta^{13}C$ units using the international PDB standards. Nitrogen-15 and $\delta^{13}C$ analyses were performed at the IAEA Analytical Laboratory at Seibersdorf, Austria. Crop residue inputs were quantified by weighing fresh residues on each plot and calculating the dry weight using the dry matter contents of sub-samples. The results were analysed using an ANOVA with $p \geq 0.05$ and linear regression.

RESULTS AND DISCUSSION

After a corn crop and addition of corn residues, the following wheat plant $\delta^{13}C$ values changed significantly with time (Table 1), being higher at two months than at one month after seeding. The greatest difference was found in the NRNT treatment (–1.05‰) while for the treatments NRT, RT and RNT the difference was lower (–0.54‰) (Table 1).

At the end of the season, the $\delta^{13}C$ values were higher than at one or two months after seeding. In all treatments, the seeds had higher $\delta^{13}C$ values than the leaves and plant residues. The RNT treatment produced the largest difference in $\delta^{13}C$ values between residues and seeds (–2.3‰) while NRNT and NRT showed the smallest difference (–1.5‰). Residue treatment resulted in a difference of –1.8‰ as well as an increase of $\delta^{13}C$ from the source (leaves) to the sink (seeds). The addition of C4 plant residues (i.e., corn residues) increased the $\delta^{13}C$ values of wheat residues and grain, but tillage had no significant effect on these values (Table 1), while the difference between the till and no-till treatments reached a maximum of 0.5‰. Regression of $\delta^{13}C$ values with wheat total N showed a relation between both factors for the first and second harvests and for grain at the end of the growing season. One month after seeding, wheat $\delta^{13}C$ values in one and 2 months after seeding were correlated with plant total N (R^2 = 0.53-0.64) and at the end of the growing season $\delta^{13}C$ values of the grain were still correlated with plant total N (R^2 = 0.53). The $\delta^{13}C$ values of wheat were significantly affected by the previous C4 corn crop and by the residues left on the surface of the soil. These values increased from 0.77 to 2.63‰ for the grain and from 0.5 to 1.54‰ for residues, the grain of NRT treatment showing the highest increase (from –29.03 to –26.4‰) followed by RT treatment (from –28.67 to –27.07‰). Dungait *et al.* (2010) found that total mean leaf $\delta^{13}C$ values for 26 plants from Manor Farm were –28.8‰ with a range spanning 7.5‰. Therefore tillage induced a much greater effect by the corn residues on wheat $\delta^{13}C$ values. The residues affected the $\delta^{13}C$ values of the following wheat and the tillage induced an additional increase due to organic matter mineralization.

The legume faba bean $\delta^{13}C$ values were less affected by addition of residues to the soil and changed only with the tillage treatment (1.25‰) (Table 1). Dungate *et al.* (2010) demonstrated that the wide range of $\delta^{13}C$ values of grassland plants could be ascribed to grazing management, interspecific and spatiotemporal influences. Total N of crops correlated well with $\delta^{13}C$ values, the R^2 being equal to 0.26 to 0.83. Correlation was best with the fourth (wheat) crop and increased from the first (corn) to the fourth crop.

The percent ^{15}N of wheat was affected significantly by the addition of residues and tillage treatments. At one month after seeding, the difference was not significant, but at one and two months after seeding, the treatment RT had the highest percent ^{15}N values (1.5 and 1.9 percent, respectively) showing the effect oftillage on residue mineralization and the higher contribution of residues to the N nutrition of wheat when the soil was tilled. When the residues were

TABLE 1. Effect of time, residues and tillage on dry weight, total N, % ^{15}N and plant $\delta^{13}C$ of wheat planted after corn in a rotation system of wheat — faba bean — wheat cropped under CA treatments. The first wheat crop was sampled at one month after seeding (M1), two months after seeding (M2) and at the end of season (grain and residues). In each column, means with different a, b, c, d letters are significantly different at p ≥ 0.05

Dry weight (kg/ha)

	Wheat		Faba bean		wheat	
	Grain	Residues	Grain	Residues	Grain	Residues
NRNT	4 357^{c}	6 130^{b}	1 987 ^{a}	3 012 ^{a}	4 997 ^{a}	6 031 ^{a}
NRT	4 336^{c}	7 298 ^{b}	2 025 ^{a}	3 685 ^{a}	4 716 ^{a}	6 495^{a}
RT	5 726^{c}	6 735 ^{b}	2 906 ^{b}	3 267 ^{a}	5 665 ^{a}	6 562^{a}
RNT	6 500 ^{b}	7 226 ^{b}	2 956 ^{b}	3 672 ^{a}	6 170 ^{b}	7 575 ^{b}

Total N (kg·N·ha^{-1})

	Wheat		Faba	wheat	
	Grain	Residues	Plant	Grain	Residues
NRNT	66.52^{a}	34^{a}	68.53 ^{a}	71.62^{a}	23.1^{a}
NRT	82.31^{b}	31.8^{a}	71.53 ^{a}	116.9 ^{b}	35.6 ^{b}
RT	91.3 ^{b}	33.6^{a}	126.7 ^{b}	91.06 ^{b}	31.1^{b}
RNT	118.5^{c}	32^{a}	105.4 ^{b}	87.63^{b}	24.8^{a}

Plant $\delta^{13}C$ (‰)

	Wheat				Faba bean	Wheat	
	M1	M2	Grain	Residues	Plant	Grain	Residues
NRNT	−30.8^{a}	−31.85^{a}	−28.71^{a}	−30.20^{a}	−28.85^{a}	−27.77^{a}	−29.7^{a}
NRT	−30.78^{a}	−31.32^{a}	−29.02^{b}	−30.51^{b}	−28.58^{a}	−26.4^{b}	−29.17^{b}
RT	−31.08^{b}	−31.62^{a}	−28.66^{a}	−30.47^{b}	−29.86^{b}	−27.07^{c}	−29.24^{b}
RNT	−30.57^{b}	−31.13^{a}	−28.56^{a}	−30.85^{c}	−28.61^{a}	−27.79^{a}	−29.31^{b}

% ^{15}N (atom % ^{15}N excess)

	Wheat				Faba	Wheat		Ray	Corn
	M1	M2	Grain	Residues	Plant	Grain	Residues	Plant	Plant
NRNT	0.928^{a}	1.505^{a}	0.939^{c}	0.988^{b}	0.144^{a}	0.246^{a}	0.209^{a}	0.416^{a}	1.261^{a}
NRT	1.170^{a}	1.193^{a}	0.831^{c}	0.882^{b}	0.364^{a}	0.28^{a}	0.275^{a}	0.548^{a}	1.251^{a}
RT	1.464^{a}	1.895^{b}	1.542^{b}	1.610^{c}	0.19^{a}	0.705^{b}	0.638^{b}	0.538^{a}	0.412^{b}
RNT	1.174^{a}	1.287^{a}	1.103^{c}	1.118^{b}	0.17^{a}	0.451^{b}	0.394^{a}	0.558^{a}	0.763^{b}

added to soil, the effect of tillage on percent ^{15}N was significant (Table 1). At the end of the season, a significant difference was found between the residues and tillage treatments. The RT treatment maintained the highest percent ^{15}N values in the grain (1.5 percent) and in the residues (1.6 percent), while RNT had the second lowest values in the grain (1.1 percent) and residues (1.1%), showing the effect of residues and tillage on the N nutrition of wheat. Tillage increased N mineralization by more than 50 percent. When no residues were left on the soil, tillage did not have a significant effect on the percent ^{15}N of wheat.

Total ^{15}N accumulated by the wheat was affected significantly by residue and tillage treatments. Total ^{15}N of the plant did not correlate with $\delta^{13}C$ at the first crops and showed a positive relation at the fourth wheat crop (R^2 = 0.63 for grain and 0.53 for residues). When residues were left on the soil, the wheat plants accumulated more N and the quantity of ^{15}N taken up increased with tillage (Table 1). Wheat accumulated ^{15}N from residues and from soil in NRNT, NRT, RT and RNT treatments. During the four cropping seasons, percent ^{15}N decreased in all treatments demonstrating high rates of OM degradation in the soil whether tilled or not tilled. The percent ^{15}N of faba bean did not show significant treatment differences. Two control plants were used to determine N_2 fixation and differences were not found in the percent ^{15}N with any treatment. Residues and tillage increased significantly the total ^{15}N in all crops, showing the positive effect of residues and tillage on N nutrition of the crops. Tillage and residues treatments did not affect BNF by faba bean (Table 2).

After corn at the wheat crop, less N was derived from fertilizer (10 percent). At the end of the cropping season, percent Ndff values showed significant differences between residues and tillage treatments. First, residues increased these values significantly (15.4 percent in grain and 16.1 percent in residues) in RT and (11 percent and 11.2 percent) in RNT compared with no residues (9.4 percent

TABLE 2. Effect of tillage and residues addition on %Ndfa of faba bean in a rotation system of wheat and faba bean planted under CA when rye grass (%$Ndfa_1$) and corn (%$Ndfa_2$) were used as control plants

Controls	%$Ndfa_1$	%$Ndfa_2$
NRNT	65.38	88.58
NRT	33.58	70.9
RT	64.68	53.88
RNT	69.53	77.72

in grain and 9.9 percent in residues) in NRNT and (8.3 percent and 8.9 percent) in NRT. Second, tillage also increased the percent Ndff, but not significantly. In the no-residue treatment, the effect of tillage on fertilizer use was lower than when residues were left on the soil. For the faba bean, the third crop, percent Ndff was low because of BNF. At the fourth crop, wheat derived less N from fertilizer and the recovery was low (2–7 percent). Dourado *et al*. (2009) found that the average recovery of fertilizer and residue in the soil after the first growing season was 38 percent (residues added) and 71.2 percent (residues removed). On average, an additional 4.8 percent of the ^{15}N fertilizer and 6.6 percent of ^{15}N residue was recovered by the crop during the 2nd and 3rd growing season (Table 3).

Small quantities of fertilizer N were recovered in the second (wheat) crop. When no residues were added to the soil, N recovery was 6.5 kg·N·ha^{-1} (4.3 percent) under NRNT and NRT treatments. When residues were added 13 kg·N·ha^{-1} (8.7 percent) were recovered under RT and 11.1 kg·N·ha^{-1} (7.4 percent) under RNT (Table 4). Recovery was higher in RT and RNT because of the additional N added to soil through mineralization of corn residues. Less N was recovered from faba bean under all treatments (between 0.66 kg·N·ha^{-1} (0.4 percent] and 1.7 kg·N·ha^{-1} (1.2 percent)). Recovery from the fourth wheat crop was between 1.5 and 5.6 kg·N·ha^{-1} (1–3.7 percent). With the residue and till treatments, N recovery from fertilizer was greater than from all the other treatments. Cumulative N recovery during the four growing seasons was 122 kg·N·ha^{-1} (81.5 percent) for RT and 116 kg·N·ha^{-1} (77.1 percent) for RNT. In the four cropping seasons recoveries under NRNT and NRT were respectively 104 (69.3 percent) and 106 kg·N·ha^{-1} (71 percent). The percentages of N not recovered by the crops were respectively 18.5 for RT, 22.9 for RNT, 31 for NRNT and 29 for NRT (Table 4). Most of the N not recovered was in the SOM.

CONCLUSIONS

The use of ^{13}C isotopic discrimination together with ^{15}N labelled fertilizer in CA experiments showed that leaving crop residues on the soil and no tillage improved soil organic C as reflected in ^{13}C signature of the crop residues in the subsequent crops) and the N nutrition of the crops as shown by ^{15}N dilution. The use of C_4 plant crop residues as a tracer of C in the subsequent crops is a good indicator of N nutrition of crops and soil organic carbon stocks. Addition of residues to soil affected ^{13}C discrimination of grasses but had no effect on legumes. Total ^{15}N of crops did not correlate with plant $\delta^{13}C$ values.

The residue and tillage treatments had the highest percent ^{15}N values and increased mineralized N by 50 percent. When the corn residues were left on the soil the subsequent wheat plants accumulated more N. Biological N_2 fixation was reduced significantly by water stress, increased by residue additions and was not affected by tillage. Most of the fertilizer N was recovered by the first crop; less N was recovered from the following crops. Nitrogen recovery increased with the addition of residues and with tillage. Most N not recovered by the crops was held in the SOM. Maintaining organic matter levels in the soil therefore remains a crucial component of sustainable agricultural practices.

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TABLE 4. Recovery of fertilizer N in crops as affected by season, residues and tillage in a rotation system of wheat and faba bean planted under CA treatments. In each column, means with different a, b, c, d letters are significantly different at p ≥ 0.05.

Recovery of fertilizer N (kg·N·ha^{-1})

	Wheat			Faba	Wheat			Total
	Grain	Residues	Plant	Plant	Grain	Residues	Plant	recovery
NRNT	4.18 ^{a}	2.25^{a}	6.43^{a}	0.66^{a}	1.18^{a}	0.32^{a}	1.5^{a}	104.0^{a}
NRT	4.58 ^{a}	1.88^{a}	6.46^{a}	1.74^{b}	2.19^{b}	0.66^{b}	2.85^{b}	106.5^{a}
RT	9.42 ^{b}	3.62^{b}	13^{b}	1.61^{b}	4.3^{c}	1.33^{c}	5.63^{c}	122.2^{b}
RNT	8.75 ^{b}	2.39c	11.1^{c}	1.2^{c}	2.65^{d}	0.65^{b}	3.3^{d}	115.7 ^{c}

Percent N recovery of fertilizer N

	Wheat			Faba	Wheat				Not
	Grain	Residue	Plant	Plant	Grain	Residue	Plant	Total	recovered
NRNT	2.79	1.5	4.29	0.44	0.79	0.22	1	69.35	30.6
NRT	3.05	1.25	4.3	1.16	1.46	0.44	1.9	71.01	29.0
RT	6.28	2.41	8.7	1.07	2.86	0.89	3.75	81.49	18.5
RNT	5.83	1.6	7.43	0.8	1.76	0.44	2.2	77.13	22.9

TABLE 3. Effect of season, residues and tillage on %Ndff of wheat and faba bean cropped in a rotation system of wheat and faba bean planted under CA treatments. The first wheat crop was sampled at one month after seeding (M1), two months after seeding (M2) and at the end of season (grain and residues). In each column, means with different a, b, c, d letters are significantly different at $p \geq 0.05$.

	Wheat				Faba bean	Wheat	
	M1	M2	Grain	Residues	Plant	Grain	Residues
NRNT	9.28[a]	15.1[a]	9.4[b]	9.88[b]	1.44[a]	2.46[a]	2.09[a]
NRT	11.7[a]	11.9[a]	8.31[b]	8.82[b]	3.65[b]	7.05[b]	6.38[b]
RT	14.6[a]	19[c]	15.4[c]	16.1[c]	1.9[a]	2.8[a]	2.75[a]
RNT	11.7[a]	12.9[a]	11[b]	11.2[b]	1.71[a]	4.52[a]	3.94[a]

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Biological Nitrogen Fixation by Soybean and Fate of Applied ^{15}N-Fertilizer in Wheat under Conservation Agriculture

M.S. Aulakh[1,*], A.K. Garg[1], J.S. Manchanda[1], G. Dercon[2] and M.L. Nguyen[2]

ABSTRACT

Nitrogen (N) is one of the key drivers of global agricultural production. Four field experiments with irrigated summer-grown soybean (*Glycine max* L.) and winter-grown wheat (*Triticum aestivum* L.) were conducted during 2006–08 to investigate whether biological N_2 fixation (BNF) by soybean can be improved under conservation agriculture (CA) where crop residues (CR) retained on the soil surface, as compared with conventional tillage (CT) and to assess the fate of N fertilizer in subsequent wheat on *Fatehpur* loamy sand soil (*Typic Haplustept*) using ^{15}N labelled fertilizer. The BNF estimated using ^{15}N isotope dilution and ^{15}N natural abundance methods, were comparable suggesting that the latter method which does not require costly ^{15}N-enriched fertilizer, could be employed to estimate BNF by legumes. Use of sorghum as a reference plant, grown in the same plot as soybean, led to up to 36 percent lower estimation of BNF than with the use of *in situ* spontaneous weeds (*Eleusine aegytiacum* L., *Euphorbia hirta* L. and *Cynodon dactylon* L.), which have a similar size and rooting depth as the soybean. The irrigated soybean in the semi-arid subtropical soils could biologically fix 81–125 kg·N·ha^{-1} (68–85 percent of total N uptake), depending upon tillage and CR management. Significant increases in BNF by soybean were recorded when CR was retained on the soil surface of CA presumably due to better rhizobia activity of caused by cooler rhizosphere environment. Recovery of applied ^{15}N in the soil–plant system at harvest of the wheat crop showed that 36–47 percent of it was utilized by the crop, 37–49 percent was left in the soil profile and 5–27 percent was lost, which was estimated as unrecovered ^{15}N. Utilization of ^{15}N was significantly lower when 25 percent more fertilizer N was applied than recommended in both CT and CA without CR. It was also lower when CR was incorporated in CT, or retained on the soil surface in CA. The recovery of ^{15}N in the soil profile at harvest of the wheat crop revealed that the majority of the residual fertilizer N was present in the first 15 cm (54–61 percent), although downward movement of ^{15}N below this level of the soil surface layer was also evident in the soil profile up to a depth of 120 cm. These results illustrate the benefits of CA with CR retained on soil surface on BNF by soybean, and similar patterns in N uptake and translocation from vegetative parts to grain and utilization of applied N by wheat in both tillage systems.

Keywords*: *Conventional tillage, reduced tillage, ^{15}N isotope dilution technique, ^{15}N natural abundance technique, ^{15}N balance, denitrification.

INTRODUCTION

Nitrogen (N) is an important nutrient for global agricultural production as 150–200 million tonnes (t) mineral N are required each year by crops (Unkovich *et al*., 2008). These large N requirements are met mainly through application of N fertilizers, soil organic matter (SOM), and biological N_2 fixation (BNF) by legumes. Improvements in the build-up of SOM, BNF and the plant use efficiency of fertilizer N, and reduction in leaching and gaseous losses of N are vital to the long-term sustainability of agricultural systems, conservation of natural resources and environmental quality. Conservation agriculture (CA) including continuous soil cover through retention of crop residues on the soil surface (CR), with no or reduced tillage, the use of cover crops and the inclusion of grain legumes or green manure crops in rotations, have shown many positive benefits for increasing agricultural productivity and systems' sustainability in several parts of the world (Hobbs, 2007). However, limited information is available to support such claims in India.

Efforts are being made to develop efficient production technology for soybean (*Glycine max* L.) to replace some of the area under the predominant summer-grown rice crop (Aulakh, Pasricha and Bahl, 2003; Aulakh *et al.,* 2010, 2012). Information about the potential of irrigated soybean for BNF in soils of the semi-arid subtropical regions of South Asia is limited. ^{15}N isotopic dilution and ^{15}N isotope abundance techniques have been widely used for estimating BNF by legumes (Boddey *et al.,* 1995; Chalk and Ladha, 1999; Unkovich *et al.,* 2008). While the ^{15}N isotopic dilution method requires costly ^{15}N-enriched fertilizer, the ^{15}N natural abundance method is based on natural abundance of ^{15}N. Therefore, if both techniques provide comparable BNF estimates, the ^{15}N natural abundance method is much cheaper to use. In addition to the potential of soybean for BNF under conventional tillage (CT) and CA without or with CR, the fate of fertilizer N applied to wheat (*Triticum aestivum* L.) under such systems is not known. Nitrate (NO_3) enrichment of groundwater beneath irrigated soils of semi-arid subtropical regions is evident from the NO_3^- analysis of tubewell waters (Bajwa, Bijay-Singh and Parminder-Singh, 1993). However no data have been reported on the movement of ^{15}N fertilizer in the profile of coarse-textured porous irrigated soils under CA and CR management systems. Such studies would become

1 Department of Soil Science, Punjab Agricultural University, Ludhiana, Punjab, India 141004
2 Soil and Water Management & Crop Nutrition Subprogramme, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria
* E-mail address of corresponding author: msaulakh2004@yahoo.co.in

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more important as porous soils are used increasingly for irrigated cropping systems (Aulakh and Bijay-Singh, 1997).

Among the various concerted efforts made to expand CA in Asia, a four-year field study was conducted with soybean-wheat rotation under CT and CA with or without CR on irrigated soil in the semi-arid subtropical region. The results on crop yields, nutrient uptake, water storage in the soil profile, and temperature dynamics were presented earlier (Aulakh *et al.*, 2012). The present study investigated if (i) BNF by soybean is improved under CA as compared with CT when CR is retained on the soil surface, (ii) both nitrogen-15 (^{15}N) dilution and ^{15}N natural abundance techniques provide comparable BNF estimates because employing the ^{15}N natural abundance method is much cheaper, and (iii) the fate of fertilizer ^{15}N applied to succeeding winter-grown wheat including N uptake and utilization by crop, its recovery and distribution in the soil profile and the losses from the soil-plant system under CA and CT is similar.

Information on these aspects will contribute greatly to the development of optimal CA management practices according to local needs and conditions.

MATERIALS AND METHODS

Experimental site, climate and soil characteristics

A field study was conducted with an annual soybean–wheat rotation at Punjab Agricultural University Research Farm, Ludhiana, India (30° 54′ N and 75° 48′ E, 247 m a.s.l. The long-term mean monthly minimum air temperature ranges from 4–5°C in January to 27–28°C in July, while the maximum temperature ranges from 17–20°C in January to 37–38°C in June. The long-term annual rainfall ranges from 600 to 1 200 mm with between 74 percent and 85 percent falling during the monsoon period from July to September. During the study period (2005 to 2008), the mean monthly minimum air temperature ranged from 4°C in January to 28°C in July, the maximum temperature ranged from 17°C in January to 40°C in June; the annual rainfall ranged from 563 to 995 mm of which 71–88 percent was received during June–September.

Subtropical regions have summer and winter crop growing seasons. Summers are characterized by high temperatures and rainfall (monsoons) whereas winters are often dry with low temperatures. In the present study, soybean was grown during summer (June to October) and wheat during winter (November to April). The experiments were established on a Fatehpur loamy sand soil, which was loamy sand up to 60 cm (sand, 833–882 g/kg; silt 36–69 g/kg and clay 82–102 g/kg soil), thereafter sandy loam up to 90 cm (sand, 730–888 g/kg; silt 36–114 g/kg and clay 76–100 g/kg soil), clay loam up to 120 cm (sand, 234 g/kg; silt 400 g/kg and clay 366 g/kg soil) and silty clay loam up to 150 cm (sand, 157 g/kg; silt 443 g/kg and clay 400 g/kg soil), and classified as *Typic Haplustept* (USDA, 1999). The important characteristics of the surface soil (0–15 cm) at the beginning of the study were: pH 8.1; electrical conductivity (1:2 soil:water ratio) 0.09 dS/m; organic C 3.0 g·C·kg^{-1} soil; and Olsen-phosphorus (P) 12 kg·P·ha^{-1} and total nitrogen (N) 665 mg/kg.

Treatments

The study involving ^{15}N labelled techniques formed part of a larger four-year field study with a soybean–wheat rotation, which had 16 treatments consisting of combinations with respect to CT and CA system, CR and fertilizer N and P in individual macro-plots of 3.15 × 8.30 m size as described in Aulakh *et al.* (2012). All treatments were replicated three times. Within the large macro-plots, micro-plots of 1 m^2 (0.8 m wide × 1.25 m long) were confined by 30-cm high rectangular metal retainers pressed to a 15-cm depth in the soil, separately for soybean and wheat crops for conducting four experiments as described below.

Experiments for measuring BNF by soybean and fate of ^{15}N-fertilizer in wheat

Experiment 1

Biological N_2 fixation by soybean was measured with the ^{15}N isotopic dilution method (Unkovich *et al.,* 2008;). To apply the isotope dilution technique, it is necessary to grow the "N_2-fixing" crop and a suitable "non-N_2-fixing" reference crop in the same ^{15}N-labelled soil and the ^{15}N enrichment of the reference plants is assumed to be equal to the ^{15}N enrichment of the N derived from the soil in the "N_2-fixing" crop. Therefore, the proportion of the unlabelled N being derived from the air via BNF in the "N-fixing" crop is proportional to the dilution of the enrichment of the N derived from the labelled soil. Boddey *et al.* (1990) suggested that as compared with a single reference plant, the use of several reference plants better accounts for temporal changes in ^{15}N enrichment of soil mineral N and produces a range of different estimates of the BNF contribution to the "N_2-fixing" crops, and that the extent of this range gives a measure of the accuracy of the estimates. Therefore, in Experiment 1, sorghum Sorghum (*Sorghum vulgare* L.) and spontaneous weeds (weed plant species that grew *in situ* in the experimental plots) were used as the multiple non-legume reference plants (Chalk, 1985; Boddey *et al.*, 1995; Chalk and Ladha, 1999). Two weed plants "Madhana" (*Eleusine indica* L. Gaerth) and weed "Badi dodhak" (*Euphorbia hirta L.*) were used in the study.

The four treatments selected for measuring BNF consisted of tillage systems with and without CR and were replicated thrice [2 (CT and CA) × 2 (0 and 6 t·wheat residue·ha^{-1}) × 3 (replications) = 12 plots]. In CT treatments, soil was tilled to a layer of 10–12 cm. In CR treatments, wheat residue (6 t·ha^{-1}) was incorporated in CT and spread on the soil surface in the CA system before seeding soybean (cv SL 295) in June 2006 in rows 45 cm apart in individual micro-plots. Furrows were opened by hand and levelled after seeding soybean with each row having 12 plants. Just prior to seeding, soybean seed was inoculated with Rhizobium culture (*Bradyrhizobium japonicum*) obtained from the Department of Microbiology of the Punjab Agricultural University. Besides the ^{15}N soybean micro-plots, additional micro-plots were established where sorghum (cv SGL 87) was seeded at the same time in three rows 30 cm apart each having 12 plants. The advantage of having the soybean plot with an adjacent non-legume reference micro-plot is that variation in soil fertility and physical conditions between them is minimized. Soybean and sorghum crops were fertilized with 10 kg·N·ha^{-1} of ^{15}N-labelled ($^{15}NH_4)_2SO_4$ (10 percent atom ^{15}N excess) and 26 kg·P·ha^{-1} (equivalent to 60 kg·P_2O_5·ha^{-1}) as KH_2PO_4 in solution form. Additional water was applied immediately to wash the fertilizer N and P down in the plough layer. As and when required, irrigation of 7.5 cm water was given by taking into consideration the water received through rainfall.

Plants of soybean, sorghum and spontaneous weeds were harvested during the last week of September 2006 at the mid-pod (R2–R3) stage of soybean because at this stage the plants usually cease fixing as well as absorbing N from the soil. An area of 80 cm × 80 cm of each ^{15}N-labelled micro-plot was harvested after leaving 20 cm on either side of the plot to avoid the roots picking up N from areas which had not been labelled. Weeds were separated species by species, providing thereby replicates of weed "Madhana" (*Eleusine*

indica L. Gaerth) and weed "Badi dodhak" (*Euphorbia hirta L.*) in all plots. In this way there were three independent reference plants (sorghum and two weeds).

Experiment 2

BNF by soybean was again measured in 2008 in the same four treatments with the ^{15}N isotope dilution method and following the procedure described for Experiment 1. However, the location of micro-plots within the large macro-plots was changed in order to avoid carryover effect of ^{15}N applied earlier in Experiment 1. At the time of harvesting soybean, plants of sorghum and spontaneous weed "Khabal" (*Cynodon dactylon* L. Pers) were collected; the plants of two weeds collected as non-N_2-fixing reference plants in Experiment 1 were not found in the micro-plots of Experiment 2.

Experiment 3

BNF by soybean was also measured in 2008 with the ^{15}N natural abundance method (Unkovich *et al.*, 2008). The samples of soybean and the reference weed "Khabal" were collected from the replicated macro-plots of four treatments as described for Experiment 1. However, the spots selected for sampling were 4 m away from the ^{15}N micro-plots used in Experiments 1 and 2.

Experiment 4

To investigate the fate of fertilizer ^{15}N applied to a wheat crop seeded after harvesting soybean and to construct a ^{15}N balance sheet, eight treatments were selected. These treatments consist of two tillage systems (CT and CA), two N and P rates [N_{120} P_{26} (120 kg N + 26 kg·P·ha^{-1}) and N_{150} P_{33} (150 kg·N + 33 kg·P·ha^{-1})] without or with CR (0 and 3 t·soybean residue·ha^{-1}). The recommended rates of fertilizer nutrients for optimum yields of wheat are 120 kg N and 26 kg·P·ha^{-1} (Aulakh *et al.*, 2000). A rate 25 percent higher than the recommended NP rate (N_{150} P_{33}) was included to verify whether modified tillage and residue management practices require more fertilizer nutrients for producing optimum yields. Twenty four micro-plots (eight treatments × three replications) were established before seeding the wheat crop in November 2006. Phosphorus as KH_2PO_4 corresponding to 26 and 33 kg·P·ha^{-1} was applied in micro-plots according to the respective treatments before seeding wheat in three rows, 22.5 cm apart, on 6 November 2006. Ammonium sulphate (5 percent ^{15}N atom excess) was applied in two equal splits, the first dose before seeding wheat and the second as a top dressing one d after first irrigation (30 d after seeding). In CR treatments, soybean residue (3 t/ha) was incorporated under CT and spread on the soil surface under CA before seeding the wheat crop.

At maturity, the wheat plants in the central rows were harvested on 17 April 2007 while leaving one row on both sides of the micro-plot and 10 cm along the front and rear side. Representative soil samples were collected from each micro-plot by combining five soil cores taken with an auger from six soil layers (0–15, 15–30, 30–60, 60–90, 90–120 and 120–150 cm), processed and ground to pass through a 2 mm sieve.

All plant samples (soybean, weeds, wheat grain and straw) collected in the four experiments were oven-dried at 60 ± 3°C and ground. Soil and plant samples from all four experiments were analysed for total N and ^{15}N enrichment using an elemental analyzer (NA1500, Carlo Erba Instruments) coupled to an isotope ratio mass spectrometer (Isoprime from GV Instruments, UK) at the IAEA Seibersdorf Laboratories, Austria.

Computation and statistical analysis

The percentage of N derived from the atmosphere (% N_{dfa}) and the amount of BNF by soybean as measured using the ^{15}N isotope dilution method were calculated as follows:

$$\%N_{dfa} = 100\ [1 - (\%\ \text{atom}\ ^{15}\text{N excess in soybean} / \%\ \text{atom}\ ^{15}\text{N excess in reference plant})]$$

$$\text{BNF (kg·N·ha}^{-1}) = [\%N_{dfa} \times \text{total N uptake·(kg/ha)}] /100$$

The $\%N_{dfa}$ by soybean as measured using the ^{15}N natural abundance method was calculated as follows:

$$\delta^{15}\text{N (‰)} = 1\ 000 \times (\%\ \text{atom}\ ^{15}\text{N of plants} - 0.3663) / (0.3663)$$

$$\%N_{dfa} = 100\ [(\delta^{15}\text{N of reference weed plant} - \delta^{15}\text{N of soybean})/(\delta^{15}\text{N of reference weed plant} - B)]$$

where B is delta ^{15}N (δ^{15}N) of N fixed by soybean nodules and transported to shoots and assumed to be –1.3‰ (Unkovich *et al.,* 2008).

Nitrogen-15 fertilizer derived by the wheat crop from fertilizer (% N_{dff}), N derived from soil ($\%N_{dfs}$), ^{15}N fertilizer left in the soil profile and ^{15}N balance sheets were calculated as follows:

$$\%N_{dff} = 100\ (\%\ \text{atom}\ ^{15}\text{N excess in plant sample} / \%\ \text{atom}\ ^{15}\text{N excess in fertilizer})$$

$$\%N_{dfs} = 100 - \%\ N_{dff}$$

$$\text{Total uptake of fertilizer}\ ^{15}\text{N (kg N ha}^{-1}) \text{ in wheat straw} = \%\ N_{dff} \times \text{total N uptake by straw (kg N ha}^{-1})$$

$$\text{Total uptake of fertilizer}\ ^{15}\text{N (kg N ha}^{-1}) \text{ in wheat grain} = \%N_{dff} \times \text{total N uptake by grain (kg N ha}^{-1})$$

$$\%\ ^{15}\text{N fertilizer utilization} = 100\ [\text{total}\ ^{15}\text{N-fertilizer uptake by wheat grain and straw (kg N ha}^{-1}) / \text{rate of}\ ^{15}\text{N fertilizer applied (kg·N·ha}^{-1})]$$

$$\%N_{dff} \text{ by soil} = 100\ (\%\ \text{atom}\ ^{15}\text{N excess in a soil layer}/\%\ \text{atom}\ ^{15}\text{N excess in fertilizer})$$

$$\text{Total N in a soil layer (kg·N·ha}^{-1}) = \%\ \text{N in a soil layer} \times \text{total mass of a soil layer (kg·soil·ha}^{-1})$$

$$\text{Total amount of}\ ^{15}\text{N fertilizer in a soil layer (kg·N·ha}^{-1}) = [\%N_{dff} \text{ by soil} \times \text{total N in soil layer (kg·N·ha}^{-1})] / 100$$

where total mass of a soil layer (kg/ha) = soil depth (m) × bulk density (Mg/m^3) × 10^3 kg/Mg × area (10^4 m^2/ha); and % recovery of ^{15}N fertilizer = 100 {[total ^{15}N-fertilizer uptake by wheat grain and straw (kg/ha) + total amount of ^{15}N-fertilizer in all soil layers (kg /ha)] / [rate of ^{15}N-fertilizer applied (kg/ha)]}

Statistical analysis of values for BNF by soybean, ^{15}N uptake and utilization by wheat and recovery ^{15}N in different layers of soil profile was carried out by ANOVA (Cochran and Cox, 1950) using a randomized block design (RBD). The effects of different treatments viz. (four for soybean experiments and eight in the wheat experiment) were evaluated using the least significant difference (LSD) test at the 0.05 level of probability. The data presented in figures are means ± standard deviation (SD) of three replications.

RESULTS

Biological nitrogen fixation by soybean

In Experiment 1, %Ndfa values for soybean with sorghum and two spontaneous weeds as non-legume reference plants were 51, 72 and 79 in CT without CR, 54, 68 and 77 in CA without CR, and 66, 79 and 85 in CA with CR retained on soil surface (Figure 1). In Experiment 2, with the soybean crop of 2008, % Ndfa by soybean using sorghum and the spontaneous weed "Khabal" varied among different treatments from 77 to 79 in CT and from 76 to 84 in CA, respectively (Figure 2).

On an average of three reference plants in Experiment 1, N_{dfa} was 67 percent in CT and 66 percent in CA without CR but increased to 77 percent in CA with CR (Figure 1). When sorghum was excluded, on an average of two reference plants the corresponding N_{dfa} was 75 percent in CT and 73 percent in CA without CR but increased to 82 percent in CA with CR. In Experiment 2, where sorghum and only one weed "Khabal" were used as reference plants, the trends of $\%N_{dfa}$ among the treatments were similar to those observed in Experiment 1 (Figure 2), again suggesting that as compared with CT, $\%N_{dfa}$ was enhanced significantly in CA when CR was retained on the surface. The results of Experiment 3, where the ^{15}N natural abundance method was used, further illustrated that N_{dfa} by soybean did not differ in CT with or without CR (72 and 73 percent, respectively but was significantly higher (81 percent) in CA when CR was retained on the soil surface (Figure 3).

Estimates of the total amount of BNF by soybean in all three experiments showed significant differences among tillage and CR treatments (Table 1). In Experiments 1 and 2 conducted using the ^{15}N isotope dilution method, BNF ranged from 61 to 115 kg/ha in CT without CR, 64 to 115 kg/ha in CA without CR and 81 to 125 kg/ha in CA with CR. In both experiments, sorghum as reference plant showed the lowest amount of BNF, and the effects of reference plants on the amount of BNF were significant in Experiment 1. Overall results from the three experiments showed that irrigated soybean grown on soils of semi-arid subtropical region fixed N_2 between 81 and 125 kg/ha, which is equivalent to between 68 percent and 85 percent of total N uptake by soybean, depending upon tillage and CR management.

Uptake and utilization of applied ^{15}N fertilizer by wheat

The amounts of ^{15}N and fertilizer N removed by wheat grain and straw based on plant ^{15}N, the proportion of ^{15}N fertilizer utilized

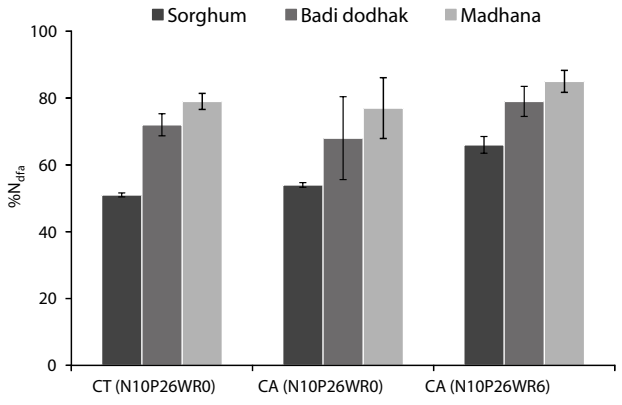


FIGURE 1. Estimates of %Ndfa by soybean under conventional tillage (CT) and conservation agriculture (CA) systems using ^{15}N isotope dilution method with three reference plants in Experiment 1. Treatment details are given in Table 1. Error bars denote standard deviations (three replicates). LSD (0.05) = 9.7.

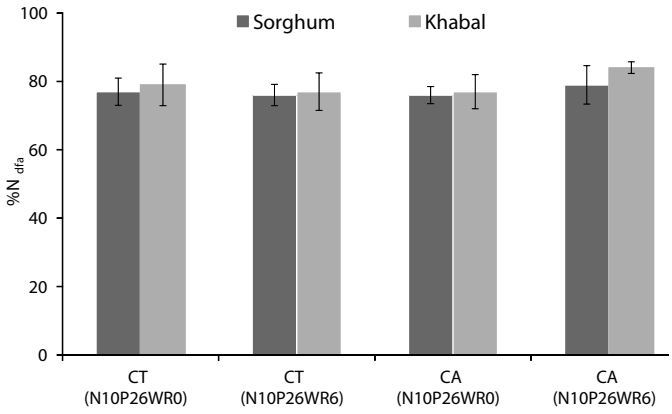


FIGURE 2. Estimates of %Ndfa by soybean under conventional tillage (CT) and conservation agriculture (CA) systems using ^{15}N isotope dilution method with two reference plants in Experiment 2. Treatment details are given in Table 1. Error bars denote standard deviations (three replicates).

TABLE 1. Biological N fixation (kg/ha) by soybean in Experiments 1, 2 and 3 under conventional tillage (CT) and conservation agriculture (CA)

Treatments	Reference plants in Experiment 1				Reference plants in Experiment 2			Experiment 3[2]
	Sorghum	Badi Dodhak	Madhana	Mean	Sorghum	Khabal	Mean	
CT N_{10} $P_{26}WR_0$[1]	61	86	95	80	112	115	114	107
CT N_{10} $P_{26}WR_6$	na	na	na	na	115	115	115	109
CA N_{10} $P_{26}WR_0$	64	81	91	79	112	115	114	109
CA N_{10} $P_{26}WR_6$	81	97	104	94	118	125	122	120
LSD (0.05)	Treatment	Reference plant			Treatment	Reference plant		Treatment
	8	8			5	ns		7

[1] N — fertilizer N (kg N/ha); P — fertilizer P (kg P/ha); WR — Wheat crop residue (t/ha)
[2] Using ^{15}N natural abundance method
na — Not available
ns — non-significant

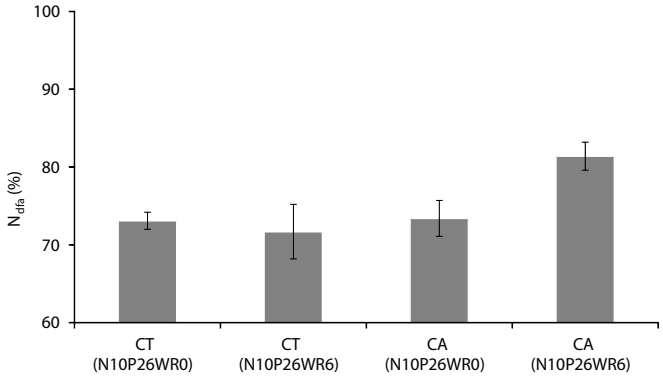


FIGURE 3. Estimates of %Ndfa by soybean under conventional tillage (CT) and conservation agriculture (CA) systems using ^{15}N natural abundance method with one reference plant in Experiment 3. Treatment details are given in Table 1. Error bars denote standard deviations (three replicates).

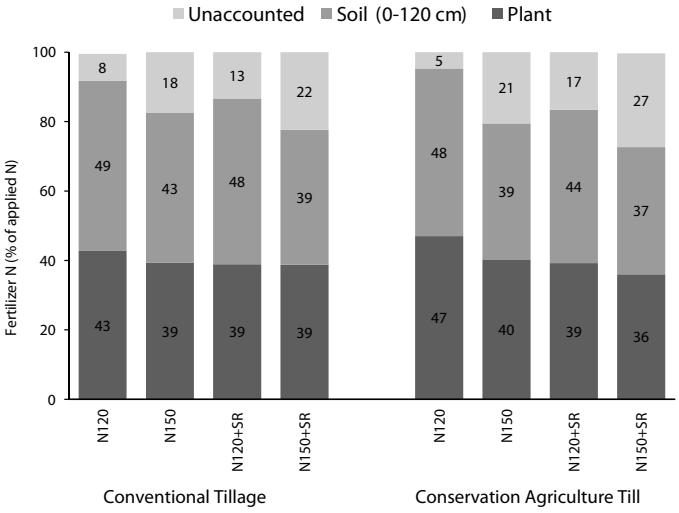


FIGURE 4. Balance sheet of ^{15}N-labelled fertilizer N in wheat in Experiment 4. Treatment details are given in Table 2. Unaccounted ^{15}N represents losses of fertilizer N from soil–plant system.

by wheat and the amounts of ^{15}N retained in different layers of soil profile up to 120 cm are presented in Table 2.

The uptake of ^{15}N fertilizer in wheat grain and straw ranged respectively from 36–44 kg·N·ha^{-1} and 11–17 kg·N·ha^{-1} in CT and between 33–43 kg·N·ha^{-1} and 14–17 kg·N·ha^{-1} in CA. Similarly, utilization of applied fertilizer ^{15}N by wheat ranged from 39 to 43 percent and from 39 to 47 percent in CT and CA, respectively.

Utilization of ^{15}N was highest with the application of 120 kg/ha without CR in CA (47 percent) followed by 120 kg/ha without CR in CT (43 percent), 120 kg/ha with CR in both CT and CA (39 percent), and was lowest with 150 kg/ha applied with CR in CA (36 percent). Conversely, the utilization of ^{15}N by wheat was significantly lower when a rate 25 percent higher than recommended N rate (N_{150}) was applied in CA with CR retained on the soil surface.

Recovery of applied ^{15}N fertilizer in the soil profile after wheat

The recovery of ^{15}N in different layers of soil profile (Table 2) at the harvest of wheat (five and half months after fertilizer application)

TABLE 2. Effect of tillage, fertilizer rate and crop residue treatments on uptake and utilization of applied ^{15}N fertilizer by wheat, and recovery in different layers of soil profiles

Treatments	^{15}N fertilizer uptake by wheat (kg/ha)			^{15}N utilization by wheat	^{15}N fertilizer recovered in soil profile (kg/ha) (depths in cm) .					
	Grain	Straw	Total	(%)	0–15	15–30	30–60	60–90	90–120	Total
Conventional tillage										
N_{120} P_{26} SR_0[1]	39.3	12.0	51.3	42.8	35.7	17.2	2.3	1.8	2.4	59.4
N_{150} P_{33} SR_0	43.8	15.2	59.0	39.3	35.6	18.8	6.0	1.3	3.0	64.7
N_{120} P_{26} SR_3	35.9	10.8	46.7	38.9	33.3	16.8	4.4	1.4	1.4	57.3
N_{150} P_{33} SR_3	41.4	16.8	58.2	38.8	33.1	16.4	5.1	1.5	2.2	58.3
CT Mean	40.1	13.7	53.8	40.0	34.4	17.3	4.5	1.5	2.3	60.0
Conservation agriculture										
N_{120} P_{26} SR_0	39.4	17.0	56.4	47.0	32.3	11.7	6.9	3.9	3.2	58.0
N_{150} P_{33} SR_0	43.4	16.8	60.2	40.1	34.0	14.5	3.6	3.7	3.2	59.0
N_{120} P_{26} SR_3	33.0	14.1	47.1	39.3	32.3	12.7	3.5	2.6	1.9	53.0
N_{150} P_{33} SR_3	38.1	15.3	53.4	35.6	29.6	17.8	4.5	1.2	1.9	55.0
CA Mean	38.5	15.8	54.3	40.5	32.1	14.2	4.6	2.9	2.6	56.3
LSD (0.05)										
Treatment	3.02	2.70	4.38	3.27	ns	ns	ns	ns	ns	ns
Tillage	ns	ns	ns	ns	ns	0.45	ns	ns	ns	ns

[1] N — fertilizer N (kg N/ha); P — fertilizer P (kg P/ha); SR — soybean crop residue (t/ha)
ns — non-significant

revealed that between 54 and 61 percent of the residual fertilizer N was present in the 0–15 cm layer. However, movement of ^{15}N below 0–15 cm soil surface layer was evident in the soil profile up to 120 cm soil depth. Of the total ^{15}N left in soil profile, between 20 and 32 percent moved down to the 15–30 cm layer, and even 1–3 percent was found in the 90–120 cm soil layer.

^{15}N balance and losses in wheat

Recovery of applied ^{15}N in the soil–plant system at the harvest of wheat showed that 36–47 percent was utilized by the crop, 37–49 percent was left in the soil profile and 5–27 percent was lost, which was estimated as unrecovered ^{15}N (Figure 4). Losses of N were between five and eight percent when 120 kg·N·ha^{-1} was applied without CR in both CT and CR but increased to 18 and 21 percent with the application of 150 kg·N·ha^{-1}. As a result of enhanced losses of applied fertilizer N, the uptake and utilization of ^{15}N were significantly reduced (Table 2).

DISCUSSION

Biological nitrogen fixation by soybean

While the $\%N_{dfa}$ values were quite comparable among all three experiments (Figures 1, 2 and 3), the amounts of BNF were substantially less in Experiment 1 than in Experiments 2 and 3. This was essentially due to the lower yields and total N uptake by soybean obtained in Experiment 1 (data not shown). The results of both Experiments 1 and 2 revealed lowest N_{dfa} by soybean when sorghum was used as the reference plant.

Earlier studies, where multiple reference plants were used, showed that the lowest $\%N_{dfa}$ by soybean was with sorghum as the reference plant. Boddey and Urquiaga (1990) reported that non-nodulating soybean, sorghum and sunflower had ^{15}N enrichments of 0.4314, 0.4272 and 0.6217 percent atom respectively, while nodulated soybean had 0.3616 percent atom. Boddey *et al.* (1995) showed that estimates of N_{dfa} by soybean cv 29W using four non-N_2 fixing reference plants varied significantly between 14 percent (okra), 16 percent (sorghum), 47 percent (non-nodulated soybean) and 50 percent (rice). The results of this study confirmed earlier observations that sorghum, having deeper roots with different root architecture than soybean, absorbs mineral N from much deeper soil depths and therefore, most likely utilizes mineral N with a different ^{15}N enrichment than the soybean roots (Boddey *et al.,* 1995). Thus, the spontaneous weeds chosen as reference plants in the present study which were in closer proximity to soybean than sorghum, had a similar root depth and grew to a similar size as soybean, thereby facilitating similar exploration of soil mineral N and presumably provided a reliable index of $\%N_{dfa}$ by soybean.

These results support the hypothesis that, on average from different reference plants, BNF was significantly higher in CA with CR retained on the soil surface. Using automated temperature monitoring probes, Aulakh *et al.* (2012) measured soil temperature at 4 and 10 cm soil depth in macro-plots of the same experiment and demonstrated that during daytime, the maximum soil temperature at 4 cm depth remained 4–8°C lower in plots with retention of CR on the soil surface in CA as compared with bare soil surface. Thus, the beneficial effect on BNF by soybean of CR retention on the soil surface was presumably due to better activity of rhizobia created by the relatively cooler environment in the rhizosphere.

Comparable results were obtained in Experiment 2 where the ^{15}N isotope dilution method was used and in Experiment 3 where the ^{15}N natural abundance method was used, with spontaneous weeds as reference plants. While the former method requires costly ^{15}N-enriched fertilizer, the latter is based on natural abundance of ^{15}N and hence is much cheaper to use.

FATE OF ^{15}N FERTILIZER APPLIED TO WHEAT

The uptake of ^{15}N fertilizer in wheat grain and straw indicated quite similar patterns in both CT and CA tillage systems for N removal from soil as well as translocation from vegetative parts to grain. Similarly, the range of ^{15}N utilization by wheat in this study (39–47 percent) was similar to that reported from earlier studies with CT at the same site (Katyal *et al*., 1987; Bijay-Singh *et al.,* 2001). The reduction in the utilization of ^{15}N where crop residue was incorporated in CT or retained on soil surface in CA could possibly be due to higher losses of N via ammonia (NH_3) volatilization and/or denitrification as discussed later. The reduced utilization of ^{15}N at 150 kg/ha suggests that fertilizer was applied in excess of crop needs.

The significantly lower uptake of fertilizer N by wheat (straw + grain) in CA with CR retained is in line with the results obtained by Aulakh *et al.* (2012) from the macro-plots of the same study. At these macro-plots, reduced wheat grain yield and total N uptake were observed due to the cooler soil surface and related delayed germination with CR retained on soil surface in CA as discussed above.

The recovery of applied ^{15}N fertilizer in different layers of the soil profile (Table 2) at the harvest of wheat revealed that the majority of the residual fertilizer N was present in the top soil layer with little evidence for effects of fertilizer N rate, tillage and crop residue management. Movement of ^{15}N downwards was not affected significantly by fertilizer rate, tillage and CR treatments. In an earlier study with a rice–wheat rotation under CT system on the nearby site, Aulakh *et al.* (2000) reported rapid distribution of fertilizer N to a soil depth of 60 cm within 1–5 d after its application. Also, they observed that significant amounts of nitrate (NO_3^-)-N from soil layers up to 60-cm depth were used by wheat following rice because of its deeper and extensive rooting system.

Losses of N were low when 120 kg·N·ha^{-1} was applied without CR in both CT and CA but increased with the application of 150 kg·N·ha^{-1}, again suggesting that excessive N application led to higher N losses as a consequence of reduced utilization of ^{15}N by the wheat crop (Table 2). The losses of N could be via NH_3 volatilization and/or denitrification as the possibility of fertilizer N losses due to leaching to deeper soil layers appears to be low (only 2–6 percent of ^{15}N in fertilizer was recovered in the 60–120 cm soil layer). Since soil pH was 8.1, there is a possibility of NH_3 volatilization when NH_4-N fertilizer is applied on the soil surface (Aulakh and Bijay-Singh, 1997). The opportunity for this N loss process to occur was greater from soil under CA than that under CT. The increased ^{15}N losses in CA with the retention of CR on the soil surface in conjunction with 120 kg·N·ha^{-1} and 150 kg·N·ha^{-1} were also presumably in part through denitrification supported by soluble carbon (C) that moves downward from surface residue. On the other hand, the increased ^{15}N losses in CT with the incorporation of CR in conjunction with 120 kg·N·ha^{-1} and 150 kg·N·ha^{-1} were mainly through denitrification as decomposition of CR creates congenial conditions by consuming oxygen from the soil and supplying organic C substrate to denitrifying organisms (Aulakh *et al.,* 1991, 2001).

CONCLUSIONS

Results of this study support several conclusions that may have important agronomic and environmental implications. Soybean fixed N ranging from 81 to 125 kg·N·ha^{-1}, equivalent to 68–85 percent

of total crop N uptake, depending upon tillage and crop management. Furthermore, significant increases in BNF by soybean (between 5 and 20 percent) were recorded when crop residue was retained on the soil surface of CA and indicate the benefits that could be accrued by adopting such systems. Thus, partial substitution of rice (which requires 120–150 kg/ha of fertilizer N as well as 3–4 times the amount of irrigation water) by soybean, especially in rotation with wheat under irrigated conditions, could lead to an environmentally and economically sustainable cropping system. Although soybean grains remove a major part of N derived from BNF, earlier studies have well documented that soybean residues have substantial N content and enrich the soil upon mineralization leading to sustainable systems (Aulakh *et al*., 1991, 2012).

The recommendation for the adoption of CA in soybean–wheat rotation in the Indo-Gangetic Plains of northwestern India is further strengthened by quite similar patterns of N uptake, translocation from vegetative parts to grain, and utilization of applied ^{15}N by wheat in both tillage systems. The amount of unaccounted ^{15}N, which may have arisen from losses of fertilizer N from the soil–plant system, was higher with 150 kg·N·ha^{-1} in both CT and CA systems, suggesting the need for future research to improve N use efficiency in winter-grown wheat. The use of isotopic techniques in the present investigations facilitated the identification of factors and practices that can form the basis of future field studies for the development of effective strategies for the management of tillage and CR for fostering sustainable and environmentally sound agricultural systems. The good performance of the cheap natural abundance-based ^{15}N techniques also offers great opportunities for further multiple site and on-farm research.

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Soil Organic Carbon Preservation and Sequestration in European Agricultural Soils: An Overview

F. Bampa[1,*], E. Aksoy[1], R.A Guicharnaud[1], R. Hiederer[1], A. Jones[1], E. Lugato[1], P. Macaigne[2], L. Montanarella[1], M. Nocita[1], P. Panagos[1] and G. Tóth[1]

ABSTRACT

The decrease of fertility in European soils is fully recognized both from the scientific arena and from institutional bodies. Soil organic matter (SOM) decline is on the agenda of the Thematic Strategy for Soil Protection of the European Commission (EC) and the implementation of pilot long-term experiments (LTE) to detect soil organic carbon (SOC) changes is considerably increasing. Several bodies have started national inventories of forested areas, the Reducing Emissions from Deforestation in Developing Countries (REDD) mechanism is non-stop aiming to reduce emissions from forests, and the role of peatlands in climate change has been widely debated. Also, the integration of agricultural soils, climate change influence and food security in Europe is a topic under investigation. Fighting hunger in a sustainable way is on the agenda of the European Union food security policy and the Joint Research Centre's (JRC) role is to build a cross-disciplinary approach between the science and policy to take actions. Studies on SOC changes and the best management for C sequestration are continuously appearing, but how to deal with a growing population and the related food demand in the context of a climate change scenario is unknown. This paper gives an overview of the activities carrie d out in the JRC–SOIL Action with a specific focus on agricultural soils and their SOC content. A short paragraph covers the potential application of nuclear and isotopic techniques to support the knowledge of SOM and C sequestration dynamics.

Key words: *organic carbon, agriculture, soil, Europe, isotopic and nuclear techniques.*

SOIL PROTECTION IN EUROPE (COM (2006) 231 FINAL)

The thematic strategy for soil protection

The decline of soil organic matter (SOM) is recognized as one of the eight soil threats (together with erosion, contamination, sealing, compaction, loss of biodiversity, salinization, impermeabilization, and landslides) expressed in the European Commission's Thematic Strategy for Soil Protection with the objective of ensuring that the soils of Europe remain healthy and capable of supporting human activities and ecosystems (EC, 2006). One of the key goals of the strategy is to maintain and improve SOM levels to assure fertility. Climate change is recognized as a common element in many of the soil threats and for this reason the European Commission (EC) intends to assess the actual contribution of soil protection to climate change mitigation and the effects of climate change on SOM content. The overall objective of the strategy is to establish a Soil Framework Directive for the protection and sustainable use of soil, by preventing further soil degradation, preserving biophysical functions and by restoring degraded soils taking into account land use. In 2012, a report (COM (2012) 46 final) was published on the implementation of the Soil Thematic Strategy and ongoing activities by the EC's Directorate General for the Environment (DG ENV). In the document the use of appropriate management practices is mentioned as a way to maintain and increase SOM content (EC, 2012a).

Land use, land use change and forestry (LULUCF)

LULUCF refers to the forestry and agricultural sector in the context of the international climate negotiations under the United Nations Framework Convention on Climate Change (UNFCCC). Land use, land use change and forestry covers greenhouse gas (GHG) emissions and removals related to soils, trees, plants, biomass and timber. Forests and agricultural lands currently cover more than three-quarters of the European Union (EU) territory and hold large stocks of carbon (C). The accounting of C stored in the forests and soils is only partly recognized in the total GHG accounting due to the lack of robust C databases and common rules on how to account for emissions and removals. The EU intended to close the gap in the GHG accounting in its climate policy, submitting a proposal to harmonize rules to account for forests and agricultural soil emissions across the EU (the first step to incorporate these major sectors without common EU-wide rules into the EU's reduction efforts). The proposal (COM (2012) 93 final) aims to make accounting for grassland management and cropland management mandatory for Member States (MS) (EC, 2012b). Appropriate land use and management practices in forestry and agriculture could limit emissions of C and enhance removals from the atmosphere. The accounting framework could lead to positive mitigation efforts in the sectors becoming more visible and at the same time provide a solid basis for further cost-effective mitigation options and sustainable growth.

1 European Commission, JRC, Institute for Environment and Sustainability, Via E. Fermi 2749, IT-21027, Ispra (VA), Italy
2 Soil Water Management and Crop Nutrition Se ction, Joint FAO/IAEA Division, IAEA, Vienna, Austria
* E-mail address of corresponding author: bampa.francesca@jrc.ec.europa.eu

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Agriculture, soils and climate change: the CAP towards 2020

A substantial proportion of land in Europe is occupied by agriculture, and consequently this sector plays a crucial role in natural resources protection, a precondition for other human activities in rural areas. Adopting inefficient and non-sustainable land uses, management and farming practices, including poorly managed intensification as well as land abandonment, have adverse impacts on natural resources (EC, 2009a). According to the available literature (monitoring programmes, long-term experiments (LTE) and modelling studies), C preservation and sequestration in the EU's agricultural soils could have some potential to mitigate the effects of climate change. Of this potential, the highest mitigation share is related to C preservation linked to preventing certain land use changes (conversion of grassland or native ecosystems to cropland) and maintaining C stocks in organic soils. The most effective strategy to prevent global SOC losses would be to halt land conversion to cropland, but this may conflict with growing global food demand unless per-area productivity of the cropland continues to grow. The adoption of best management practices within all different land use categories could solve this conflict. (Kimble, Lal, and Follett, 2002) Implementing these technical mitigation strategies at the EU level would require changes to current agricultural practices. For example, changes in the cropping systems (already to some extent part of cross compliance minimum requirements), conversion of land use or the adoption of practices that leads to yield decreases. Other measures may be the production of neutral or imply long-term gains in terms of soil fertility and additional benefits (Schils *et al.,* 2008). Since 2009, with the White Paper, the EC and specifically the Directorate General for Agriculture and Rural Development started to take into account the role of European agricultural soils in climate change at the policy level. One of the two working documents of the White Paper, (SEC (2009) 417), explored the effect of projected climatic changes on crop yields, livestock management and the location of production, with a focus also on depletion in SOM and soil C preservation capacity (EC, 2009b). The other document, (SEC (2009) 1093 final), matched the reduction of GHG emissions coping with the changing climate to ensure synergies between adaptation and mitigation for co-benefits (soil and tillage practices, protection and management of pastures, organic farming) (EC, 2009c). The EC is actually preparing the groundwork for the new Common Agricultural Policy (CAP) post-2013. The new policy aims to assure (1) a viable food production, (2) a sustainable management of natural resources, and (3) climate action and a balanced territorial development. The communication on the new CAP, (COM (2010) 672 final), describes how farming practices could limit soil depletion, water shortages, pollution, C sequestration and loss of biodiversity (EC, 2010).

Soil action at the Joint Research Centre

The Joint Research Centre (JRC) is the EC's in-house science service aiming to serve society, stimulate innovation and support legislation. The JRC has the mission to provide scientific advice and technical know-how to support the conception, development, implementation and monitoring of EU policies. One of the seven institutes of the JRC is the Institute of Environment and Sustainability (IES) which deals with EU policies for the protection of the environment and the sustainable management of natural resources. The SOIL Action, which belongs to the Land Resource Management Unit of IES, aims to develop policies relevant to soil data and information systems. This activity has been running for more than 20 years and has developed the main reference data on the European soils at various levels of detail including:

- Establishment of the European Soil Data Centre (ESDAC) as a single point for all soil data;
- Development of procedures and methods for data collection, quality assessment and control, management and storage, distribution to the EC and external users, fully complying with INSPIRE (Infrastructure for Spatial Information in Europe) Directive principles;
- Research and development of advanced modelling techniques, indicators and scenario analyses in relation to the main threats to soil;
- Scientific support and technical assistance to other Commission services, European bodies and MS regarding soil information and negotiations;
- Extension of the coverage of the European Soil Information System (EUSIS) towards a fully operational Global Soil Information System (GLOSIS).

Focusing on SOC, the SOIL Action is involved in several issues and more specifically the assessment of SOC content and stocks; the collection and harmonization of the EU MS data; monitoring of the SOC decline and sequestration potential; the establishment of a proposal for a common sampling methodology aiming at C estimates; the refinement of pedotransfer rules (PTRs) and functions (PTFs); the mapping land cover/land use changes; the assessment of the effect of agricultural management practices on SOC; and a research study on the potentiality of biochar application, etc. The final aim of the SOIL Action will be to develop tools that will allow for the rapid, cost-effective and precise measurement of SOC content on a regular basis in Europe allowing for easy verification and reporting of SOC changes. Of particular importance is also the development of new advanced digital soil mapping techniques based on geo-statistical analysis and new measurement techniques.

European Soil Data Centre (ESDAC)

ESDAC is the thematic platform for soil related data in Europe. It was established through a decision taken at the end of 2005 by the EC's DG ENV, DG JRC, DG Eurostat (ESTAT) and the European Environment Agency (EEA) to establish ten environmental data centres in Europe. As a new development within the Seventh Framework Programme (FP7) for research of the EU (2007–2013), the SOIL Action has established ESDAC as an INSPIRE system on soil data and information operated by a network of national and regional data centres. The primary responsibility of ESDAC, as the single focal point, is to organize the availability and quality of the soil data required for policy making. Data come from projects run inside the EU institutions but also from collaborative projects between the JRC and several research partners (FAO, ISRIC world soil information data centre and the European Soil Bureau Network, etc.). The European Soil Data Centre can support EU policies related to soil as many key data sources are held by it and readily accessible to policy makers, the scientific community and to the public at large (Panagos *et al*., 2012).

European Environment Information and Observation NETwork (EIONET)

At the European level, there is a serious lack of reliable geo-referenced, measured and harmonized data on soil properties and specifically on SOC from systematic sampling programmes. At present, the most homogeneous and complete data on the SOM and SOC contents of European soils remain those that can be extracted and/or

derived from the European Soil Database (ESDB), in combination with associated databases on land cover, climate and topography. The latter database is the only comprehensive source of data on the soils of Europe harmonized according to the FAO standard international classification. In the context of the development of ESDAC, the European Environment Information and Observation NETwork (EIONET) has been established with the aim of providing timely and quality-assured data, information and expertise to assess the state of the environment in Europe, and the pressures acting upon it. The European Environment Information and Observation NETwork consists of representative organizations from 38 European countries (including the 27 MS of the EU plus other European countries). The network is built on national focal points that co-ordinate primary contact points and national reference centres for specific areas such as soil, water, waste, etc. The Directorate General for the Environment and EEA have identified the decline in SOM and soil losses by erosion as priorities in relation to the collection of the EU policy relevant soil data. The need for data is urgent and several efforts to establish agreed datasets for the EU countries have been made in the past. To support modelling activities and to display variations for these soil degradation indicators, EEA and the JRC jointly decided that all soil data management activities carried out by EEA in collaboration with EIONET would be transferred to the JRC. The latter, through EIONET, performed a data collection exercise in 2010 among MS with the final objective of creating a European wide dataset for SOC and soil erosion according to a grid based approach. There was no legal obligation for the EIONET MS to participate and PCPs and NRCs for soil contributed on a voluntary basis. The technical specifications of the data requested followed the standard 1 km grid defined by the INSPIRE Directive and the data submitted by participating countries could be based on measurements or be the best "estimate". Coming from different data sources, the data collected were harmonized to account for different methods of soil analyses, scales and time periods. As shown in Figure 1, for the SOC content (in percent), data were received from 12 EIONET Members (32 percent of the total EIONET network), but only five of them provided data for more than 50 percent of their geographical coverage (Panagos *et al.,* 2013).

Topsoil organic carbon content map (OCTOP)

The geographical distribution of the SOC content has been mapped for the EU and the JRC is undertaking SOC sequestration potential investigations (Jones *et al*, 2005). The European soil portal provides the map of topsoil organic carbon content (OCTOP) of soils in Europe, shown in Figure 2. The estimation of SOC and the management of SOC sequestration from soils and more specifically from agricultural soils have become a priority issue and needs to be addressed not just at the scientific level, but also within the policy arena. Already several projects supported by the EC took into consideration the use of agricultural conservation practices in relation to the main soil protection objectives, and provide a stocktaking of the current situation with regards to the policy measures that address (or contribute to) soil conservation within a EU-wide perspective (EC 2009a).

The JRC has a research activity on the implementation of the CAP policy options with the land-use modelling platform. The study estimated changes in SOC stocks from changes in land use as an indicator to evaluate the impact of various options of agricultural policies in support to DG AGRI and DG ENV. The changes in land use were defined based on scenarios of land use changes between the base year (2010) until a projected year (2020). The changes in SOC stocks are estimated following the Tier 1 approach of the IPCC (Lavalle *et al.*, 2011).

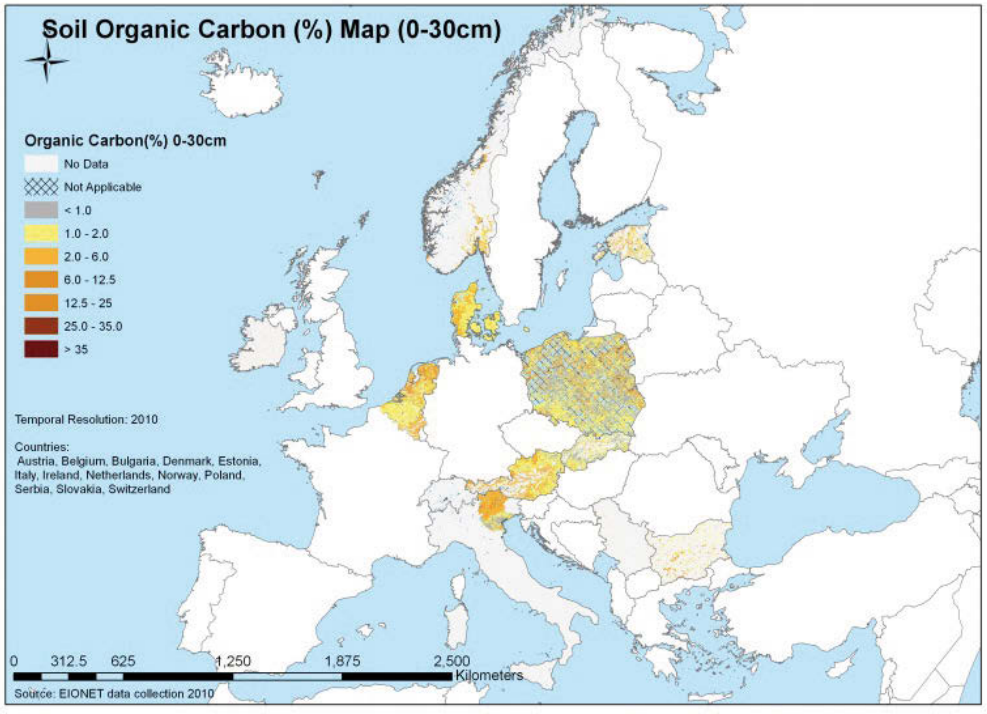


FIGURE 1. Results obtained from the 2010 SOC data collection exercise (*Panagos et al.,* 2013).

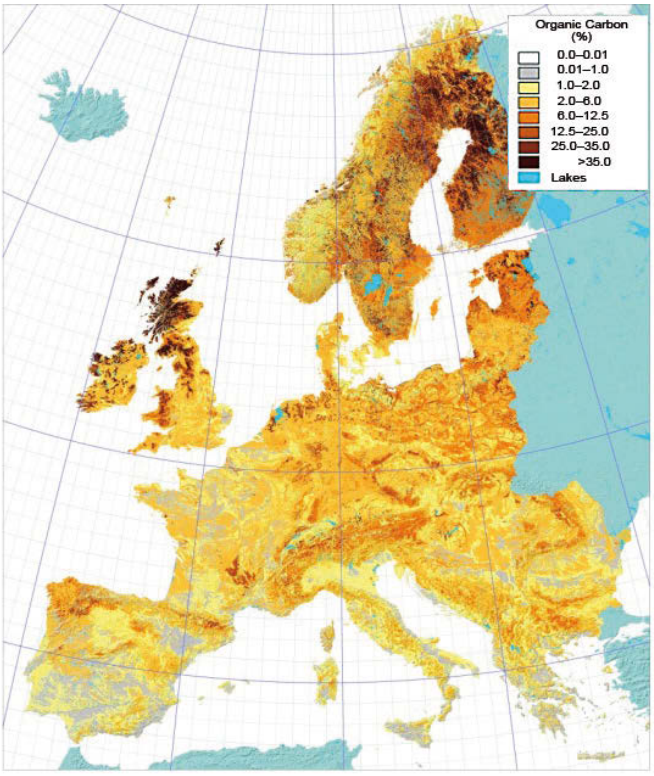


FIGURE 2. OC content (in percent) in the surface horizon (0-30 cm) of soils (*Jones et al.*, 2005).

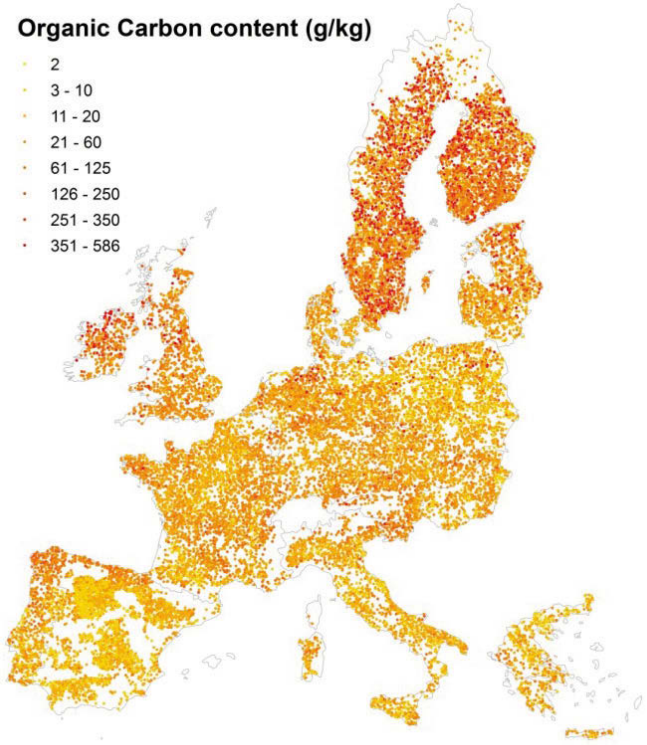


FIGURE 3. Distribution of 19 515 points and their level of topsoil OC concentration (g/kg) (*JRC*, 2011).

Land use/cover statistical area frame survey (LUCAS) project

Land represents a key actor for policy makers and European ecosystem planning, and for this reason there is a need for data: on changes in land cover, in biophysical attributes of the earth's surface and in land use affected by human actions. The capability to monitor those changes is linked to the availability of information on the coverage and use of the land. The LUCAS project is a field survey carried out on a sample of about 235 000 geo-referenced points spread over Europe. As such it is essentially a monitoring tool to follow the status of landscape diversity, to provide harmonized information on land cover and land use and to monitor changes in management and coverage in the European territory. The selection of the points was based on a regular 2 × 2 km grid covering the EU territory and defined as the intersection of around 1 million geo-referenced points. Each point was classified into seven land-cover classes using orthophotos or satellite images. From the stratified master sample, a sub-sample of 235 000 points was extracted to be classified by field observations in 2006 according to the full land nomenclature. The pilot phase (2000–007) involved 13 to 15 MS, with the first survey being held in 2001 to test the methodology at the EU level with a restricted budget. The focus of the survey was agricultural land with a sampling rate of 50 percent for arable land and permanent crops, 40 percent for grassland and 10 percent for non-agricultural land. The latest survey took place in 2009 in 23 EU MS. During the campaign, the field surveyors collected data on land cover/land use plus landscape photographs. In addition, linear elements and land-cover changes along a 250 m walk eastwards from each point (transect) were recorded.

LUCAS soil 2009

In 2009, within the LUCAS general project, an exercise coordinated directly by the JRC–SOIL Action called LUCAS Soil 2009 was performed to support the thematic strategy for soil protection. Its main aim was to produce the first coherent soil sampling exercise and harmonized set of analysis in the EU. This exercise is organized jointly by Eurostat, the DG ENV and the JRC and includes a topsoil module to the survey involving a subset of around 20 000 sites that were sampled in 25 MS (EU-27 except Romania and Bulgaria). The soil samples were collected by the LUCAS surveyors at points of their visit and dispatched to a central laboratory for the analysis of physical, chemical and multispectral properties (particle size distribution, pH, OC, carbonates, nitrogen, phosphorus and potassium [NPK], cation exchange capacity (CEC) and visible and near infrared diffuse reflectance). The same soil sampling exercise was carried out in Bulgaria and Romania in the early summer of 2012. The samples, weighing around 11 t in total, are now stored in the European Soil Archive Facility, at the JRC -Ispra. The points were extracted from the general LUCAS for topsoil sampling as being representative for soils of Europe and its 27 MS, stratified according to the topography and land use, and giving priority to points located on agricultural land. The LUCAS Soil 2009 dataset represents the latest and most comprehensive and harmonized information on physico-chemical properties of topsoil from the EU-27. The data on land use/land cover from the general LUCAS and the parameters collected from the soil sampling exercise are under evaluation, showing links between land use/cover classes, land management and the physico-chemical properties. Figure 3 shows the distribution of LUCAS points for SOC content. The LUCAS project provides new data for the estimation of topsoil C content at European level with a special focus on agricultural land.

A quality assessment of data was performed on the dataset, taking the main climatic zones, regions, land cover classes and management practices into consideration. Results highlight important linkages among these factors and help to understand and quantify the potential of European croplands with regards to C content and other major soil functions and indicators of soil health. Woodland and shrubland show the highest level of SOC in all climatic regions; this trend is in line with the common understanding of high values of SOC in forest compared to other land-cover classes. The lowest levels of SOC are observed in the Mediterranean climatic region; this general trend confirms that SOC content in northern regions is higher than in southern parts of the continent. Levels of SOC in arable land and the boreal climatic region are at least three times higher than in the other climatic regions. The map of OC content in topsoils in Europe from the LUCAS soil 2009 survey is the first step towards the interpolation of the LUCAS SOC points for creating a map using geo-statistical methods with a specific focus on agricultural topsoils (Jones *et al.*, 2005). Another evaluation in the LUCAS dataset is the C and nitrogen (N) limitation in soils. Preliminary results show that forested soils tend to be N limited and plant available C is rather a more limiting factor in the other soils than N. No specific trend is observed between climatic regions. The SOIL Action is performing other soil survey campaigns aimed at the collection of updated SOC data for Europe. For forest soils these data are already completed and are part of the data collected within the BioSoil demonstration project carried out in the course of the Forest Focus1 Regulation.

LUCAS spectral library for SOC prediction

In the context of global environmental change, the estimation of C fluxes between soils and the atmosphere has been the object of a growing number of studies. This has been motivated notably by the possibility to sequester carbon dioxide (CO_2) into soils by increasing SOC stocks and by the role of SOC in maintaining soil quality (Lal, 2004). The spatial variability of SOC masks its slow accumulation or depletion, and the sampling density required to detect a change in SOC content is often very high and thus labour intensive and costly (Shepherd and Walsh, 2002). Visible near infrared diffuse reflectance spectroscopy (Vis-NIR DRS) is a fast, cheap and efficient tool for the prediction of SOC at fine scales (Stevens *et al.*, 2008; Morgan *et al*, 2009). However, when applied to regional or country scales, Vis-NIR DRS does not provide sufficient accuracy as an alternative to standard laboratory soil analyses for SOC monitoring (Brown *et al.*, 2006). Within the framework of the LUCAS project, soil samples were scanned with a Vis-NIR spectrometer in the same laboratory. The scope of this research was to predict SOC content at the European scale using the LUCAS spectral library. A modified local partial least square regression (l-PLS) was implemented including, in addition to spectral distance, other potentially useful covariates (geography, texture, etc.) to select a group of predicting neighbours for each unknown sample. The dataset was split into mineral soils under cropland, under grassland, under woodland and organic soils due to the extremely diverse spectral response of the four classes. Four of every class training (70 percent) and test (30 percent) sets were created to calibrate and validate the SOC prediction models. The results showed very good prediction ability for mineral soils under cropland and under grassland, with a root mean square errors (RMSEs) of 3.6 and 7.2 g·C/kg respectively, while mineral soils under woodland and organic soil predictions were less accurate (RMSEs of respectively 11.9 and 51.1 g·C/kg). The RMSE was lower (except for organic soils) when sand content was used as a covariate in the selection of the l-PLS predicting neighbours. Despite the enormous spatial variability of European soils, the modified l-PLS algorithm developed was able to produce stable calibrations and accurate predictions. It is essential to invest in spectral libraries built according to sampling strategies based on soil types, and a standardized laboratory protocol. Vis-NIR DRS spectroscopy is a powerful and cost effective tool to predict SOC content at regional/continental scales, and should be converted from a pure research tool into a reference operational method to decrease the uncertainties of SOC monitoring and terrestrial ecosystem C fluxes at all scales.

LUCAS in the future

The land use/cover statistical area frame survey design has been initiated in Iceland (an EU candidate country), with soil samples planned to be collected in 2012 and 2013. In subsequent years, a large contribution is expected from other candidate countries. Preliminary studies on the database show the applicability of the LUCAS exercise for monitoring and accounting of the soil properties at the EU level. In addition, the data covering chemical and physical properties can be correlated with the general LUCAS data on land use/land cover and the impact of land-use/cover changes over time monitored. For this reason the LUCAS topsoil survey exercise represents a potential EU soil monitoring reporting verification system. If this exercise could be carried on over time, it would be possible to detect changes, for example, in SOC stocks and subsequently to evaluate the potential of EU agricultural soils for C preservation and sequestration.

CAPRESE project

There is a lack of knowledge as to how SOC-based mitigation options in agriculture are distributed across different agricultural production systems, and how the implementation of such mitigation options affects present land use practices (Jones *et al.*, 2005). SOIL Action is underway to focus on a new assessment of the current state of SOC levels in the agricultural soils of the EU. A review of current literature on the science of SOC fluxes in relation to current land covers/uses and policies is on-going, with particular attention being given to land changes and land conversion status. The literature review is also addressing and evaluating a range of agricultural management mitigation options and their theoretical impacts in stabilizing or enhancing SOC stocks (e.g. land-use change, tillage methods, cropping systems, irrigation methods, nutrient management, cross-compliance, rural development measures, etc.) in interaction with soil characteristics and climatic conditions. The final results will be presented in a report that will also evaluate the possible constraints underlying the full application of mitigation options. In the context of this review, a new project for DG AGRI started in 2012. The CArbon PREservation and SEquestration in agricultural soils — options and implications for agricultural production (CAPRESE) project aims to provide the necessary background that will contribute to the mid- to long-term development of policies addressing climate change soil-related aspects in European agriculture. Particular objectives of the study are: review of the potential climate change mitigation actions for agricultural soils across the EU; potential impact of selected mitigation measures in relation to OC levels; the effect of selected soil management measures on production patterns for different agricultural products; associated costs and support mechanisms to monitor and quantify the effects of the measures on the greenhouse gas balance; support for future evaluation of CAP measures; mitigation target scenarios for agricultural soils; and development of recommendations.

Soil transformation into European catchments (SoilTrec) projects

SoilTrec is one of the FP7 projects in SOIL Action. The "Spatial prediction of soil organic carbon of Crete & Koiliaris CZO by using geostatistics" study is one of the SoilTrEC projects, the purposes of which are: predicting SOC distribution of Koiliaris CZO and Crete by using geostatistics; developing up-scaling rules for Koiliaris CZO and Crete; developing a current OC map; and detecting C changes in space (land-use types) and in time. Soil organic carbon distribution on Koiliaris CZO and Crete were predicted using soil samples and environmental predictors (slope, aspect, elevation, CORINE land-cover classification, geological formations, World Reference Base for Soil Resources (WRB) soil classification, texture, average temperature and precipitation) with the Regression-Kriging (RK) method. These studies showed that the RK method is useful and reliable for predicting SOC. A significant correlation was found between the covariates and the OC dependent variable. The combination of a local dataset and LUCAS samples was advantageous for calibrating the land-use based soil data. The incorporation of associated local soil data improved the SOC estimates of the Crete map. The current SOC map of Crete was developed using the RK method. Currently, the project is focusing on a new CZO for the Damma Glacier in Switzerland for detecting SOC with the same method (Aksoy *et al.,* 2011; Aksoy, Panagos and Montanarella, 2012*).*

APPLICATIONS OF NUCLEAR AND ISOTOPIC TECHNIQUES TO SUPPORT THE KNOWLEDGE OF SOIL ORGANIC MATTER AND CARBON SEQUESTRATION DYNAMICS

Isotopic techniques are high precision tools that can provide an insight into soil processes and help to better understand, define and quantify the decline in OM and the C sequestration processes occurring in soils. The delta carbon-13 ($\delta^{13}C$) and delta nitrogen-15 ($\delta^{15}N$) isotopic signatures of SOM fractions can be used to identify C stabilization mechanisms and determine how long C molecules persist in soil (Kayler *et al.,* 2011*)*. Methods using the stable isotope ^{13}C or the fall-out radionuclide carbon-14 (^{14}C) are being used increasingly to study SOM turnover (Six and Jastrow, 2002). These methods are very useful in this field of research, as isotopes are the only tools which yield information over relatively short time periods (months and years), which is particularly relevant to study the impact of land-use management on SOM. The differences in the $\delta^{13}C$ isotopic signatures between C3 (e.g. wheat) and C4 plants (e.g. corn) can be used as fingerprints with simple mixing models to estimate the respective contribution to SOM and the residence time in soil of each C organic input. For a specific ecosystem, the natural $\delta^{13}C$ isotopic signature of the CO_2 released from the soil provides information about the processes driving CO_2 exchanges and the sources of the emissions (Pataki *et al.,* 2003).

EUROPEAN SOIL PORTAL AND NETWORKS

European soil portal

The SOIL Action manages the European Soil Portal (http://eusoils.jrc.ec.europa.eu) which contributes to a thematic data infrastructure for soils in Europe and acts as a web platform for ESDAC. It presents data and information on the European soils and tries to provide links to national or global datasets. In the portal it is possible to find: an inventory and access to the soil data currently held by the JRC, a library of scanned maps and a collection of prepared maps derived from the existing soil databases and user's applications to interact on-line with the data and all the SOIL Action activities.

ESBN network

The European soil portal website serves as a vehicle to promote the activities of the European Soil Bureau Network (ESBN). It was created in 1996 as a network of national soil science institutions and is operated at the JRC by the SOIL Action. Its main tasks are to collect, harmonize, organize and distribute soil information for Europe.

The SOIL Action is involved in many other worldwide networks and collaborations such as the Global Soil Partnership, Harmonized World Soil Database, Intergovernmental Panel on Climate Change, Global Soil Map.net, Global Soil Biodiversity Initiative, Sino-EU Panel on Land and Soil, etc.

CONCLUSIONS

The SOIL Action team and activities are very dynamic and influenced on one side by scientific passion and on the other side by the policy requests of the EC. The agenda of C sequestration has become a priority and the way to co-benefit soil protection and production with a cost-competitive mitigation potential is a new area of investigation.

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Global Monitoring of Soil Resources for Agriculture: Feasible Options for Early Implementation

M.G. Kibblewhite[1,*] and P.H. Bellamy[1]

ABSTRACT

Information on the status and trends in the condition of global soil resources for agriculture is urgently needed to drive policy for their protection and to support future food security. Current information on the extent and condition of global soil resources is inadequate and poorly collated. This paper reviews technical options and the feasibility of implementing a global soil monitoring system. Remote sensing methods are available for measuring indicators of change in the extent of soil resources (e.g. land area under arable production; area sealed by urban and infrastructure development). A soil monitoring network (SMN) based on physical sampling of strata corresponding to soil types and bio-geographical regions appears best suited to global ground-based soil monitoring. Reliable, agreed methods are available for estimating some forms of degradation (e.g. soil erosion by water, soil organic matter decline and salinisation). Thus there is an existing technical basis for establishing a global soil monitoring system and investment in this should be an urgent priority for the international community.

Key words: *soil degradation, food security, monitoring design*

INTRODUCTION

There is widespread and urgent concern in the scientific community (Lal, 2010) about the degradation of global soil resources and its impact on future food security, but this is not yet matched by concerted action by the international community to protect soil resources. This paper addresses the following questions: Why is soil monitoring a global priority? What needs to be monitored? Which options for a global monitoring programme are technically ready for implementation relatively quickly?

Why is soil monitoring a global priority?

Soil resources underpin the productive capacity of land. Their natural regeneration takes longer than human life times and their degradation is not always reversible, e.g. when accompanied by desertification. Those soils that are most productive are also often those most threatened, either by non-sustainable agricultural practices or extension of the built environment, or both of these. Major expansion of the agricultural land area allowed exploitation of new soil resources in the last century but there is now a net decrease in the land area in production, as the land available for new expansion has reduced and existing agricultural areas are degraded. Figure 1 shows the change in the total area of arable and permanent crops from 1961 to 2009. Simultaneously with the decline in the area of agricultural land, the demand for food and biofuels is rising and this is expected to encourage a long-term increase in food prices. The commoditization of food combined with established trading institutions and logistics support a global market in food. A consequence is that losses in supply due to soil degradation anywhere have the potential to affect global prices and so food prices for citizens who are far distant from the actual degradation.

Therefore there is a need for global governance of soil resources to ensure that these are conserved to support food availability and constrain food prices for all of humanity, especially the poor. A critical requirement for this governance is a global monitoring programme providing quantitative data of known and documented quality about trends in soil status. This programme is essential to identify which soil resources are most at risk from degradation, estimate the economic and social consequences of soil degradation, support arguments for appropriate policy measures and ensure that there is necessary investment in soil protection. Information from soil monitoring is a prerequisite for convincing politicians that unacceptable risks to food security are presented by the current lack of global soil protection. Furthermore, investment will not be targeted to where it is required without information on where soil resources are at most risk.

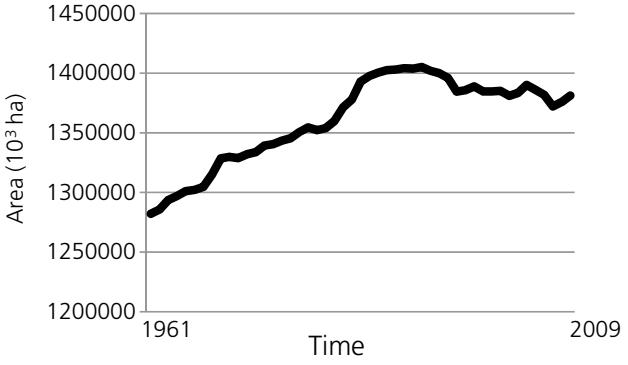


FIGURE 1. Global area of arable and permanent crops — 1961 to 2009 (10^3 ha) (FAOSTAT).

1 National Soil Resources Institute, Cranfield University, Cranfield, MK43 0AL, United Kingdom
* E-mail address of corresponding author: markkibblewhite@agritechnologyaction.com

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What should be monitored?

Information is needed both about the extent of productive soil resources and their condition. The spatial extent of productive soils may be assessed most directly from remote-sensed data on land cover and national returns on areas of land in agricultural production. Assessing the condition of soil resources means answering the question: "What is the state of the soil and how is it changing?". An integrative measure of soil status could be soil quality or soil health. Neither is ideal for global soil monitoring: soil quality (Karlen *et al*., 1997) refers to "fitness for purpose" for a defined use, but soils can support a wide range of agricultural goods and services and over time different ones are prioritized so the perceived quality of soil may alter independently of any state change. Soil health is conceptually attractive as it refers to the functioning of the soil as a living system (Kibblewhite, Ritz and Swift, 2008). However, current knowledge is probably inadequate to identify reliable indicators of this functionality and particularly to relate these to agricultural productivity. An established and more promising approach focuses on indicators that quantify the rates and consequences of soil degradation processes, e.g. soil erosion by water and wind, decline of soil organic matter, compaction, contamination, salinization, loss of soil biodiversity and sealing. A comprehensive review of potential indicators for major types of soil degradation (Huber *et al*., 2008) identified a core set of indicators (Table 1) that have mature, formal and agreed testing procedures and protocols or that are accessible via remote-sensing data. These could be employed with little or no further development. While not complete (e.g. for soil erosion by wind), the set is complete enough to support early implementation of soil monitoring, ideally with global, continental, regional and country reporting categories, and a reporting frequency of less than 10 years to match policy-making and review cycles.

Options for soil monitoring

Spatial extent of soil resources

Land cover data obtained through satellite-based remote sensing can provide accessible and reliable information on of soil resources in agricultural production systems and the changes to these resources over time as land is brought into agricultural production. Land cover data can also be used to provide information on soil resources being lost to surface sealing through extension of built environment (European Environment Agency, 2011). Therefore there is both a sound technical basis for monitoring the extent of productive soil resources and the availability of data to support this monitoring.

Condition of soil resources

Some on-going estimates of soil degradation can be obtained using remote sensing. For example, soil erosion by water can be estimated with land cover as the driving variable (Kirkby *et al*., 2008), but most types of degradation require ground monitoring, not least because the soil surface is often obscured by vegetation. Different design options are available for soil monitoring networks (SMNs) (Arrouays *et al*., 2012). They need to be sufficiently dense in space and time to provide information about soil state and change that has a low enough uncertainty to be useful. Rates of soil degradation may only be detectable across regions at multi-annual timescales even with higher spatial sampling densities. Many existing national and regional SMNs employ a regular grid-based design to provide an unbiased sample of land characteristics (soil types, land cover, etc.), but extending this approach globally could present insurmountable logistical problems (e.g. access to remote and contested regions) as well as high costs. Collection of data to support estimation of trends in the state of the soil at the continental scale may be achieved most efficiently by sampling strata defined by major soil groups and bio-geographical regions. As well as offering a more efficient sampling strategy, this approach should be able to accommodate logistical and other implementation problems more easily than a grid-based one. If sampling of some locations is not possible, data on trends can still be reported albeit with less confidence. Further investigation and agreement are probably required on within-site sampling protocols, including on whether to sample according to depth or pedogenic horizons.

Taxonomic definitions of soil types are contested but some internationally agreed ones are available (IUSS Working Group WRB, 2006), as well as data on their spatial distribution, the global coverage will hopefully be improved in the near future using digital soil mapping techniques (Sanchez *et al*., 2009). This information is probably sufficient to identify sampling locations. Existing well-described sites should be used where this is possible and appropriate but investment will be needed to qualify sites with local data and by field-based investigation. The number of sites required, even with strata limited to soil type and bio-geographical region, will be substantial. In excess of 4 000 sites were found to be necessary to detect changes in topsoil organic carbon in Europe according to soil type and land use (Morvan *et al*., 2008); by extrapolation, perhaps at least ten times this number of sites will be required for global monitoring.

CONCLUSIONS

Soil resources underpin agricultural productivity and food security. There is an urgent requirement for a global soil monitoring system to provide reliable information to policy makers on trends in the state of soil. Without such information, protection of soil will not be given the correct priority relative to other natural resources.

Remote-sensed data are available now to support monitoring of the extent of soil resources and where these are being lost and at which rates.

TABLE 1. Qualified indicators for degradation of soil

Degradation	Indicator	Unit
Erosion by water	Estimated soil loss by rill, inter-rill and sheet erosion	$t \cdot ha^{-1} \cdot y^{-1}$
Soil organic matter status	Topsoil organic carbon content (measured)	g/kg
Soil organic carbon stocks	Soil organic carbon stocks (measured)	t/ha
Diffuse contamination	Metal contents of soil	%
Soil sealing	Sealed area	ha; ha/y
Land consumption	Land take	%; ha
Salinization	Salt profile	%; dS/m

Indicators related to key soil degradation processes, erosion by water, decline in topsoil organic carbon, soil sealing are mature and supported by agreed testing procedures.

Early investment in the development of a design for a global SMN is required. Initial consideration indicates that a stratified design covering soil type and bio-geographical regions is preferable to one based on a regular grid. A possible estimate of the number of required sites is between 50 000 and 100 000.

Establishing a global soil monitoring system will take several years and information on trends in soil state will only become available following re-sampling. Realistically, it will be at least a decade and possibly two decades before a global soil monitoring system can start to report. Given the increasing pressure on soil resources for food production, the need for urgent action to establish this system is clear.

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Legumes in Crop Rotations Reduce Soil Nitrous Oxide Emissions Compared with Fertilized Non-Legume Rotations

G.D. Schwenke[1,*], D.F. Herridge[2], K.G. McMullen[1] and B.M. Haigh[1]

ABSTRACT

Soil nitrous oxide (N_2O) emissions were measured from a range of dryland crops and crop rotations in the northern grains region of Australia. The objective was to compare N_2O emissions associated with the growth and post-harvest residue decomposition of a nitrogen (N_2)-fixing legume crop with that from N fertilized non-legume crops. From 2009 to 2012 a dryland crop rotation experiment was conducted on a black Vertosol (cracking clay soil) representative of the main soil type used for grain growing in the region. Crop rotation treatments were: canola + N_wheat + N_barley + N (CaWB), chickpea_wheat + N_barley (CpWB), chickpea_wheat_chickpea (CpWCp), and chickpea_sorghum + N (CpS). Soil emissions of N_2O were monitored in the field seven to eight times per day using an automated system of chambers connected to a gas chromatograph. Soil mineral N and plant N uptake were measured by regular field sampling. During the project, extremes of cold, hot, wet and dry weather were experienced that were often well below or above long-term averages for the site. Cumulative N_2O emissions from the four rotations were in the order CaWB > CpS = CpWB > CpWCp. Emissions from CaWB (1 523 g·N_2O-N·ha^{-1}), where all crops were N fertilized, were more than twice those of CpWCp (614 g·N_2O-N·ha^{-1}), where legume N_2 fixation was the external N source. As a proportion of anthropogenic N input, legumes emitted less N_2O than N fertilized non-legumes. Most emissions from N fertilized crops occurred during early crop growth, while most emissions from legumes occurred during post-harvest decomposition of crop residues. These differences should be taken into account when devising strategies to reduce N_2O emissions from cropping.

Key words: *nitrogen, chickpea, wheat, sorghum, barley.*

INTRODUCTION

The concentration of the nitrous oxide (N_2O), a greenhouse gas in the atmosphere is increasing, largely as a consequence of increased anthropogenic nitrogen (N) inputs into the soil associated with animal and crop production. Emissions of N_2O from the soil originate from the biological processes of nitrification and denitrification that utilize inorganic N from any source (Bremner, 1997). The susceptibility for N_2O loss is determined by the interaction between the rate of supply of inorganic N, the N demand from crops and environmental conditions (Dalal *et al.*, 2003).

There is little evidence that the process of biological N_2 fixation itself contributes directly to total N_2O emissions (Jensen *et al.*, 2012), but legume crop residues are high in N and are readily mineralized in soil, albeit in a "slow-release" fashion. In contrast, inorganic N fertilizer adds N to the soil in an immediately-available form. Inclusion of N_2-fixing legume crops into an otherwise cereal dominated crop rotation should reduce the potential for N_2O losses through a reduction of soil inorganic N during the post-application period. Associated with reduced fertilizer N inputs will be reduced carbon dioxide (CO_2) emissions from N fertilizer manufacture, transport and urea dissolution. This research aims to quantify the potential for reducing soil N_2O emissions from dryland cropping by sourcing N from legume N_2 fixation rather than relying on fertilizer N.

MATERIALS AND METHODS

The experiment was located on a cracking clay soil (Black Vertosol; Isbell, 2002) at the NSW Department of Primary Industries experimental station near Tamworth, NSW, Australia. The surface 0–0.1 m of the soil was 44 percent clay, pH 8.0 (1:5 soil:water), 1.9 percent soil organic carbon, and had a bulk density of 1.0 Mg/m^3. The soil type is typical of the dominant soil used for dryland cropping throughout the northern grains region of Australia. The following crops were grown in the experiment: canola (*Brassica napus* cv. Hyola 50), chickpea (*Cicer arietinum* var. PBA Hatrick), wheat (*Triticum aestivum* var. Crusader), sorghum (*Sorghum bicolor* var. MR43), and barley (*Hordeum vulgare* var. Shepherd). Plot size was 6 m wide by 12 m long. All plots were sown using a zero-till planter at row spacings of 0.25 m (wheat, barley), 0.5 m (canola, chickpea) and 0.75 m (sorghum). Nitrogen fertilizer rates were based on the projected crop demand minus the soil mineral N supply indicated by pre-sowing soil core testing to 1.5 m. The N fertilizer used was urea, which was applied as a side band at planting at a depth of 0.5–0.1 m in the soil. The trial was a randomized complete block design with four replicates of six treatments (crop rotations). Greenhouse gas measurements were made in three replicates of four crop rotation treatments, namely:

- canola + N_wheat + N_barley+N [CaWB]
- chickpea_wheat + N_barley [CpWB]
- chickpea_wheat_chickpea [CpWCp]
- chickpea_sorghum + N [CpS]

As sorghum is a summer season crop, the change from winter to summer crops within the rotation meant only two crops were possible in the three-year study period for that treatment. The "+N"

1 Tamworth Agricultural Institute, New South Wales Department of Primary Industries, Tamworth NSW 2340, Australia
2 Primary Industries Innovation Centre, University of New England, Armidale NSW 2350, Australia
* E-mail address of corresponding author: graeme.schwenke@dpi.nsw.gov.au

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indicates treatments that had N fertilizer added. Fertilizer N rates were: 80 kg·N·ha^{-1} for canola and wheat, 40 kg·N·ha^{-1} for sorghum, and 60 kg·N·ha^{-1} for barley. After grain harvest by small plot harvester, all plant residue material from a plot was redistributed evenly across that same plot. Weeds, insect pests and plant diseases were controlled chemically using appropriate compounds at approved rates when necessary.

Above-ground whole plant samples (1 m row per plot) were collected monthly during crop growth by hand cutting, their dry matter determined, then analysed for total N concentration (percent N) by combustion analysis (EA1112, Thermo Finnigan). Total biomass N was calculated as biomass dry matter multiplied by biomass percent N. To determine the percentage of legume plant N derived from atmosphere (%Ndfa), samples collected at peak biomass were analysed for delta ^{15}N (δ^{15}N) by mass spectrometry after combustion analysis for total N. The natural abundance of ^{15}N of above-ground plant tissues of legumes was compared with that of a non-legume crop growing nearby. Percent Ndfa and total N fixed were calculated according to Unkovich *et al.* (2008). Grain samples from the plot harvester were analysed for percent moisture by oven drying and percent N by combustion analysis. Grain protein was calculated by multiplying measured grain N by a factor of 5.7 and then standardizing results based on a moisture content of 8 percent for chickpea and canola, and 12 percent for wheat, sorghum and barley.

Automatic gas measuring chambers, as described by Scheer *et al.* (2011) were used to measure N_2O emissions from soil seven to eight times per d. One chamber (0.5 m × 0.5 m × 0.2 m height) was deployed in each of three replicate plots of two treatments, CaWB and CpWB, from June 2009 until April 2010, when the system was upgraded to cover three replicates of four crop rotation treatments for the remainder of the experiment. Chamber height was increased using extensions to cover the erect crops as they grew. Chambers were secured to bases pushed 0.1 m into the soil.

Every three hours, the automatically-operated lids of all chambers in a replicate block were closed for one hour, during which time four separate samples of air were collected 15 min apart and analysed immediately by a gas chromatograph (8610C, SRI Instruments, California, USA) fitted with an electron capture detector for N_2O measurement. After one hour, the closed chambers opened and all chambers in the next replicate block closed for the hour-long period of measurement, and so on. The N_2O concentration in the four air samples collected from each chamber during each closure period was regressed against closure time. Patterns of accumulation of soil-emitted N_2O within a closed chamber during a measurement period have been reported as being either linear or non-linear. A routine developed by Pedersen, Petersen and Schelde (2010) was used that selects the most appropriate model for flux estimation based on the actual data from each measurement period. The routine first fitted a non-linear model to the data. Where this fit was not statistically significant, the linear model was then attempted. If neither model was statistically significant, a slope of zero was assigned. The slope of the selected regression was integrated back to time zero, then used in the calculation of N_2O flux as described by Scheer *et al.* (2011). Chamber temperature was measured using thermocouple probes, and barometric pressure data for Tamworth was obtained from the Australian Bureau of Meteorology.

Soil sampling for mineral N content (ammonium (NH_4^+) and nitrate (NO_3^-) was done by compositing five cores (0.05 m diameter) taken across each plot for surface and sub-surface samples (0–0.1 m and 0.1–0.2 m), and through compositing two cores per plot for deeper samples (0.2–0.3 m, 0.3–0.6 m, 0.6–0.9 m, 0.9–1.2 m, and 1.2–1.5 m). Surface and sub-surface samples were collected approximately monthly with a foot sampler, while deeper cores were only collected pre-sowing and post-harvest using a vehicle-mounted hydraulic powered coring machine. Soil water content was determined by gravimetric analysis at each time of sampling. Ammonium and NO_3^-N in filtered (Whatman 42) soil extracts (2 M KCl) were determined by standard colorimetric analyses using a flow injection analyser (Lachat Instruments, Colorado, USA).

Nitrous oxide emissions factors (EFs) were calculated by dividing the amount of N lost through N_2O emissions (output) by the amount of N input as fertilizer or fixed legume N. Where possible, these calculations took into account the background N_2O emitted from the same soil with no anthropogenic additions of N.

Statistical comparisons of treatment results were carried using Genstat v 14, with individual means tested for difference using the least significant difference test at a probability level of five percent.

RESULTS

Crop production

Grain yields and proteins for each crop in each of the four rotation treatments are summarized in Table 1. These were comparable with district averages, especially given the erratic rainfall recorded during the study period (Figure 1). During the 35-month project period, there were nine months (26 percent) where monthly rainfall was less than half the monthly average for the site, and another five months (14 percent) where it was well above the long-term average. These extremes influenced crop production by reducing plant growth in dry times, by reducing yield potential when wet conditions delayed sowing and by reducing yield recovery when harvest was delayed by very wet periods.

In 2009, canola generally yielded more than chickpea (Table 1), but there was no difference in protein content between the oilseed and the legume. There was no significant effect of applied fertilizer N on grain yields of canola (CaWB vs canola with nil N in 2009; results not shown), wheat (CaWB and CpWB vs CpWCp in 2010), nor barley (CaWB vs CpWCp in 2011). However, the application of N fertilizer did increase significantly grain protein of canola (protein for canola with nil N was 22.4 percent), wheat, and barley (Table 1). The addition of N fertilizer also increased the concentration of N in crop biomass during the growing season and the N content of the crop residues remaining after harvest.

Plant biomass N

The patterns of accumulation of above-ground (shoot) biomass N in all crops in the crop rotation trial between 2009 and 2012 are presented in Figure 2. In 2009, it is apparent that the N fertilized canola crop was N-rich; much more so than the chickpea crop. Biomass N decreased between the peak in October and the harvest in November because the November biomass mean does not include the harvested oilseed/grain N removed from the paddock. Grain N removal averaged 75 kg·N·ha^{-1} for chickpea and 120 kg·N·ha^{-1} for the canola. From Figure 2 it can be seen that the difference in biomass N between the peak for canola and the amount remaining in the plant biomass after grain harvest is greater than grain N offtake. Some of the N from the crop's peak biomass was leaf litter by the time of harvest as most canola leaves had fallen as the plant senesced. Some of the N from these fallen leaves had already mineralized in the soil as seen by the increase in mineral N seen in late 2009 in the canola soil results (Figure 3).

Plant N uptake in the 2010 wheat showed a similar level of N in the biomass as in the 2009 chickpea crop. There was no statistical difference in biomass N between fertilized and non-fertilized wheat plots ($p > 0.05$). Only at harvest time was biomass N in the fertilized

TABLE 1. Grain yield (t/ha) and protein (%) in four-treatments, three-year crop rotation experiment. Means in a row followed by the same letter in brackets were not significantly different (p = 0.05); n.s. indicates no significant treatment difference (p > 0.05)

Year	Rotation treatment / Crop / N fertilizer			
	CaWB	CpWB	CpWCp	CpS
2009	Canola+N	Chickpea	Chickpea	Chickpea
	1.8 t ha^{-1} (b)	1.4 t/ha (a)	1.3 t/ha (a)	1.7 t/ha (ab)
	23.8%	21.8%	21.6%	22.2% (n.s.)
2010	Wheat + N	Wheat + N	Wheat	
	3.1 t ha^{-1}	3.0 t/ha	3.1 t/ha (n.s.)	
	14.2% (b)	14.1% (b)	13.5% (a)	Sorghum + N
2011	Barley + N	Barley	Chickpea	9.6%
	3.7 t ha^{-1} (b)	3.8 t/ha (b)	2.0 t/ha (a)	
	13.3% (b)	10.5% (a)	23.5% (c)	

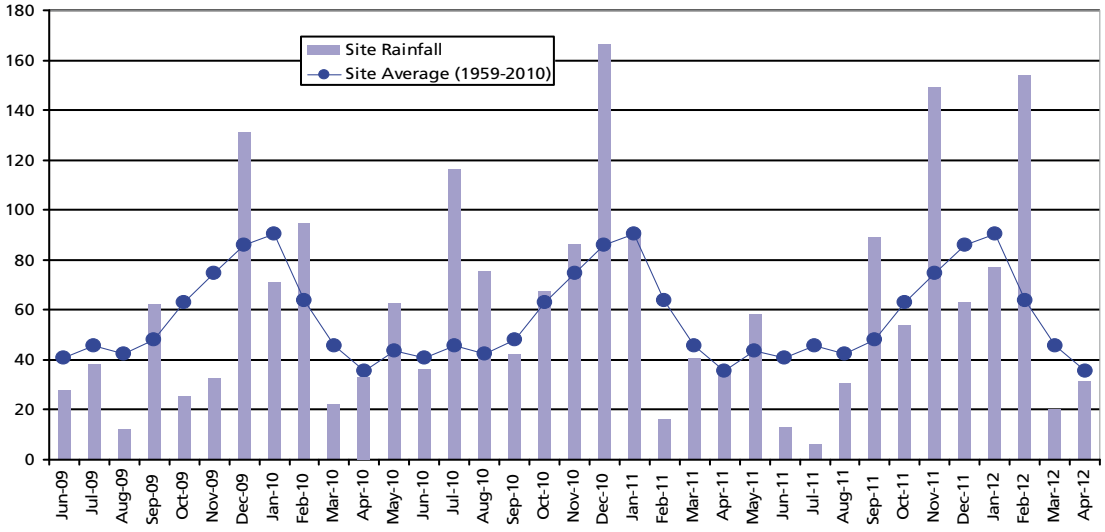


FIGURE 1. Mean monthly rainfall (mm) at the Tamworth experimental site during the experimental period, together with the monthly site average taken from 50 years of historical data collected at the site.

wheat following chickpea slightly greater than that in the fertilized wheat following canola ($p<0.05$). In 2011, there were much greater differences in biomass N between the chickpea (lowest), non-fertilized barley (after fertilized wheat), and the fertilized barley (highest). However, at harvest time, the N remaining in crop residues showed almost opposite trends with most in the chickpea plots and least in the non-fertilized barley plots where there was also less N to go into the grain, as evidenced by the lower protein results (Table 1).

Legume N_2 fixation

Chickpea plants grown in 2009 had 18% Ndfa. This is low compared with the Australian average of 41 percent (Unkovich, Baldock and Peoples, 2010), probably due to dry conditions during late crop growth and abundant mineral N in the soil. Combining Ndfa with the measured plant biomass and multiplying by two to approximate the N in the below-ground biomass of the plant (Unkovich, Baldock and Peoples, 2010), total N fixed by the crop was 49 kg·N·ha^{-1}. Since this is extra N to that already present in the soil, it can be counted as an anthropogenic input of N in the calculation of emissions factors (EF) for legume-derived N. The estimated %Ndfa for the 2011 chickpea was much higher at 37 percent. However, the plants grew less biomass than in 2009, so the total N input from N_2 fixation was just 41 kg·N·ha^{-1}.

Soil mineral N

Figure 3 shows the soil mineral N in the surface 0–0.1 m and 0.1–0.2 m layers sampled approximately at monthly interval throughout the project period. Nitrate and NH_4^+ N forms are combined in this figure. While NO_3^- was generally the dominant soil N form, NH_4^+ was also high for certain samplings due to recent additions of fertilizer N or plant residues. In 2009, the high mineral N in the canola + N plots is due to the added fertilizer. From November 2009 to May 2010, mineral N increased through mineralization of the crop residues — more so in the canola + N treatment which had dropped its leaves much earlier than the chickpea. There is a dip in the mineral N of the canola + N plots in January 2010 after heavy rains which likely caused denitrification and NO_3^- leaching. Mineral N increased subsequently as crop residues continued to mineralize, but decreased again in June 2010 after more heavy rains.

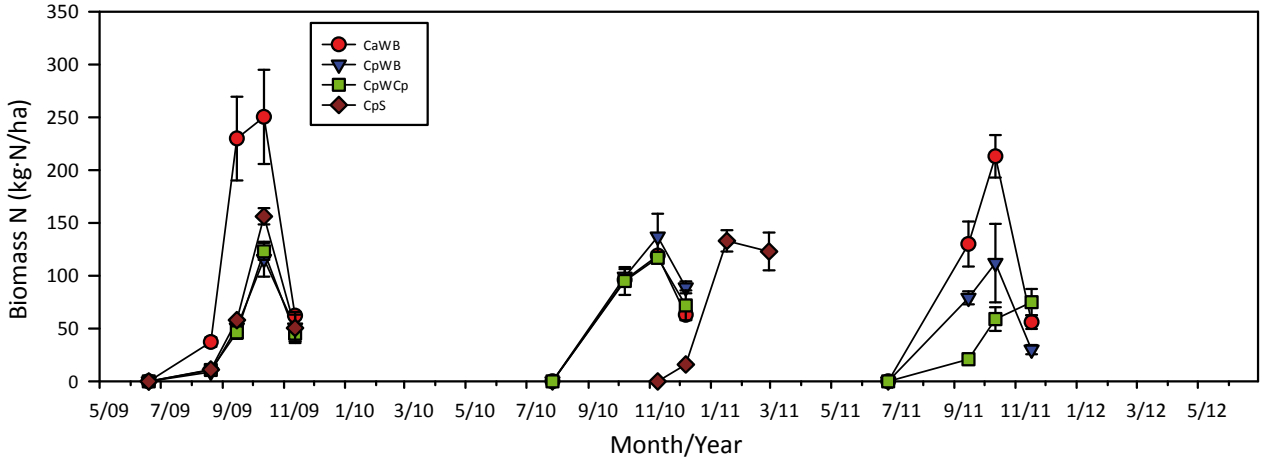


FIGURE 2. Above-ground biomass N in the crops of the four crop rotation treatments. Points are means of three replicate plots with bars indicating standard errors.

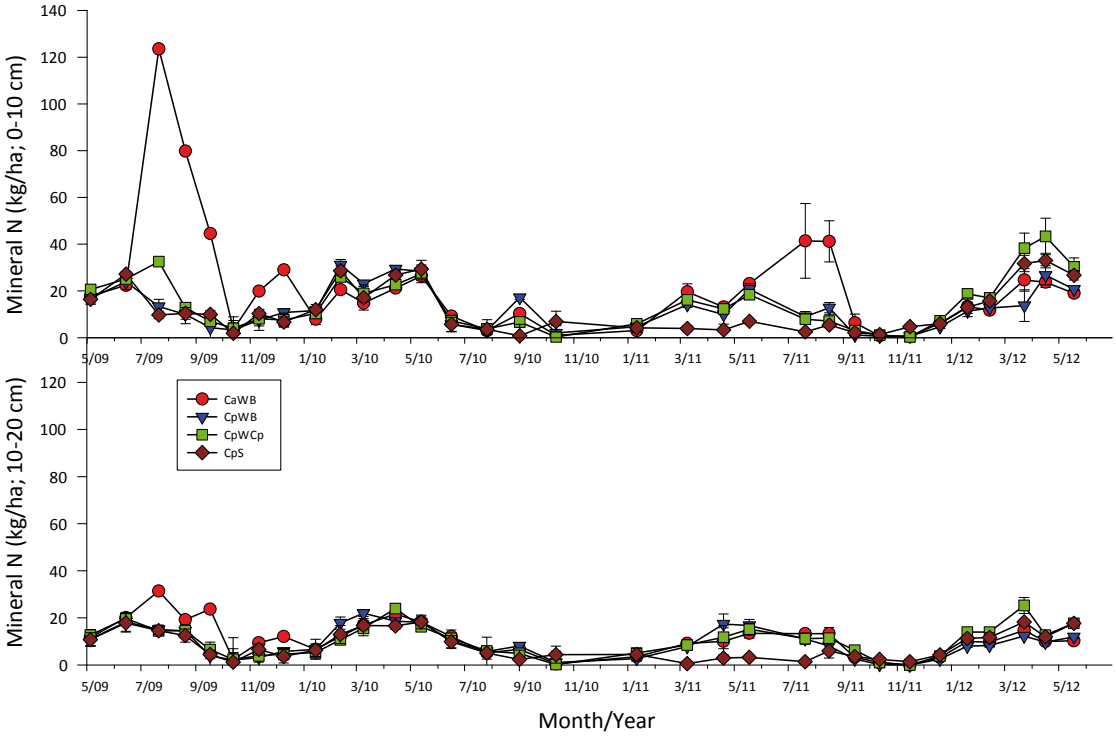


FIGURE 3. Changes in mineral N (NO_3^-+ NH_4^+ N) with time in the soil of the four crop rotation treatments. Points are means of three replicate plots with bars indicating standard errors.

The addition of fertilizer N to two of the three wheat treatments in late July 2010 led to only modest increases in mineral N in the 0–0.1 m sample depth. Again, heavy rainfall and saturated soil conditions probably led to N losses through denitrification and leaching beyond the sampling depths. Mineral N was barely detectable in the October 2010 sampling after these rains. Soil sampling was not possible in the next two months due to continued wet weather. Evidence for NO_3^- leaching during the prolonged wet conditions is shown by the significant difference in protein but not grain yield measured between the fertilized and non-fertilized wheat crops (Table 1). It is likely that some of the leached NO_3^- was accessed late in the wheat growing season when the plant was filling the grain.

After the wheat harvest in December 2010, all treatments except CpS (growing sorghum), increased in mineral N during late summer–early autumn as crop residues mineralized. Another increase occurred in July 2011 in the plots that were fertilized with N. However, the difference between N fertilized and non-fertilized treatments had disappeared by September 2011 due to plant uptake (see Figure 2). In 2012, mineral N again accumulated in the soil through continued mineralization of crop residues, with highest accumulation in the post-chickpea fallow.

Measurements of mineral N in the whole soil profile to 1.5 m (not shown) highlighted the strong accumulation of mineral N at the soil surface. Despite this, there was usually some N available throughout

the profile and sometimes a small accumulation below the surface due to NO_3^- leaching. Decomposing N-rich canola and chickpea residues, coupled with NO_3^- leaching led to mineral N differences down to 0.3 m depth.

Nitrous oxide emissions

Cumulative emissions of N_2O for all crop rotations from June 2009 to May 2012 are presented in Figure 4. Daily rainfall at the site is shown on the same graph to highlight the strong influence that rainfall had on N_2O emissions in this dryland cropping system. Cumulative N_2O emissions from the four rotations were in the order CaWB > CpS = CpWB > CpWCp, with more than twice the emissions from CaWB (1 523 g·N_2O-N·ha^{-1}) where all crops in the rotation were fertilized with N, compared with CpWCp (614 g·N_2O-N·ha^{-1}) where legume N_2 fixation was the only external N source. The patterns of N_2O emissions across the measurement period were sporadic. Long periods of nil to barely detectable emissions were interspersed with brief periods of high emission activity after heavy rain on saturated soil with either freshly-decomposing crop residue or recently added N fertilizer.

In 2009, emissions from the soil with chickpea were barely detectable during the growing season, while the soil with canola and fertilized with 80 kg·N·ha^{-1} responded to the N input and moist conditions by releasing N_2O. Between half and one third of the total emissions measured in the first 12 months occurred in just two heavy rainfall events during the summer post-harvest fallow (January–February 2010). The higher losses from the canola treatment compared with the chickpea treatment were likely due to mineralization of N from the leaves that the canola had dropped before harvest (see Figures 3 and 4). In contrast, chickpea, which had less biomass N than the canola to start with, only dropped its leaves around harvest time, after which it was mostly dry until the heavy rainfall in early January and again in February. Between the early 2009 emissions and the sowing of the following winter crop there were very few emission events despite some very wet soil conditions during May–June 2010. The mineral N measurements in the soil surface (Figure 3) indicate some losses during this time, but these did not lead to significant N_2O emissions.

Emissions of N_2O were very high soon after wheat sowing and fertilizer N application in late July 2010. Sowing was followed by 68 mm of rain in the subsequent three d. The saturated soil conditions meant that the conversion of urea to NH_4^+ then NO_3^- and its subsequent denitrification all occurred in a matter of h to d after the application of the urea. Despite identical amounts of N fertilizer applied to the CpWB and the CaWB plots, N_2O emissions in canola plots were higher, probably due to the earlier inputs of substantially greater amounts of residue N compared with chickpea.

Similarly, 60 mm of rainfall in the week following sorghum sowing in October 2010 led to significant N_2O emissions in the CpS treatment. Although only half the rate of fertilizer N was added to the sorghum (40 kg·N·ha^{-1}) compared with the 80 kg·N·ha^{-1} applied to the wheat, the magnitude of N_2O emissions from the sorghum plots were similar, so the rate of gaseous loss was double in the sorghum plots.

During 2011, N_2O emissions were consistently low throughout much of the year, with only isolated emission activity in response to rainfall. Of particular note was the lack of emissions following the addition of 60 kg fertilizer N/ha to barley in the CaWB treatment, sown in May 2011. This is despite the addition of the fertilizer clearly raising the concentration of mineral N in the surface soil (see Figure 3). It was a very dry start to the 2011 growing season (see Figures 1 and 4) with June, July and August all well below the long-term average rainfall. By September, when rainfall did return to normal, the wheat crop had taken much of the added fertilizer N into

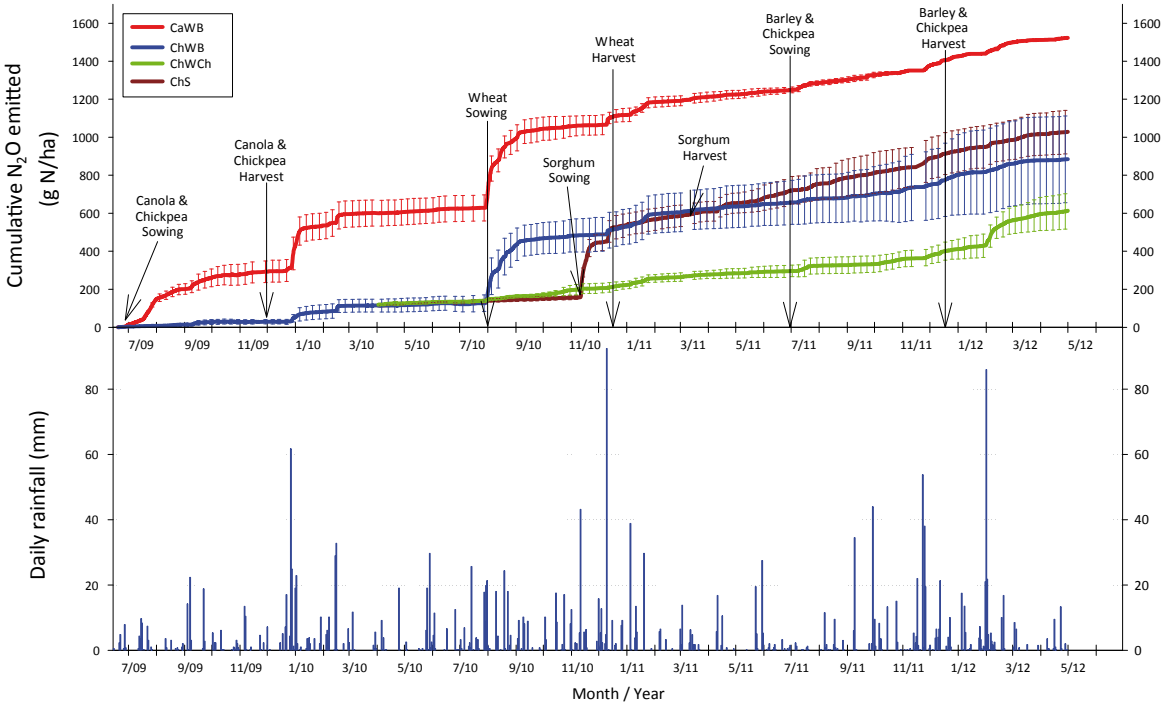


FIGURE 4. Cumulative emissions of N_2O-N for the four crop rotation treatments for the entire experimental period (top graph). Lines are means of three replicate plots with bars indicating standard errors. Bottom graph shows daily rainfall recorded at the site during the same period.

the biomass (see Figure 2), so that it was no longer susceptible to loss as N_2O. Heavy rainfall in November 2011 did cause N_2O emission activity, but the restricted supply of available NO_3^- for denitrification meant losses were not large.

After the 2011 winter crop harvest in December, warm moist conditions continued to aid mineralization of the crop residues until heavy rainfall in early February led to pronounced emissions of N_2O from the post-chickpea fallow. The N rich legume in this instance had mineralized more than the low N barley crop residues (see Figure 3), while the post-sorghum fallow had also mineralized more and consequently lost more than the barley residue plots. Again, there was no difference in losses between the post barley + N plots and those without N, as there was little difference in the harvest residue N contents (see Figure 2). This is likely because the + N treatment had channelled much of the additional N into grain protein which was removed from the paddock.

Nitrous oxide emissions factors

Emissions factors for all the experimental crops are listed in Table 2. Since there were just six chambers for the first crop, measurement of background emissions from a non-N fertilized canola crop or a non N_2-fixing chickpea crop was not possible in 2009. However, background emissions were measured for 2010 wheat and sorghum, and for the 2011 barley and chickpea crops. The smallest EF's were for the N fertilized barley in 2011 and N_2-fixing chickpea in 2009 and 2011, while the largest EF was for N fertilized sorghum in 2010–11. The wheat and sorghum EFs were largely determined by saturated soil conditions immediately after sowing and N fertilizer application. In contrast, losses of N_2O from chickpea plots were negligible during crop growth, but greater during the post-harvest fallow when plant residues were decomposing. The 2011 barley results indicate that N_2O emissions were reduced to a minimum when the mineral N from fertilizer was utilized by the crop and not subject to denitrifying conditions. These results showcase the range of emission scenarios possible in a highly variable rainfall environment coupled with a non-strategic fertilizer application programme.

The continuity of measurements during the three-year project period made it possible to compare total N_2O emissions across the four crop rotation treatments. No background emissions were subtracted from the total emissions in calculating these factors, but the background would have been the same for all treatments. Table 3 shows that the whole of rotation EF's were the same for the three winter–crop rotations (CaWB, CpWB, CpWCp), whereas the EF for the mixed winter-summer rotation (CpS) was 70% higher than winter-crop rotation. The latter result is particularly significant because this rotation had a similar total N input as the CpWCp treatment, but had two-thirds more N_2O emissions.

DISCUSSION

There have been several studies of N_2O emissions from N fertilized dryland wheat on cracking clay soils in Australia, with yearly total N_2O emissions from crops and crop sequences similar to those in this experiment ranging from 0.50 kg·N_2O-N·ha^{-1} in western Victoria (Officer *et al.*, 2008) to 0.90 kg·N_2O-N·ha^{-1} in southern Queensland (Wang *et al.*, 2011). By comparison, 0.60 kg·N_2O-N·ha^{-1} was measured here for fertilized wheat. The EF's for wheat (0.46–0.59 percent) were less than the three-year average for no-till, stubble retained wheat figure of 0.77 percent measured by Wang *et al.* (2011), but greater than the figure of 0.14 percent for the N fertilized, non-irrigated treatment in the study by Officer *et al.* (2008). The figure

TABLE 2. N_2O emissions factors for all crops in the four-treatment, three-year crop rotation experiment

Crop	Total N added*	Crop N_2O Emissions Factor (%)	
	(kg·N/ha)	(crop growth only)	(crop growth plus post crop fallow)
canola (2009)†	80	0.36 ± 0.07	0.78 ± 0.09
chickpea (2009)†	49	0.06 ± 0.02	0.26 ± 0.08
wheat (after canola)	80	0.51 ± 0.05	0.59 ± 0.07
wheat (after chickpea)	80	0.39 ± 0.07	0.46 ± 0.11
sorghum (after chickpea)	40	0.92 ± 0.12	1.31 ± 0.24
barley (2011)	60	0.07 ± 0.03	0.08 ± 0.02
chickpea (2011)	41	–0.04 ± 0.05	0.22 ± 0.16

* N added as urea fertilizer (canola, wheat, sorghum, barley) or N_2 fixation (chickpea)

† No background emissions data were available for calculation of canola 2009, or chickpea 2009

TABLE 3. N_2O emissions factors for the four crop rotations

Crop rotation treatment	Total N added*	Total N_2O-N emitted	Rotation N_2O Emissions Factor†
	(kg·N·ha^{-1})	(g·N·ha^{-1})	(%)
CaWB	80 + 80 + 60 = 220	1 523 ± 5	0.69 ± 0.00
CpWB	49 + 80 + 0 = 129	885 ± 228	0.69 ± 0.18
CpWCp	49 + 0 + 41 = 90	614 ± 93	0.68 ± 0.10
CpS	49 + 40 = 89	1 028 ± 114	1.16 ± 0.13

* N added as urea fertilizer (canola, wheat, sorghum, barley) or N_2 fixation (chickpea)

† No background emissions were subtracted

reported here came from a single year's measurement that was strongly influenced by heavy rainfall immediately after N fertilizer application into an already wet soil, so it is likely that more years of measurement would produce an average EF for wheat that is lower, as evidenced by the EF of 0.08 percent calculated for the barley crop grown in much drier conditions the following year.

Only Barton *et al.* (2011) have published Australian data on N_2O emissions during and after a dryland legume crop growing season. They found effectively nil additional N_2O emissions in the 12 months from planting a lupin crop (crop plus post-harvest fallow), once background emissions had been subtracted. However, their study was with lupins grown on a sandy soil in an arid Mediterranean climate (annual rainfall < 300 mm), whereas the present study was on a cracking clay soil in a subtropical, moderate rainfall region (annual average 680 mm). A review by Jensen *et al*. (2012) provided a mean N_2O emission value for chickpea (crop plus post-harvest fallow) of 0.06 kg·N_2O-N·ha^{-1} (range 0.06–0.16), but they did not indicate whether these figures were corrected for background emissions. In comparison, 0.13 kg·N_2O-N·ha^{-1} was measured here for 2009 chickpea (uncorrected), and 0.32 (0.10 after correction) kg·N_2O-N·ha^{-1} for 2011 chickpea. In this study, N_2O emissions during chickpea growth were negligible, but relatively high emissions were recorded during the post-harvest fallow period. For legume crops, the biologically-fixed N input into the system happens as the plant growth demands it. Any available soil mineral N is typically used by the legume first before it fixes additional N, hence during the growing season there is very little soil NO_3^- N available for potential denitrification losses. For legumes it is the release of N from the decomposing crop residues that is potentially a significant contributor to N_2O emissions (Jensen *et al.*, 2012), as seen in the 2010 summer fallow after 2009 chickpea, and the 2012 summer fallow after the 2011 chickpeas.

With the absence of Australian published data, the current Australian EF for N_2-fixing crops and pastures remains at the Inter-Governmental Panel on Climate Change (IPCC) default of 1.25 percent of N fixed (Australian Government, 2012). The measurements here in two dryland chickpea crops have shown this figure to be a significant overestimate, with an EF of 0.26 ± 0.08 percent in 2009 and 0.22 ± 0.16 percent in 2011. The 2009 EF would actually be lower than this as there were no background measurements at the same time with which to correct it. If the mean annual background measurements from the other years in the project were used instead, then this EF would have been –0.10 ± 0.05 percent. However, background emissions during the first year would likely have been lower, given that year was much drier than the following two years. Nevertheless, it is considered that the current EF for dryland pulse crops needs to be lower, in line with the results obtained here and those of Barton *et al.* (2011).

CONCLUSIONS

Both overall N_2O emissions and emissions as a proportion of anthropogenic N inputs were reduced by incorporating chickpea, an N_2-fixing legume, into an annual crop rotation in the place of non-legume crops receiving N fertilized. Also, the timings of N_2O loss from fertilizer application to winter cereal crops and of N input to the soil-plant system through legume N_2 fixation are different, and should be considered separately to mitigate emissions from the grains industry. For a winter cereal crop, the danger period is between application of the fertilizer at sowing and its uptake by the growing crop over the ensuing months of post-planting. In contrast, N_2O emissions during legume growing season in a dryland crop rotation are negligible, as the growing plant uses the available mineral N in the soil before fixing additional N to meet crop N demand. For an annual winter legume crop it is the period after grain harvest (during summer and autumn), the risk is high for N_2O emission. Heavy rainfall and saturated soils during this period can trigger emission losses from the mineral N derived from the decomposition and mineralization of legume crop residues.

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Nuclear Technologies in Global Warming: Assessing the Greenhouse Gas Effects Caused by Huge Biofuel Production in Indonesia

S.H. Waluyo[1,*]

ABSTRACT

Indonesia has developed a huge oil palm (OP) plantation industry and its contribution to global warming is unavoidable. Being the country with the largest OP production and the highest annual deforestation rate, Indonesia could contribute significantly to increasing greenhouse gas (GHG) emissions and exacerbate global warming. Oil palm production obviously puts heavy pressure on ecological processes such as land use, GHG balances, regional water and nutrient balances, erosion and biodiversity. A comprehensive understanding and proper assessment of the ecological changes arising from OP production are essential to ensure that OP cultivation has positive and sustainable impacts on climate. Measurements using stable isotopes of hydrogen (H), carbon (C), nitrogen (N), oxygen (O), and sulphur (S) and their ratios at natural abundances in the environment can address such large-scale ecological changes, detect the impacts at an early stage, assist in mitigation and give essential information that needs to be considered by government in formulating national biofuel policies. Studies of individual components of ecosystem C budgets and their environmental control can improve understanding of ecosystem function and its potential response to climate change. Measurement and analysis of C isotope ratios of leaf and atmospheric carbon dioxide (CO_2) samples can provide integrated information about important plant physiological characteristics spatially and temporally, such as variation in C isotope discrimination in natural forest, OP monoculture and pasture or other agricultural practices. Further, employing an approach using multiple isotopes to assess simultaneously the ratios of deuterium:hydrogen (D:H), oxygen-18:oxygen-16 (^{18}O:^{16}O) and/or carbon-13:carbon-12 (^{13}C:^{12}C) in different compounds, and the delta hydrogen-2 (δ^2H) and delta oxygen-18 ($\delta^{18}O$) of water (H_20), the delta carbon-13 ($\delta^{13}C$) and delta nitrogen-15 ($\delta^{15}N$) values of dissolved compounds in stream discharge from watersheds and in the tissues of organisms provide a unique means to investigate the coupling of H_2O and C fluxes at various temporal and spatial scales, and to develop sustainable land-water management practices. Water scarcity is the key limiting factor for OP production in many contexts. Major allocations of water for OP production have the greatest impact on local water resource balances, disturbing stream flows and availability of fresh water. In addition, there will be loss of biodiversity and food-fuel competition as a result of PO development. Lastly, elimination of natural food by land-use change will harm food security and increase food prices. Developing an isotope monitoring network and spatial modelling based on isotopic measurements of atmospheric inputs, ecosystem outputs, changes between inputs and outputs within ecosystems and sentinel organisms as integrators and indicators of ecological change are very usefull to detect and understand ecological changes at a continental scale. Understanding the underlying or indirect causes of deforestation and the development of an isotope monitoring network are crucial for informing environmental policy makers in Indonesia about managing efforts for mitigation to cope with the global climate change within the Reduced Emissions from Deforestation and Forest Degradation (REDD) scheme in which Indonesia has pledged to reduce GHG emissions by 41 percent by 2020. Employment of nuclear isotopic techniques (NITs) in Indonesia could contribute greatly to better conservation of forest, agricultural and peat land resources through improving soil and water conservation practicesand reducing GHG emissions.

Key words: *oil palm production, greenhouse gases, climate change, forest and peat land conversion, isotope techniques, environmental and ecosystem effects.*

BIOFUEL DEVELOPMENT IN INDONESIA

Indonesia is one of the new big emerging biofuel countries. The President of Indonesia has strongly supported to the strengthening of national energy security by seeking alternative energy sources. One of the alternatives is development of biofuels, since the raw materials are abundantly available around the country (oil palm, jatropha, sugar, cassava, maize etc). The use of five percent biofuel in the national energy mix is targeted by 2025. This policy has already been started since 2006 with Presidential Instruction No. 1, 2006, Presidential Regulation No. 5, 2006 and Presidential Decree No. 10, 2006 (Caroko *et al.*, 2011).

Oil palm (OP) has the most potential for biofuel production in Indonesia. Indonesia is the largest producer of crude palm oil (CPO) in the world, producing almost half of the world's palm oil. Production in Indonesia increased sharply from 7 Mt in 2000 to around 23 Mt in 2011, with OP plantations expanding rapidly by 11.8 percent per year. Huge oil palm plantations were developed in the last decade on the islands of Sumatra, Kalimantan and Papua (Figure 1). Nearly 11 Mha was allocated in 2011 and it is planned to expand this up to 20 Mha (Colchester *et al.*, 2006; Schoneveld, 2010; Caroko *et al.*, 2011).

Two-thirds of the current expansion of OP cultivation is based on the conversion of rainforests and one third on previously cultivated or until now fallow land. Of the converted rainforest areas, one

1 Center for the Application of Isotopes and Radiation Technology, National Nuclear Energy Agency, PasarJumat, Jakarta, Indonesia
* E-mail address of corresponding author: shwaluyo@yahoo.com

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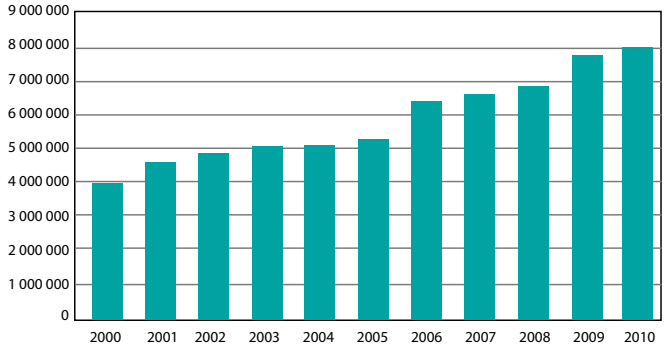


FIGURE 1. Oil palm plantation areas (ha) in Indonesia (Ministry of Industry, Republic of Indonesia).

quarter has peat soil with a high C content, and the expected share from peat soils is expected to be 50 percent by 2030 (Bringezu *et al*., 2009).

Indonesia has experienced extraordinary land cover changes over the past few decades. The deforestation rate was 2 percent (≈1.87 Mha/yr) between 2000 and 2006, and this is believed to be the highest in the world. As a result, the forest area decreased from 119.7 Mha in 1985 to only 88.5 Mha in 2005. Assuming the current trend continues, the total forest area of Indonesia would be reduced by 29 percent compared with 2005, and would cover only about 49 percent of the original area in 1990 (Hooijer *et al*., 2006; Bringezu *et al*., 2009; Ekadinata *et al*., 2011) leading to severe environmental consequences such as soil erosion and degradation, water pollution, loss of bio diversity and greenhouse gas emissions.

Greenhouse gas emissions in oil palm production

Greenhouse gas emissions occur at all points in OP production cycle (Murdiyarso *et al*., 2010; Achten and Verchot**,** 2011). Carbon dioxide is the most important GHG emitted from drained peat lands (Figure 2), contributing 98 percent or more of the total combined global warming potential (GWP). Significant amounts of stored C are lost at all stages of land use conversion and plantation management processes. Annual carbon dioxide (CO_2) emissions from direct land conversion alone are estimated at around 1.83 Gt CO_2, and are even higher under OP plantation on peatland (Ravindranath *et al*., 2009). Forest conversion on mineral soils to promote continued OP cultivation causes a net release of approximately 650 Mg·CO_2-eq·ha^{-1}. Peat forest conversions release over 1 300 Mg CO_2-eq per 25-yr cycle, and continuous decomposition augments the emission of 800 Mg CO_2-eq each cycle per ha (Germer and Sauerborn, 2007). Fargione *et al.* (2008) calculated that the conversion from forest peatland to palm oil releases 3 452 t·CO_2·ha^{-1} and that it would take >420 yr to replenish C losses caused by habitat conversion.

Methane (CH_4) fluxes in drained tropical peatland are insignificant relative to losses of CO_2, both in terms of the mass of C lost and overall climatic impact. Current research indicates that CH_4 emissions can be very high in drainage canals and form a substantial part of the GHG emissions in tropical peat lands that are converted to plantations (canals make up 3–5 percent of total plantation areas). However, this potentially important source of CH_4 remains to be quantified. Carbon lost through leaching seems to be prominent in OP plantations since particulate organic carbon (POC) and dissolved organic carbon (DOC) can easily flow away from the land. Rates of peat nitrous oxide (N_2O) fluxes in OP plantations also remain uncertain, and there is limited data concerning the magnitude and dynamics of emissions, particularly following fertilizer application. Heavy fertilization may stimulate decomposition and soil temperatures which are generally higher in plantations than in forest, and may increase microbial activity influencing denitrification. According to the Intergovernmental Panel on Climate Change (IPCC) guidelines, one percent of fertilizer applied is emitted as N_2O-N with GWP of 296 times greater than CO_2. Based on a literature review, GHG emissions related to the use of artificial fertilizers and pesticides are in

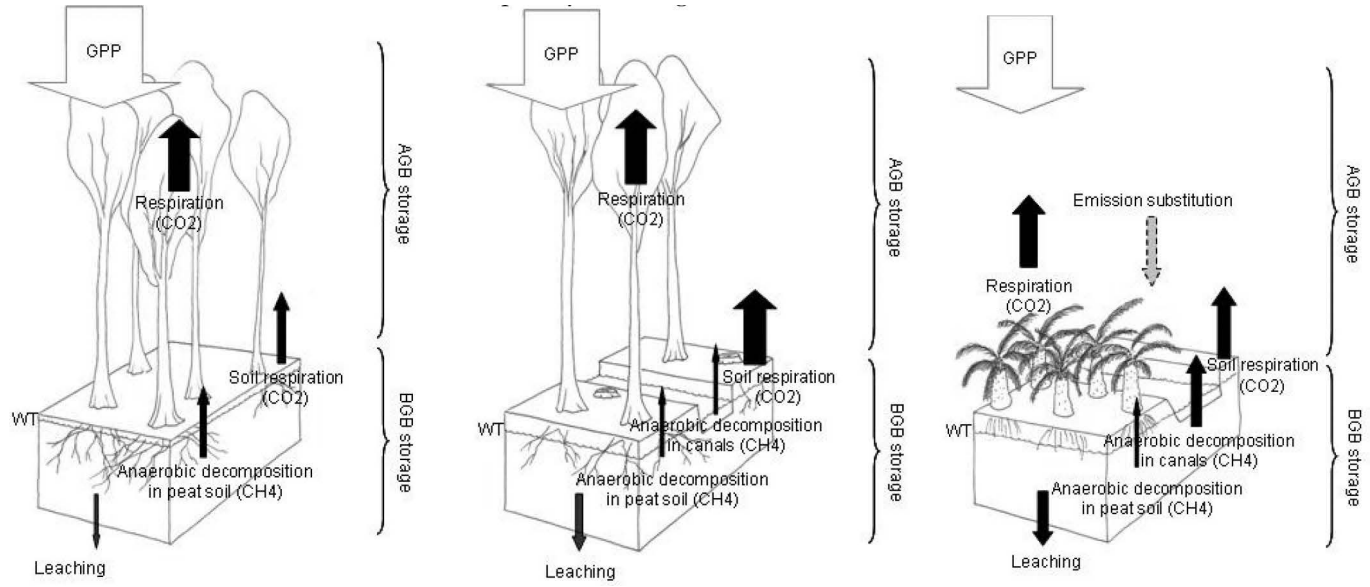


FIGURE 2. Land-based C fluxes on primary/and rained (left), on drained + partly logged peat swamp forest ecosystem (middle) and on oil palm plantation peat (right) (Verwer, van de Meer and Nabuurs, 2008).

the order of 1 000–1 500 kg·CO_2-eq·ha^{-1}·yr^{-1}. While based on average yields (3.2–4.0 t·PO·ha^{-1}·yr^{-1}), GHG emissions per t of CPO are in the order of 250–470 kg·CO_2-eq. It is worth restating that it is only the C released from decomposition of historically accumulated peat that is of relevance to global C emissions and anthropogenic climate change (Germer and Saurborn; 2007; Brinkmann Consultancy, 2009; Page *et al.*, 2011).

Indonesia is the world's third largest emitter of GHGs after the United States and China. Emissions are heavily dominated by deforestation, and more than half of the emissions come from the land-use, land-use change and forestry (LULUCF) sector (USAID Indonesia, 2008; Amron *et al.*, 2010; Ekadinata and Dewi, 2011). Almost 84 percent (57.4 Gt C) of the peat land C resources are located in Indonesia, while conversion of tropical peat forest to OP plantation increases GHG emissions significantly (Page *et al.,* 2011). To mitigate global climate change, the Government of Indonesia has declared its commitment to reduce GHG emissions by 26 percent by 2020 by a business as usual approach and by a further 15 percent with international support. Most of the reduction is targeted to come from the LULUCF.

Impacts of oil palm production on water resources

Large-scale deforestation will tend to change the regional water balance through reducing the evapotranspiration flux to the atmosphere. This will be accentuated as deforestation continues and an increasingly higher ratio of forest edge to undisturbed forest. A dense plant cover and relatively high surface air temperatures make the "biological water pump" very effective, returning a major part of precipitation back to the atmosphere. Deforestation makes the biological water pump weak, and will cause more water to run off to rivers and the local temperatures will rise. The effects of the reduction in the amount of water returned to the atmosphere will likely vary according to the size of the cleared area. However, from a meteorological point of view, the effects of deforestation may be somewhat greater in scope than the actual size of the deforested area (IAEA, 1990; Nguyen *et al.*, 2011).

Scarcity of water has proven to be the key limiting factor for OP production in many contexts. During the growing period, OP needs about 5.6 mm water·d^{-1}·$tree^{-1}$ (equal to 150–200 L·d^{-1}·$tree^{-1}$) (Kartika and Sosiawan, 2010). Further, the processing of feedstocks into biofuels can use large quantities of water, mainly for washing plants and seeds and for evaporative cooling. Massive irrigation infrastructure has to be provided for commercial yield levels and major groundwater resources around the OP plantation of local communities have been diverted. Therefore, OP plantation decreases water resources, and mainly the groundwater resources which are required by local communities, although the potential for expansion of irrigated areas appears to be high in some areas on the basis of available water resources and land.

Oil palm plantations also cause deterioration of water quality. Converting forest to OP fields may exacerbate problems such as soil erosion, sedimentation and excess nutrient N (nitrogen) and P (phosphorus) run-off into surface waters and infiltration into groundwater from increased fertilizer application. Cultivation also requires a lot of pesticides for optimum production, which often leach into rivers, contaminating the water. Currently, around 25 different pesticides are used. The most commonly used weedkiller is paraquat-dichloride, which is very toxic and accumulates in soil with repeated applications. Its toxicity and accumulation negatively affect the ability to use the land as a source for food production. Water quality is also worsened by the overflow or dumping of untreated POME into waterways, which threatens community health and reduces aquatic diversity (Colbran and Eide, 2008).

Impacts of oil palm production on soil resources

Forest ecosystems have a finely balanced nutrient system and deforestation will affect the dynamics of soil resources of N, sulphur (S) and other nutrients. Increased biofuel production will be achieved through improved land productivity and through expansion of cultivated area using existing cropland as well as less productive land. However, it is more likely that biofuels will intensify the pressure on fertile lands where higher returns can be achieved.

Establishment of OP plantations by clearing vegetation and constructing roads and drainage canals will reduce the permeability of the land, cause a loss of soil fauna activity and compact the land, all of which increase top soil run-off and cause soil erosion and affect the fertility and quality of soils. The top soil of OP plantations are prone to erosion which reduces soil fertility, and the use of fertilizers and pesticides will contaminate water resources while sediment loads in rivers and streams will increase significantly (Soyka, Palmer and Engel, 2007; Williams *et al.*, 2007; Murdiyarso *et al.*, 2010). The real danger will ultimately fall on humans and arise from elimination of a natural food while major re-allocations of water will impact negatively on food security, food prices and availability of fresh water.

In addition, there will be loss of biodiversity mostly as a result of habitat loss, increased invasive species and nutrient pollution. Nutrient emissions to water and air resulting from intensive OP cropping will impact species composition in aquatic and terrestrial systems.

Social and economic pressures will also be increased to provide fresh water, food, fuel and wood products for subsistence use or for export, and soil degradation, erosion and leaching of nutrients may reduce the subsequent ability of the ecosystem to act as a C sink.

Methods for assessing ecological changes caused by greenhouse gas effects

Forest ecosystems are controlled primarily by the interactions between water, oxygen, nutrients, carbon and microflora. These environmental structures can be modified by changes in land use due to OP plantation. Carbon dynamics are modulated primarily by the biota, especially through photosynthesis and respiration processes. It is essential to understand how plants affect the C cycle and ecosystem functioning (Prentice, 2001; Malhi, 2002). Quantifying and mitigating the potential of large-scale OP cultivation on carbon emissions is important to reduce its impact on climate change.

Information about the effects of deforestation on GHG emissions has basically come from methods of measurement such as flux chambers (Figure 3), eddy covariance, DOC and POC and subsidence monitoring for estimating C and GHG fluxes and budgets in tropical countries. It is important to note that each technique has its advantages and disadvantages, largely relating to the spatial and temporal scales of measurement. However, it is critical to have a clear understanding of exactly which components of the C and GHG budgets are measured by each method and, perhaps more importantly, which components are not measured or cannot be differentiated adequately (Page *et al.*, 2011).

One important approach is the measurement of stable isotope ratios present at natural abundances in the environment. Stable isotope measurements of key elements (H, C, N, O, and S) are an important component of ecological monitoring. Their advantages derive from their ability to integrate source and process information as well as their often greater sensitivity to ecological perturbations than elemental or compound concentrations or fluxes in nature.



FIGURE 3. Closed chamber measurements being made in the field; large static chamber (left) and dynamic chamber and CO_2 analyzer (Page *et al*., 2011).

Indeed, stable isotope measurements can capture fundamentally different aspects and dimensions of ecosystem change that cannot be realized with the conventional types of environmental measurements (Williams *et al*., 2007; Nguyen *et al*., 2011).

Natural variation in the stable isotope ratios of light elements in both biotic and abiotic components of ecological systems occur as a result of biological and physical fractionation events within an ecosystem. One important consequence of this is that sources of elements and material fluxes can be traced at large scales since different sources often have different isotope ratios based on natural fractionations in the environment. The isotope ratios of organic and inorganic substances also provide a temporal integration of significant metabolic and geochemical processes on the landscape. Further, the isotope ratios of well-mixed environmental reservoirs as reflected, for example, in the delta hydrogen-2 (δ^2H) and delta oxygen-18 ($\delta^{18}O$) of water, the delta carbon-13 ($\delta^{13}C$) and delta nitrogen-15 ($\delta^{15}N$) of dissolved compounds in stream discharges from watersheds and in the tissues of organisms, represent an integration of source inputs that extend over large spatial scales and the processing of elements within ecosystems.

NUCLEAR TECHNOLOGIES IN ASSESSING GREENHOUSE GAS EFFECTS

To date, deforestation for OP production in Indonesia is the overwhelming cause of ecosystem degradation (IAEA, 1990; Dawson and Siegewolf, 2007; Nguyen *et al*., 2011). The major links between C, H and O in atmospheric and terrestrial ecosystems are shown in Figure 4.

One particularly powerful approach is to employ multiple isotope approaches to assess/measure simultaneously the deuterium:hydrogen (D:H), (^{13}C:^{12}C) and/or (^{18}O:^{16}O) ratios in different compounds to provide a unique means to investigate the coupling of water and C fluxes at various temporal and spatial scales. The use of stable isotopes has yielded significant knowledge breakthroughs such as partitioning of CO_2 (using carbon-13 [^{13}C]) fluxes in terrestrial ecosystems between photosynthesis and respiration (^{13}C and ^{18}O), separating autotrophic and heterotrophic respiration in soils (using ^{13}C), and quantifying atmospheric N_2 inputs (^{15}N) and their impacts on ecosystem functions (Ghosh and Brand, 2003; Martinelli *et al*., 2007; Werner *et al*., 2007).

Isotope monitoring can also play an important role as an early warning of the effects of global warming. Analysis of C isotopes, for example, can help to explain what happens to the man-made GHGs in the atmosphere while N and S isotopes can reveal the connections between industrially produced oxides and acid rain. The ^{18}O and D

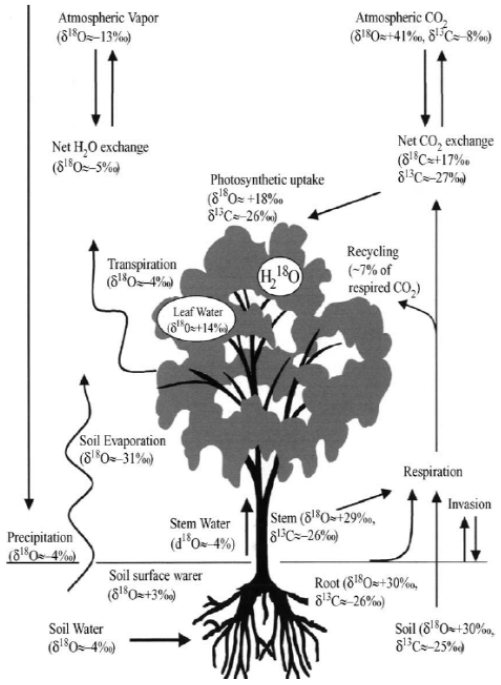


FIGURE 4. Isotopic composition of C, O and H pools in terrestrial ecosystems. The values are approximations and will vary considerably with geographical location and environmental conditions. The actual data in the figure are from Israel (Ghosh and Brand, 2003).

isotopes in water are also very useful indicators of climate-related parameters such as surface air temperature, relative humidity and amount of precipitation.

Ecosystem C budgets are controlled by the balance between C uptake during photosynthesis and C loss during respiration (Prentice, 2001; Malhi, 2002; Kodama *et al*., 2008; Brugnoli *et al*., 2010). Within an ecosystem, both photosynthesis and respiration occur in a range of different species and functional groups, so the environmental control of C exchange processes is quite different in these distinct ecosystem components. The study of individual components of the ecosystem C budget and their environmental control would improve our understanding of ecosystem function and the system's potential response to climate variation. The measurement and analysis of C isotope ratios in leaf and atmospheric CO_2 samples can provide information that integrates important plant physiological characteristics spatially and temporally. Detailed mechanistic models have been developed that successfully explain the isotope effects occurring during photosynthetic gas exchange at the leaf level, making it is possible to interpret variation in C isotope discrimination resulting from differences in plant photosynthetic pathway and environmental conditions. For example, important physiological characteristics such as water use efficiency, stomatal limitation of photosynthesis, optimal stomatal behaviour and leaf N use efficiency are related directly to the value of leaf intercellular and ambient CO_2 ratios. Variation in environmental conditions causes change in the ratio of photosynthesis to stomatal conductance, and associated changes in leaf isotopic signatures (delta C, $\delta^{13}C$) have also been documented. Using measurement of $\delta^{13}C$ of leaf tissue and CO_2 released by respiration, Ometto *et al*. (2002) found that converting forest to pasture causes significant changes to ecosystem C isotope discrimination (Figure 5).

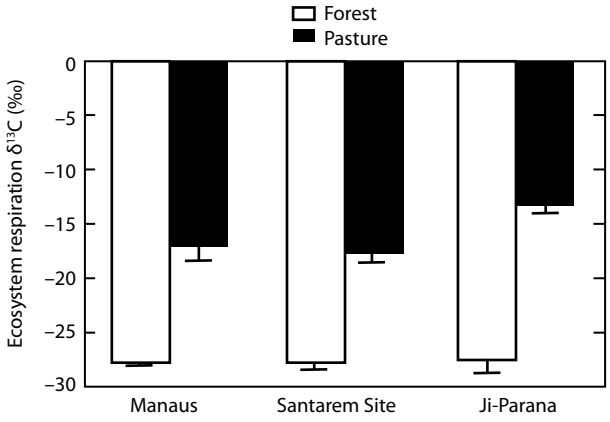


FIGURE 5. Comparison of the average carbon isotope composition of total ecosystem respiration measured in forest and pasture sites at three locations in Brazilian Amazon Basin (Ometto *et al*., 2002).

Cernusak, Farquhar and Pate (2005) used $\delta^{18}O$ and $\delta^{13}C$ measurements to study post-photosynthetic variations of *Eucalyptus globulus* Labill. This approach is related quantitatively to plant photosynthetic performance, e.g. leaf physiological responses to environmental changes. Kodama *et al*. (2008) found a strong 24-h periodicity in $\delta^{13}C$ of organic matter in leaf and twig phloem sap which was strongly dampened as carbohydrates were transported down the trunk. Periodicity reappeared in the $\delta^{13}C$ of trunk-respired CO_2, which seemed to originate from respiratory fractionation rather than from changes in $\delta^{13}C$ of the organic substrate. Ekblad and Hogberg (2001) found that the natural abundance of ^{13}C in CO_2 respired from forest soils demonstrated the speed of the link between tree photosynthesis and root respiration. It took 1–4 d for the C from canopy photosynthesis of 20–25 m trees to become available for root/rhizosphere respiration. Coletta *et al*. (2009) reported that C and N biogeochemical cycles in savannas are strongly regulated by the seasonal distribution of precipitation and pulses of nutrients released during the wetting of the dry soil, and are critical to the dynamics of microorganisms and vegetation. The $\delta^{13}C$ values showed a consistent relationship with canopy height, revealing the importance canopy structure in determining the C isotopic signature of the vegetation. Carbon isotopic variations associated with the length of the dry season indicated the importance of recently fixed C to the integrated isotopic signature of the leaf organic C. Variations in foliar C and N isotope ratios were consistent with highly diverse vegetation with high energy availability but low availability of water and N (Matinelli *et al*., 2007).

Isotope tracer techniques also show promise for quantifying the impacts of urban processes on the isotopic composition of the atmosphere and the partitioning the urban CO_2 sources into their component parts (Pataki, Bowling and Ehleringer, 2003). Further, the isotope ratios of well-mixed environmental reservoirs such as the $\delta^{2}H$ and $\delta^{18}O$ of water and the $\delta^{13}C$ and $\delta^{15}N$ of dissolved compounds in stream discharge from watersheds and in the tissues of organisms, represent an integration of source inputs that extends over large spatial scales and the processing (mixing, losses, biogeochemical transformations) of elements within ecosystems. Therefore employing the approach in different compounds provides a unique means to investigate the coupling of water and C fluxes at various temporal and spatial scales.

Carbon isotopes (^{12}C, ^{13}C and ^{14}C) may hold a key to determining the source of the increased C in the atmosphere by distinguishing the C cycles from deforestation, oceanic and fossil fuel. Plants prefer ^{12}C to ^{13}C and therefore photosynthetic CO_2 is much lower in ^{13}C than in the CO_2 that comes from other sources (e.g. animal respiration). Declining ^{14}C:^{12}C and ^{13}C:^{12}C ratios parallel the reported increase of atmospheric CO_2 and which are linked to the fact that fossil fuels, forests and soil C come from photosynthetic C which is low in ^{13}C, while increased CO_2 due to warming of the oceans would not be followed by reductions in the ratios of ^{13}C and ^{14}C to ^{12}C. There are other clues that suggest the source of increased CO_2 is not related to the warming of and subsequent release of CO_2 from the ocean. For example, there has been a decline in the oxygen concentration of the atmosphere; therefore if ocean warming was responsible for the CO_2 increase, an increase in atmospheric O_2 should also be observed because O_2 is also released as the water is warmed. The ocean is a sink for atmospheric C, and the C content of the oceans has increased by 118 ± 19 Pg C in the last 200 yr. If atmospheric CO_2 was the result of oceans releasing CO_2 to the atmosphere, the CO_2 in the ocean should not be rising as a result of ocean warming.

Global climate change will alter water availability in many ecosystems worldwide with marked impacts on biogeochemical cycles, as water represents one of the key factors constraining ecosystem productivity. Thus, a mechanistic understanding of the linkage between C and water cycles within the soil–plant–atmosphere continuum is needed to identify past and future climate and land-use change effects on ecosystem functioning (Heimann and Reichstein, 2008; Werner *et al*., 2011).

Tritium is especially useful in studying the dynamics of water movement in different compartments of the hydrosphere, both on the local and global scales, while the heavy stable isotopes of deuterium and ^{18}O can provide information about steady-state characteristics of the water cycle. Isotope data gathered from isotope monitoring of river water in main channels and in floodplains is expected to be very helpful for determining surface water routing, particularly to determine the proportions of water stored in the main channel and floodplains and to derive rates of transfer between them at various seasons and in each segment of the river valley. Preliminary isotope analyses suggest that up to 30 percent of water in the main channel is derived from water which has passed through the floodplain. Environmental isotope data also yield site-specific information about water transport and storage in the unsaturated zone. Numerous studies have shown that tritium is a very powerful tracer of H_2O movement in the unsaturated zone. In a study carried out in Brazil, it was possible to evaluate the average infiltration velocity and evapotranspiration flux for both the undisturbed forest and cleared area in the region. However, while isotope tracing provides information about the dynamics of H_2O movement in the unsaturated zone, the storage of water in the soil is commonly determined using neutron gauges (Nguyen *et al*., 2011).

Sediment (and associated nutrients and chemicals) play important roles in degrading water quality and causing a range of other environmental problems. Sediment transport is the key to understanding the movement and fate of many nutrients (e.g. N and P), and of contaminants and organic C mobilization within the watershed (Figure 6). Soil erosion and associated deposition can cause redistribution in a differential manner of both soil particles and soil organic matter (SOM) along the landscape, and ultimately may result in C losses from the watershed as emissions of CO_2 and CH_4 and/or deposition/burial in sediment sinks and neighbouring aquatic systems. Fallout radionuclides (FRNs) such as caesium-137 (^{137}Cs), lead-210 (^{210}Pb) and beryllium-7 (^{7}Be), naturally occurring radionuclides (NOR) such as potassium-40 (^{40}K), radon-226 (^{226}Ra) and thallium-232 (^{232}Th), compound-specific stable isotope (CSSI) and conventional (modelling) techniques can be used to measure actual rates of soil erosion

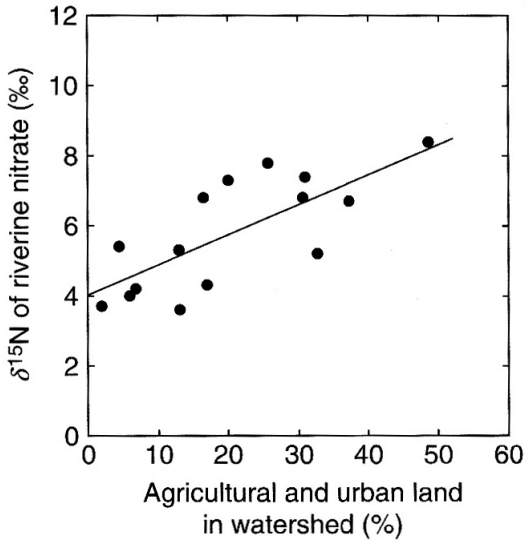


FIGURE 6. Mean N isotope ratio of nitrate in rivers draining 16 watersheds (Williams *et al.*, 2007).

and sedimentation. Analysis of ^{210}Pb and ^{137}Cs stratigraphies in sediment cores has been used to determine the histories of sedimentation in floodplain lakes in Brazil (IAEA, 1990; Nguyen *et al.*, 2011).

The history and pathway of water in different parts of the hydrological cycle can be followed by the abundance of the stable heavy isotopes of ^{2}H and ^{18}O. In this way, water in different environments develops isotopic "fingerprints" with which it can be identified and its origins traced. Consequently, isotopic technique can determine the origins and ages of different water bodies; provide an estimate of the degree of mixing; determine the location and proportion of water recharge; measure recharge and indicate the velocity of groundwater flow. Recharge of groundwater is one critical aspect in water resource management, and isotopes can help determine both the area and the rate of recharge. The area can be identified by measuring ^{2}H and ^{18}O concentrations and correlating them with the altitude at which precipitation could have infiltrated the ground. The rate can also be measured by tracing levels of radioactive tritium in soil at various depths. The tritium peak method has been applied all over the world and in many different climates. In many instances, the tritium "peak" can be found at considerable depths, which indicates the distance travelled by the moisture since being deposited as tritium fallout in 1963. In moist climates where infiltration is high, artificial tritium can be injected as a tracer to determine the rate of recharge. Profiles of either environmental or artificial tritium can also give a measure of the movement of pollutants such as nitrates and pesticides from agriculture.

Polluted groundwater may remain in aquifers for centuries, even millennia, and is very difficult if not impossible to clean up. Isotope techniques can assess the vulnerability of groundwater to pollution from the surface by determining how rapidly it moves and where it is being recharged. Surface sources of pollution can then be determined, e.g. natural, industrial, agricultural or domestic. Isotope techniques can also identify incipient pollution, providing an early warning when the chemical or biological indicators do not give cause for concern (Williams *et al.*, 2007).

New instruments for sustainable use and conservation of peat forest such as REDD need more in-depth knowledge of C stocks and flows of peat land systems. Full ecosystem C balance data are needed to determine to what extent C accumulation still occurs in peatland ecosystems and to what extent peat formation is limited under oil plantations. The full ecosystem C balance for different land use types such as undrained peat forest and disturbed forest and agricultural ecosystems can be estimated quite precisely with eddy covariance measurements above the forest canopy (incorporation of C fluxes of both respiration and decomposition). Soil CO_2 fluxes are generally measured using the closed chamber method, while the eddy covariance tower can measure incoming and outgoing gas fluxes above the forest canopy and give more reliable estimation of ecosystem production and C dynamics. Leaching can be estimated using surface water samples from the catchment area and extracting the organic material.

Growing international concern over the adverse impacts of climate change and associated environmental stresses on sustainable development and poverty has ensured that climate change policy issues remain a central focus of most large funding agencies. Measurement of natural stable isotope ratios and the availability of large-scale isotope networks (Hemming *et al.*, 2007) are well placed to provide some of the key information required by policy makers, particularly in the field of C and water cycle management. It is therefore recommended that international agencies assist Indonesia to be a better conserver of its forest and peatland resources, either through forest conservation or improved soil and water management.

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Effect of Elevated Carbon Dioxide on Nitrogen Dynamics and Greenhouse Gas Emissions in Grain Crop and Legume Pasture Systems: FACE Experiments and a Meta-Analysis

D. Chen[1,*], S.K. Lam[1], A.J. Weatherley[1], R.M. Norton[1,2], R. Armstrong[3], E. Lin[4] and A. R. Mosier[1]

ABSTRACT

By 2070, atmospheric carbon dioxide concentration [CO_2] is expected to be double that observed in 1950. In this higher [CO_2] world, the sustainability of global crop production may be in jeopardy unless current nitrogen (N) management strategies are changed because of potential interactions between elevated atmospheric [CO_2] and soil N dynamics. However, these interactions are poorly understood especially in semi-arid cropping systems. In this paper, experimental results are presented on the effects of elevated [CO_2] on crop N demand, fertilizer N recovery, symbiotic N_2 fixation and greenhouse gas emissions from cropping systems in southern Australia and northern China using free-air CO_2 enrichment (FACE) facilities. Also discussed are the findings of a meta-analytic review of current literature which estimated quantitatively the effects of elevated [CO_2] on soil N dynamics in grain crop and legume pasture systems. Results of experiments reported here and the meta-analysis suggest that under future elevated CO_2 atmospheres (i) there will be an increase in crop demand for N, (ii) higher fertilizer N application rates and greater use of legume intercropping using locally appropriate agricultural management practices to meet the additional crop N demand, and (iii) increases in the terrestrial C sink may be less than expected since there will be a significant increase in greenhouse gas emissions (CO_2-equivalent) associated with an increase in atmospheric [CO_2].

Key words: *elevated [CO_2], fertilizer N recovery, grain N removal, meta-analysis, greenhouse gas emissions.*

INTRODUCTION

Atmospheric carbon dioxide concentration ([CO_2]) has increased from 280 µmol/mol at the beginning of the Industrial Revolution to the current level of 397 µmol/mol (NOAA, 2013). If CO_2 emissions continue to rise at their present rate, [CO_2] is estimated to reach about 550 µmol/mol by 2050 and 700 µmol/mol by the end of this century (IPCC, 2007). Elevated [CO_2] can, in turn, affect agricultural greenhouse gas emissions via changes to carbon (C) and nitrogen (N) cycles in the plant–soil system (van Groenigen *et al.*, 2006). Agriculture accounted for 16 percent of net national emissions from Australia in 2009 (Department of Climate Change and Energy Efficiency, 2011), and 15 percent from China in 2005 (Wang, Huang and Rozelle, 2010). Elevated [CO_2] has been estimated to increase the emission of nitrous oxide (N_2O) and methane (CH_4) from terrestrial ecosystems by a total of 1.12 Pg CO_2 equivalent/yr due to enhanced C substrate availability (Hungate *et al.*, 1997; Baggs *et al.*, 2003; Inubushi, Cheng and Aonuma, 2003) and/or improved soil moisture (Leakey *et al.*, 2009), thereby offsetting 17 percent of the predicted increase in the entire terrestrial C sink (van Groenigen *et al.*, 2011).

Increasing [CO_2] reduces stomatal conductance and transpiration and improves water use efficiency; it also stimulates photosynthetic processes, often resulting in increased crop growth and yield (Kimball, 1983; Drake *et al.*, 1997; Ainsworth and Long, 2005). When C_3 plants are grown in an elevated [CO_2], total N uptake and N removal in grain generally increase (Kimball, Kobayashi and Bindi, 2002). This increase in crop demand for N would be expected to gradually reduce soil N reserves unless replenished.

Depletion of soil N in agroecosystems can be compensated for by N fertilizer application although recovery by crops rarely exceeds 40 percent under ambient [CO_2] (Chen *et al.*, 2008). Studies of the effect of elevated [CO_2] on fertilizer N recovery in crops have generally been inconclusive and contradictory, showing positive (Martín-Olmedo, Rees and Grace, 2002; Weerakoon, Ingram and Moss, 2005), or neutral effects (Torbert *et al.*, 2004; Kim *et al.*, 2011). Symbiotically fixed N_2 derived from crop and pasture legumes provides an alternative to additional fertilizer N use (Unkovich, Pate and Sanford, 1997; Chalk, 1998). Elevated [CO_2] generally increases overall biomass and the amount of symbiotically fixed N_2 due to increases in nodule size, nodule number per plant or, less likely, specific nitrogenase activity (Rogers *et al.*, 2009).

1 Melbourne School of Land and Environment, The University of Melbourne, Victoria 3010, Australia
2 International Plant Nutrition Institute, 54 Florence Street, Horsham, Victoria 3400, Australia
3 Department of Primary Industries, Private Bag 260, Victoria 3401, Australia
4 Key Laboratory of Ministry of Agriculture on Agro-environment and Climate Change; Agro-Environment and Sustainable Development Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China
* E-mail address of corresponding author: delichen@unimelb.edu.au

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This paper examines the effect of elevated [CO_2] on the fluxes of N_2O, CH_4 and CO_2 from wheat-based cropping systems under semi-arid environments, such as in southern Australia and northern China and its interaction with agricultural practice, including the utilization of applied N fertilizer and symbiotic N_2 fixation. It summarizes published data using free-air CO_2 enrichment (FACE) facilities and extends the work with a meta-analysis of 108 published experiments examining N dynamics under elevated [CO_2]. This information provides new insight into the efficient management of fertilizer N and thus the sustainability of crop production under future high [CO_2].

MATERIALS AND METHODS

The field experiments were conducted at Horsham (*36°45′ S, 142°07′ E), Victoria, Australia* from June to December in 2008 and 2009, and at Changping (40°10′ N, 116°14′ E), Beijing, China from October 2008 to October 2009, using free-air CO_2 enrichment (FACE) facilities. The targeted elevated [CO_2] was 550 µmol/mol for both sites. Detailed methodology has been described elsewhere for the experiments on fertilizer ^{15}N recovery (Lam *et al*., 2012a, 2012c), symbiotic N_2 fixation (Lam *et al*., 2012d), greenhouse gas emissions (Lam *et al*., 2011, 2013) and the meta-analysis (Lam *et al*., 2012b). Briefly, to determine the recovery of fertilizer N in the wheat–soil system, nitrogen-15 (^{15}N)-labelled (10.22 atom %) granular urea was applied (at a rate according to local practice) to field micro-plots. Symbiotic N_2 fixation in soybean was assessed using the ^{15}N natural abundance method. The total C, total N and delta (δ)^{15}N of plant and soil samples were analysed by isotope ratio mass spectrometry. The fluxes of N_2O, CO_2 and CH_4 were measured by closed static chambers at various key growth stages of a wheat crop. The gas samples were analysed by gas chromatography.

Meta-analysis was conducted based on the natural log of the response ratio (r = response at elevated [CO_2]:response at ambient [CO_2]) as a metric for analyses (Rosenberg, Adams and Gurevitch, 2000). Mean effect sizes and 95 percent confidence intervals were generated by bootstrapping (4 999 iterations) (Adams, Gurevitch and Rosenberg, 1997) using MetaWin 2.1 (Rosenberg, Adams and Gurevitch, 2000).

RESULTS

Fertilizer N recovery

The recovery of fertilizer N by the wheat crop or in the soil was not affected by elevated [CO_2] in the FACE experiments in Australia and China. The [CO_2]-induced increase in plant N uptake (18–44 percent) was satisfied mostly by increased uptake of indigenous N (19–50 percent) at both sites (Tables 1 and 2). Irrespective of [CO_2], fertilizer N recovery by wheat grown under FACE was stimulated (13–609 percent) by supplementary irrigation (higher rainfall scenario) in Horsham (Lam *et al*., 2012a), but reduced (47 percent) by high N application in Changping (Lam *et al*., 2012c).

Symbiotic nitrogen fixation

Under FACE conditions in Changping, elevated [CO_2] increased both the proportion (from 59 to 79 percent) and the amount (from 166 to 275 kg·N·ha^{-1}) of shoot N derived from the atmosphere (Ndfa) by soybean cultivar Zhonghuang 13, but had no significant effect on either parameter for the other cultivar Zhonghuang 35 (Figure 1).

Greenhouse gas emissions

Elevated [CO_2] increased the emission of N_2O (108 percent), CO_2 (29 percent) and CH_4 (from –0.14 to 3.45 µg·C·m^{-2}·h^{-1}) from soil in Horsham (Table 3), with changes being greater during the wheat vegetative stage than later in the growing season. Supplementary irrigation (higher rainfall scenario) reduced N_2O emission by 36 percent when averaged across [CO_2] treatments. Supplementary irrigation increased CO_2 flux by 26 percent at ambient [CO2], but not at elevated [CO_2], and had no impact on CH_4 flux (Table 3). At the Changping site, elevated [CO_2] increased N_2O (60 percent) and CO_2 (15 percent) emission, but had no significant effect on CH_4 flux (Table 4).

Meta-analysis

Elevated [CO_2] increased grain N removal (17 percent) of C_3 non-legumes, legumes and C_4 crops. This increase resulted from an overall increase (27 percent) in grain yield but a reduction (8 percent) in grain N concentration (Figure 2). The C:N ratio of residues from C_3 non-legumes and legumes increased under elevated [CO_2] by 16 percent and 8 percent, respectively, but the increase in residue C:N ratio

Table 1. The effect of elevated [CO_2] on the plant total N (kg/ha) derived from fertilizer N and soil N in Horsham, Australia, under different irrigation regimes (adapted from Lam *et al.,* 2012a)

	Ndff	Ndfs	Total N	Ndff	Ndfs	Total N	Ndff	Ndfs	Total N
		2008NS			2008LS			2009NS	
Rainfed									
Ambient [CO_2]	23.0	149.5	172.5	2.0	67.8	69.8	19.2	103.1	122.3
Elevated [CO_2]	23.4	179.8	203.2	2.1	72.3	74.4	21.1	154.7	175.8
Supplementary irrigated									
Ambient [CO_2]	--	--	--	12.6	111.0	123.6	23.7	194.3	218.0
Elevated [CO_2]	--	--	--	16.5	129.1	145.6	22.1	195.6	217.7
[CO_2]	ns	*	*	ns	ns	ns	ns	0.08	0.09
Irrigation regime	--	--	--	***	***	***	0.06	***	***
[CO_2] × Irrigation regime	--	--	--	ns	ns	ns	ns	0.09	0.09

Ndff — N derived from fertilizer; Ndfs — N derived from soil. 2008NS, 2008 normal sowing time; 2008LS, 2008 late sowing time; and 2009NS, 2009 normal sowing time

Significant effects are indicated as *p < 0.05, **p < 0.01 and ***p < 0.001; ns — not significant

TABLE 2. The effect of elevated [CO_2] and N input on the plant total N (kg/ha) derived from fertilizer N and soil N at stem elongation in Changping, China (adapted from Lam *et al.*, 2012c)

	Ndff	Ndfs	Total N
Low N input (25 kg·N·ha^{-1})			
Ambient [CO_2]	3.65	181.6	185.3
Elevated [CO_2]	3.88	187.4	191.3
High N input (95 kg·N·ha^{-1})			
Ambient [CO_2]	7.86	148.2	156.1
Elevated [CO_2]	8.06	168.0	176.0
[CO_2]	ns	0.08	0.09
N input	*	ns	ns
[CO_2] × N input	ns	ns	ns

Ndff: = N derived from fertilizer; Ndfs: = N derived from soil

Significant effects are indicated as * $p < 0.05$; ns = not significant

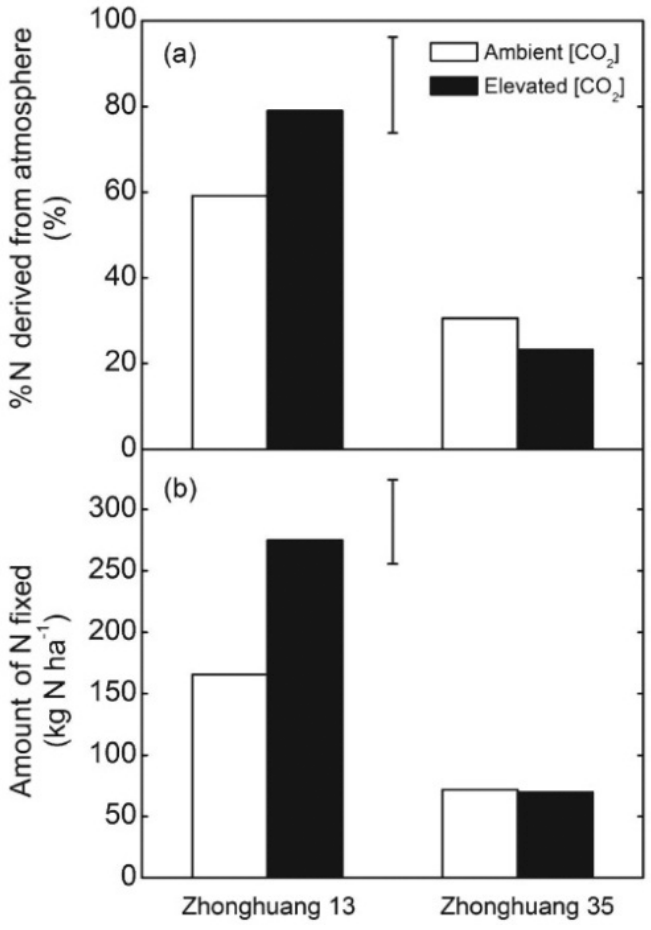


FIGURE 1. Effect of elevated [CO_2] on (a) %Ndfa and, (b) amount of N_2 fixed in the above-ground parts of two soybean cultivars (adapted from Lam *et al.*, 2012d). Each data point represents the mean of three replicates. Vertical bar indicates least significant difference (LSD) ($p = 0.05$).

of C_4 crops (9 percent) was not significant. Elevated [CO_2] increased the recovery of fertilizer N in crops by 17 percent (Lam *et al.*, 2012b). Under elevated [CO_2], there was a 38 percent increase in the amount of N_2 fixed from the atmosphere, which was accompanied by greater whole plant nodule number (33 percent), nodule mass (39 percent), nitrogenase activity (37 percent) and %N derived from the atmosphere (10 percent; non-significant) (Lam *et al.*, 2012b). Elevated [CO_2] increased the emissions of CO_2 (19 percent), CH_4 from rice paddies (38 percent) and N_2O (27 percent) (Figure 3).

DISCUSSION

The need for N replenishment in cropping systems under elevated [CO_2]

The meta-analysis of current literature and FACE experiments indicate that elevated [CO_2] generally increases plant N uptake and grain N removal from cropping systems. Furthermore, elevated [CO_2] resulted in greater production of crop residues with a higher C:N ratio (especially for C_3 non-legumes), leading to increased N immobilization in the soil (Torbert *et al.*, 2000). These [CO_2]-induced changes suggest that extra N will be required for cropping systems to maintain soil N availability and sustain grain yield (Lam *et al.*, 2012b). This is especially important in dryland cropping systems where water availability will often limit the cycling of N. In these systems, the "CO_2 fertilization effect" on crop growth and N demand will most often be realized when rainfall is between average and moderately below average

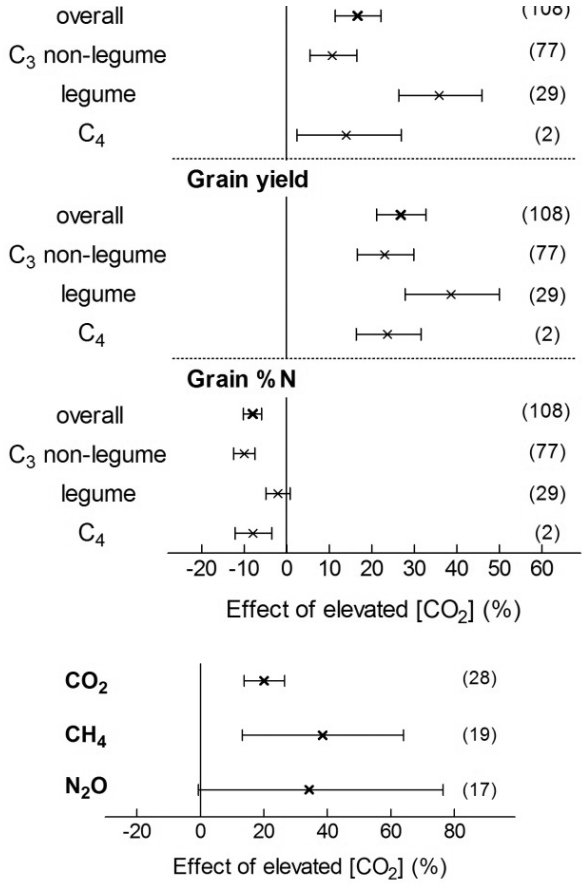


FIGURE 2. Effect of elevated [CO_2] on (a) grain N removal, (b) grain yield and (c) grain %N (adapted and modified from Lam *et al.*, 2012b). Means and 95 percent confidence intervals are depicted. Numbers of experimental observations are in parentheses.

TABLE 3. Interaction between [CO_2] and irrigation on the emission of N_2O, CO_2 and CH_4 averaged across the experimental period in Horsham, Australia (adapted from Lam *et al.*, 2013)

	N_2O (μg $N \cdot m^{-2} \cdot h^{-1}$)	CO_2 (mg·C·m^{-2}·h^{-1})[a]	CH_4 (μg·C·m^{-2}·h^{-1})
Ambient [CO_2]			
Rainfed	27.7	259.7	–0.56
Supplementary irrigated	15.6	327.6	0.29
Elevated [CO_2]			
Rainfed	53.3	379.7	7.06
Supplementary irrigated	36.5	378.7	–0.24
[CO_2] (C)	**	***	ns
Irrigation regime (I)	†	†	ns
C × I	ns	†	ns

[a] CO_2 fluxes included both plant and soil respiration as plants were inside the measurement chambers

Significant effects are indicated as † $p < 0.1$, ** $p < 0.01$ and *** $p < 0.001$; ns — not significant

TABLE 4. Emission of N_2O, CO_2 and CH_4 as affected by ambient and elevated [CO_2], averaged across N application rates and irrigation events in Changping, China (adapted from Lam *et al.*, 2011)

	N_2O (μg $N \cdot m^{-2} \cdot h^{-1}$)	CO_2 (mg·C·m^{-2}·h^{-1})[a]	CH_4 (μg·C·m^{-2}·h^{-1})
Ambient [CO_2]	24.0	37.3	–5.6
Elevated [CO_2]	38.4	42.8	–4.2
[CO_2]	*	*	ns

Significant effects are indicated as * $p < 0.05$; ns — not significant

(Lam *et al.*, 2012a). While [CO_2]-induced increase in N uptake is associated with a slightly greater increase in grain yield under these rainfall conditions, improved N management practice (higher N application rate or using enhanced efficiency fertilizers) will be required to satisfy the increased demand for N.

Measures to optimize the benefits of N replenishment strategy under elevated [CO_2]

Using the ^{15}N labelling technique, it was demonstrated that both higher rates of fertilizer N application and greater use of legume intercropping can compensate for the enhanced rate of grain N removal under future elevated CO_2 environments (Lam *et al.*, 2012b). There are several strategies to optimize the benefits of N replenishment under elevated [CO_2]. Firstly, while irrigation increases plant uptake of fertilizer N (Lam *et al.*, 2012a), irrigation is unavailable in dryland cropping systems in southern Australia and many other semi-arid farming systems. Farmers will need to take even greater account of likely seasonal rainfall conditions when making fertilizer management decisions to enhance recovery, e.g. using split fertilizer applications (Chen *et al.*, 2008). Secondly, an optimum N application rate should be determined. Excessive N application reduces wheat growth (van Herwaarden *et al.*, 1998) and the recovery of fertilizer N in the plant regardless of [CO_2] (Lam *et al.*, 2012c). This defeats the purpose of compensating for the additional N removed in grains under elevated [CO_2], and is therefore not recommended. Thirdly, legume species, cultivars and rhizobacteria should be selected according to their ability to fix more N_2 under elevated [CO_2] (Lam *et al.*, 2012d; Matsunami *et al.*, 2009). Nonetheless, [CO_2]-induced increases in grain N removal by pulse legumes should also be considered (Lam *et al.*, 2012b).

Agriculture's potential contribution to future climate change

As a result of additional C input to soil under elevated [CO_2], there will be a positive relation between the elevation of [CO_2] and emission of greenhouse gases of CO_2 and N_2O from semi-arid cropping systems (Lam *et al.*, 2011, 2012b, 2013). This will partly negate the expected increase in global terrestrial C sinks expected under elevated [CO_2] (van Groenigen *et al.*, 2011).

During the vegetative stage of crop growth there are rapid changes in soil C and N. Under elevated [CO_2] the extent of stimulation of greenhouse gas emissions may be reduced if this growth stage is shortened, e.g. by future warmer temperatures increasing the rate of crop maturation, or through choice of crop. This stimulation of greenhouse gas emissions may also be lower in irrigated systems (Lam *et al.*, 2013) if water supply is sufficient to facilitate the reduction of N_2O to N_2 (Weier *et al.*, 1993; Ciarlo *et al.*, 2008). This process is favoured by the higher C substrate availability under elevated [CO_2] (Baggs *et al.*, 2003). The greater [CO_2] effect on N_2O emission under high N compared with low N input (Lam *et al.*, 2011) reaffirms that excessive application of N fertilizer should be avoided in semi-arid cropping systems in future elevated CO_2 environments.

CONCLUSIONS

Elevated [CO_2] reduced grain N concentration, but increased N demand and removal in grain cropping systems. Extra N will be required to maintain soil N availability (avoid gradual decline of soil N) and sustain crop yield. The extra N could come from increased rates of fertilizer N application, or greater use of legume intercropping and legume cover crops. Increases in agricultural greenhouse gas

emissions will negate part of the predicted increase in the terrestrial C sink.

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SESSION 5

MANAGING AGRICULTURAL WATER FOR CLIMATE CHANGE ADAPTATION

Enhancing the Contribution of Isotopic Techniques to the Expansion of Precision Irrigation

E. Fereres[1,*] and L.K. Heng[2]

ABSTRACT

In the future, irrigated agriculture will take place under water scarcity, as insufficient water for irrigation is becoming the norm rather than the exception. There is a need therefore to increase the precision of water application with irrigation management. Successful application of the precision irrigation (PI) concept requires one to know the crop water requirements with a certain degree of accuracy and to be able to monitor effectively the water status of the root zone. This paper reviews the use of remote sensing from satellites to characterize irrigation performance for benchmarking areas in need of improvement. The use of remote sensing techniques has progressed substantially in recent years through their capability to detect vegetation properties with very high resolution. Similarly, a new approach which uses cosmic-ray neutrons involving measuring background fast neutrons radiation from cosmic rays and those generated within the soil, moderated by hydrogen atoms in water, is showing promise for obtaining information about area-wide temporal changes in water content in relation to satellite remote sensing observations.

Key words: *precision agriculture, irrigation management, remote sensing, cosmic ray.*

INTRODUCTION

Nearly 70 percent of all freshwater abstractions worldwide are used in irrigation. As demands from other sectors increase and the options for developing new supplies tend to diminish, the need for saving water in irrigation is more imperative than ever before. Recent modernization efforts in the irrigation sector in many countries have shown that it is possible to reduce irrigation water use, albeit with increasing energy usage. Modernization focuses on minimizing conveyance losses by converting open channels to pipe networks, and on changing the method of irrigation from gravity to pressurized systems (sprinkler or drip). All these engineering changes increase the potential for highly efficient irrigation, but that potential can only be realized fully if the management of water is adequate.

Irrigation water management is a complex activity and one that often causes problems for growers in the context of improving irrigation efficiency. Adequate amounts applied at appropriate times are the ingredients for efficient irrigation. This is easier said than done, and most irrigators around the world use their qualitative skills to determine time and amount of irrigation rather than technical, quantitative procedures. However, in recent decades there have been substantial advances in irrigation management towards increasing the precision of water application.

Although the diversity of world agriculture is very significant, in all cases irrigated production can be related to one of three water resource situations. Firstly, where water is abundant and reliable, supplies are guaranteed each year. Here, the optimal amount of irrigation is dictated by energy costs and/or competition with other sectors, including the environment. Irrigation usually exceeds crop water requirements, excess water being applied as an insurance policy to avoid the risks associated with crop stress (water deficit), and hence substantial water savings are feasible. Secondly, there are situations where water supplies vary from being unconstrained to being restricted during periodic droughts. Adaptation in this context requires fine tuning of the irrigation equipment and its management. Here, there is scope for optimizing management and for substantial water savings in years of ample supply and by adopting better coping strategies in drought years. Finally, there is the situation of chronic water scarcity, mainly in the arid and semi-arid areas around the world, caused by a number of factors such irrigation over-expansion, competition from other sectors, notably the greater attention to environmental flows and unsustainable over-exploitation of groundwater resources. In this third situation, emphasis must be placed on optimizing use of the limited supplies by concentrating the available water on high-value crops and by using deficit irrigation (Fereres and Soriano, 2007) in a sustainable fashion. In all cases, science, engineering and management are the needed ingredients to achieve efficient use of water in agricultural systems.

PRECISION IRRIGATION

Recent droughts in many parts of the world and the threats of climate change with the uncertainties in future regional rainfall regimes, emphasize the need to have new irrigation strategies whereby increased precision in the use of water must play a central role in support of water conservation and environmental protection. Precision irrigation (PI) is defined here as the efficient, timely and correct amount of water delivered to fields to maximize crop yield and quality, and to minimize environmental impacts, including the application of variable amounts of water over a field in response to spatial crop and soil heterogeneities. There are two important prerequisites for successful application of PI: one is to know the crop water requirements (ET) with a certain degree of accuracy, and the other is to be able to monitor effectively the water status of the root zone. Much effort has been devoted to research for developing specific PI technologies based on new hardware (such as sensors) and/or new software (such as decision-support models), but its impact has been limited so far.

The first step for optimizing on-farm water management is using irrigation scheduling (IS) techniques to determine precisely the date and amount of irrigation (Fereres, 1996). A collection of techni-

1 IAS-CSIC and University of Cordoba, Spain
2 International Atomic Energy Agency, Wagramerstrasse 5, PO Box 100, 1400 Vienna, Austria
* E-mail address of corresponding author: ag1fecae@uco.es

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 259–261

cal procedures and tools has been developed from analysis of the soil–plant–atmosphere continuum allowing the depth and frequency of irrigation applications to be forecast. The most robust technique for IS is the soil water budget. Here, irrigation timing is computed by adding the crop ET losses minus effective rainfall until a soil water level termed the allowable depletion is reached. Nowadays, agrometeorological weather station networks provide the information needed for calculating reference evapotranspiration (ETo) from meteorological variables, while crop coefficient (Kc) values for the major water demanding crops have also been determined (FAO, 1998). Computer programs have been developed for calculating the water balance of fields, and irrigation scheduling services have been developed, mostly by public agencies but also by private consultants. The soil water budget method, despite being widely used, has some uncertainties. These include assumptions about the relations between ETo and ETc (crop evapotranspiration), particularly in the case of woody crops, and on the representativeness of the point measurements of soil water considering the spatial variations in soil water across fields. Also, there is uncertainty about the optimum frequency of irrigation, which depends upon several field and irrigation system design characteristics and which, in turn, is very difficult to take into account in simple models. Nevertheless, the frequency of irrigation is a crucial aspect of irrigation scheduling, determining largely the overall on-farm irrigation efficiency.

Soil water status determinations can be carried out to visualize the effects of the irrigation regime on soil water availability. There is now a new generation of soil water sensors which track soil water status continuously rather than providing intermittent measurements as offered by the traditional instruments such as the tensiometer (Leib *et al.*, 2003). Unfortunately, these new developments have not resolved the quantification of volumetric soil water content with depth, a parameter that is still most reliably measured with the neutron probe, particularly under saline soil conditions (IAEA, 2008). However, obtaining a representative estimate of the soil water content of a whole field is a difficult task when using point sensors, due to the very large soil water variability resulting from variations in soil properties and the fact that under localized irrigation, the soil surface is not uniformly wet. The strong spatial heterogeneity of soil water status even in what are considered uniform soils, combined with the variations in the distribution of irrigation water applications and the uncertainties of rooting depth and densities, all contribute to create a heterogeneous environment that farmers have to manage as accurately as possible. In developed countries, this problem has become more pronounced in the last decades due to the increased size of management units, in an attempt to reduce production costs by managing uniformly large field units. The complexities involved in dealing with the variability problem are such that, until very recently, the common solution chosen by irrigators was to apply water in excess so that the risk of inducing water deficits in some parts of the field is minimized. Because of the difficulties that farmers and technicians have had in characterizing the variability, significant uncertainty is introduced and often irrigation management decisions may have substantial errors.

To advance solutions for coping with the variability problem, i.e. to implement PI under field conditions, what is needed is to be able to characterize the variation across a field, and also to have the option of applying variable amounts of water within that field. The objective would then be to apply water at variable depths under non-uniform crop growing conditions to match the requirements of every area of the field, while minimizing the environmental consequences arising from uniform irrigation over a variable field. The technologies for variable water application are already available in self-propelled sprinkler systems and can lead to significant water conservation (Sadler *et al.*, 2005). Significant efforts in the engineering of irrigation systems have been undertaken recently to offer the flexibility of applying spatially variable amounts of water (and agrochemicals) for the different pressurized methods, including micro-irrigation (Evans and Sadler, 2007). These new PI capabilities should enable growers to increase productivity and minimize the negative environmental impacts of irrigation.

While the engineering solutions for PI are underway, there is still the need to both characterize and monitor the variability as well as to interpret the causes of variations in crop growth and development. The characterization of irrigation performance through remote sensing (Santos *et al.*, 2010) is a promising area, as it enables performance to be evaluated quickly and inexpensively; it can also identify areas in need of improvement. The use of remote sensing techniques has progressed substantially in recent years through the development of capabilities for detecting a number of vegetation properties with very high resolution (Zarco-Tejada *et al.*, 2009). High-resolution images cannot be acquired from current satellites, and a number of initiatives have been launched recently to obtain these from aerial vehicles flying closer to the ground

ISOTOPIC TECHNIQUES FOR PRECISION IRRIGATION MANAGEMENT

Effective irrigation management requires accurate knowledge of crop water requirements (ETc) and knowledge of the average water status of the crop–soil system and its variation among and within management units. Isotopic techniques (using oxygen-18 and deuterium isotopes) that quantify the separation of evaporation (E) and transpiration (T) are important research tools to determine the relative magnitudes of E and T in different situations (Williams *et al.*, 2004; Heng *et al.*, 2013, these proceedings). Evaporation rates vary widely from 50 percent to 10 percent of ETc or less under conditions of complete radiation interception by the crop canopy (Villalobos and Fereres, 1990). Isotopic techniques based on oxygen-18 can be used to compute the magnitude of E in many situations, e.g. where subsurface drip irrigation is economically viable and can lead to water savings relative to other irrigation methods.

ASSESSING SOIL WATER OVER LARGE AREAS: THE COSMIC-RAY SOIL MOISTURE OBSERVING SYSTEM (COSMOS)

Making point observations of soil water or plant water status to assess the "average" water status of large areas and thereby reduce the variability problem is not feasible. The cosmic-ray neutron probe (Zreda *et al.*, 2008; Shuttleworth *et al.*, 2010) is a new instrument that can provide measurements of "area-average" soil water content over a circle of about 700 m diameter and over depths varying between 15 and 70 cm (Zreda *et al.*, 2008, 2012; Franz *et al.*, 2012). Monitoring areas at these scales allows the integration of variations caused by differences in soil–crop properties and in the distribution of irrigation water. A sequence of observations over time also permits the computation of the components of the field water balance if the appropriate inputs and outputs are recorded.

To advance solutions for coping with the variability problem one needs, firstly, to characterize the variation across a field and, if unmanageable as a single unit, then to be able to apply variable amounts of water within that field. The common decision to irrigate based on averaging field indicators and integration of some farm constraints should give way to the idea of using sub-field areas to decide and then integrate field and farm constraints. Such sub-fields should have "uniform" characteristics and potentialities, and should

be watered differentially from others, as is already practised for seeding, fertilizing or in pesticide applications in precision farming.

CONCLUSIONS

An integrated approach involving the use of remote sensing, field and large-scale soil moisture sensing devices such as the soil moisture neutron probe and the new cosmic-ray neutron method is needed to improve the application of PI for accurate determination of area-wide crop water requirements and the water status of the root zone. The new PI capabilities should enable growers to evaluate performance in a fast and inexpensive way, leading to increased productivity and to reduce environmental impacts of irrigation

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Partitioning Wheat Transpiration and Soil Evaporation with Eddy Covariance, Stable Isotope and Micro-Lysimeter Methods in the North China Plain

D. Gong[1, 2], X. Mei[1,*], J.Shi[3], W. Hao[1] and L. Heng[4]

ABSTRACT

The dynamics of water vapour delta oxygen-18 ($\delta^{18}O$) at five different heights were monitored continuously in a wheat field by a stable water vapour isotope analysis system. Combined with a Keeling plot curve this was used to partition evapotranspiration (ET) into its components of evaporation (E) and transpiration (T) which were compared with estimations using the eddy covariance system and a micro-lysimeter (i.e. EC-MLS method). There was significant agreement between the ratio E:ET estimated by the stable isotopic and conventional methods (R^2 = 0.8468, n = 27), indicating that the combination of the isotopic Keeling plot method with *in situ* continuous measurements of stable isotope composition of water vapour accurately partitioned ET in the wheat field. The stable isotope composition of atmospheric water vapour (δv) in the wheat field decreased significantly after irrigation events and was linearly related to vapour pressure deficit (VPD) and solar radiation (Rn) with correlation coefficients (R) of 0.696 (n = 1250, $\alpha < 0.001$) and 0.704 (n = 1250, $\alpha < 0.001$), respectively. The stable isotope composition of water vapour arising from soil evaporation (δ_E) also had significant isotopic fractionation effects which were alleviated by low soil water content and VPD.

Key words: *evapotranspiration, stable isotope, eddy covariance, soil evaporation, Keeling plot, partition.*

INTRODUCTION

At farmland scale, evapotranspiration (ET) can be measured easily by conventional methods such as the Bowen ratio (Angus and Watts, 1984), eddy covariance (Wilson *et al.*, 2001), the gradient system and weighting macro-lysimeters (Liu, Zhang and Zhang, 2002). However, it is difficult to distinguish its components: plant transpiration (T) and soil evaporation (E), which are controlled by different mechanisms and to different degrees by biotic and abiotic factors (Raz-Yaseef *et al.*, 2012). Soil evaporation is equal to about 30 percent of ET during the whole plant development period under the common irrigation pattern used in the North China Plain (Liu, Zhang and Zhang, 2002). Partitioning ET accurately into these two components can enhance understanding of water loss processes at the interfaces of the soil–plant–atmosphere continuum (SPAC), which helps us to explore solutions to improve crop water productivity.

Conventional methodologies for separating ET include (i) a combination of soil lysimeters for E and sap flow sensors/chambers for plant T (Gong *et al.*, 2007); (ii) an eddy covariance system/Bowen ratio system/gradient system/weighting macro-lysimeter for ET and soil lysimeters for E (Liu, Zhang and You, 1998); (iii) an eddy covariance system/Bowen ratio system/gradient system/weighting macro-lysimeter for ET and sap flow sensors/chamber system for plant T; and (iv) theoretical methods such as the Shuttleworth-Wallace model (Hu *et al.*, 2009), the dual crop coefficients method (Er-Raki *et al.*, 2010) and time series analysis (Scanlon and Kustas, 2010). The first approach suffers from poor spatial representation (Wang *et al.*, 2010), the second and third methods must solve the problem of transforming scale from point to farmland, and the last ones are confronted with the difficulty of parameter uncertainties.

Incorporating measurements of water isotopic concentration in soil, plant and air vapour can address the limitations of these conventional methods. Significantly, if the isotopic signal of T reaches steady state, the isotopic enrichment of leaf water can be omitted and the isotopic concentration of the plant T vapour equates with that of the local soil water absorbed by roots. Nevertheless, the lighter H_2 oxygen-16 ($H_2{}^{16}O$) molecules, which have a higher vapour pressure and binary diffusivity compared with the heavier isotopologues (HDO or $H_2{}^{18}O$), evaporate more readily from soil, leaving the soil water pools more enriched in delta (δ) ^{18}O and delta deuterium (δD). This results in a significant difference between the isotopic concentration of water vapour from T and E. The different isotopic signals of T and E can provide the basis to separate the total water flux at farmland scales (Wang and Yakir, 2000).

Since Yakir and Wang's (1996) pioneering work on stable isotope methods for partitioning ET, the cold-trap technique has been used for collecting air moisture and stable isotope analysis. However, the traditional cold-trap method is time consuming and labour intensive, and has limited applications to short period (several days),

1 State Engineering Laboratory of Efficient Water Use of Crops and Disaster Prevention and Loss Reduction, Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agriculture Science, Beijing 100081, P.R. China
2 State Key Laboratory of Hydro-science and Engineering, Department of Hydraulic Engineering, Tsinghua University, Beijing 100084, P. R. China
3 Key Laboratory of Agricultural Soil and Water Engineering in Arid and Semiarid Areas, Ministry of Education, Northwest A & F University, Yangling 712100, P. R. China
4 Soil and Water Management and Crop Nutrition Section, Joint FAO/IAEA Division, International Atomic Energy Agency, A-1400 Vienna, Austria
* E-mail address of corresponding author: meixr@ieda.org.cn

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small-scale (chamber scale) studies with low time precision (daily). At present, real-time and continuous measurements of $\delta^{18}O$ and δD in air vapour and liquid water by tunable diode laser absorption spectroscopy provide an opportunity to perform *in situ* and continuous ET partitioning on a diurnal timescale (Zhang *et al*., 2010).

The main objective of this study was to develop and evaluate an isotopic method for separating plant T and soil E using *in situ* continual measurements from a laser-based isotope system and the Keeling plot approach, and to verify this method using measurements from an eddy covariance system and micro-lysimeters.

MATERIALS AND METHODS

Experimental site and plant materials

The experiment was conducted on a winter wheat field at the National Precision Agriculture Station, Changping, Beijing, China (40°11′ N, 116°26′ E, 43 m a lt.), (Figure 1). The climate is temperate (average temperature 12°C and mean annual rainfall 600 mm). The growth period of winter wheat is usually dry with about 120 mm rainfall and a prominent northwest wind. The soil at the station is classified as fluvo-aquic with a loam texture and an average bulk density of 1.42 g/cm^3. At the root zone (0–100 cm), the volumetric soil water content at field capacity and wilting point were 33 percent and 16 percent, respectively. The field experimental plot area was about 4.5 ha (length 150 m, width 300 m), which fulfills the installation requirements of the eddy covariance system. The wheat was sown in October 2010 at a density of 600 000 seeds/ha. During the period of observation from April–June 2011, the total precipitation was only about 60 mm, and the wheat was irrigated two times using a sprinkler system. The amounts of irrigation water were about 75 mm and 45 mm. Rainfall and the quantities of irrigation and soil water content (at 0–200 cm depth) at the study site are shown in Figure 2. The two large increments in soil water content were due to the irrigation events.

Measurements of evapotranspiration and soil evaporation at farmland scale

An eddy covariance (EC) system was installed in the centre of the plot 1.5 m above the wheat canopy to monitor ET. Fluxes of latent (LE) and sensible heat (H) were monitored using respectively a LI-7500 H_2O/CO_2 analyzer and a CAST3 three-dimensional sonic anemometer. Net radiation (Rn) over the field was measured at a height of 4.2 m by a four-way net radiometer (CNR1, Kipp & Zonen Inc., Delftechpark, The Netherlands). Soil heat flux was measured using heat flux plates with a constant thermal conductivity (HFT3, Campbell Scientific Inc., Logan, UT, USA).The accuracy of the EC system measurements was evaluated based on the energy balance principle.

Soil E was measured with micro-lysimeters (Boast and Robertson, 1982) during dry periods. Considering there was uneven coverage of the winter wheat foliage which gives rise to heterogeneity in the incoming radiation and rainfall at the soil surface, seven micro-lysimeters were installed randomly in the windy upstream direction of the eddy covariance system to measure daily soil evaporation. These had an internal diameter of 7.0 cm and a depth of 17.5 cm. The bottom of each micro-lysimeter was capped with a steel plate that did not permit free drainage of water. In this experiment, soil in the micro-lysimeters was replaced every two or three days to avoid any divergence from the surrounding soil due to cessation of root extraction and water exchange with subsoil.

Measurements of stable isotope ratios of vapour, soil and plant water

Air δD, $\delta^{18}O$ isotope signatures and vapour concentrations at different heights (5, 50, 80, 120 and 160 cm) were monitored using a cavity ring down spectroscopy stable isotopic water vapour analysis system (Picarro Inc.). Soil samples at different depths (2, 5, 10 and 15 cm) on three sites and five plant stem samples were collected at intervals of 2–3 days to measure isotopic values of water using an elemental analyzer.

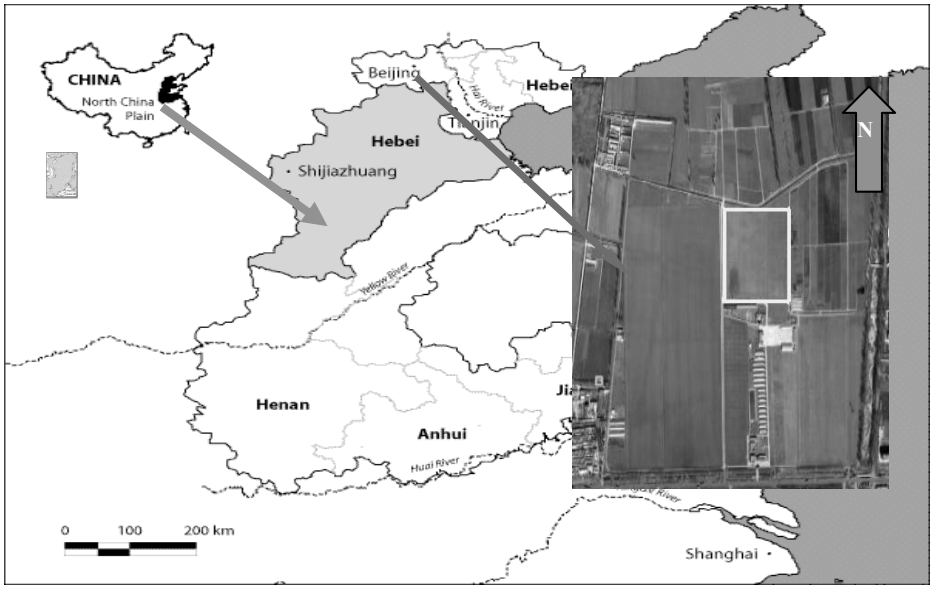


FIGURE 1. Schematic diagram of the experimental station and experimental plot.

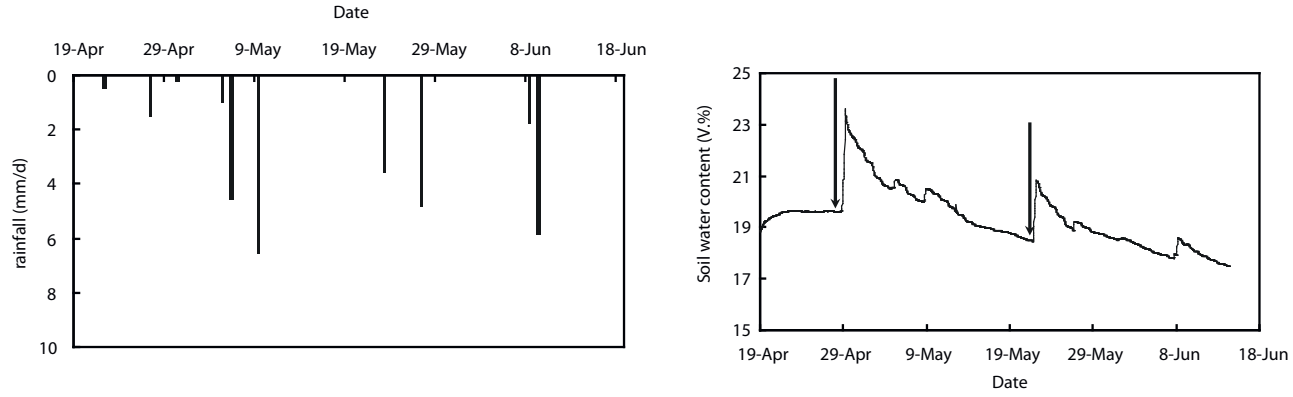


FIGURE 2. Daily rainfall (left), irrigation (right, arrows downward), and soil water dynamics at Changping (April–June 2011).

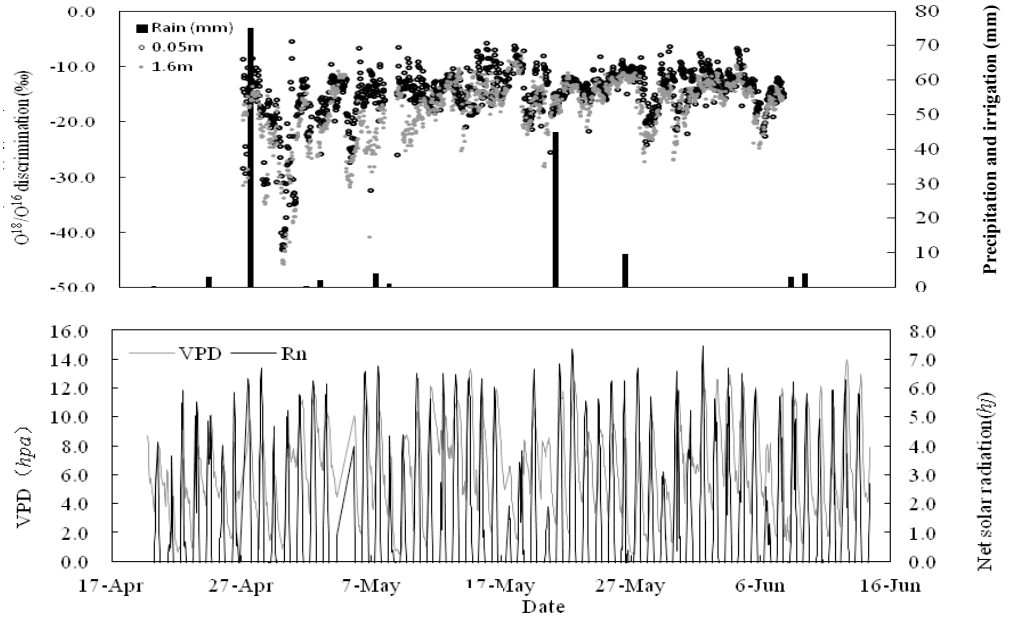


Figure 3. The dynamics of vapour stable composition ($\delta^{18}O$) during the experimental period.

Canopy cover (C_c) measurements

Canopy cover was measured using a LI-191 Line Quantum Sensor (LI-COR Inc., Lincoln, NB, USA). Rather than using multiple detectors arranged linearly over its 1 m length, the LI-191 uses a 1 m-long quartz rod under a diffuser to conduct light to a single, high-quality quantum sensor. Photosynthetic photon flux densities from beneath the canopy ($PPFD_b$) and above the canopy ($PPFD_a$) were measured by this sensor at five different sites. Canopy cover (C_c) was calculated using the equation:

$$C_c = 1 - (PPFD_b/PPFD_a) \quad (1)$$

Meteorological measurements

Meteorological data were measured in a standard automatic weather station nearby the experiment plots. Variables measured included global radiation, air temperature, air humidity, rainfall, and wind speed at 2 m above ground.

Theory description

The general approach for partitioning *ET* into its components is based on simple isotopic mass balance and that the contribution to atmospheric moisture from the farmland surface arises from soil *E* and plant *T*:

$$ET = E + T \quad (2)$$

If these two components are isotopically distinct, the isotopic mass balance is:

$$\delta_{ET} = \delta_E + \delta_T \quad (3)$$

where δ is the isotopic composition and subscripts ET, E, and T denote respectively evapotranspiration, evaporation and transpiration.

Assuming that $F_S = E/ET$, and substituting $T = ET - E$ from Equation (2) into Equation (3) and rearranging, Fs is obtained as:

$$F_S = \frac{\delta_{ET} - \delta_T}{\delta_E - \delta_T} \quad (4)$$

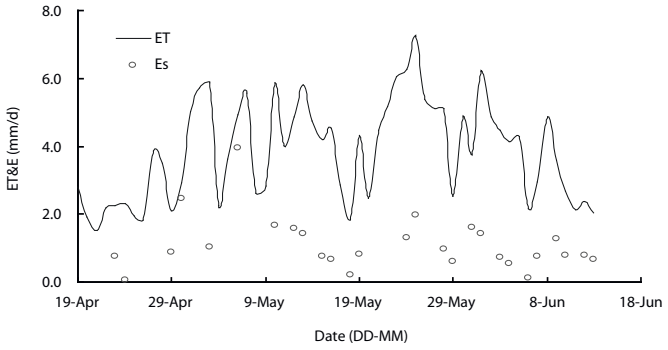


FIGURE 4. Dynamics of evapotranspiration (ET) and soil evaporation (E) estimated by eddy covariance and micro-lysimeters during the experimental period.

where δ_{ET} is estimated using the Keeling mixing model (Keeling, 1961); δ_E is estimated using the Graig and Gordon (1965) model, and δ_T is estimated from the isotopic values of stem water.

RESULTS AND DISCUSSION

Temporal dynamics of $\delta^{18}O$ composition in air vapour at different heights

Figure 3 shows that the $\delta^{18}O$ composition of air vapour in the winter wheat field changed with rainfall and irrigation (R and I), vapour pressure deficit (VPD) and net solar radiation (R_n) at two heights (0.05 m and 1.60 m) from April to June in 2011. The $\delta^{18}O$ of the lower layer (0.05 m) was greater than that of higher layer (1.60 m) because the $\delta^{18}O$ composition of the vapour derived from soil evaporation and the atmospheric background are very different. The difference between the low and high layers was about 0~10.0‰, and was related to the weather conditions. These were similar to the isotopic composition distribution of vapour in a forest (Lee, Kim and Smith, 2007). The stable isotope composition of vapour $\delta^{18}O$ was about –45.00‰ ~ –5.00‰ during the observation period. In the rainfall and irrigation periods the average daily stable isotope values of vapour $\delta^{18}O$ decreased rapidly, and then increased gradually to the maximum because of the isotopic fractionation effects of soil water evaporation. This is consistent with the findings of Yuan *et al*. (2010). Statistical analyses of the results showed that the $\delta^{18}O$ composition of air vapour at the two heights correlated significantly with VPD and R_n with average correlation coefficients of about 0.696 ($n = 1250$, $\alpha < 0.001$) and 0.704 ($n = 1250$, $\alpha < 0.001$).

The ratio of soil evaporation to evapotranspiration estimated by eddy covariance and micro-lysimeters

Results for evapotranspiration (ET) and soil E estimated by the eddy covariance system and using micro-lysimeters are shown in Figure 4. Evapotranspiration increased during the initial stages of wheat development, stabilized in the middle of the growth period and decreased during the late growth period. During the whole observation period the maximum and minimum rates of ET were 7.2 mm/day (May 25) and 1.5 mm/day (April 21), respectively, while the corresponding values for E were 2.4 mm/day and 0.4 mm/day.

The ratio of plant transpiration to evapotranspiration estimated by the isotopic method and its comparison with the EC-MLS method

Gradients in atmospheric moisture content and isotopic composition through the profile of the canopy boundary layer were observed during the experimental period. Linear regressions were fitted to mid-day (11.00–15.00 h) data to estimate δ_{ET}, the isotopic composition of the ET flux (Figure 5). There was a significant relationship between inversion of atmospheric moisture content and isotopic composition of water vapour, suggesting that the Keeling plot method for estimating the isotopic composition of the ET flux from continuous

Table 1. Parameters of the Craig-Gordon model and estimates of evaporation flux δ_E on selected days

Date	$\alpha_{L\text{-}V}$	$\varepsilon_{L\text{-}V}$	h	$\Delta\xi$(‰)	δ_v(‰)	δ_S(‰)	δ_E(‰)
4–23	0.9800	19.957	0.26	21.105	–27.48	–5.32	–46.83
4–30	0.9883	11.702	0.51	14.020	–18.54	–10.05	–48.16
5–6	0.9867	13.274	0.47	15.132	–12.96	–9.33	–47.39
5–9	0.9882	11.798	0.43	16.160	–15.75	–10.11	–43.51
5–18	0.9932	6.753	0.67	9.536	–11.73	–5.47	–53.02
5–22	0.9914	8.565	0.61	11.220	–15.59	–12.42	–42.71
5–28	0.9924	7.571	0.67	9.521	–11.48	–9.70	–37.45
5–31	0.9948	5.219	0.65	9.990	–18.65	–10.63	–48.12
6–3	0.9956	4.433	0.35	18.519	–15.68	–6.63	–33.56
6–6	0.9964	3.566	0.71	8.286	–11.04	–9.05	–36.58
6–9	0.9967	3.298	0.78	6.264	–11.05	–5.92	–55.82
6–12	0.9974	2.631	0.48	14.687	–18.47	–10.87	–29.89

Note: $\alpha_{v\text{-}l}$ is the equilibrium fractionation factor for liquid–vapour exchange of H_2O; $\varepsilon_{v\text{-}l}$ is another form of $\alpha_{v\text{-}l}$; h is the soil relative humidity; $\Delta\xi$ is kinetic fractionation factor; δ_v is the vapour water $\delta^{18}O$ isotopic value measured by a stable water vapour isotope analysis system (Picarro Inc.); δ_s is the average isotopic values of water at the soil surface; δ_E is fitting value of water vapour from soil evaporation.

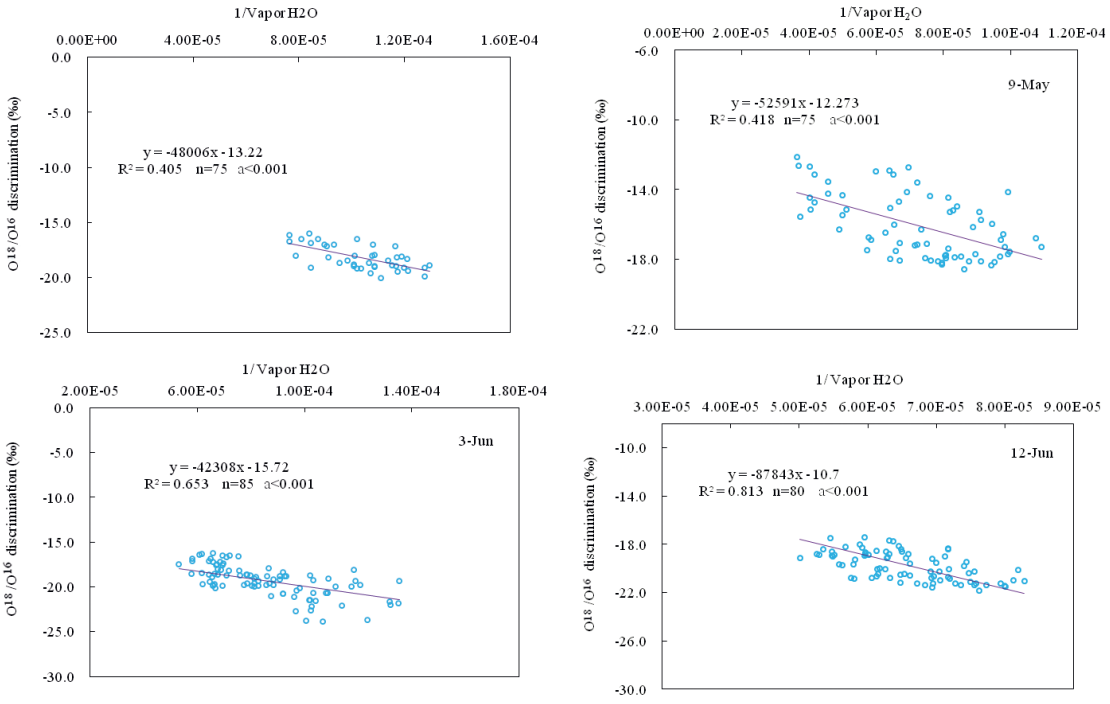


Figure 5. Daytime Keeling plots of water vapour monitored for $\delta^{18}O$ by a cavity ring-down spectroscopy (Picarro Inc.) at different heights above the ground on selected days.

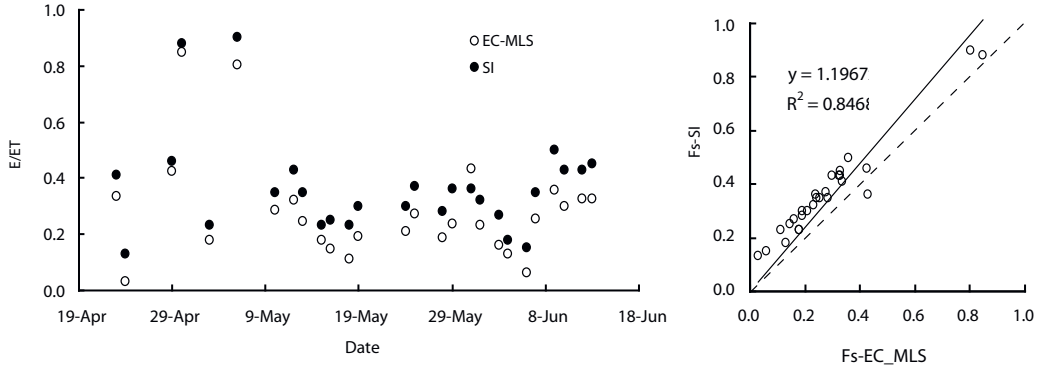


FIGURE 6. Seasonal changes in the ratios of E to ET (F_s) estimated by conventional (o) and isotopic (●) methods (left), and their inter-relationship (right).

measurements using the water vapour stable isotopic analysis system was feasible in the wheat fields of the North China Plain.

The stable isotope composition of soil evaporation (δ_E) in the wheat field and various other parameters were estimated from the isotope fractionation coefficients and the Craig-Gordon model for stable isotope composition of the soil evaporation (Table 1). The values of δ_E of soil evaporation were mostly about -30 ‰ ~–50 ‰, which is smaller than δ_S of the soil water, indicating that there was obvious isotope fractionation during the soil evaporation process. These results are consistent with the findings of Yuan *et al.* (2010).

The stable isotope compositions of wheat field evapotranspiration (δ_{ET}), soil evaporation (δ_E) and crop stems (δ_T), were used to calculate E/ET according to Equation (4), and the results compared with values estimated by the conventional method (Figure 6, left). The E/ET ratios during the early and late growing seasons were higher than that in the mid growing season because of the smaller canopy cover in the early and late seasons. The E/ET ratios estimated by the stable isotopic method were almost 20 percent higher than by combining the eddy covariance and mini-lysimeter methods. There was significant agreement between the E/ET ratio estimates from the stable isotopic and conventional methods (R^2 = 0.8468, n = 27) (Figure 6, right). These results are consistent with the findings of Zhang (2009).

The relationship between E:ET and canopy cover (C_c)

The ratio (E/ET) is controlled by canopy cover, the wetting area of the soil surface layer and weather conditions. Figure 7 shows the curve fitted between E/ET estimates by the conventional method and from crop canopy cover (C_c). There was a significant negative linear relationship between average E/ET and C_c (correlation coefficients, R^2 = 0.936, n = 7), indicating that E/ET decreased with increasing C_c because the amount of solar radiation reaching the soil surface decreased as the crop leaf area increased.

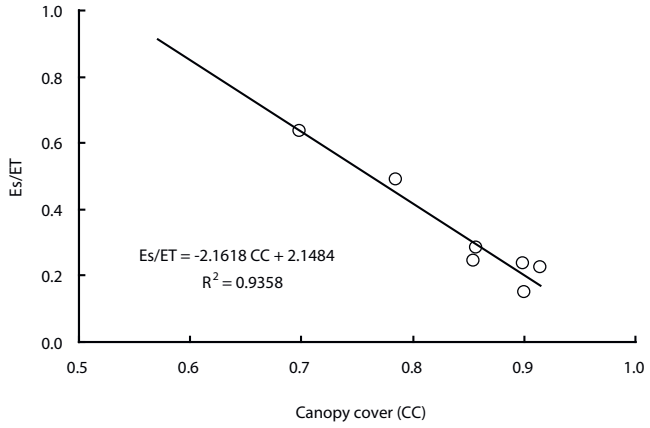


FIGURE 7. Relationship between E/ET estimated by the eddy covariance micro-lysimeter method and from canopy cover (C_c).

CONCLUSIONS

This study has shown that E/ET ratios esti 1mated by an on-line stable isotopic air vapour analysis system using a cavity ring-down spectroscopy were comparable with those obtained by the conventional method, demonstrating that the partition of ET into E and T components by the isotopic method is feasible and reliable in the North Plain of China. The isotopic method gives rapid and reliable results, but whether it provides the precision required by researchers needs further investigation.

ACKNOWLEDGMENTS

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Isotopic and Conventional Techniques to Improve the Irrigation Practice in Order to Enhance Agriculture Production under Water Limiting Conditions in Morocco

N. Amenzou[1,*], H. Marah[1], F. Raibi[1], J. Ezzahar[1], S. Khabba[2], J. Lionel[3] and M. Ismaili[4]

ABSTRACT

Sound and efficient irrigation practices are important for achieving sustainable management of water resources for agriculture in arid and semi-arid regions. An experiment was conducted to monitor seasonal water consumption of citrus plants irrigated by a drip irrigation system at the Agafay station in the middle of Morocco. For this purpose, an eddy-covariance (EC) system, a meteorological station and a fluxmeter were employed, as well as measurements of soil moisture and temperature which were made available continuously during the experimental period. The oxygen-18 stable isotope was used to partition evapotranspiration (ET) fluxes, i.e. a Keeling Plot with data from five layers inside a mandarin orchard being generated to assess ecosystem isotopic flux. The results suggest that loss by percolation constituted about 38 percent of the cumulative amounts of irrigation and rainfall. Partitioning of ET showed that transpiration dominated the evaporation during the two sampling days. This result confirms that the method of irrigation applied by the farmer was very appropriate for conditions in the orchard, but that it is necessary to re-examine both the amount and timing of water applied through irrigation to minimize losses by percolation.

Key words: *evapotranspiration partitioning, water losses, stable isotopes, percolation, fluxmeter.*

INTRODUCTION

Arid and semi-arid regions constitute roughly one third of the total earth surface. In these regions, water scarcity is one of the main limiting factors for economic growth. The impact of such water scarcity is amplified by inefficient irrigation practices, especially since about 85 percent of available water is used for irrigation in these regions. Sound and efficient irrigation practices are therefore important for achieving sustainable management of water resources in these regions. In this regard, a better understanding of the water balance is essential for exploring water-saving practices and to avoid contamination of groundwater. The most important components of water balance in semi-arid areas are evapotranspiration (ET) and deep percolation. Methods such as lysimetric and sap flow measurements and micrometeorological techniques are used to measure or estimate ET. These do, however, have limitations. Stable isotopic tracer methods offer new opportunities to study the components of ET at field scales, from the leaf to ecosystem levels, and can partition ET from different compartments of the ecosystem that incorporate water vapour.

MATERIALS AND METHODS

Study site

The study site was a mandarin (Afourer variety) orchard planted in July 2000. It is located approximately 30 km southwest of Marrakech city, Morocco (31°50′ 27″N, 008°25′ 02″W). This area has a semi-arid Mediterranean climate, characterized by low and irregular rainfall (annual average of about 240 mm) and a higher reference evapotranspiration (ET_0) of 1 600 mm per year. The trees were planted in a regular square pattern (4 m × 6 m), and were maintained in well-watered conditions by drip irrigation, supplied every day. Fertilization, pest and weed control were performed. The soils have high sand and low clay contents (18 percent clay, 32 percent silt, and 50 percent sand).

Meteorological data and reference evapotranspiration

The site was equipped with a set of standard meteorological instruments to measure wind speed and direction (model Wp200, R.M. Young Co., USA) as well as air temperature and humidity (model HMP45AC, Vaisala Oyj Finland) at four heights. Net radiation over the vegetation and soil was measured using net radiometers (model CNR1, Kipp and Zonen, The Netherlands and the Q7 net radiometer, REBS Inc., USA). Soil heat flux was measured using soil heat flux plates (Hukseflux). Water content reflectometers (CS616, Campbell Scientific Ltd., USA) were installed at depths of 5, 10, 20, 30, 40, 60 and 80 cm in order to measure the soil humidity profile. Measurements were taken at 1 Hz, and averages stored at 30-min intervals on CR23X data loggers (Campbell Scientific Ltd., USA).

Eddy covariance measurements

An eddy-covariance (EC) system was installed over the citrus field to provide continuous measurements of vertical fluxes of heat (H_{EC}) and water vapour (L_vE_{EC}). The EC system consisted of commercially avail-

1 Water and Climate Unit, CNESTEN, Rabat, Morocco
2 Cadi Ayyad University, Marrakech, Morocco
3 Institut de Recherche pour le Développement (IRD), Morocco
4 Faculty of Science, Meknès, Morocco
* E-mail address of corresponding author: amenzou@cnesten.org.ma

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able instrumentation: a 3D sonic anemometer (CSAT3, Campbell Scientific Ltd., USA) and a fast response hygrometer (Campbell Scientific Inc., USA). Raw data were collected at a rate of 20 Hz and recorded using CR5000 data loggers (Campbell Scientific Ltd., USA). The half-hourly fluxes were later calculated off-line using EC processing software 'ECpack', after performing all required corrections for planar fit, humidity and oxygen (KH_2O), frequency response for slow apparatus and path length integration (Van Dijk, Moene and De Bruin, 2004).

Infiltration measurements using Fluxmeter

Besides the standard meteorological measurements, one flux meter was installed at a depth of 80 cm which corresponds to the root zone to quantify the water loss by deep percolation.

Stable isotope measurements

Soil and plant water and vapour collection

Using a hand auger, soil was sampled from the surface to a depth of 10 cm. Sampled branches of mandarin trees were 0.5–1.0 cm in diameter and 1–2 cm in length, and from each the bark was removed. Every plant sample was composed of 2–3 stems from different individuals. Soil and plant samples were placed into screw-cap glass vials (5 ml) and sealed with parafilm, then stored at about 2°C.

Water vapour was collected at five heights: 0.1 m, 1.75 m, 2.95 m, 4.45 m and 8.12 m. Sampling was carried out at 10:00, 11:00, 13:00, 14:00 and 15:00 h during the collection period mentioned above. For each group, vapour was collected using a vacuum pump for one hour with a flow rate of 250 mL/min. The air was circulated through a set of 45 cm long glass traps (modified from Helliker *et al.,* 2002) which were immersed in a mixture of ethanol and liquid nitrogen (at about –80°C). Traps were made of 9 mm diameter Pyrex glass attached to 6–9 mm diameter Cajon Ultra-Torr adapters which were framed in 9 mm diameter Swagelok Union Tee. After sampling the traps were sealed with parafilm and stored at about 2°C.

Probes of model HMP45AC, Vaisala Oyj, Finland for measuring the air temperature (T_a, in Kelvin) and relative humidity (h,) were placed near the vapour sampling inlets at 5 min intervals. Using T_a, h and atmospheric pressure (Pa, in hPa), water vapour concentration was calculated using Equation 1 (shown below; McRae, 1980):

$$H_2O \text{ (mmol/mol)} = \frac{10h\left[P_a \exp(13.3185\, t - 1.99760\, t^2 - 0.6445\, t^3 - 0.1299\, t^4)\right]}{P_a} \quad (1)$$

where P_a = standard atmosphere pressure (about 1 013.25 hP_a) and $t = 1 - (373.15/T_a)$.

Keeling Plots were generated using the inverse of the average vapour concentration ($1/[H_2O]$) at each height as independent variables and isotopic composition of water vapour (delta oxygen-18 or deuterium, $\delta^{18}O$ or δD) collected at the corresponding height as dependent variables.

Stable isotope and data analysis

Soil and plant water were extracted by cryogenic vacuum distillation (Ehleringer, Roden and Dawson, 2000). Water samples were analysed isotopically at the National Center of Sciences and Nuclear Techniques (CNESTEN) by DLT-100 laser spectroscopy (±1 standard deviation). The standard deviation for repeated analysis of laboratory standards was 0.2‰ for ^{18}O and 1‰ for D. Concentrations of these isotopes are expressed in per mil (‰) as deviations from an international standard (V-SMOW) and using the delta (δ) notation as follows:

$$\delta‰ = [(R_s/R_{st}) - 1] \times 1000 \quad (2)$$

where R_s and R_{st} were the ratios of the heavy to light isotopes in the sample and the standard, respectively.

RESULTS AND DISCUSSION

Evolution of climatic conditions

Figure 1 presents the variations in climatic variables during the 2009 growing season at the Agafay site. The lowest values for Ta occurred during the winter (4.4°C) and the highest were in the summer (43.5°C). Atmospheric humidity was low (56 percent), and global radiation was high in summer (606 Wm^2) and low in the winter (35 W/m^2). The wind speed was stable during all seasons (average 1.1 m/s). Rainfall was quite uneven and variable throughout the year.

Seasonal variations in the reference evapotranspiration ET_0 of well-watered grass were calculated using the FAO Penman–Monteith equation (FAO, 1998) using the meteorological parameters collected over the study site. The ET_0 pattern was characteristic of semi-arid continental climates, with an average accumulated annual ET_0 of 1355 mm. The lowest values occurred during the winter and

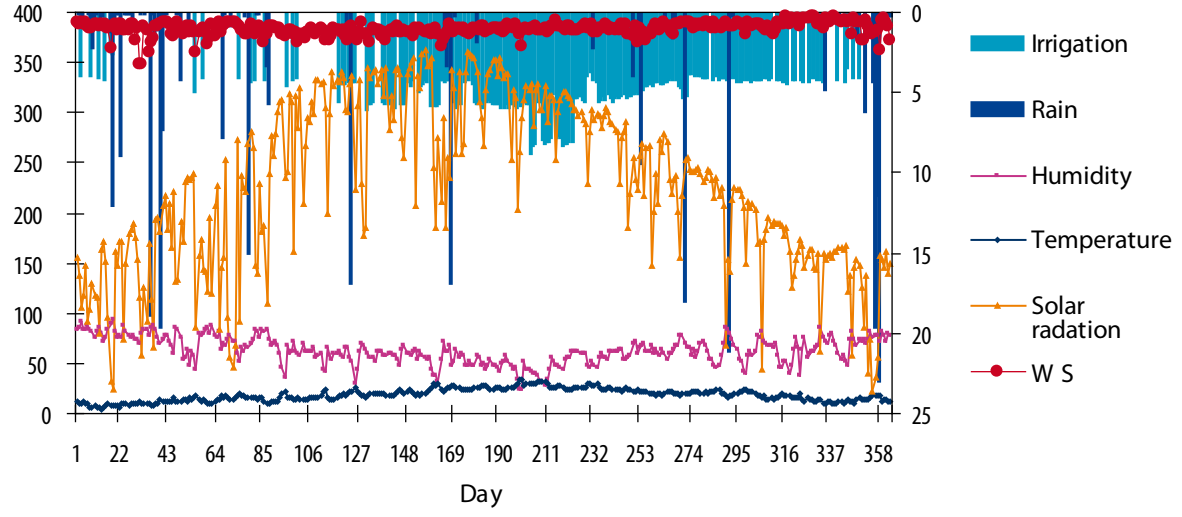


FIGURE 1. Variations in environmental conditions during the growing season of 2009.

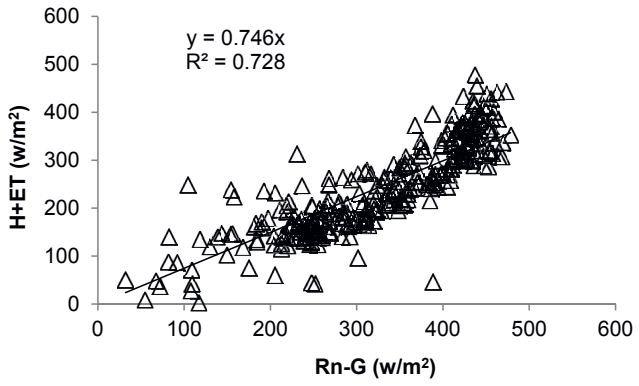


FIGURE 2. Daily energy balance closure.

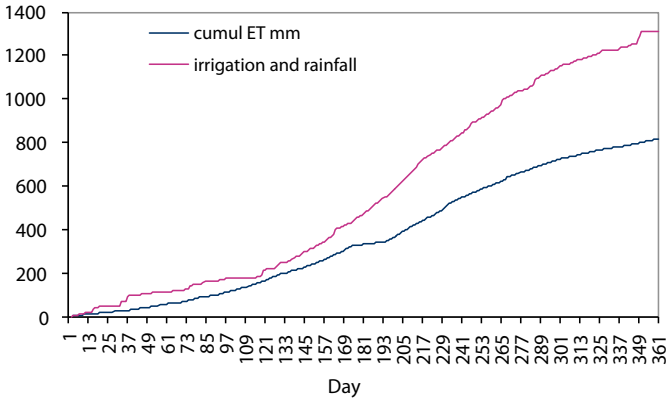


FIGURE 3. Cumulative evapotranspiration (ET) compared with the sum of the amounts of precipitation and irrigation.

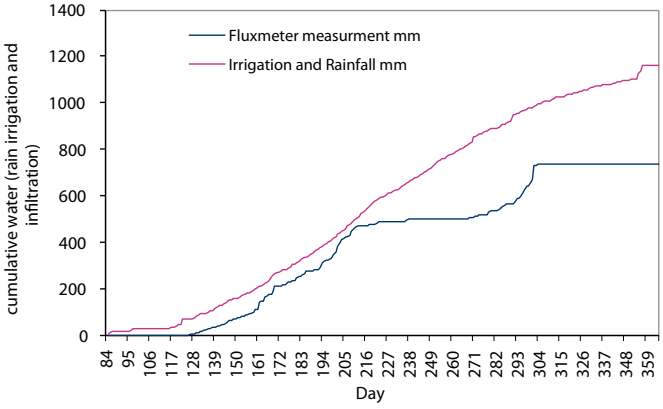


FIGURE 4. Cumulative water drainage compared with the sum of precipitation and irrigation.

autumn (0.05 mm/day) and the highest values were in the summer (11.07 mm/day).

Flux data quality assessment

Energy balance closure is an important indicator of the performance of an EC system. By assuming the principle of conservation of energy, the energy balance closure (*EC*) is defined as:

$$EC = R_n - H - ET - G \quad (3)$$

where R_n = net radiation; G = soil heat flux; and H and ET = sensible and latent heat fluxes derived from the EC.

Figure 2 presents a cross plot between measured ($R_n - G$) and the sum of the turbulent fluxes ($H + ET$). The difference in terms of the source areas of the different instruments has the greatest impact on the closure of the energy balance, especially over sparsely vegetated surfaces. The source area sampled by EC is much larger than that of net radiation and soil heat flux, and it can change rapidly depending on wind speed and direction and on surface conditions. However, compared with values reported in the literature, the closure can be considered as fairly acceptable.

Losses by infiltration.

Losses through infiltration were evaluated using the water balance equation method and directly using a fluxmeter.

Water balance measurements (EC system)

This method consists of comparing the cumulative evapotranspiration measured by EC and the sum of the cumulative amounts of irrigation and rainfall. Total rainfall during the experiment was 295 mm, while the average annual rainfall in the Tensift river basin is 240 mm. Figure 3 shows that about 495 mm was lost by infiltration and runoff during this season, representing 38 percent of the sum of irrigation and rain.

Direct and indirect estimation of infiltration

Values for cumulative infiltration and the cumulative totals for irrigation and rain (Figure 4) show that water lost by deep infiltration was about 425 mm, representing 32 percent of the sum of cumulative rain and irrigation. The difference between direct measurement of percolation and that derived from the water balance can be explained by surface runoff during rain events. This result is consistent with that obtained using the water balance equation, and reveals that the farmer applied a large amount of water. Therefore, it is necessary to re-examine the amount of water applied and the timing of irrigation.

Partitioning evapotranspiration components

The stable isotopic composition of water vapour, stem and soil water

Isotopic compositions of soil water (δ_S) ranged from -6.33‰ to -4.36‰ for $\delta^{18}O$, and from –54.31‰ to –30.2‰ for δD. Isotopic ratios of stem water (δT_S) ranged from –6.19‰ to –5.27‰ for $\delta^{18}O$, and from –45.25‰ to –43.47‰ for δD. Isotopic compositions of vapour (δa) ranged from –11.32‰ to –7.81‰ for $\delta^{18}O$, and from –73.05‰ to –59.68‰ for δD. These results indicate that the isotopic values of water vapour evaporating from the soil surface (δ_E, soil evaporation) were more isotopically depleted relative to vapour generated by plant transpiration (δ_T) during the two sampling days. All samples (vapour, soil water, stem water, irrigation water) were situated around the local meteoric water line (LMWL). The regression line of all samples intersected the LMWL at the point that presented the origin of all samples.

Keeling plot analysis

The isotopic ratio of atmospheric water vapour at a certain altitude can be described using Equation 4 by considering the mixing of evapotranspired water vapour and free atmospheric water vapour (Keeling, 1958; Moreira *et al.,* 1997). This relationship is linear and

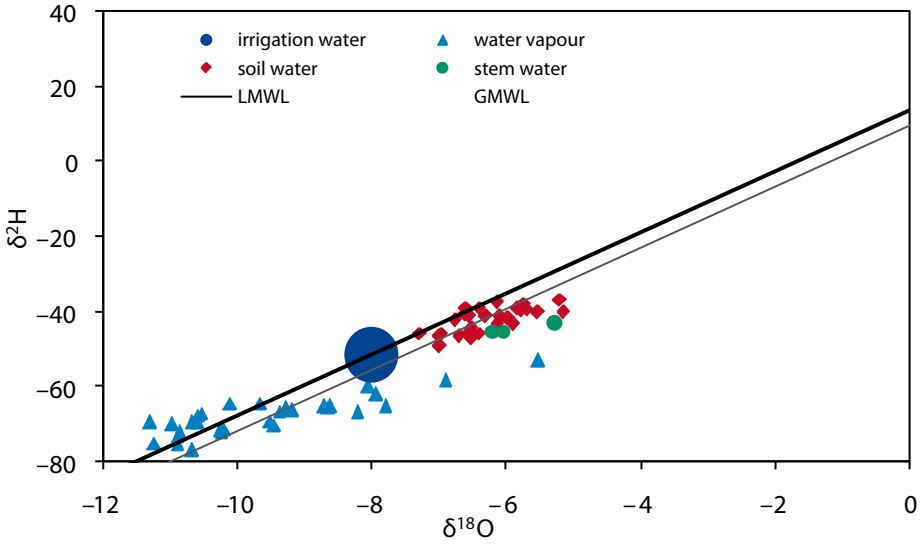


FIGURE 5. $\delta^{18}O$ versus δD in atmospheric water vapour, irrigation water, stem water and soil water at the Agafay site.

when used with water vapour the *y*-intercept reflects the source isotopic composition of the evapotranspiration flux:

$$\delta_{ebl} = C_a (\delta_a - \delta_{ET}) \frac{1}{C_{ebl}} + \delta_{ET} \quad (4)$$

where δ_{ebl} = isotopic composition of vapour collected from the ecosystem boundary layer; C_a — atmospheric vapour concentration; C_{ebl} — vapour concentration in the ecosystem boundary layer; δ_a —isotopic composition of the atmospheric background; and δ_{ET} — the isotopic composition of the evapotranspiration flux.

The Keeling plot approach is based on the assumption that the atmospheric concentration of vapour in an ecosystem combines the inputs of two major sources: the background vapour from the atmosphere and vapour added by sources within the ecosystem. It is further assumed that the only loss of water vapour from the ecosystem is by turbulent mixing with the background atmosphere.

The isotopic ratio of evaporated water vapour from the soil surface is described by considering the fractionation process (Craig and Gordon, 1965) as:

$$\delta_E = \frac{\alpha^* \delta_{surf} - h\delta_{atm} - \varepsilon_{eq} - (1-h)\varepsilon_k}{(1-h) + (1-h)\varepsilon_k \frac{\varepsilon_k}{100}} \quad (5)$$

where δ_E — isotopic composition of soil evaporation flux; α^* —the temperature-dependent equilibrium fractionation factor; ε_k — kinetic fractionation factor; *h* — relative humidity normalized to the temperature at the evaporation surface in soil; δ_{atm} —isotopic composition of atmospheric vapour; and δ_{surf} — isotopic composition of water at the evaporation surface in soil.

In this paper, $\alpha^* = 1/\alpha^+$ (Gat and Matsui, 1996) and α^+ can be calculated by Equations 5 and 6 provided by Majoube (1971):

$$^{18}O\alpha^* = [1.137\,(10^6/T^2) - 0.4156\,(10^3/T) - 2.0667]1000 + 1 \quad (6)$$

$$D\alpha^* = [24.844\,(10^6/T^2) - 76.248\,(10^3/T) - 52.612]1000 + 1 \quad (7)$$

where *T* = soil temperature recorded at 5 cm depth in degrees Kelvin and ε_k is estimated using the diffusivity ratios of 1.0251 for H_2O:HDO and 1.0281 for H_2O:$H_2{}^{18}O$ (Merlivat, 1978).

The contribution of transpiration to evapotranspiration (F_T) was estimated by Yakir and Sternberg (2000) as:

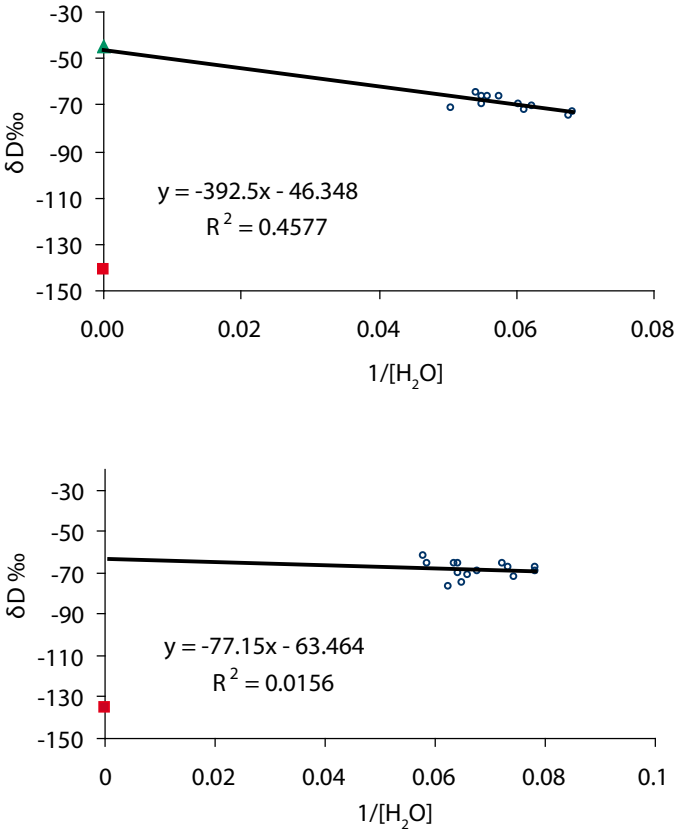


FIGURE 6. Relationship between δD at different levels above ground and the inverse of air absolute humidity at the Agafay site (A 16/07/2009, B 17/07/2009) (E = evaporation, T= transpiration).

Table 1. Slope and intercept of the regression lines between δD values of water vapour collected at different heights and the inverse of the corresponding vapour concentration. The intercept indicates the isotopic compositions of evapotranspiration (δET).

	δs	δa	δ_T	δ_{ET}	R^2	p	n	δ_E	T/ET
16/07/2009	-41.4	-65.1	-44.6	-46.3	0.457	0.022*	11	-140.5	0.982
17/07/2009	-46.14	-68.5	-45.1	-63.5	0.015	0.67	14	-134.9	0.795

* The significance level is 0.05

$$F_T = \frac{\delta_{ET} - \delta_E}{\delta_T - \delta_E} \quad (8)$$

Figure 6 shows the relationship between the δ_D values of ambient vapour and the inverse of its absolute humidity while Table 1 shows the mathematical treatment for data obtained from Figure 6, including the slope and intercept of the regression equations between the δ_D values of vapour and the inverse of absolute humidity.

A significant correlation between isotopic values and the inverse of vapour concentrations was observed only for the first day of sampling. The intercepts of the regression lines at Agafay show a high transpiration contribution for the mandarin vapour, suggesting that this source plays an important role in the water cycle.

Considering mandarin crop transpiration as one source and soil evaporation as another, the fractional contribution of plant transpiration to total ET (T/ET) varied between 98 percent and 79.5 percent for δD during the two days of sampling. Consequently, transpiration dominated the evaporation, a result confirming that the irrigation method applied by the farmer was very appropriate for the conditions in the orchard and considering evaporation as the only source of water loss.

CONCLUSIONS

Stable isotope contents and Keeling plots allowed the partitioning of ET into different flux components in a citrus orchard irrigated with drip irrigation. The results obtained on two sampling days during July 2009 indicated that more than 80 percent of ET was generated by plant transpiration, from which it can be concluded that evaporation was negligible. However, loss by drainage was more important, contributing about 38 percent of total losses from the total cumulative irrigation and rainfall. This percolation, which depends closely on the irrigation, was accentuated by the addition of rainfall.

These results confirm that by considering only evaporative losses the efficiency of the irrigation system applied on the Agafay station was high. However, the results also showed that large quantities of water were lost by percolation, infiltration and runoff. The farmer should therefore re-consider both the amount of water applied and the timing of irrigation in order to minimize such losses.

ACKNOWLEDGMENTS

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Effects of Climate and Soil Management on Crop Water Use Efficiency: The Role of Modelling

E. Wang[1,*], Z. Zhao[1,2], C. Chen[3] and C.J. Smith[1]

ABSTRACT

Water scarcity is the major limitation to crop production in many parts of the world. The water use efficiency (WUE) by which crops use rainfall and irrigation water to produce grains is regulated by climate and soil conditions during the crop growing season. This paper briefly depicts how crop WUE is regulated by climate and soil, and demonstrates that crop modelling provides an effective means to benchmark crop WUE as influenced by climate change and soil management practices. Comparison of the modelled WUE against what is observed in the field enables the assessment of WUE and yield gaps, and possible management options to increase both WUE and yield. While water loss by evaporation from soil is a major factor reducing crop WUE, and it increases in drier climates, improvement in technologies to quantify crop transpiration and soil evaporation, and measures to reduce soil evaporation can help to identify approaches to increase crop WUE.

Key words: *Water productivity, wheat, Murray Darling Basin, North China Plain, transpiration, evaporation.*

INTRODUCTION

Water is a major limiting factor to crop production in many parts of the world, particularly in semi-arid and arid regions, which occupy around one third of the Earth's land surface. Although low water availability is often associated with high climate (rainfall) variability, it also coincides with abundant radiation and high crop yield potential on good soil under irrigation. Improved management of climate variability and soil fertility then becomes essential to ensure efficient use of the limited water resources. This, however, is a challenging task because crop yield is a result of integration of climate, soil and management conditions over the whole growing season. The overall crop water use efficiency (WUE, i.e. crop yield produced per mm of water used) depends not only the amount, but also the timing of water supply (rainfall or irrigation), and the capacity of the soil to hold water for plant use.

Soil–plant systems modelling can play a key role in understanding the interactions between climate and soil as they impact on WUE. A crop model integrates the current understanding of crop physiological processes, and is able to dynamically simulate crop growth, development and yield formation in response to seasonal changes in weather, soil and management conditions. It provides an effective means to identify constraints to crop yield and to evaluate management strategies for increasing crop productivity and WUE. This paper briefly explains how crop WUE is regulated by climate and soil conditions and demonstrates how crop modelling helps to define the crop yield potential and the water use efficiency frontier (WUE maximum), and to identify yield constraints and management strategies to increase crop WUE.

MATERIALS AND METHODS

Roles of climate and soil to regulate WUE

Crops transpire water (H_2O) while they assimilate carbon dioxide (CO_2) mainly through stomatal control, to produce biomass and grain. Combining the diffusion equations of H_2O and CO_2 through leaf, canopy characteristics and bio-composition of a crop, it can be shown that crop transpiration efficiency (ε, the amount of water transpired to produce unit amount of biomass, B_m) is a function of crop type, daytime vapour pressure deficit (D) and CO_2 concentration in the air (C_a) (Sinclair, Tanner and Bennett, 1984; Wang *et al.*, 2004):

$$\varepsilon = \frac{B_m}{T} = abc\frac{C_a}{D} = \beta\frac{C_a}{D} \quad (1)$$

where a is a constant derived from the molecular weight of H_2O and CO_2 and canopy leaf structure; b is the conversion coefficient of hexose to plant biomass; and c accounts for the ratio of CO_2 partial pressure inside and outside leaf. $\beta = abc$ is then a crop-dependent coefficient.

Equation 1 is easily applied on a daily basis. If B_m and T are the total biomass and transpiration of the whole growing season, β and D need to be representative for the whole season, their calculation procedures become more complex. Nonetheless, the equation still applies. It turns out that $\beta = abc$, is relatively constant for a given crop species, thus the biomass produced (B_m) or crop grain yield (Y) is directly proportional to the amount of water transpired (T) and *vice versa*. If $Y = H_iB_m$, where H_i is the harvest index, then:

$$Y = H_iB_m = H_iT\varepsilon = T\left(H_i\beta\frac{C_a}{D}\right) \quad (2)$$

If crop WUE is defined as grain yield per unit water input (the sum of precipitation P and irrigation I), $f(T)$ and $f(ET)$ are the fraction of crop transpiration in total evapotranspiration (ET) and the fraction of ET in total water inputs ($P+I$), it gives:

$$WUE = \frac{Y}{P+I} = H_i\varepsilon\left(\frac{T}{ET}\right)\left(\frac{ET}{P+I}\right) = H_i\beta\frac{C_a}{D}f(T)f(ET) \quad (3)$$

It follows that: (i) WUE is directly related to transpiration efficiency (ε), therefore it increases with atmospheric CO_2 concentration but decreases with the dryness of the air (D), (ii) WUE is crop-dependent

1 CSIRO Land and Water, Black Mountain, Canberra, ACT 2601, Australia
2 China Agricultural University, Beijing 100093, China
3 University of Technology, Sydney, NSW 2007, Australia
* E-mail address of corresponding author: enli.wang @csiro.au

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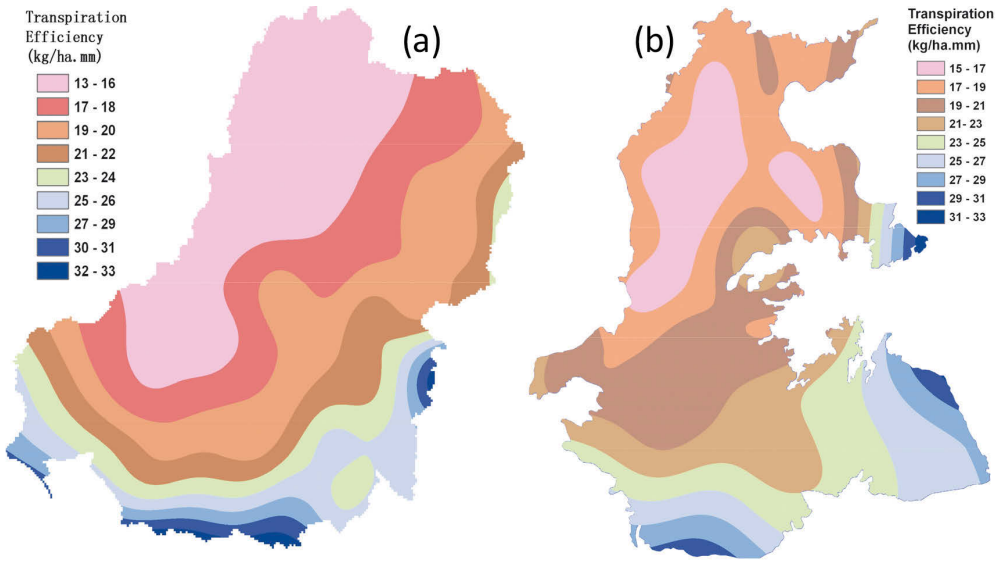


FIGURE 1. Average whole season transpiration efficiency of wheat crop at Murray Darling Basin of Australia (a) and the North China Plain (b).

(β, H_i) and increases with crop harvest index (H_i), and (iii) WUE can be increased through increasing the fraction of transpiration *f*(*T*) and evapotranspiration and *f*(*ET*).

Experimental results also show that higher CO_2 concentrations tend to increase leaf and plant level WUE (Eamus, 1991). Breeding efforts for new crop varieties have increased crop harvest index (H_i) continuously leading to significant increases in crop yields and WUE (Liu *et al.,* 2010; Liu *et al.,* 2012).

Soil regulates WUE through its capacity to store or hold water for plant use. Soils that enable more water to infiltrate and that have larger plant available water holding capacity (PAWC) reduce water losses by surface runoff and deep drainage, thus increasing *ET* and crop transpiration (*T*), i.e. both *f*(*T*) and *f*(*ET*), particularly in areas with higher rainfall variability (Wang *et al.*, 2009). Management practices to reduce soil evaporation (*E*) further improve WUE through increasing *T*.

Crop modelling

The Agricultural Production Systems Simulator APSIM (Wang *et al.*, 2002; Keating *et al.*, 2003) was used to simulate wheat grain yield and WUE across climatic regions and on several soil types to demonstrate how modelling can help define the WUE frontiers. The wheat module in APSIM (APSIM-Wheat; Wang *et al.*, 2003), simulates wheat growth, development and yield formation on a daily time step in response to climatic and soil conditions and management interventions. It has been tested extensively in Australia (Wang *et al.*, 2003; Hochman, Holzworth and Hunt, 2009) and China (Chen, Wang and Yu, 2010), and has shown good performance in predicting wheat yield and water use under different rainfall conditions and irrigation levels.

The Murray Darling Basin (MDB) in Australia and the North China Plain (NCP) were used as study areas to sample the climate impact on WUE. The Agricultural Production Systems Simulator was run with a commonly used wheat cultivar "Janz" in MDB and "Zhixuan 1" in NCP to grow on a representative soil with PAWC (to 150 cm depth) of 233 mm in MDB and 350 mm in NCP, respectively. Wheat was simulated in a single cropping system (sowing wheat every yr) in MDB for 101 years from 1891 to 2002, while in a double cropping system (wheat–maize) in NCP for 50 years from 1961 to 2010. Inter annual rainfall variability is high in both MDB and NCP. Average annual rainfall in MDB decreased from >1 000 mm in the east to <300 mm in the west areas, while in NCP it decreased from > 900 mm in the southeast to <500 mm in the northwest during the simulation periods. The simulation results enabled the whole season crop transpiration efficiency (ε) to be defined. For each site, it was calculated as the slope of regression line between the grain yield (*Y*) and the growing season transpiration (*T*) from the simulation results, representing average transpiration efficiency over the yr of simulation.

In MDB, wheat is normally grown in dryland conditions, and the yield directly responds to rainfall. The impact of different soils (PAWC range of 70–260 mm) on wheat yield was investigated at a selected site Young (wheat season rainfall 406 mm). In NCP, on the other hand, most wheat is grown under irrigation. The wheat yield response to different levels of irrigation water supply was also studied (range of 60–420 mm of irrigation) at Luancheng (wheat season rainfall 146 mm).

RESULTS AND DISCUSSION

The whole season crop transpiration efficiency (ε) is the grain yield produced per mm of water transpired by the wheat crop. It represents the maximum value of crop WUE under the current climate if all water (*P* + *I*) can be used by plant via transpiration. It can be used as a benchmark of crop WUE across the study regions. In both MDB and NCP, it had a similar range of 15–33 kg·grain·ha^{-1}·mm^{-1} (Figure 1), and decreased from southeast to northwest regions, roughly following the spatial distribution pattern of wheat season rainfall.

The spatial pattern of the whole season transpiration efficiency clearly shows the climate regulation of WUE. This pattern indicates that much more water is required in drier areas to produce per kg of wheat grain. In the humid area of both MDB and NCP (ε = 30 kg grain·ha^{-1}·mm^{-1}), to produce a wheat grain yield of 5 Mg/ha, 167 mm of water needs to be transpired by the crop. In the driest area (ε = 15 kg·grain·ha^{-1}·mm^{-1}), twice as much water (333 mm) would be needed to produce the same wheat grain yield.

The role of soil in regulating WUE is illustrated by the simulation results with different soils at Young in MDB. Under the same climate and wheat season rainfall (average of 406 mm), soils with higher

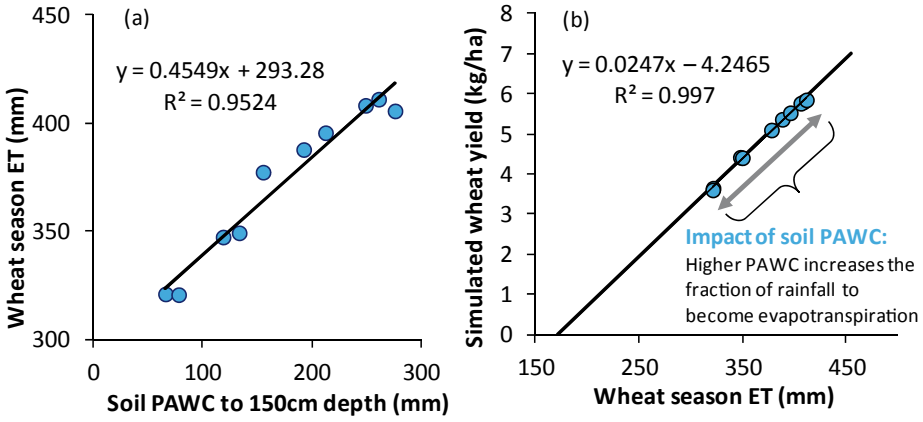


FIGURE 2. Impact of PAWC of soils on wheat growing season evapotranspiration (ET) (a) and the relationship between dryland wheat yield and wheat season ET (b) at Young, NSW, Australia. Dots are the average values calculated based on 101 yr of simulation results. Lines are regression lines. In (b) the slope of the regression line is the whole season transpiration efficiency of wheat.

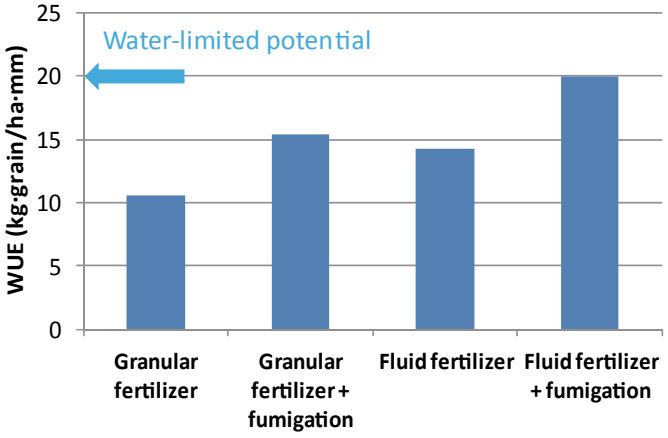


FIGURE 3. Impact of application of granular fertilizer, liquid fertilizer and fumigation on wheat WUE. Results are from an experiment conducted at Eyre Peninsula South Australia.

PAWC hold more water from the variable rainfall for a longer time to allow crop water uptake, leading to increased crop season evapotranspiration (*ET*) and subsequently higher wheat grain yield (Figure 2). Increase of soil PAWC from 80 mm to 260 mm (representing a change from a shallow soil to a deep soil) would result in a 28 percent increase in wheat season *ET* (Figure 2a), corresponding to a 62 percent increase in wheat grain yield and whole season wheat WUE (Figure 2b).

Changes in soil PAWC did not change the crop transpiration efficiency (ε = the slope of the line in Figure 2b). Rather, it increased crop season *ET* and subsequently crop transpiration (*T*). As a result, both *f*(*T*) and *f*(*ET*) in equation (3) were increased.

Once a benchmark WUE is defined based on the local climatic and soil conditions, it can be used to identify other constraints to crop yield and to explore management options that can reduce or eliminate these constraints to increase crop yield and WUE. Figure 3 shows an example where the benchmark WUE was 20 kg·gain·ha^{-1}·mm^{-1}, but both nutrient supply and soil disease limit crop yield. Use of liquid fertilizers plus fumigation of the soil enabled the WUE to reach the benchmark value.

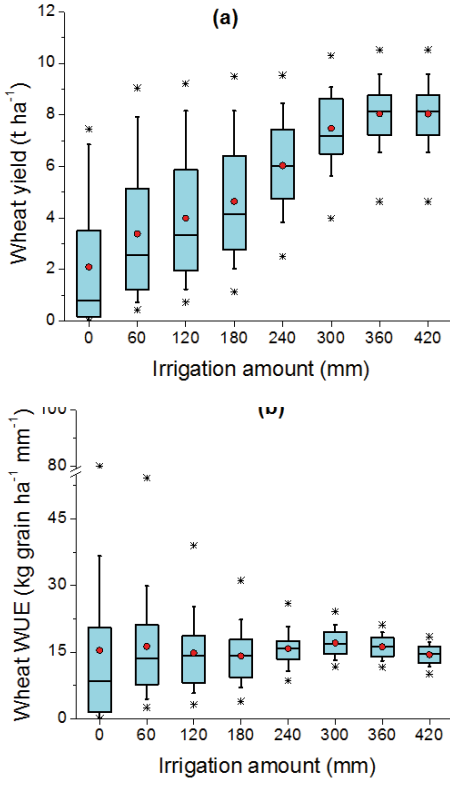


FIGURE 4. Impact of irrigation on simulated wheat yield (a) and wheat whole season WUE (b) at Luancheng in NCP. In (a) the box plots show the 0, 10, 25, 50, 75, 90 and 100 percentiles. The circles in the box plots show the average.

Figure 4 shows the change in simulated wheat yield in response to irrigation water supply at Luancheng in NCP. Inter-annual climate (rainfall) variability had a significant impact on wheat yield as shown by the ranges of the box plots (Figure 4a). Increasing irrigation resulted in both increased average wheat yield and in reduced yield variability between the yr. The results clearly show that up to 360 mm of irrigation water would be needed to achieve the maximum wheat yield (Figure 4a). However, WUE would decrease if irrigation

water supply exceeded 300 mm (Figure 4b). The results in Figure 4 enable optimization of irrigation scheduling depending on whether to achieve maximum yield or maximum WUE, or maximum profit if grain and water prices were available.

Evaporation loss from soil was simulated to be a major loss that reduced the wheat WUE. The fraction of evaporation in total crop evapotranspiration increased from around 30 percent in high rainfall areas to 70–80 percent in low rainfall areas under dryland conditions in both MDB and NCP. Therefore, preventing evaporation loss through mulching or other measures remains an effective means to increase WUE; the drier the area is, the more effective is reducing evaporation to increase grain yield. Using isotopes of hydrogen-2 (^{2}H) and oxygen-18 (^{18}O) to partition evapotranspiration into evaporation and transpiration can help to quantify what is achievable under different cropping systems across different climatic regions.

CONCLUSIONS

In semi-arid and arid areas, crop yield is directly proportional to the amount of water transpired by the crop. The crop transpiration efficiency is regulated by climate through the CO_2 concentration in the air and air dryness (vapour pressure deficit). Soil regulates crop WUE through its capacity to store rainfall (and irrigation) for use by crop and through its nutrient levels to support crop growth. Crop modelling provides an effective means to benchmark crop WUE under various climatic and soil conditions. Comparison of benchmarks with what is observed in the field enables the identification of WUE and yield gaps, and management options to increase both yield and WUE. While water loss from evaporation from soil is a major factor reducing crop WUE, improvement in technologies to quantify crop transpiration and soil evaporation, and measures to reduce evaporation from soil can help to identify approaches to increase crop WUE.

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Environmental Sustainability of Vegetable Production above a Shallow Aquifer

V. Zupanc[1,*], M. Šturm[2], S. Lojen[2], N. Maršić-Kacjan[1], J. Adu-Gyamfi[3], B. Bračič-Železnik[4], J. Urbanc[5] and M. Pintar[1]

ABSTRACT

Irrigation and fertilization techniques were evaluated over a two-year study period in terms of yield and environmental sustainability at a benchmark site near water protection zones above a shallow aquifer. For vegetables with a short growing period, three irrigation and fertilization treatments were applied in 2006 and 2007: (i) fertilization and 100 percent drip irrigation (fertigation), (ii) the farmer's practice (sprinkler irrigation) and fertilization, and (iii) control (the farmer's practice of irrigation, no fertilization). Equivalents of 80 and 200 kg/ha of nitrogen (N), 50 and 80 kg/ha of phosphorus (P), and 120 and 300 kg/ha of potassium (K) were added for endive and cabbage, respectively. Nitrogen-15 labelled fertilizer was used as a tracer. Results showed that environmentally sustainable practices (split application of nutrients compared with broadcast incorporating fertilization) in a humid climate can and should be a practice of choice on soils in water protection zones. The findings showed that fertigation is an effective way of minimizing nitrate leaching, and should be considered for vegetable production in or close to groundwater protection zones.

Key words: *irrigation, nitrate leaching, environmental sustainability, fertigation.*

INTRODUCTION

With impending climate change, agriculture will face extremely variable climatic conditions — periods of exceptionally high air temperatures and drought followed by periods of extremely high precipitations. Water deficit impedes nutrient uptake (Pandey, Maranville and Chetima, 2000), and unused fertilizer moves with deep percolation toward aquifers. Under such climatic conditions shallow aquifers are vulnerable to nitrate pollution (Almasri and Kaluarachchi, 2005; Burkart and Stoner, 2008), due to the proximity of the groundwater table, high nitrogen (N) fertilizer inputs in intensive vegetable production areas as well as inputs from urban and industrial pollution (Egboka, 1984). In order to preserve groundwater as a drinking water source, legislation requires strict measures on agriculture, severely limiting or banning fertilization of agricultural land on water protection zones. Effective irrigation, temporally and spatially adjusted to plant demands, decreases nitrate leaching to groundwater (Zupanc *et al*, 2011) and could enable agricultural production above water protection zones.

In an experiment conducted in the vicinity of a water protection zone in Slovenia, different irrigation and fertilization techniques for production of vegetables with a shorter growing period were tested under controlled conditions in a humid climate.

MATERIALS AND METHODS

On the alluvial plains of Ljubljansko polje, Slovenia (46°5′ N, 14°36′ E), three irrigation and fertilization treatments were applied to vegetables with a short growing period: (i) fertilization and 100 percent drip irrigation (fertigation), (ii) the farmer's practice of irrigation (sprinkler irrigation using water stored in plastic tanks) and fertilization (broadcast), and (iii) control (the farmer's practice of irrigation but no fertilization). The equivalents of 80 and 200 kg/ha of nitrogen (N), 50 and 80 kg/ha of phosphorus (P) and 120 and 300 kg/ha of potassium (K) were applied respectively to endive *(Cichorium endivia L.)* in 2006 and to cabbage *(Brassica oleracea* var. *capitata*) in 2007. Nitrogen-15 (^{15}N) labelled fertilizer as KNO_3 was used as a tracer. Fertilized plots (6.5 m^2) were divided into three sub-plots (2.6 m^2), and the labelled fertilizer was applied in the middle of the sub-plot (Šturm *et al.*, 2010). The labelled KNO_3 and the unlabelled water soluble $Ca(NO_3)_2$ were dissolved in tap water, then applied as a solution with a final relative ^{15}N concentration of 3.52 ± 0.04 atom percent. For the plots with the farmer's practice of fertilization, unlabelled $Ca(NO_3)_2$ (0.365 at. percent ^{15}N) was broadcast, followed by application of the labelled fertilizer as a solution. It was assumed that $Ca(NO_3)_2$, which was applied as a broadcast application the day before transplanting, was dissolved after the irrigation within a few hours and mixed with the labelled fertilizer in the soil (Zupanc *et al.*, 2011).

The irrigation regime was adjusted to the actual weather conditions in the field. Due to the wet weather in August and September 2006, the N fertilizer was applied to endive only twice, once before planting and once as a solution with 20 mm of water 23 d after transplanting. Fertilizer for cabbage was applied four times for the fertigation treatment: once before planting, and three times during the growing period via solution at 57, 66, and 75 d after transplanting (Šturm *et al.*, 2010). For the farmers' practice, each crop was irrigated with 50 mm of water one d before and 50 mm of water one d after transplanting using a tank sprinkler.

Soil was a gleyic Fluvisol and endogleyic Fluvisol, with a loam and sandy loam texture (Table 1), and a layer > 80 percent of sand

1 University of Ljubljana, Biotechnical Faculty, Agronomy Department, Ljubljana, Slovenia
2 Jožef Stefan Institute, Department of Environmental Sciences, Ljubljana, Slovenia
3 Soil and Water Management & Crop Nutrition Laboratory, International Atomic Energy Agency, Vienna, Austria
4 Drinking Water and Sewerage System Public Utility, Ljubljana, Slovenia
5 Geological Survey of Slovenia, Ljubljana, Slovenia
* E-mail address of corresponding author: vesna.zupanc@bf.uni-lj.si

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TABLE 1. Soil layer depth (cm), total N (%), soil texture and bulk density (g/cm^3) on the experimental site above a shallow aquifer in Slovenia

Depth (cm)	N total (%)	Sand (%)	Silt (%)	Clay (%)	Bulk density (g/cm)
0–20	0.12–0.18	37.7–39.1	47.9–48.8	13.0–13.8	1.34
20–35	0.05–0.12	40.2–56.9	33.3–36.2	9.8–11.2	1.42
35–60	—	83.6–87.4	9.1–13.2	2.6–3.2	1.38
60–100	—	57.2–71.4	23.0–36.2	3.5–8.6	1.27

appearing at the depth of 30 cm. Physical and chemical properties of the soil are presented in Table 1.

Soil water balance was calculated from continuous soil water measurements at different depths (10, 30, 50, 70 and 100 cm) using a time domain reflectometer (TDR) (Trase®, Soil Moisture, USA). Soil water storage *W* (mm) was calculated from soil water content measurements (volumetric water content) over the total soil depth.

Soil water was sampled below the vegetable root zone with soil moisture suction cups at a depth of 50 cm (at a suction of 33 kPa). Samples were analysed for nitrate N concentration and soluble and total N using standard methods (ISO/DIS 14255 and ISO 13878). The nitrate concentration results are expressed in mg/L N. For N isotope analysis nitrate was isolated from the soil water as ammonium sulphate using the micro diffusion method (Brooks and Stark, 1989). The isotopic composition of the samples was determined using a continuous flow isotope ratio mass spectrometer with an ANCA-SL preparation module (PDZ Europa Ltd., UK.). Nitrogen-15:nitrogen-14 ratios are reported in atom percent (atom %) ^{15}N excess, which is the value obtained by subtracting the ^{15}N concentration of the reference material — air N_2 (0.3663 atom % ^{15}N) from the measured sample concentration as follows (Kendall, 1998):

$$^{15}\text{N excess} = \text{measured } ^{15}\text{N concentration value–natural abundance [atom \%]} \quad (1)$$

The analytical precision of the isotopic measurements depends on the level of enrichment and was estimated to be ±0.002 atom %^{15}N for the samples below one atom %^{15}N and ±0.004 atom %^{15}N for samples above one atom %^{15}N, based on replicate measurements of the reference materials and samples. The following reference materials were used: IAEA 305-B (0.0503 atom %^{15}N), IAEA PLANT RM (1.187 atom %^{15}N) and IAEA-311 (2.05 atom %^{15}N). The N content of soil water was calculated from *W* (mm) and the nitrate concentration measured in the suction cups (mg/L), converted to kg/ha. Nitrate N losses were calculated from average nitrate concentration in the suction cups between two samplings and the amount of deep percolation (mm) between two samplings, converted to kg/ha, then summed over the growing period of the individual crop (Zupanc *et al.*, 2011).

For the final N uptake, plants were sampled at the end of each growing period for determination of dry matter yield and plant N uptake (i.e. N yield), calculated using the following equations (IAEA, Training Course Series No.14, 2001):

$$\text{Dry matter yield (kg/ha)} = \text{plant fresh mass(kg)} \times \frac{10\,000\,(\text{m}^2/\text{ha})}{\text{area harvested}\,(\text{m}^2)} \times \frac{\text{plant dry mass(kg)}}{\text{plant fresh mass(kg)}} \quad (2)$$

$$\text{N yield (kg/ha)} = \text{dry matter yield (kg/ha)} \times \frac{\%\,\text{N}}{100} \quad (3)$$

Total N yield (kg/ha) was calculated by multiplying the dry matter yield of plant parts and their mean N concentration.

Nitrogen balance was calculated for the growing period of individual crops as the difference between N input and crop uptake on one side, and the difference in the N content of the soil water and the N loss on the other side. Nitrogen input (kg/ha) was the total N fertilizer added on the soil surface with either fertigation or broadcast application. Difference in the N content in soil water was calculated as the difference between nitrate concentration at beginning and at the end of the crop growing period.

Statistical analysis was conducted for a completely randomized design with three replications. Differences in nitrate concentration in soil water and in ^{15}N atom % excess were evaluated using repeated measure ANOVA for the dates when data for all treatments were available for a sampling event. Main effects were considered significant at $p \leq 0.05$. All statistical analyses were performed using SPSS 17.0 (SPSS, Inc., Chicago, USA).

RESULTS AND DISCUSSION

Due to the wet conditions in 2006, improved practices could not be tested. Nevertheless, there were large differences between the three treatments in terms of N losses through leaching, which were highest for the farmer's practice (Table 2) where as much as 160 kg N/ha was lost compared with only 36 kg N/ha for fertigation treatment. Relatively high N losses through leaching also occurred in the control treatment (116 kg N/ha). The high leaching was due to the high rainfall coupled with the large amount of irrigation being applied to both the farmer's practice and control treatments, as well to the high nitrate concentration in the suction cups (69, 50, and 23 mg/L for the farmer's practice, control and fertigation treatments, respectively). During the cabbage growing period nitrate N losses were negligible for all treatments as there was no deep drainage due to insufficient irrigation being applied (Table 2).

There were no statistical differences in N uptake between treatments for endive, an average of 65 kg N/ha being taken up by the crop (Table 2). On the other hand, N uptake for cabbage was statistically higher for the farmer's practice (246 kg/ha), extracting from the soil more N than was added by the farmer (Table 2). During the endive growing period, N balance was negative for all treatments as a consequence of the extremely unfavourable wet conditions and heavy deep drainage. For cabbage, N balance was negative for the farmer's practice as well as for the control treatment.

The highest ^{15}N enrichments of nitrate in soil water as well as the highest variability in ^{15}N were determined under the farmer's fertilization practice plots (Figure 1) in the wet part of the year (end of September–December for both 2006 and 2007), confirming the highest leaching in that treatment.

Split fertilizer application and applying the right amount of water at the correct time leads to better nutrient uptake and minimizes nitrate leaching compared with broadcast fertilization. This finding is consistent with findings of Logan (1993) and D'Arcy and Frost (2001)

TABLE 2. Rainfall, irrigation, ET_{crop}, estimated nitrate N leaching losses, N input, crop N uptake (n = 9) and N balance for fertigation, farmer's practice and control treatments at Sneberje, Slovenia

Parameter§	Endive 2006[1]			Cabbage 2007[1]		
	Fertigation	Farmer's practice	Control	Fertigation	Farmer's practice	Control
Rainfall	355	355	355	184	184	184
Irrigation	20	100	100	110	100	100
ET_{crop}	220	214	214	377	350	350
Estimated nitrate-N leaching losses	36	160	116	0	0	0
N input	79	80	0	200	200	0
Crop N uptake[2]	63[a]	69[a]	65[a]	169[b]	246[a]	84[c]
N balance	–20	–149	–181	31	–46	–84

1 Growing period; Endive = 10.08.–26.10.2006; Cabbage, 11.04.–26.6.2007

2 Different superscript letters (a,b,c) denote a statistically significant difference among treatments at $p < 0.05$, n = 9

§ Units of rainfall, irrigation, ET_{crop} (mm); units of N (kg/ha)

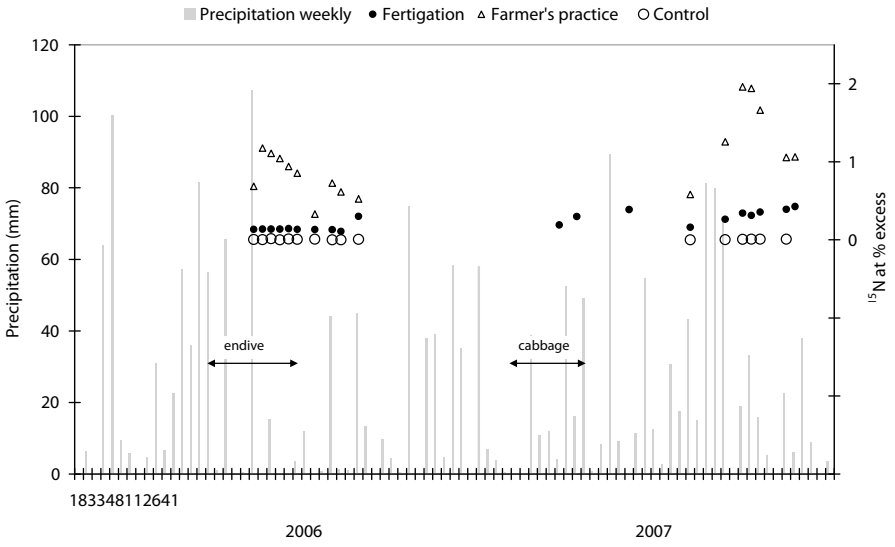


FIGURE 1. ^{15}N (atom % excess) in soil water collected with suction cups at 50 cm depth and weekly precipitation in Sneberje, Slovenia (2006–2007) for fertigation, farmer's practice and control treatments for endive (2006) and cabbage (2007).

and is the basis of 'best-management-practices' recommended to farmers in many countries (Morari, Lutago and Borin, 2004). Economic analysis combining agricultural and environmental measurements showed that the best management practice was not sufficient to satisfy the nitrate concentration constraint every year (Lacroix, Beaudoin and Makowski, 2005). Nevertheless, in water protection zones with severe restrictions on or prohibition of fertilizer applications, environmentally friendly techniques such as fertigation could be the solution for agricultural practices, enabling farmers to make profitable use of the land.

CONCLUSIONS

The results presented here provide guidelines for fertigation in the production of vegetables with a shorter growing period (i.e. lettuce and Brassicaceae), grown on areas where potentially high groundwater pollution is possible due to the soil texture and structure. With the help of nuclear techniques it was possible to identify environmentally more sustainable practices such as split application of nutrients compared with broadcast incorporating fertilization, which can and should be a practice of choice in water protection zones. Fertigation should therefore be considered as an environmentally friendly practice for vegetable production on or close to groundwater protection zones. Due to the anticipated higher energy and time inputs and lower yield outputs, environmentally friendly techniques should be supported by legislation, finance and education.

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Improving Irrigation Practice to Reduce Risk of Nitrogen Percolation into Deeper Aquifers in Vegetable Cultivation in Suburban Ha Noi, Viet Nam

D.N. Dang[1,*], N.H. Anh[2], T. T. Nguyen[3], A.M. Dang[3] and L.A. Ha[3]

ABSTRACT

This research demonstrates the advantages of drip irrigation with scheduling (DIS) over furrow irrigation (FI) for improving water use efficiency (WUE) and reducing the risk of potential nitrogen (N) contamination of groundwater in cabbage cultivation on alluvial soils of the Red River, North Viet Nam. Compared with FI, the DIS practice improved the irrigation WUE of the vegetable from 2.11 ± 0.35 to 5.23 ± 0.41 kg/m^3 during the autumn–winter (October–December), and from 2.15 ± 0.27 to 5.31 ± 0.35 kg/m^3 during the winter–spring (February–April) cropping seasons. Overall, DIS saved between 42 percent and 46 percent of water in comparison with FI during the autumn–winter and winter–spring seasons, respectively. It appears that with FI, ammonium (NH_4^+) percolates beyond the rooting depth of the crop, but in DIS it does not. Percolation could potentially cause groundwater contamination with NH_4^+. The delta nitrogen-15 ($\delta^{15}N$) values of ammonium in FI increased with increasing NH_4^+ concentration implying that there were at least two sources of N release, namely from inorganic fertilizer and from the manure applied. The $\delta^{15}N$ of the soil nitrate (NO_3^-) in FI was almost unchanged with increasing NO_3^- concentration, varying from 1‰ to 5‰, suggesting that NO_3^- was derived only from inorganic fertilizer. With DIS, soil NH_4^+ was found to be from the manure whereas NO_3^- was from inorganic fertilizers.

Key words: *cabbage, drip irrigation, scheduling, furrow irrigation, water use efficiency, nitrogen-15.*

INTRODUCTION

Vegetables are one of the most important foods of the Vietnamese people, particularly so in the city of Ha Noi where about 2600 tonnes (t) of various kinds of vegetable are consumed daily (DARD, 2011). Vegetables are currently produced on 3255 ha of land on the city's suburbs to supply 60 percent of the demand, the remaining comes from surrounding provinces. Among the 85 kinds of vegetables (spinach, cucumber, tomato, kohlrabi, beans, etc.), cabbage occupies around 20 percent of the production and trade on the market (DARD, 2011).

To have high levels of vegetable production, local farmers apply high rates of nitrogen (N), phosphorus (P), potassium (K) and urea fertilizers. For each cabbage season about 660 kg N–P–K and 300 kg urea are spread over one ha of land (DARD, 2009). Furrow irrigation (FI) is widely practised in Viet Nam for vegetable production and this is believed to be the cause of surface and groundwater quality deterioration. Surface and groundwater in the city of Ha Noi are currently polluted with N (Norrman *et al.*, 2008). These authors have implicated as the reasons the overuse of inorganic fertilizers in agriculture and direct discharge of water waste from the city without further treatment. Recently, an annual report to the National Assembly from the Ministry of Natural Resources and Environment (MONRE) determined that a combination of inappropriate irrigation practices and heavy abstraction of groundwater for both irrigation and supplying the population were the main reasons for the deterioration in water quality (MONRE, 2009).

Against this background, it should be noted that studies on water and fertilizer use efficiency (WUE and FUE) in agronomy, and particularly in vegetable production in the country are still very limited. The aim of this study was therefore to compare the advantages of drip irrigation with scheduling (DIS) over the traditional furrow irrigation (FI) practice in cabbage production in suburban Ha Noi city for improving WUE, i.e. improving the profitability of production while preventing N fertilizer residues from percolating into the deeper soil profile and risking groundwater contamination. The results could assist agricultural managers in Viet Nam in developing measures for proper fertilizer use and appropriate irrigation practices for maintaining high crop productivity and protecting the environment.

EXPERIMENTAL DESIGN

The experiment was conducted in Nam Hong village, Dong Anh – a northeast district of Ha Noi city where vegetable production to supply the capital is the only activity of all farmers. Cabbage, a broad-leaf vegetable that needs more water than any other crop was supplied by the Petoseed Company (USA), the preferred variety by farmers of the village. The seasons and years of planting were autumn–winter (September–December) and winter–spring (February–April) of 2006, 2007 and 2008. Cabbage was transplanted on the ancient alluvial soil of the Red River, North Viet Nam. The texture of the soil in the rooting zone was 13 percent sand, 52 percent silt and 35 percent

1 Vietnam Atomic Energy Institute, 59 Ly ThuongKiet, Ha Noi, Viet Nam
2 Ha Noi Institute for Vegetable and Fruits Researches, Gia Lam, HaNoi, Viet Nam
3 Institute for Nuclear Science and Technology, 179 Hoang Quoc Viet, Ha Noi, Viet Nam
* E-mail address of corresponding author: dangducnhan50@gmail.com

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clay. Before planting the soil was treated with organic manure (chicken muck), and with N–P–K and urea fertilizers. The rate of manure application was 10 t/ha, while the inorganic fertilizers were applied to the crop four times during each season with a total of 360 kg urea, 510 kg superphosphate, and 270 kg potassium sulphate as follow: 20 percent of the total amount before planting, a second application of 25 percent of the total amount at the rooting period, a third application of 33 percent of the total amount when the canopy occupied 10 percent of the land, and last application of 12 percent during head formation.

The land area for the experiment was 450 m^2 and was split into two plots each of 225 m^2. The first plot was set up for traditional FI and the second for advanced DIS. Soil moisture was monitored using a neutron probe (PB-205, Fieldtech, Japan).

Irrigation scheduling and calculations

The time for watering in the DIS plot was established based on the assumption that the crop needed to be watered if the refill point (RP) was down to half of the available soil water content (ASWC), i.e.

$$RP = 0.5 \times AWSC \quad (1)$$

and

$$ASWC = FC - WP \quad (2)$$

where *FC* — field capacity, and *WP* — wilting point.

For the alluvial soil of the Red River, the *FC* and the *WP* of cabbage were experimentally determined (this study) to be 25 percent and 9 percent, respectively. The *ASWC* of the soil should be 16 percent. The *FC* and *WP* for a crop (mm) were estimated as the product of its root zone depth (*RZD*) in mm and *FC* expressed in percentage units, i.e.

$$FC\ (\text{mm}) = RZD\ (\text{mm}) \times FC\ (\text{percent}) \quad (3)$$

$$WP\ (\text{mm}) = RZD\ (\text{mm}) \times WP\ (\text{percent}) \quad (4)$$

The *RZD* of cabbage was assigned a value of 450 mm as recommended by Simonne, Duke and Haman (2007). Since there is usually no rain over the Ha Noi city area between September and April, the amount of water needed in the DIS could be estimated as:

$$I = RP + ET_c \quad (5)$$

where ET_c — crop evapotranspiration, calculated as:

$$ET_c = K_c \times ET_o \quad (6)$$

The crop coefficient (K_c) in Equation 6 for cabbage was taken from MAFF (2001). According to this publication, a value of $Kc_{ini} = 0.7$ was assigned for the period from planting to the time when the crop canopy covered 10 percent of the land; the $Kc_{mid} = 1.05$ was assigned for the period when 10–80 percent of the land was covered by the crop canopy and the $Kc_{end} = 0.95$ was used for the time between when 80 percent of the land was covered by the canopy and harvesting. The ET_o of the field was estimated using the Penman-Monteith model (Allen, 2000). The meteorological parameters of the locality were measured using a mini-meteorological station (Pro Vantage 2, USA).

With FI, the soil moisture was maintained at between 23 and 24 percent (i.e. almost at the FC point) from the rooting time until harvest, as was the local farming practice. To compare the irrigation WUE of the vegetables under the DIS and FI, the total amount of irrigated water (I) in both practices along with the vegetable yield were recorded. The amount of irrigated water was measured using a water meter. Vegetable yield was determined through two measures, namely the total biological (Y_{bio}) and edible (Y_{ed}) yields. The former was the total weight of vegetable harvested and included the weight of both green leaves and the head. The latter was the weight of the head only. This provided two values for WUE. Irrigation WUE was estimated according to Howell (2001).

$$WUE_{Bio} = \frac{Y_{Bio}}{I}\ (\text{kg/m}^3) \quad (7)$$

and

$$WUE_{ed} = \frac{Y_{ed}}{I}\ (\text{kg/m}^3) \quad (8)$$

In this paper only the results for WUE_{ed} are presented.

Soil analyses

To elucidate the possibility of ammonium (NH_4^+) and nitrate (NO_3^-) percolating into deeper soil profiles, concentrations of these substances between the soil surface and a depth of 100 cm were determined each 10 cm before planting and after harvesting. The soil cores were taken representatively from both fields using a corer (Eijkelkamp, The Netherlands). Ammonium and NO_3^- in soil samples (5 g air-dried) were extracted three times at room temperature for four hours using 2 molar (M) potassium chloride (KCl). The soil:solution ratio was 1:5 (w:w). The extracts were combined, filtered through 0.45 mm Millipore filter and then analysed using appropriate techniques (Bremner and Mulvaney, 1982). Ion-chromatography (Dionex 600, USA) was used to determine $NH_4^+$and NO_3^- .

Delta nitrogen-15 determination

The sources of N released in the soil were identified based on the $d^{15}N$ values in the NH_4^+ and NO_3^- present in the soil samples (Mayer *et al*, 2002; Jin *et al*., 2004). A diffusion technique was used to trap NH_4^+ in the KCl extracts. To do this, a piece of Whatman filter (f10 mm) impregnated with potassium bisulphate ($KHSO_4$) (trap) and covered tightly on both sides with a thin Teflon film was allowed to float on the surface of the solution-extract. The extract was then alkalified with NaOH to pH 12 and the flask containing extract with the trap was immediately capped tightly and left for a week with occasional shaking. In this way, evolved NH_3 diffuses through the Teflon film and reacts quantitatively with the salt to form potassium ammonium sulphate [$K(NH_4)SO_4$]. This double salt decomposes with copper oxide (CuO) in the elemental analyser to convert NH_4^+into N_2 gas before entering the ion source of an isotope ratio mass spectrometer for the ^{15}N determination.

Nitrate in the extract was first reduced to NH_4^+ by the Devada reductive reagent magnesium oxide (MgO) under acidic conditions and an NH_4^+ trapping procedure was followed similar to that described above. The apparatus and procedure for the diffusion method used for $\delta^{15}N$ determinations were as described by Liu and Mulvaney (1992). The delta ($\delta^{15}N$) is expressed as:

$$\delta^{15}N = \left(\frac{R_{^{15}N,sample}}{R_{^{15}N,std}} - 1\right) \times 1000 \quad (9)$$

where — abundance of ^{15}N isotope in the sample to be analysed, and — abundance of ^{15}N isotope in the standard used for determination of $\delta^{15}N$. Atmospheric N was used as the standard for the $\delta^{15}N$ determination.

The $\delta^{15}N$ values of soil NH_4^+ and NO_3^- samples were determined using an isotope ratio mass spectrometer (IR MS, Micro mass, UK) with an uncertainty ($1\sigma \leq \pm 0.2‰$).

Table 1. Influence of irrigation practices on development parameters of cabbage

Irrigation practice	Growing span from planting to (days)			
	Rooting	Canopy covered 10% land	Head formation	Harvest
FI	10±2	41±3	50±2	92±3
DIS	10±2	40±3	51±3	92±3
F test (n=15)	NS*	NS	NS	NS

NS: no significant difference at p<0.01

Table 2. Influence of irrigation practices on biological parameters of cabbage

Irrigation practice	H_{pl} (cm)	D_c (cm)	No. green leaves	No. leaves in a head	H_h (cm)	D_h (cm)
DIS	30.2±5.7^b	66.2±3.8^b	37.0±4.6^a	37.2±3.5^a	13.8±2.1^a	22.0±3.6^a
FI	31.1±3.6^a	61.1±6.2^c	36.5±3.8^a	25.0±4.6^b	13.1±2.7^b	21.8±3.5^a
F test (n=15)	23.4**	59.18**	NS	170.8**	4.76*	NS

Notes: H_{pl} — plant height; D_c — canopy diameter; H_h — head height; and D_h — the head diameter. Figures marked with the same letter for the DIS and FI practices imply no significant difference (ns) by F-test, but those with different letters imply that there is a difference between the two figures at significance levels of 5 percent and 1 percent respectively, with one and two asterisks.

TABLE 3. Influence of irrigation practices on the productivity and WUE_{ed} of cabbage

Treatment	A–W season (2006–2008)				W–S season (2006–2008)			
	Density (plants/m^2)	Y_{ed} (t/ha)	Irrigated water, I (m^3/ha)	WUE_{ed} (kg/m^3)	Density (plants/m)	Y_{ed} (t/ha)	Irrigated water, I (m^3/ha)	WUE_{ed} (kg/m^3)
Furrow irrigation	2.6±0.4	31. 7±0.5	12.7×10^3	2.11±0.35	2.7±0.3	25.8±0.7	12.5x10^3	2.15±0.27
Drip irrigation	2.5±0.4	39.2 ±0.3	7.5×10^3	5.23±0.41	2.5±0.4	36.1±0.3	6.8×10^3	5.31±0.35

RESULTS AND DISCUSSION

Influence of irrigation practices on the development of cabbage

Tables 1 and 2 show the influence of the DIS and traditional FI practices on the development and biological parameters of cabbage (*Petoseed* var.). Results are the mean values and standard deviations for each parameter obtained for five harvesting seasons: three for autumn–winter (A–W, October–December) and two for winter–spring (W–S, January–March) during the period 2006–2008.

As seen from Table 1, DIS did not affect the growing span of the vegetable, the time span from planting to harvesting of the crop being almost the same in the both DIS and FI and the crop could be harvested after 90–95 d. However, DIS did impact on the major biological parameters of cabbage (Table 2). Usually, farmers believe that a high soil moisture level makes nutrients more available to crops, and therefore that furrow irrigation should be a better practice to supply more nutrients to crops leading to higher productivity. In fact, as shown (Table 2), high moisture (23–24 percent in the FI) reduced the number of leaves in the head (edible leaves) although the head diameter was the same for the crop under both irrigation practices. This means that under DIS the leaves in the cabbage head were more compactly arranged than under FI, and as a consequence the productivity of the vegetable under DIS would be higher than under FI.

Influence of irrigation practices on the productivity of cabbage

Table 3 shows the influence of the different irrigation practices on the productivity of cabbage produced in the A–W and W–S seasons along with WUE_{ed} under the two irrigation practices.

Although the density of plants was the same with both irrigation practices (2.5 and 2.6 plants/m^2), the edible yield of the vegetable under DIS was 24 and 40 percent higher than that under the traditional FI for A–W and W–S seasons. At the same time, the DIS practice led to 41 and 46 percent saving water used for irrigation during A–W and W–S seasons (Table 3). The amount of irrigated water used in the W–S season was somewhat less than in the A–W season because sometimes during the W–S season drizzle made the soil moist and irrigation was managed infrequently. WUE_{ed} was estimated based on the Y_{ed} and I (Table 3). For the A–W season, the WUE_{ed} of cabbage improved from 2.11 kg/m^3 under FI to 5.23 kg/m^3 under the DIS, in the W–S season the corresponding figures were 2.15 kg/m^3 and 5.31 kg/m^3. These differences probably arose because the number of edible leaves in the head under DIS was almost 50 percent more than under the FI practice (Table 2). The lower productivity of vegetable under FI could also be explained by the fact that the soil under FI became too wet since soil moisture levels were almost at an FC of 25 percent, thereby preventing the cabbage to develop its secondary roots for more effective absorption of water and nutrition. Also, in some cases under wet conditions a number of diseases may occur within the root system like *Phytophthora* root rot of chile pepper grown under FI (Xie *et al.,* 1999).

From these results, one can estimate the amount of water that could be saved in cabbage production on 20 percent of the 3255 ha used for cultivation in the city of Ha Noi if DIS technology were applied. The figure was around 3.55 x 10^6 m^3 each cropping season. Additionally, using DIS technology local famers could gain more profit from cabbage production because of extra yields as much as 4 900 and 6 700 t, worth US$1.2 and US$1.6 million respectively for the A–W and W–S seasons, assuming the market price was US cents 0.25/kg.

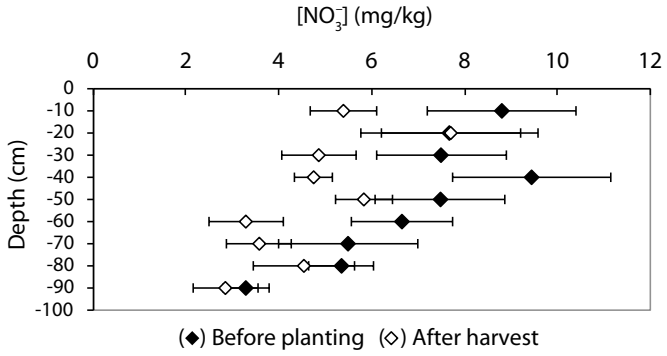


FIGURE 1. Variation of nitrate concentration (±1σ) along the soil profile with FI of cabbage.

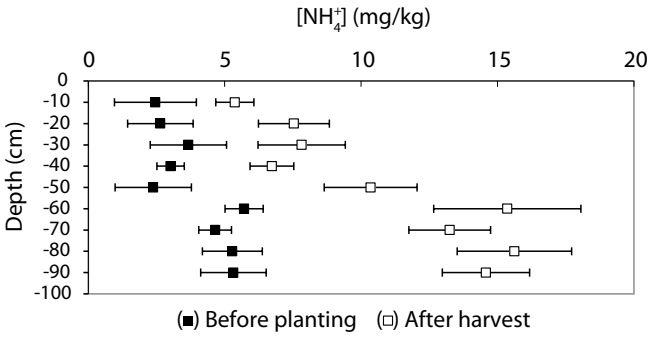


FIGURE 2. Variation of ammonium concentration (±1σ) along the soil profile with FI of cabbage.

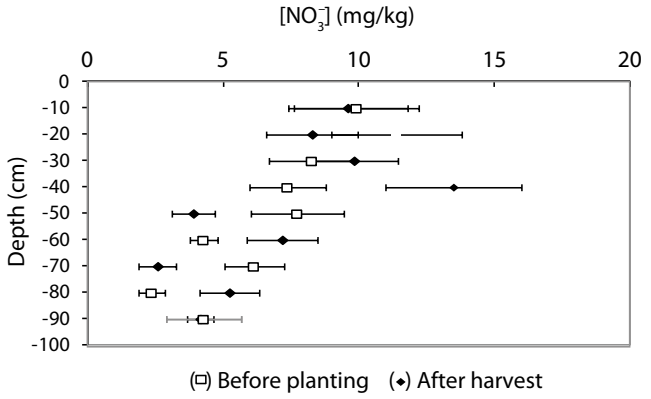


FIGURE 3. Variation of nitrate concentration (±1σ) along soil profile with DIS of cabbage.

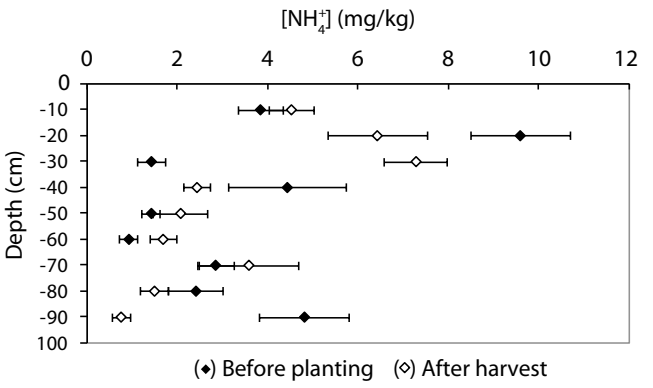


FIGURE 4. Variation of ammonium concentration (±1σ) along soil profile with DIS of cabbage.

Percolation of nitrogen fertilizer beyond crop rooting depth under different irrigation practices

Figures 1 and 2 depict the variation in NO_3^- and NH_4^+ concentrations along the soil profile with FI, while Figures 3 and 4 illustrate the variations with DIS. The data covered all five harvests between 2006 and 2008.

Figures 1 and 3 indicate that NO_3^- did not percolate into the deeper soil layers under either irrigation practice, with concentrations ranging from 2 to10 mg/kg soil. The highest NO_3^- concentration was found in the surface soil, but levels decreased with the soil depth. In contrast to NO_3^-, NH_4^+ under the FI seemed to percolate into the deeper soil profile (Figure 2), but not to the same extent in the DIS (Figure 3). Under FI, at a depth of 80 cm from the surface, the soil NH_4^+ concentration before planting was found to be 5.0 ± 1.0 mg/kg and it increased to 15.7 ± 1.0 mg/kg after harvest, almost three times higher than the NH_4^+ concentration in the surface soil. These findings suggest that the environment in the soil under the two irrigation practices as well as the sources of NO_3^- and NH_4^+ in the soil were different. Under FI the soil environment probably was reductive as the moisture level was high at all times (i.e. around an FC of 25 percent), which is not ideal for enabling air to diffuse into the soil and NH_4^+ to be oxidized. Under DIS, the environment in the soil was most probably oxidative since the soil moisture was low (10–15 percent), facilitating air diffusion and thereby oxidation of NH_4^+ in the rooting zone. The balance between soil and vegetable N (results not show here) and the total amount of N-nutrient applied initially to the crop showed that most N was volatilized, making the fertilizer use efficiency of the crop very low (between 20–30 percent only).

Sources of N-contaminant in soil under different irrigation practices

Figures 5 and 6 depict the relationship between ^{15}N composition ($\delta^{15}N$, ‰) and NO_3^- and NH_4^+ concentrations in soil under the FI and DIS practices, respectively. As seen from Figure 5 the composition of ^{15}N in soil NO_3^- under FI ranged from 3 to 7‰ and there was no clear trend for the relationship between $\delta^{15}N$ (nitrate) and concentration. This implies that the soil NO_3^- originated from the inorganic fertilizer applied because both the urea and the N in the N-P-K were synthesized from the air (Mayer *et al.*, 2002). In contrast to NO_3^-, the ^{15}N composition in NH_4^+ increased with its increasing concentration of NH_4^+ in soil (Figure 5). This suggests that NH_4^+ in the soil was from at least two different sources, probably from the organic manure and inorganic fertilizer (Mayer *et al.,* 2002). Assuming that soil NH_4^+ concentrations under FI followed an additive model, the contribution of each individual source was estimated to be 50 percent at an NH_4^+ concentration of 15 mg/kg and an assumed ^{15}N composition in inorganic fertilizer and manure to be respectively 5‰ and 25‰ (Mayer *et al.,* 2002).

Under DIS, the ^{15}N composition in NH_4^+ declined with increasing soil NH_4^+ concentration (Figure 6). In this case, it appears that the manure applied was the major source of soil NH_4^+ as ammonification of organic matter leads to depletion of the heavy isotope of N (Mayer *et al.,* 2002). However, the ^{15}N composition in NO_3^- under DIS did not vary with concentration, and was within 5‰–7‰ indicating that the soil NO_3^- under DIS was from the inorganic fertilizer applied, like that observed under FI.

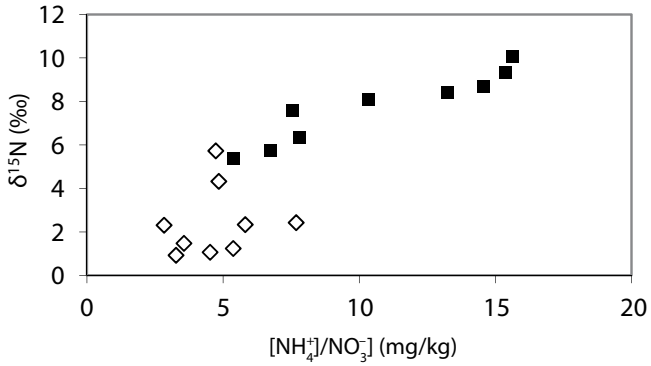


FIGURE 5. Relationship between ^{15}N composition and nitrate (◊) and ammonium (■) concentrations in soil under FI of cabbage.

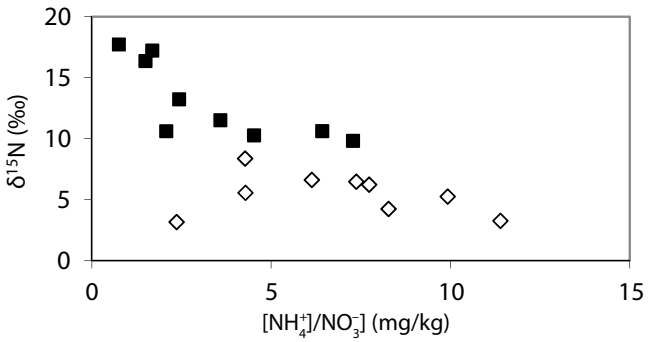


FIGURE 6. Relationship between ^{15}N composition and nitrate (◊) and ammonium (■) concentrations under DIS of cabbage.

CONCLUSIONS

Drip irrigation with scheduling improved the WUE of cabbage produced on the ancient alluvial soil of the Red River in suburban Ha Noi city, Viet Nam by up to 150 percent compared with the traditional FI practice in both autumn–winter and winter–summer seasons. Additionally, local farmers could gain an extra-profit by applying the former irrigation practice $US2.9 million per year from cabbage production on 651 ha of their land. Drip irrigation reduced the risk of groundwater contamination by N originating from fertilizers. The soil nitrate under both irrigation practices was from inorganic fertilizers (N-P-K, and urea), while ammonium under FI was from both inorganic and organic fertilizers; however, under the DIS it originated mainly from organic manure.

Proper use of N fertilizers and management of irrigation water in agriculture should be given particular attention in Viet Nam to maintain highly productive crops and to minimize the risk of water resources deterioration because of agrochemical residues percolating into groundwater.

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Improving Yield and Water Use Efficiency of Wheat (*Triticum aestivum* L.) by Regulating Plant-Available Water during Crop Development under Semi-arid Conditions in Pakistan

K. Mahmood[1,*], W. Ishaque[1] and L.K. Heng[2]

ABSTRACT

Scarcity of irrigation water in Pakistan warrants adoption of appropriate practices for maximizing crop water use efficiency that at present is far lower compared with other countries. The objective of this research was to enhance water use efficiency (WUE) and optimize irrigation scheduling for optimal wheat yield under water scarce conditions. Field experiments with different irrigation regimes: rain-fed, optimal irrigation and regulated deficit irrigation at different growth stages were conducted on a deep loam soil for four crop seasons (2008–2012). The results showed that regulated deficit irrigation at less sensitive crop stage(s) could allow up to 25 percent water saving without compromising economic yield. AquaCrop simulations were quite reliable for predictions of crop development (canopy cover and in-season biomass), crop yield, soil water dynamics and thus water productivity under different irrigation regimes. Since water saving through deficit irrigation would allow irrigation of additional crop area, the AquaCrop model can be a useful tool for assessing crop requirements and devising irrigation strategies to enhance water productivity under different scenarios.

Key words: *regulated deficit irrigation, wheat, water use efficiency, AquaCrop model, crop development and yield, soil water.*

INTRODUCTION

Pakistan has an arid/semi-arid climate in most of its area where annual pan evaporation (1 600 mm) exceeds rainfall by four to five-fold. Consequently, crop production relies heavily on irrigation, about 60 percent of which is contributed from the Indus river system (Iqbal and Munir, 2006). The main source of irrigation water from canals is insufficient to meet crop requirements particularly during the winter season. This water deficiency is met mostly with poor quality groundwater leading to alarming depletion of aquifers and also soil degradation due salinization of productive land. Hence, better irrigation water management is of prime importance to increase crop water use efficiency, enhance yield, reduce soil degradation and ensure food security under changing climate scenarios.

The limited scope for expansion of the cultivated area for crop production and water scarcity necessitate practical measures to maximize crop water use efficiency (WUE) while minimizing any adverse impact on yields, soil and environment. Higher WUE in agriculture can be achieved by maximizing crop water productivity using efficient irrigation techniques such as drip and deficit irrigation. The important practice of deficit irrigation to achieve higher water productivity and reducing irrigation water use has not received sufficient attention in research (Fereres and Soriano, 2007). Geerts and Raes (2009) reviewed selected research from around the world covering a range of crops and summarized the advantages and disadvantages of deficit irrigation. With this practice, reductions in the quantity and timing of irrigation may save water without reducing significantly the quantity or quality of the crop yield. Applying deficit irrigation and other irrigation management technologies based on quantitative estimation of irrigation demand has been demonstrated successfully and is now widely used in Andalucia, Spain (Fereres, Orgaz and Gonzalez-Dugo, 2011).

Deficit irrigation requires accurate estimation of potential crop evapotranspiration (ETc), which is difficult to compute accurately without computer models which can predict crop growth and evapotranspiration partitioning into soil evaporation and transpiration components (Gallardo *et al*., 1996). The recently developed FAO AquaCrop model has the ability to separate deep percolation, evaporation and transpiration components of the total water budget during a crop growing season. This model simulates crop yields as a function of water consumption under stored soil water/rain-fed, deficit, and full irrigation conditions (Steduto *et al*., 2009a; Farahani, Izzi and Oweis, 2009) and is an important tool for estimating crop water productivity under different irrigation management strategies for improving WUE in agriculture (Heng *et al*., 2009; Steduto *et al*., 2009b).

Very little research on deficit irrigation has been carried out in Pakistan. The purpose of this study was therefore to evaluate irrigation techniques on wheat yield and WUE and to devise strategies for water saving without compromising crop productivity using simulation modelling approach.

MATERIALS AND METHODS

Study area

This research was conducted at the experimental farm of the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan (31°23' N, 73° 2' E, 184 m asl). The climate of the area is

1 Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan
2 International Atomic Energy Agency (IAEA), Vienna, Austria
* E-mail address of corresponding author: kmahmoodniab@yahoo.com

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semi-arid, with average annual rainfall of about 350 mm, of which approximately 80 percent occurs during the monsoon season from July to September. The area also receives winter showers of less intensity from December to February during the wheat growing season (November–April). Mean daily minimum temperatures range from 15 to 31°C, and maximum temperature from 32 to 48°C. Soil of the study site is *Typic Ustochrepts* with loam texture, deep, alkaline calcareous in nature, poor in organic matter (< 1 percent) and total soil nitrogen (< 0.05 percent). Selected physical properties of the soil (0–95 cm) are given in Table 1.

Experimental design and crop management

A series of field experiments was conducted for four consecutive crop seasons during 2008–2012, to determine the yield and WUE of wheat (*Triticum aestivum* L.) under varying irrigation water applications in a randomized complete block design with four replicates. Similar cultural practices were followed during different crop years. Pre-sowing irrigation of (≈75 mm) was applied uniformly to all plots. Seed at 125 kg/ha (90 percent germination) was sown with a drill. Nitrogen as urea at 125 kg/ha was applied in two splits, half at sowing and half top-dressed at the early tillering stage (1st irrigation). Phosphorus was applied as basal dose at 250 kg/ha in the form of di-ammonium phosphate (DAP, 46 percent P_2O_5) at sowing time.

In the initial trial, wheat was grown with different irrigation levels, i.e. rain-fed (O–O–O–O) and 100 percent ETc (I–I–I–I) in 2008–2009. Subsequently, during the years 2009–2010 and 2010–2011, the irrigation treatments included: 100 percent ETc in four irrigations at four growth stages of the crop (I–I–I–I) and one irrigation missed at each of these stages, i.e. crown root initiation/tillering, booting, flowering and grain formation stages, respectively, to determine the critical crop growth stages. Tillering and flowering were identified as more sensitive crop growth stages to irrigation application with higher reductions (>10 percent) in biomass and grain yield (data not shown). Based on the these results, regulated deficit irrigation treatments at comparatively less sensitive stages (booting and grain filling) were applied in 2011–2012, which included: 50 percent irrigation at the booting stage (I–0.5I–I–I), at grain filling (I–I–I–0.5I) and at both booting and grain filling stages (I–0.5I–I–0.5I).

Crop evapotranspiration (ETc) and irrigation

Crop evapotranspiration (ETc) was calculated using the water balance approach which considers irrigation, rainfall, soil water depletion, deep drainage and runoff. To assess the changes in soil water content in the root zone during crop growth, water status at different soil depths was measured using a neutron moisture meter (NMM) calibrated on-site. Calibration equations relating the count ratio (CR) to the volumetric soil water content (θ) were obtained using a non-weighted, least squares regression technique (Table 2). Water contents of the top 15 cm were also determined gravimetrically.

Soil water contents at sowing and harvest of the crop were determined gravimetrically at 15 cm increments over 0–95 cm of the soil profile. Irrigation water, collected in a tank (32 m^3 [4 x 4 x 2 m]), was pumped through a pipe system and the amount applied to each treatment sub-plot was measured using a flow meter connected between the pump and delivery pipe line. This practice ensured full and uniform water coverage/distribution in the sub-plots and no surface runoff occurred at any time during the crop growth. Irrigation treatments were started immediately after sowing by withholding or applying the irrigation for different treatments, as and when required.

Weather data including daily maximum and minimum air temperatures (°C), humidity, wind speed and daily hours of sunshine (h) were obtained from the University of Agriculture, Faisalabad, while rainfall (mm) was measured at the experimental site. The reference crop evapotranspiration (ETo) was estimated using the ETo Calculator (Version 3.1 http://www.fao.org/nr/water/eto.html) developed by FAO's Land and Water Division, Rome, Italy. Reference crop evapotranspiration, maximum and minimum temperature files were further used in the AquaCrop model. The crop data file for the AquaCrop model contained crop-specific parameters related to development (initial canopy cover, canopy development, flowering and yield formation, and root depth), production (water productivity, harvest index) and biomass stress (soil fertility, soil salinity, soil fertility/salinity). The crop development stages were defined when 50 percent of the plants showed visual signs of the stage being considered. For this purpose, 10 plants from each treatment and replicate were tagged after emergence of the crop to study/record the development stages as number of days taken from emergence to (i) tillering, (ii) anthesis, (iii) end flowering, (iv) senescence, and (v) maturity.

In-season aboveground biomass was determined at different growth stages by clipping the plants at the soil surface within a randomly selected area (1 m × 1 m) in each treatment plot. The corresponding canopy cover development (CC percent) was monitored using digital photographs. The photographs were taken high enough (1–1.5 m) above the canopy at mid-day and digitized using a JAVA program (Image-J) for calculation of canopy cover. Built-in values for initial canopy cover and water productivity were used. Soil fertility and salinity stresses for biomass production were not considered. The crop was harvested at maturity and total biomass, grain yield and harvest index were determined. From the biomass, grain yield and soil water balance, biomass-based and grain-based WUE (i.e., WUEb and WUEg, respectively) were calculated.

Soil, water and irrigation files were prepared and measured/estimated crop parameters were inserted in the model. Default values for canopy cover per seedling, water stress factor for canopy expansion, soil depth contributing to seed germination, deepening shape factor and mid-season crop coefficients were used.

The model was calibrated with the non-stressed treatment data from 2008–2009. To check the accuracy of simulations, the model

TABLE 1. Selected physical properties of soil of the experimental field

Depth (cm)	Texture	Bulk Density (g/cm^3)	θ FC (m^3/m^3)	θ WP (m^3/m^3)	Ksat (mm/hr)
0–15	Loam	1.56	29.8	13.1	4.50
15–35	Loam	1.39	27.4	12.6	5.53
35–55	Loam	1.37	27.0	12.6	5.89
55–75	Loam	1.43	24.5	12.0	8.12
75–95	Loam	1.46	22.8	11.3	10.40

θ — volumetric water; FC — field capacity; WP — wilting point; Ksat — saturated hydraulic conductivity

TABLE 2. Calibration equations of NMM for different soil depths

Depth (cm)	Equation	R^2
0–15	θ = 0.578 CR – 0.242	0.90
15–35	θ = 0.465 CR – 0.189	0.92
35–55	θ = 0.397 CR – 0.139	0.90
55–75	θ = 0.280 CR – 0.038	0.83
75–95	θ = 0.265 CR – 0.020	0.80

AquaCrop calibration and validation

was run with data recorded for irrigated and rain-fed treatments during the years 2009–2010 and 2010–2011 and for all the treatments in the year 2011–2012. During this process, available data on grain yield, total crop biomass and maximum canopy cover were compared with simulated values. Simulation performance was evaluated by calculating different statistic indices like root mean square error (RMSE) (Wallach and Goffinet, 1989) and modelling efficiency (EF) in all the treatments. Time-course simulations of crop biomass and canopy cover were assessed by an index of agreement (d) (Willmott, 1981) that is an aggregate overall indicator. These parameters were calculated as follows:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}[S_i - M_i]}{n}}$$

$$EF = 1 - \frac{\sum_{i=1}^{n}(M_i - S_i)^2}{\sum_{i=1}^{n}\left(M_i - \bar{M}\right)^2}$$

$$d = 1 - \frac{\sum_{i=1}^{n}[M_i - S_i]^2}{\sum_{i=1}^{n}\left[\left|S_i - \bar{M}\right| + \left|M_i - \bar{M}\right|\right]^2}$$

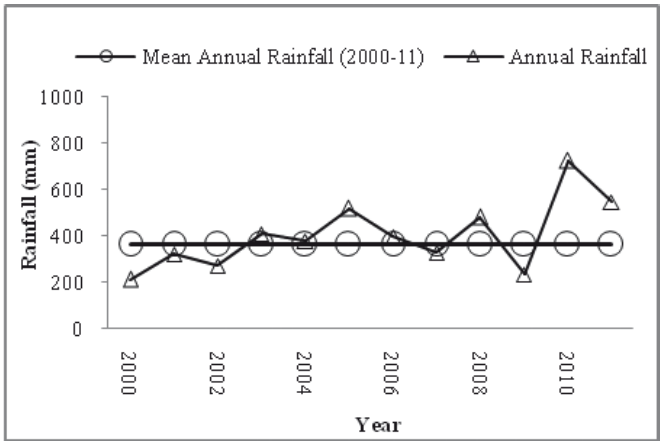
where *Si* and *Mi* = predicted and measured values for variables studied; and *n* = number of observations.

Linear regression analysis between simulated and observed grain yield and biomass at harvest was conducted to evaluate the performance of the model. Model performance improved as R^2 and d values approach unity while RMSE values are nearer to zero.

RESULTS AND DISCUSSION

Climatic parameters

Patterns of rainfall and air temperature at the study site over the last ten years are shown in Figure 1. Normally May is the driest month of the year with an average relative humidity 30 percent that may rise to over 70 percent during monsoon (July and August) and then decreases gradually in October and November. Due to winter rains, the weather becomes more humid with maximum mean relative humidity (> 70 percent) recorded for the month of January. The maximum temperature in summer may occasionally reach up to 50°C in June with average maximum and minimum temperatures of 45°C and 28°C, respectively. Average daily evaporation varies from 2 mm in January to a maximum average of 11.4 mm in May and June with annual excess of pan evaporation in the range of about 1 600 mm over rainfall. During the wheat growing season (November–April), temperature may, at times, fall below freezing point in December and January with an average minimum temperature of ≈ 2°C. Based on the 10-year mean, approximately 72 mm rainfall occurs in the wheat



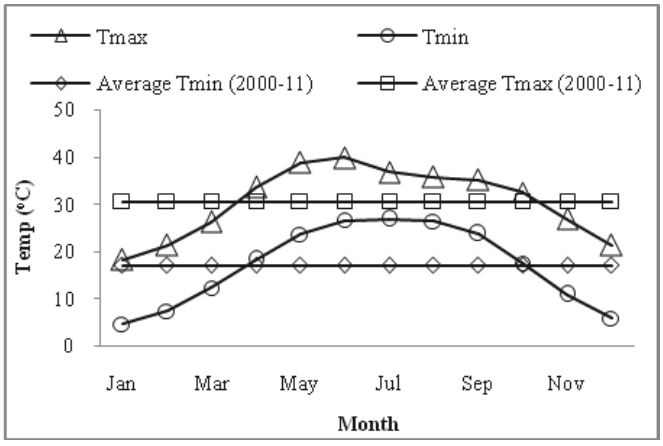


FIGURE 1. Patterns of rainfall and air temperature at the study site (2000–2011).

growing season. However, during the study period, the highest rainfall was recorded in 2008–2009 (65 mm) and the lowest in 2011–2012 (13 mm). Most of the rainfall during the 2008–2009 cropping season was in early November (25 mm) just before sowing and mid-April (20 mm), making only about 20 mm utilized by the crop. Similarly, the contribution of rainfall to plant consumed water between 2009 and 2012 was < 40 mm.

Yield and water use efficiency (measured)

Aboveground biomass and grain yield of wheat were significantly higher in irrigated than the rain-fed treatment in all four years (Table 3). Under rain-fed conditions, comparable values for biomass, grain yield, and HI (harvest index) were recorded in all the study years. Average biomass, 6.08 t/ha and grain yield 2.0 t/ha gave a harvest index of 33 percent. However, significantly lower WUEb and WUEg values were obtained in the 2009–2010 and 2011–2012 seasons due to poor rainfall in early April when the crop was near to maturity. These rainfall events affected the total water balance but did not contribute to increases in total biomass or grain yield. Water use efficiency was significantly higher under rain-fed than irrigated conditions but this was related to drastic decreases in biomass and grain yields of the rain-fed crop (Table 3). Average reductions in WUEb and WUEg under irrigated condition were, respectively, ≈ 16 percent and 8 percent compared with those under rain-fed condition.

Biomass ranged from about 12–15.3 t/ha (average 14.2 t/ha) and grain yield 4.68–5.70 t/ha (average 5.3 t/ha) in irrigated treatments during the four-year study (Table 3). Under regulated deficit

TABLE 3. Yield and water use efficiency of wheat grown under different irrigation levels during four crop years (2008–2012). Values are means of four replicates

Crop	Treatment	Biomass (t/ha)	Grain Yield (t/ha)	HI (%)	WUEb (kg/ha·mm^{-1})	WUEg (kg·ha^{-1}·mm^{-1})
2008–09	I–I–I–I	13.51	5.10	36.67	53.85	19.72
2009–10	Rain-fed	5.83	2.10	36.02	39.58	14.30
	I–I–I–I	11.98	4.68	39.07	33.60	13.05
2010–11	Rain-fed	6.07	1.89	31.13	55.20	17.20
	I–I–I–I	15.30	5.70	37.30	48.30	18.00
2011–12	Rain-fed	6.53b	1.97b	30.00b	45.20a	13.70b
	I–I–I–I	15.03a*	5.46a	36.70a	41.70ab	15.20a
	I–0.5I–I–I	14.20a	5.23a	36.70a	41.80ab	15.40a
	I–I–I–0.5I	14.36a	5.30a	37.00a	40.50b	15.00ab
	1–0.5I–I–0.5I	14.10a	5.13a	36.70a	42.20ab	15.30a

*Values followed by same letter in a column are not significantly different (crop 2011–2012)

irrigation, preliminary results showed insignificant differences among the treatments for biomass, grain yield and harvest index. However, the lowest WUEb (40.5 kg·ha^{-1}·mm^{-1}) was obtained when irrigation was reduced at the grain filling stage (I–I–I–0.5I). In comparison with other treatments, similar WUEg values ≈ 15.3–15.4 kg·ha^{-1}·mm^{-1} were found when irrigation was reduced at the booting stage (I–0.5I–I–I) or at both the booting and grain filling stages (1–0.5I–I–0.5I).

AquaCrop simulations

Model calibration

The AquaCrop model was calibrated against measured data for in-season biomass, canopy cover, biomass at harvest and grain yield under the non-stressed irrigated treatment for the year 2008–2009. The calibration procedure involved adjustments in crop phenology including plant population, days taken by the crop to emergence, full canopy cover, flowering, senescence and harvest. During calibration, in-season biomass and canopy cover predicted with AquaCrop closely followed the observed values with a reasonable root mean square error (RMSE), index of agreement (d) and modelling efficiency (E). Low RMSE values (0.47 for biomass and 9.0 for CC) along with high d and E (closer to 1) demonstrate the adequacy of model calibration (Figure 2) for simulating in-season biomass and canopy cover.

Soil water and water balance

Fluctuations in volumetric soil water measured with neutron probe (NMM) for different depths for the rainfed and fully irrigated treatments for the 2011–2012 season are given in Figure 3. No appreciable water movement in any cropping year was observed beyond 75 cm of soil depth under irrigation treatment, indicating that all irrigation water applied was either lost in the form of soil evaporation or transpired by the crop. The model predictions were quite satisfactory for simulating soil water content in the soil depths studied with a smaller range in RMSE (2.58–3.74) and d (0.94–0.84). Coefficients of regression (R^2) also indicated reasonable correlations between simulated and observed water contents ranging from 0.89 to 0.76. However, model predictions for soil water contents were relatively higher for different soil depths compared with observed values in all the years. The spatial variability within the individual soil columns may cause some differences in the measured values especially in the water stress treatments (Heng *et al.*, 2009). In the present study, overall simulated and measured soil water contents were closely correlated for the four-year data (Figure 3).

Total soil water balance based on the water budget approach (Figure 4) showed some irrigation water loss by the model in the form of drainage under irrigated treatment during all the cropping years. As a result, the model under-predicted the total water consumed in the form of evaporation and transpiration by the crop. On the other hand, no deep percolation losses were observed beyond the root zone (Figure 3). The accuracy of model prediction to simulate total water consumed by the crop was improved (R^2 = 0.90) when the simulated drainage component was added to the total predicted evaporation and transpiration components of water balance (Figure 4).

Yield and water use efficiency (simulated)

The deviations of simulated values for biomass and grain yield from measured values are expressed as percentages of measured values (Table 4) and show reasonable predictions by the model. The deviations between measured and simulated biomass at harvest (1.26 percent) and grain yield (0.98 percent) were less than 2 percent during calibration. For the 2009–2010 crop, values of in-season biomass and root growth (depth) predicted by AquaCrop were higher with RMSE 1.46 (Figure 2) and higher differences in end-season biomass, i.e. 19.9 percent for rain-fed and 7.35 percent for irrigated conditions. The differences require reassessment of the validity of the model.

The RMSE, d and E values for irrigated (I–I–I–I) and rain-fed (O–O–O–O) treatments (Figure 2) showed that overall prediction of crop growth parameters were simulated well for irrigated treatments. However, some over-estimations of biomass and canopy growth with higher deviations in final biomass and grain yields were observed under rain-fed treatment in the years 2009–2011 (Table 4). A similar trend was observed for this treatment in 2011–2012. This over-estimation was due to higher predicted in-season biomass and canopy growth.

For different regulated (deficit) irrigation treatments during 2011–2012, the model predicted in-season biomass accurately in all the treatments with mean RMSE, d and E values of 0.64, 0.98 and 0.97, respectively. These predictions were comparatively less accurate for canopy growth, with higher RMSE = 12.45 when irrigation was reduced at grain formation (I–I–I–0.5I) and lower RMSE = 6.32 at half irrigation at both booting and grain formation stages (I–0.5I–

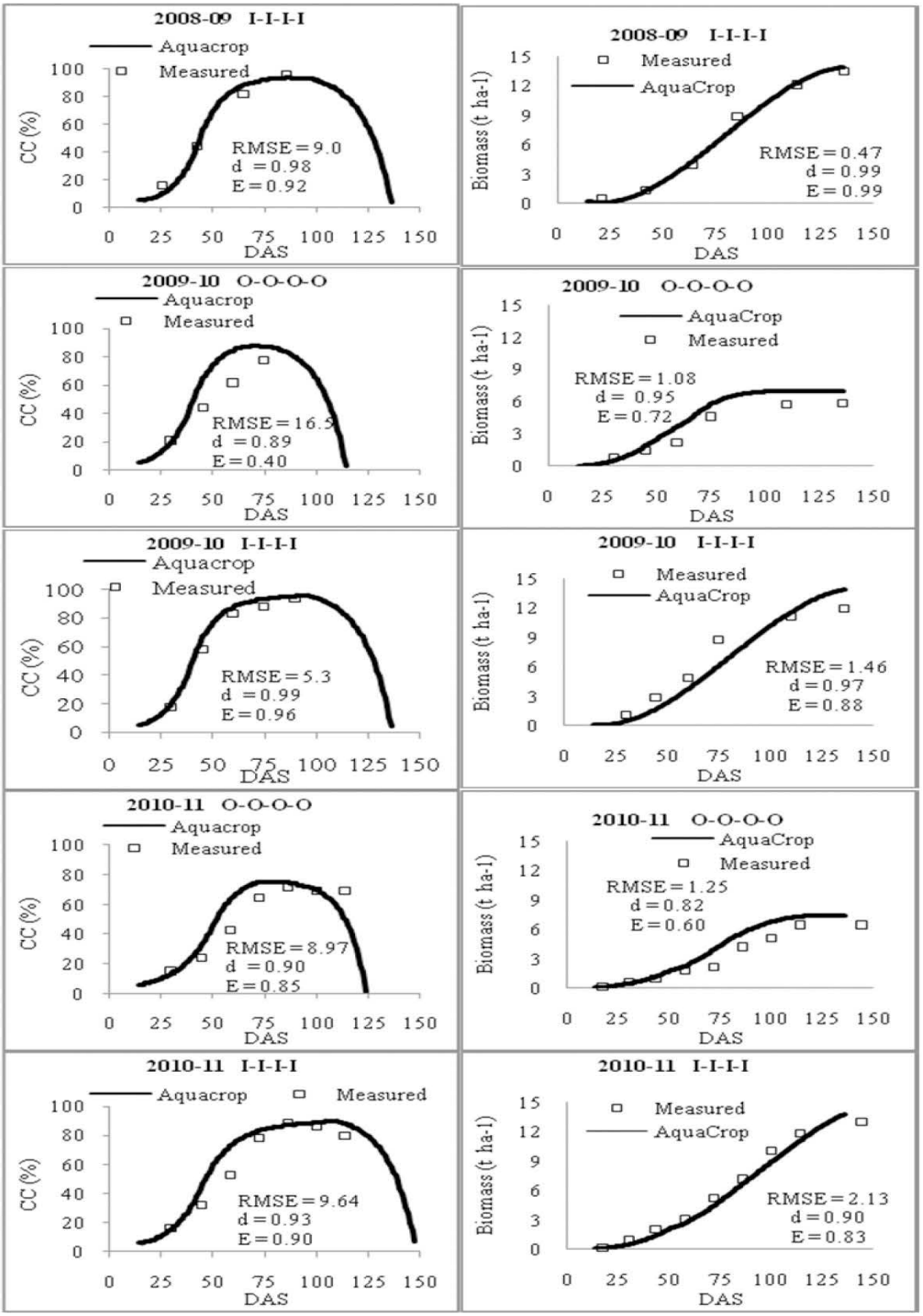


FIGURE 2. Simulated (AquaCrop) and measured values of in-season biomass and canopy cover along with statistical indicators of RMSE, d and E showing adequacy of model fitting during calibration (2008–2009) and validation (2009–2010 and 2010–2011) under irrigated (I–I–I–I) and rain-fed (O–O–O–O) conditions.

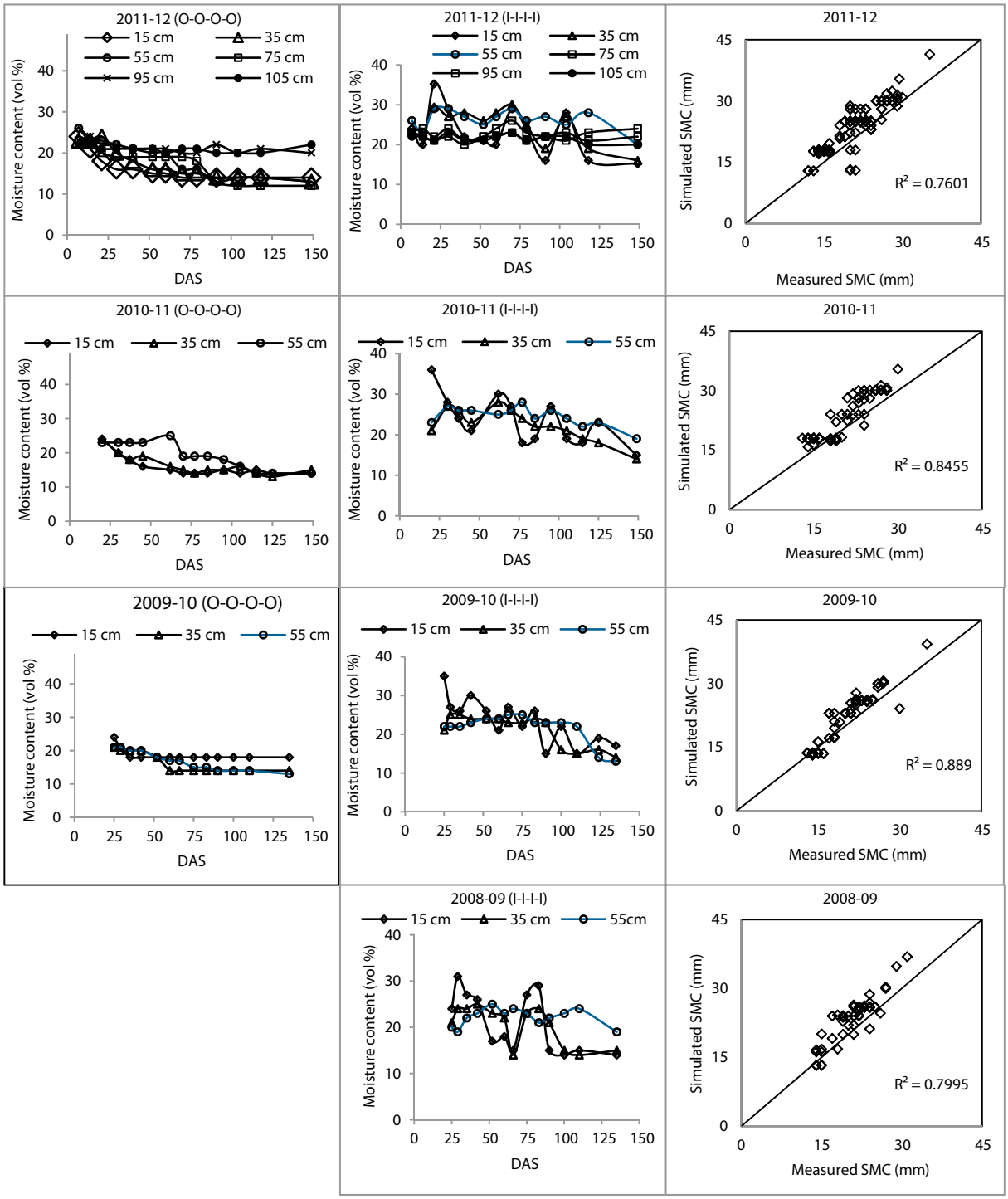


FIGURE 3. Fluctuations in soil water at different depths over the cropping season (expressed as days after sowing (DAS)) (left and centre), and the comparison between measured and simulated soil water content (SMC) (right)) along with statistical indicators for 2008–2012.

I–0.5I). Modelling efficiency was also lower at O–O–O–O (rain-fed) and I–0.5I–I–0.5I irrigation, and deviations between simulated and observed values were within 10 percent of measured values (Table 4). Since examining yield responses to different water applications in field and controlled experiments is laborious and cannot cover all possible combinations of factors influencing crop growth, modelling can be a useful tool to study and develop appropriate deficit irrigation strategies (Geerts and Raes, 2009).

As observed in Figure 2, AquaCrop was able to simulate accurately the canopy cover (CC) development in the 2009–2010 and 2010–2011 cropping seasons. Simulated values of CC indicated slightly faster canopy development compared with the measured CC values. The overall relationship between measured and simulated values was very good with $R^2 > 0.90$ and a sufficient mean index of agreement (0.92). A similar trend was observed for in-season biomass and total biomass at harvest and grain yield (Table 4). The deviation between simulated and measured values was < 10 percent for

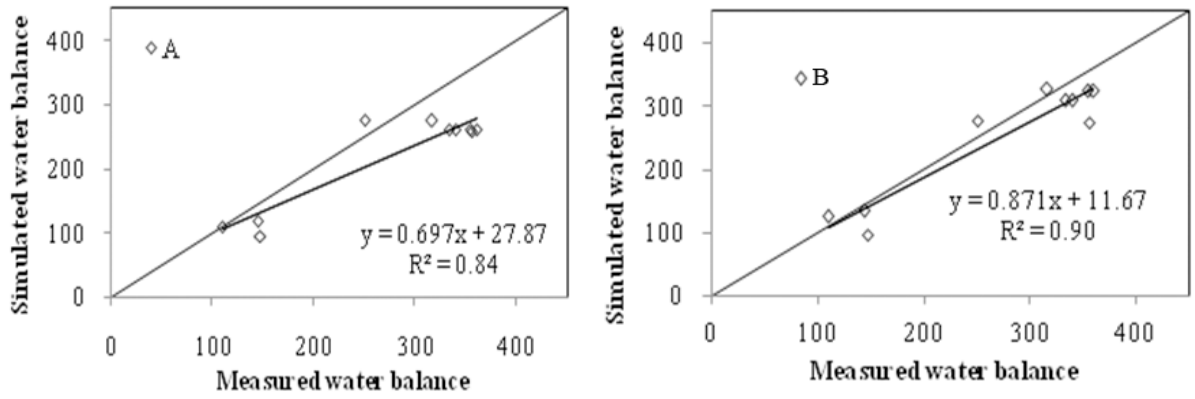


FIGURE 4. Total measured and simulated (AquaCrop) water balance (A: drainage excluded, B: drainage included) under different irrigation treatments during four-year study (2008–2012).

TABLE 4. Measured and AquaCrop simulated values of biomass and grain yield of wheat under different irrigation regimes during crop seasons 2008–2012

Crop	Treatment	Biomass (t/ha)			Grain Yield (t/ha)		
		M	S	D (%)	M	S	D (%)
2008–09	I–I–I–I	13.51	13.68	1.3	5.10	5.15	1.0
2009–10	Rain-fed	5.83	6.99	19.9	2.10	2.39	13.8
	I–I–I–I	11.98	12.86	7.4	4.68	4.89	4.5
2010–11	Rain-fed	6.07	7.38	19.1	1.89	2.10	4.9
	I–I–I–I	15.30	14.07	-2.5	5.70	5.51	5.0
2011–12	Rain-fed	6.53	7.27	11.9	1.97	2.25	12.5
	I–I–I–I	15.03	13.70	-8.7	5.46	5.42	-1.5
	I–0.5I–I–I	14.20	13.70	-3.5	5.23	5.42	4.2
	I–I–I–0.5I	14.36	13.70	-4.9	5.30	5.42	2.3
	1–0.5I–I–0.5I	14.10	13.69	-2.9	5.13	5.42	6.3

M = measured, S = simulated and D = percentage deviation between measured and simulated

both biomass and grain yield under irrigated treatment. In rain-fed conditions, higher deviations for biomass (19.9 percent) and grain yield (13.6 percent) were recorded in 2009–2010, but the deviation was reduced to 4.9 percent for grain yield in 2010–2011. The smaller deviations between measured and simulated biomass and grain yields (2010–2011) were due to better control of soil water balance and adjusting the rooting depth of the crop by *in-situ* measurements of wheat root growth under different irrigation treatments.

Evaporation and transpiration components

Crop growth models allow a combined assessment of different factors affecting yield (Liu *et al*., 2007), and also allow differentiating between evaporation and transpiration components of evapotranspiration. The patterns of evapotranspiration estimated using AquaCrop in the present study are presented in Figure 5. Soil evaporation was the main component of water loss during the early growth stage due to the low canopy cover; however, after the fourth week, crop transpiration increased and accounted for the majority of the total ET as canopy cover increased. Transpiration remained higher during the vegetative growth and flowering stages and started declining at grain filling. The initiation of leaf senescence during the grain filling stage resulted in a decrease in transpiration with a corresponding increase in the evaporation component (Figure 5). An extensive sampling of soil, air and plant water at different crop growth stages was carried out to validate the AquaCrop evaporation and transpiration patterns with isotopic methods. The attempted Keeling plot gave variable estimates of evaporation and transpiration components; some data need reassessment and are not discussed here.

CONCLUSIONS

The present study showed that crop yield and water use efficiency of wheat could be improved by regulating plant-available water through irrigation at crop stages that are more sensitive (tillering and grain formation) to water deficit. The study demonstrated that up to 25 percent of the irrigation water can be saved by deficit irrigation when irrigation is applied at the less sensitive crop stage(s), thus allowing irrigating additional crop area. The proportion of water lost as soil evaporation (E) versus plant transpiration (T) relative to the total sum of evapotranspiration (ET) were quantified for the different irrigation treatments, such information can help to evaluate the effectiveness of land and water management practices that influence E and T components. The AquaCrop model can be a useful tool for assessing crop water requirements and devising regulated deficit irrigation strategies to enhance water productivity under different scenarios in water scarce areas.

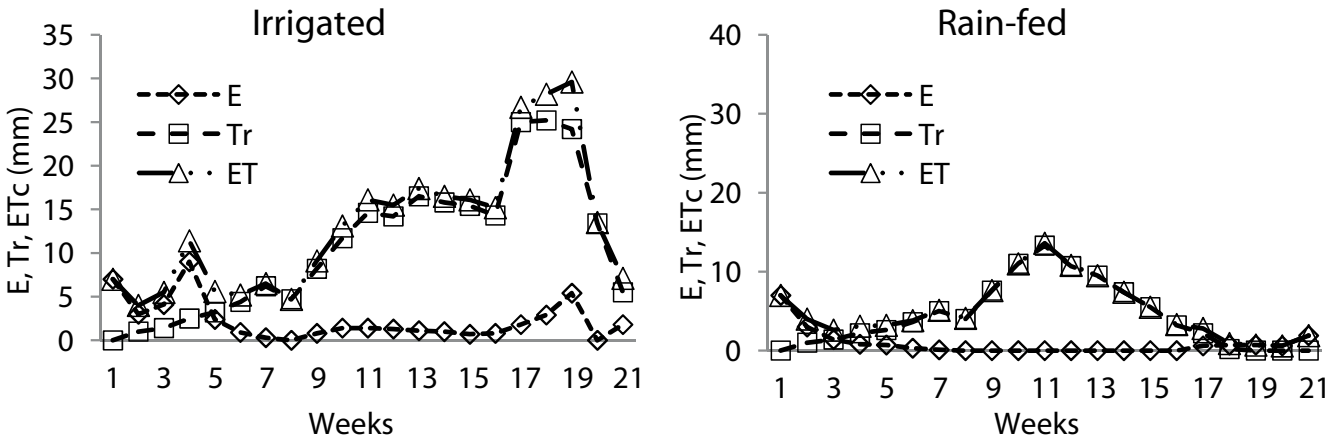


FIGURE 5. Pattern of wheat crop evapotranspiration and its components under rain-fed and irrigated conditions as determined by AquaCrop (2010–2011).

ACKNOWLEDGEMENTS

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Yield, Water and Nitrogen Use by Drip-irrigated Cabbage Grown under Different Levels of Applied Water

D.K. Asare[1,*], I.T. Larteh[1], J.O. Frimpong[1], M. Yaro[1], K.E. Banson[1], E.O. Ayeh[1] and L.K. Heng[2]

ABSTRACT

Drip irrigation, known for its efficient delivery of water to the root zone of crops, could be used for efficient management of scarce water that limits cabbage production and income levels for vegetable farmers during the dry season in Ghana. This study was conducted to evaluate the productivity, water use efficiency (WUE) and fertilizer nitrogen (N) use efficiency (FNUE) of cabbage cultivars KK-Cross and Oxylus, grown using small-scale drip irrigation system under variable water application levels in a coastal savannah environment of Ghana during the dry season. Total fresh yield (TFY), total dry matter (TDM), WUE and FNUE of cabbage cultivars decreased with decreasing levels of applied water. Linear models adequately described the relationship between TFY and seasonal crop evapotranspiration (ETc) as well as between TDM and ETc for these cultivars. The NFUE and WUE were linearly correlated with the KK-Cross cultivar; however, the models could not adequately describe the relationship between NFUE and WUE for Oxylus. The cabbage productivity under water limiting conditions could be enhanced through efficient use of applied N and water by adopting good water management strategies as ensured by the drip irrigation technology, there is also the need to formulate management strategies, including reducing farm size to match limited available water and to ensure efficient use of resources and high productivity by drip-irrigated cabbage.

Key words: *cabbage, water use efficiency, nitrogen use efficiency, drip irrigation, yield.*

INTRODUCTION

Cabbage production is a profitable venture during the dry season in the coastal savannah environment of Ghana but production levels are generally low (Vordzorgbe, 1997) due to factors that include inadequate availability of irrigation water and inefficient irrigation practices. Drip irrigation has the potential to increase water use efficiency (WUE) in vegetable production (Locascio, 2005) thereby reducing water use by 30–70 percent while increasing crop yields by more than 50 percent (Al-Rawahy, Abdel-Rahman and Al-Kalbani, 2004). Benefits from the application of low volumes of water to plant root zones include reduced soil surface evaporation losses and improved irrigation uniformity (Schwankl, Edstrom and Hopmans, 1996), and making drip irrigation suitable for the production of high value crops (Sanders, 1991), particularly under inadequate water supply. Knowledge of cabbage responses to different levels of applied water, and the associated use of applied nitrogen (N), could guide irrigation management strategies for optimizing cabbage yield and ensuring high efficiency of applied water and N use for the resource-poor farmers. This study was therefore undertaken to assess yield and water and N use efficiencies of two cabbage cultivars grown at different levels of applied water using a small-scale drip irrigation system.

MATERIALS AND METHODS

A field experiment was conducted at the Research Farm of the Biotechnology and Nuclear Agriculture Research Institute (BNARI), Ghana Atomic Energy Commission (GAEC). The experimental site is 76 m above sea level and situated on latitude 05°, 40′ N and longitude 0°, 13′ W in the coastal savannah environment of Ghana. Annual rainfall at the site ranges between 700 and 1 000 mm (Morris, Tripp and Dankyi, 1999). Additionally, the soil at the site is a well-drained sandy loam savannah Ochrosol (ferric acrisol) derived from quartzite schist (FAO/UNESCO, 1994), and known locally as the Haatso series.

A split-plot experimental design in three replicates was used, with the main plot being the levels of applied water (100, 85, 70, 55 and 40 percent of required water), and cabbage cultivars being the sub-plot. A small-scale drip irrigation system, occupying a total area of 525 m^2, was used to irrigate the cabbage cultivars at the various levels of applied water used for the study. A micro-plot, measuring 6 m x 0.8 m was established in each sub-plot for nitrogen-15 (^{15}N) fertilizer application and soil moisture monitored using a neutron probe (CPN Hydroprobe model 503DR).

Cabbage seedlings were transplanted at a spacing of 0.8 m x 0.6 m in each sub-plot on November 22, 2010 and harvested on February 19, 2011. Fertilizers applied were 120 kg/ha N, 100 kg/ha of potassium (K) and 50 kg/ha of phosphorus (P), one-third of which being applied two weeks after transplanting and the remaining two-thirds six weeks after transplanting of seedlings.

The potential crop evapotranspiration, ET_c, was estimated as:

$$ET_C = K_c \times ET_0 \quad (1)$$

where K_c is crop coefficient; ET_o is reference evapotranspiration (mm/day), computed using the previous day's daily weather variables

1 Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, Legon-Accra, Ghana
2 Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, IAEA, Vienna, Austria
* E-mail address of corresponding author: daniel_asare@yahoo.com

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based on the Penman-Monteith model (FAO, 1998), as given in Equation 2 (mm/day):

$$ET_0 = \frac{0.408\,\Delta(Rn - G) + \frac{890\gamma U(ea - ed)}{T + 273}}{\Delta + \gamma(1 + 0.339U)} \quad (2)$$

where Δ is slope of the saturated vapour pressure function (kPa/°C); γ is psychometric constant (kPa/°C); *Rn* is net solar radiation ($MJ \cdot m^{-2} \cdot d^{-1}$); *U* is wind speed (m/s) at 2.0 m height above the ground surface; *T* is mean daily temperature (°C); (*ea-ed*) is atmospheric vapour pressure deficit; and *G* is soil heat flux density ($MJ \cdot m^{-2} \cdot d^{-1}$), estimated according to Hargreaves and Merkley (2004) as:

$$G = 0.38(T_{day} - T3) \quad (3)$$

where T_{day} is mean air temperature on the day of calculation (°C); and *T3* is mean daily air temperatures of the previous three days (°C).

Soil moisture data was used to determine the actual crop water use (ET_a) (mm), based on the water balance approach:

$$ET_a = P + I \pm \Delta S \pm D \pm R \quad (4)$$

where *P* is precipitation (mm); *I* is irrigation (mm); *ΔS* is change in moisture stored in the soil profile (mm); *D* is deep drainage or capillary rise below the 100 cm soil profile (mm); and *R* is run-off (mm).

Run-off, deep drainage and capillary rise were assumed to be negligible and, therefore, set to zero since the experiment was conducted under dry conditions and water application was controlled using the small-scale drip irrigation system.

Sampling and analyses

Four cabbage plants (above ground and below ground) were harvested from each micro-plot and the samples oven-dried at 70°C for dry matter determination. Dry matter samples were also used for total N and ^{15}N analyses at the IAEA Laboratories in Seibersdorf, near Vienna, Austria. Additionally, cabbage plants were sampled at harvest from a 4.8 m^2 area in each sub-plot to determine total fresh yield.

Water and nitrogen use efficiencies

Water use efficiency (WUE) was estimated as follows on the basis of both total fresh yield (TFY) and total dry matter (TDM):

$$WUE_{TFY} = \frac{TFY}{ETc} \quad (5)$$

and

$$WUE_{TFY} = \frac{TDM}{ETc} \quad (6)$$

where WUE_{TFY} and WUE_{TDM} ($kg \cdot ha^{-1} \cdot mm^{-1}$) is WUEs based respectively, on *TFY* and *TDM*; and *ETc* is seasonal actual evapotranspiration (mm).

Fertilizer *N* use efficiency, *FNUE* (%), was estimated as:

$$FNUE = \frac{N_{YIELD} \times \%Ndff}{N_{APPLIED}} \quad (7)$$

and

$$\%Ndff = \frac{(\%N15AEPS) \times 100}{\%N15AEF} \quad (8)$$

where N_{YIELD} is total N uptake by plant (kg/ha); $N_{APPLIED}$ is amount of fertilizer applied (kg/ha); *%Ndff* is fraction of *N* in plant sample derived from the applied fertilizer; *%N15AEPS* is percent of ^{15}N atom excess in plant; and *%N15AEF* is percent ^{15}N atom excess in the fertilizer.

Statistical analyses

Total fresh yield (TFY), total dry matter (TDM), WUE_{TDM}, and FNUE were subjected to analysis of variance (ANOVA) based on the split-plot design and the least significant difference (LSD) used to separate means when significant differences were observed. The GENSTATS statistical package was employed in the analysis of the data. Also, linear regression and correlation analyses were used to assess the relationships between TFY and ETc, TDM and ETc and between NFUE and WUE.

RESULTS

Total fresh yield (TFY)

The five levels of water application (100, 85, 70, 55, and 40% of the optimal required level) were equivalent to 260.9, 222.5, 184.1, 145.7 and 107.3 mm, respectively of water applied. Water application levels were found to affect significantly the TFY of the cabbage cultivars, with the 100 percent water application level producing the highest TFY at 44.0 tons (t)/ha, followed by 33.0 t/ha for the 85 percent water application level (Table 1). Seventy percent and 55 percent water levels produced statistically similar TFYs of 20.4 t/ha and 19.3 t/ha, respectively. The 40 percent water application level produced the lowest TFY with 11.4 t/ha. For cabbage cultivars, the K-K Cross produced significantly higher TFYs (47.43 t/ha and 24.97 t/ha respectively at the 100 percent and 70 percent water application levels compared with corresponding values of 40.41 t/ha and 15.83 t/ha for Oxylus (Table 1). Furthermore, reducing the level of applied water by 15 percent from the 100 percent optimal level resulted in 33.5 percent and 15 percent decreases in TFY for KK-Cross and Oxylus, respectively. Similarly, TFYs produced by KK-Cross and Oxylus at 40 percent water application level were 24.5 percent and 27.6 percent, respectively of the TFY produced at the 100 percent water application level.

Total dry matter (TDM)

The 100 percent water application level produced the highest TDM of 4.23 t/ha ($p < 0.001$), followed by 3.27 t/ha, 2.05 t/ha, 2.07 t/ha and 1.37 t/ha produced by the 85, 70, 55 and 40 percent water application levels, respectively. The cabbage cultivars KK-Cross and Oxylus produced statistically different ($p < 0.05$) levels of TDM under both 100 percent and 85 percent water application levels (Table 1), with TDM for KK-Cross (4.59 t/ha) being higher than that for Oxylus at the 100 percent water application level (3.86 t/ha) while Oxylus produced a higher TDM (3.52 t/ha) than KK-Cross (3.02 t/ha) at the 85 percent water application level. In contrast, both cabbage cultivars produced statistically similar levels of TDM at each of the other water application levels (Table 1).

Water use efficiency based on total fresh yield (WUE_{TFY})

Water application levels significantly affected ($p < 0.001$) water use efficiency of the cabbage cultivars, WUE_{TFY} values at 100 percent and 85 percent being 131.80 $kg \cdot ha^{-1} \cdot mm^{-1}$ and 118.40 $kg \cdot ha^{-1} \cdot mm^{-1}$ and statistically similar (Table 1). Additionally, WUE_{TFY} at 70 percent and 55 percent water applications levels were respectively 86.40 $kg \cdot ha^{-1} \cdot mm^{-1}$ and 95.10 $kg \cdot ha^{-1} \cdot mm^{-1}$, but statistically similar, while the 40 percent water application level produced the lowest WUE_{TFY} value (63.20 $kg \cdot ha^{-1} \cdot mm^{-1}$). The KK-Cross cultivar had significantly higher WUE_{TFY} ($p < 0.009$) than Oxylus at the 100 percent

water application level, but the cabbage cultivars had statistically similar WUE_{TFY} values at each of the other application levels (Table 1).

Water use efficiency based on total dry matter (WUE_{TDM})

Water use efficiencies based on TDM were statistically similar at all levels of water application. Comparatively, Oxylus had a significantly higher ($p < 0.05$) WUE_{TDM} value than KK-Cross only at the 85 percent level of applied water (14.37 and 11.65 $kg \cdot ha^{-1} \cdot mm^{-1}$, respectively) (Table 1).

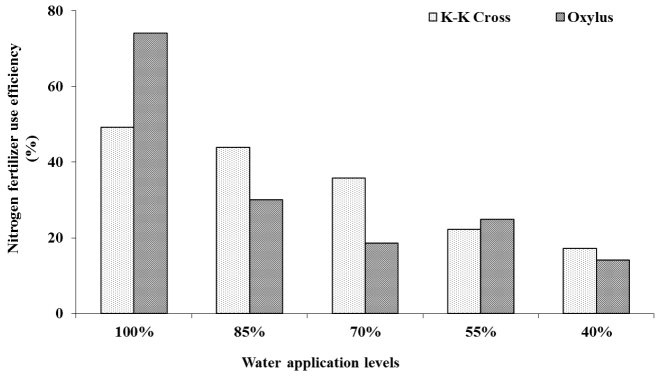


FIGURE 1. Nitrogen fertilizer use efficiency (NFUE) of cabbage cultivars KK-Cross and Oxylus at different levels of applied water.

Nitrogen fertilizer use efficiency (NFUE)

A significant difference ($p \leq 0.007$) was observed in NFUE values for the water application levels. At the 100 percent level of water application it produced the highest NFUE of 61.7 percent (Figure 1). Values for other water application levels were statistically similar and KK-Cross and Oxylus had statistically similar NFUE values at each water application level. Nitrogen use efficiency decreased from 60 percent at 100 percent water application level to as low as 18 percent at 40 percent water application level (Figure 1).

Linear regression and correlation analyses

Total fresh yield and TDM increased with increasing ETc whereas NFUE generally increased with increasing WUE_{TFY} and WUE_{TDM}. Linear models adequately described the functional relationship between TFY and ETc for the cabbage cultivars, as more than 60 percent of the data used for the analyses were accounted for by a linear model (Table 2). Similar results were obtained for the relationship between TDM and ETc for both cabbage cultivars (Table 2) as well as between NFUE and WUE_{TFY} and between NFUE and WUE_{TDM} for only the KK-Cross (Table 2). However, the relationship between NFUE and WUE_{TFY} and WUE_{TDM} for Oxylus could not be described adequately by a linear model, as shown by the poor R^2 value (Table 2).

DISCUSSION

Total fresh yield levels for the drip irrigated cabbage cultivars, which ranged between 11.15 t/ha and 47.43 t/ha across the 40–100 percent water application levels, were within the range of the mean world's cabbage yield level of 10–40 t/ha (de Lannoy, 2001) but lower than the 69 t/ha reported by Jangandi, Shekar and Shridhara (2000) and the 106 t/ha reported by Tiware, Singh and Mal (2003) for cultivars grown under plastic mulches with drip irrigation. The average values

TABLE 1. Total fresh yield (TFY), total dry matter (TDM), water use efficiencies based on total fresh yield (WUE_{TFY}) and total dry matter (WUE_{TDM}) for KK-Cross and Oxylus at varying levels of applied water. Adjacent values with same letters under a major heading are not significantly different.

Levels of applied water (%)	TFY (t/ha)		TDM (t/ha)		WUE_{TFY} ($kg \cdot ha^{-1} \cdot mm^{-1}$)		WUE_{TDM} ($kg \cdot ha^{-1} \cdot mm^{-1}$)	
	KK-Cross	Oxylus	KK-Cross	Oxylus	KK-Cross	Oxylus	KK-Cross	Oxylus
100	47.43a	40.41b	4.59a	3.86b	129.80a	106.90b	12.54a	10.22a
85	31.56a	34.34a	3.02a	3.52b	123.20a	140.4a	11.65a	14.37b
70	24.97a	15.83b	2.11a	1.99a	104.40a	68.50b	8.86a	8.60a
55	19.03a	19.58a	1.88a	2.25a	91.50a	98.70a	9.06a	11.33a
40	11.64a	11.15a	1.27a	1.47a	62.1a	64.30a	6.76a	8.48a

TABLE 2. Linear relationships between TFY and ETc, betweenTDM and ETc and between NFUE and WUE_{TFY} (WUE_{TDM})

No.	Relationship	Cabbage cultivar	Linear model	R^2
1	TFY vs. ETc	KK-Cross	$TFY = 0.155 \times ETc - 12.184$	0.657
		Oxylus	$TFY = 0.132 \times ETc - 8.288$	0.674
2	TDM vs. ETc	KK-Cross	$TDM = 0.016 \times ETc - 1.380$	0.747
		Oxylus	$TDM = 0.100 \times ETc + 0.079$	0.615
3	NFUE vs. WUE_{TFY}	KK-Cross	$NFUE = 0.488 \times WUE_{TFY} - 16.206$	0.930
		Oxylus	$NFUE = 0.334 \times WUE_{TFY} + 0.411$	0.186
4	NFUE vs. WUE_{TDM}	KK-Cross	$NFUE = 5.461 \times WUE_{TDM} - 19.698$	0.858
		Oxylus	$NFUE = 1.551 \times WUE_{TDM} + 15.961$	0.024

obtained were, however, close to the values of 30 t/ha obtained under drip irrigation with poultry manure (Ijoyah and Sophie, 2009), 32–37 t/ha under drip irrigation (Ijoyah and Rakotomavo, 2007) and 15–46 t/ha under sprinkler irrigation (Imtiyaz, 2000), and much higher than the 5.5 t/ha reported by Ogbodo, Okorie and Utobo (2009) for cabbage grown under rain-fed conditions. This suggests that drip irrigation has the potential to enhance the productivity of cabbage under limited water application levels.

Total dry matter is an indicator of a resource use by crops (Garnier *et al.,* 2001). Here, TDM levels of the cabbage cultivars ranged from 1.27 t/ha to 4.6 t/ha across the water application levels, higher than the 0.30–0.80 t/ha reported by Ogbodo, Okorie and Utobo (2009) for cabbage grown under rain-fed conditions, but generally within the range 1.5–10.5 t/ha reported by Franczuk *et al.* (2009) for cabbage grown under mulches.

Water use efficiency (WUE) is an important agricultural crop index (Hunsaker *et al.* 1996) which can be used to assess how soil water has been used for the production of total dry biomass and economic yield. The observed WUE_{TDM}, which ranged from 6.76 $kg{\cdot}ha^{-1}{\cdot}mm^{-1}$ to 14.37 $kg{\cdot}ha^{-1}{\cdot}mm^{-1}$, was generally lower than the range 12–50 $kg{\cdot}ha^{-1}{\cdot}mm^{-1}$ reported by Beletse, du Plooy and Mogatlane (2009), while values of WUE_{TFY} (range 64.30 to 140.40 $kg{\cdot}ha^{-1}{\cdot}mm^{-1}$) were higher than the 39–66 $kg{\cdot}ha^{-1}{\cdot}mm^{-1}$ reported by Imtiyaz *et al.* (2000). However, the values of WUE_{TFY} obtained in this study were far lower than the 427 $kg{\cdot}ha^{-1}{\cdot}mm^{-1}$reported by Tiware, Singh and Mal (2003) for cabbage grown under drip irrigation using mulch; the difference could be due to a greater availability of soil water due to reduced soil surface evaporation from the use of surface mulch.

The NFUE value of 73.0 percent for Oxylus at the 100 percent water application level was higher than the value of 42.0 percent reported by Sturm *et al.* (2010) for cabbage grown under the practice of tank sprinkler irrigation. It was also higher than the values of 46.8 percent and 39.4 percent reported by Bing *et al.* (2005) for Chinese cabbage receiving 75 and 150 $kg{\cdot}N{\cdot}ha^{-1}$, respectively. However, the NFUE value of 48.0 percent for the K-K Cross cultivar at 100 percent water application level is in agreement with values reported by Bing *et al.* (2005) and Sturm *et al.* (2010). Furthermore, the range of NFUE values for K-K Cross (17.2–49.2 percent) and Oxylus (14.2–74.1 percent) across the levels of applied water considered in this study, are above the range of values of 18.1–24.6 percent reported by Bing *et al.* (2007) for Chinese cabbage grown in an open field.

The significantly higher NFUE for Oxylus compared with K-K Cross were due to the higher level of applied N taken by Oxylus. Similarly, the higher NFUE for K-K Cross at 85 and 70 percent water application levels compared with values for Oxylus resulted from higher uptake of applied N. The comparatively decreasing NFUE with decreasing levels of applied water observed here further emphasizes the need to ensure an adequate level of moisture in the soil through appropriate water management strategies in order to enhance the recovery of applied N fertilizer and ensure enhanced crop productivity.

CONCLUSIONS

Productivity, ETc, WUE and NFUE for cabbage cultivars K-K Cross and Oxylus were affected by levels of applied water. Reducing the level of water to 85 percent of optimal amount reduced the yield of these cultivars by around 33 percent and 15 percent, respectively. This suggests that a sharp decrease in the productivity of KK-Cross can arise from a slight reduction in the amount of water application. While the cabbage cultivars generally had similar WUEs at each level of water application, the K-K Cross specifically had significantly higher WUE_{TFY} values compared with Oxylus. NFUE generally increased with increasing WUE, emphasizing the importance of ensuring adequate soil moisture through appropriate water management strategies for enhanced crop productivity, WUE and NFUE. Additionally, the significant correlation between productivity and WUE emphasized the need to adopt improved water management practices that could simultaneously enhance productivity.

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Water Use Efficiency of Coffee (*Robusta*) Under Mulch and Drip Irrigation on the Tay Nguyen Plateau, Viet Nam

D.N. Dang[1,*], H.S. Duong[2], M.C. Khuong[2], L.K. Heng[3], and M.L. Nguyen[3]

ABSTRACT

This paper attempts to separate the transpiration and evaporation components from total evapotranspiration (ET) and compares the water use efficiency (WUE) of coffee (*Robusta*) under furrow and drip irrigation practices, with and without mulching. The experiments were conducted on a 10-year old coffee plantation on a clay soil of the Tay Nguyen Plateau, in the central part of Viet Nam. The plantation is relatively homogeneous in terms of crop height, with a leaf area index (LAI) ranging from six to seven. The study showed that transpiration was the highest contribution (95 ± 5 percent) to ET during flowering (February–March), and the lowest (47 ± 3 percent) during the mature and canopy reformation stages (October–November), as separated by the isotopic technique. Drip irrigation (DrI) combined with plant residues (mulch) increased the WUE of coffee up to 2.13 kg clean bean per m^3 of irrigated water (kg/m^3), while WUEs under DrI and furrow irrigation (FI) without mulch were only 1.93 and 1.78 kg/m^3, respectively. Due to the improvement in WUE, the local farmers are making extra profit from the application of DrI with mulching from their coffee crop. Local farmers in the Tay Nguyen Plateau are now advised to use DrI with mulch from plant residues to all coffee plantations.

Key words: *coffee, water use efficiency, evapotranspiration, isotopic mass balance, drip irrigation, mulch.*

INTRODUCTION

Water is needed for plants to produce biomass and the role of water for getting high crop yields is recognized by farmers worldwide. In Viet Nam, wherever water is abundant farmers usually irrigate their crops excessively without thinking about the negative effects of over-irrigation such as runoff of fertilizer to the surface water causing eutrophication or leaching into deep aquifers leading to groundwater contamination. On the other hand, whenever water is scarce, for example during the dry season, farmers do not apply any measures to maintain or conserve soil moisture. The concept of water use efficiency (WUE) is still not familiar to Vietnamese farmers.

To improve WUE and produce the highest crop yield with the minimum amount of irrigated water, can be achieved through a number of approaches, e.g. deficit irrigation (DI) (FAO, 2002; Fereres and Soriano, 2007) and through covering the soil with plant residues or mulching (Edwards *et al.,* 2000; McIntyre *et al.*, 2000). All these practices are, in fact, aimed at minimizing evaporation (E) while maximizing the transpiration (T) components of total plant evapotranspiration (ET). To develop technological approaches for improving the WUE of a crop, it is therefore important to know the contributions of the E and/or T components to the ET of a crop, particularly in areas where water resources are scarce.

Currently, Viet Nam is the second biggest coffee exporter in the world. In 2011, 1.2 million metric tons (t) of coffee beans were exported, valued at $US2.7 billion on the London coffee market (VCA, 2011). Coffee is cultivated mainly on clay soil on the Tay Nguyen Plateau in the central part of Viet Nam, with a total area of 290 000 ha, at an elevation of 600–650 m above sea level (asl). Average air temperature is (23 ± 7)°C and rainfall is (2000 ± 120) mm but not evenly distributed over the year (NMB, 2011). Over the last few decades the climate in the country has become increasingly variable, e.g. extreme events such as heavier rain in the rainy season and typhoons from the Pacific Ocean hitting the country with unusual trajectories compared with those in the past, and have become more frequent (Ngu and Hieu, 2009). During the rainy season (April–August), rainfall over the Tay Nguyen Plateau is usually heavy, while during the dry season (September–March) there is almost no rain, requiring farmers to irrigate their coffee crop often during this period. The irrigation practice used by local farmers is furrow irrigation (FI). This has a low WUE, causing losses of soil and nutrients due to erosion and percolation of nutrients into the deeper soil profile and threatening groundwater quality deterioration.

The aim of the work reported here was to investigate cultivation practices that could improve the WUE of coffee plants, i.e. maximizing T while minimizing the E component of the crop. The investigations involved the following studies: (i) using an isotopic technique to separate E and T from the total ET of coffee plantation at different stages of their development cycle (mature, bean development, bean formation, flowering and bud development, and (ii) comparison of WUE of coffee plantations under drip irrigation (DrI) with mulch and that under the traditional furrow irrigation (FI) without mulch. The first study was to evaluate at what stage in the coffee development cycle the crop needed water most, i.e. when the T component was the highest. The second study aimed to establish whether DrI with scheduling in combination with mulch (abbreviated DrIS&M) had any advantage over the traditional FI and no mulch practice. It was hoped that the cooperation with the local farmers in conduct-

1 Vietnam Atomic Energy Institute, 59 Ly ThuongKiet, Ha Noi, Viet Nam
2 Institute for Water Resources and Environment, 117, Chua Boc, Ha Noi, Viet Nam
3 International Atomic Energy Agency, Wagramerstrasse 5, PO Box 100, 1400 Vienna, Austria
* E-mail address of the corresponding author: dangducnhan50@gmail.com

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ing this investigation would be the way to prove that application of advanced agronomic practices could improve the WUE of their coffee crop and increase their profit.

To our knowledge this is the first time that investigation of E and T separation and of WUE and agronomic practices that could improve the WUE of coffee plants was conducted in Viet Nam.

EXPERIMENTAL

Location

The experiment was conducted on a coffee plantation located in the town of Dak Ha in the Dak Ha district of Kon Tum Province (14°32'329N, 107°56'893E). The plantation is at an elevation of (630 ± 20) m asl, occupies an area of more than 100 ha and it belongs to several owners. The coffee variety is *Robusta*, planted on a clay soil in rows of 3 m × 3 m. The crop is 10 years old which is the most productive age. The trees were treated in such a way that their canopies just covered each other. The leaf area index (LAI) of the trees was between six and seven. Water used for irrigation was taken from a canal originating from a lake located around 2 km from the plantation. The canal passes under the foothill of the plantation.

Methods

The Keeling Plot method (Wang and Yakir, 2000; Williams *et al*., 2004) was applied to separate E and T from the ET of the coffee trees. This method is based on the assumption that the uptake of water by plant roots occurs without isotope fractionation so that the isotopic composition of the atmospheric moisture above the canopy would be different from that of the moisture within the canopy and from the sources of evapotranspiration. The isotopic mass balance of each component is given as (Yakir and Sternberg, 2000):

$$\delta_{under} = C_{above}(\delta_{above} - \delta_{ET})\frac{1}{C_{under}} + \delta_{ET} \quad (1)$$

where δ_{under}, δ_{above}, and δ_{ET} are the isotopic compositions of either deuterium (^2H) or oxygen-18 (^{18}O) in ‰ in the air moisture under, above the canopy, and in the atmospheric moisture or evapotranspiration, respectively, and C_{under} and C_{above} are the moisture content (mmole/m^3) under and above the canopy layers, respectively.

If the moisture content within the canopy (C_{under}) and its isotopic composition, e.g. of $\delta^{18}O$ (d_{under}) are known then one can construct a graph of δ_{under} *vs* $1/C_{under}$, known as the "Keeling Plot". From this, the value of δ_{ET} can be derived as the intercept of the graph, and the contribution of the T component in percent (F_T, %)) to ET can be calculated as:

$$F_T, \% = \frac{\delta_{ET} - \delta_E}{\delta_T - \delta_E} x100 \quad (2)$$

where δ_E and δ_T denote the isotopic composition of soil evaporation and plant transpiration, respectively. The contribution of the E component to ET will be $F_E = (100 - F_T)$.

The values of δ_E and δ_T were, respectively, the isotopic compositions of $\delta^{18}O$ or δ^2H in soil water within the rooting zone and in the moisture of plant tissue. In this case, plant tissues were the skin of secondary branches of the coffee trees.

Atmospheric moisture along the canopy was collected using cryogenic traps and its isotopic composition (δ^2H and $\delta^{18}O$) was determined in the Laboratory of Isotope Hydrology, Institute for Nuclear Sciences and Technologies (INST) in Ha Noi. The air moisture content along the coffee canopy (C_{under}, mmole/m^3) was determined using a psychometric device that was designed and constructed by INST engineers. Details of the device are described below. In the experiment with mulch, the farmer was instructed to leave all branches and leaves after the canopy reforming stage on the land and not to clean up as is the normal practice. The thickness of the mulch was 5–8 cm.

Scheduling with DrI was based on the assumption that the plant needed to be watered when the refill point (RP) was down to half of the available soil water content (ASWC):

$$RP = 0.5\ ASWC \quad (3)$$

and ASWC was estimated as:

$$ASWC = FC - WP \quad (4)$$

where *FC* is the field capacity and *WP* is the permanent wilting point.

For a clay soil, the recommended ASWC ranges from 10-25 percent (FAO, 1985). The FC of clay soil in the coffee plantation was determined experimentally as 27 percent. The ASWC of the soil and rooting zone of the coffee plants was considered to be 12 percent and 2000 mm, respectively.

The amount of irrigation water in the DrIS was estimated for each watering time so that the trees were watered just enough to reach FC. With FI, it was left to the farmer to manage the timing and amount of water provided according to normal practice. Fertilizers used as well as the fertilization rate for the crop in the experiment were managed also by the farmer himself.

Soil moisture was measured and monitored using a neutron probe (PB-205, Fieldtech, Japan). To compare the WUE of the coffee crop under different management practices, (DrI&M and DrIS& no mulch *versus* FI), the experiment was conducted for three years (2009 to 2011). For each year, the total amount of irrigated water, [I (m^3/ha)] was recorded using water meters. The yield (Y) of the crop (kg/a) harvested during each harvesting season was recorded after processing to bean and air-drying. Irrigation WUE (Howell, 2001) was calculated as:

$$WUE = \frac{Y}{I}\ (kg/m^3) \quad (5)$$

Instrumentation

Air temperature measurement and estimation of atmospheric moisture content

A "multi temperature device" was designed and assembled by engineers of the INST to estimate atmospheric moisture. As the height of the coffee trees was 2.2–2.5 m, the device was constructed with 12 chromel/alumel thermocouples installed at six positions along the canopy, i.e. at ground level 0 cm, 20 cm, 60 cm, 120 cm, 170 cm and 280 cm above the ground. The thermocouples were installed in pairs, i.e. at each sampling position one sensor measured "dry" while the other measured "wet" bulb temperatures. The wet bulbs were mounted on a piece of material immersed in a cup of water. This device functions like a traditional psychrometer used to determine air humidity in meteorological observations. The specific feature of the device is that it allows continuous recording of "dry" and "wet" temperatures as well as over variable time periods, e.g. 30 sec or 1 min etc. To do so, the thermocouples were connected to an electronic circuit that records the electrical signals appearing at the junction. Software was installed in a computer to convert the electrical signals into temperatures in Celsius degrees (°C). The device was calibrated in the Heat & Pressure Laboratory of the Vietnam National Metrological Institute and the accuracy of measurements was ±0.1°C over the range of 15–40°C.

Estimates of atmospheric moisture at each sampling position were based on the "dry" and "wet" temperatures using the Calcula-

tor for the Properties of Moist Air Program (http://www.natmus.dk/cons/tp/atmcalc/atmocalc.htp).

Collection of local meteorological data

The local meteorological data during the time of the experiment was recorded by a mini weather station, Vantage Pro2, supplied by the Davis Inst. Co. (California, USA). The device can record and create graphs of air temperature (^{o}C), relative air humidity (%), solar radiation (W/m), dew point (^{o}C), wind direction, wind speed (m/min), rain rate (mm/h) and total daily rain (mm), atmospheric pressure (mb), potential evapotranspiration ET_0 (mm) etc.

Atmospheric moisture collection

Atmospheric moisture was collected at six positions along the coffee canopy using cryogenic traps. The cooling agent used was liquid nitrogen stored in a Dewar flask covered by a polyurethane foam cap through which six glass tubes with caps were inserted for trapping the moisture. Figure 1 depicts the scheme of one the cryogenic traps used.

The moisture from six sampling positions along the coffee canopy condensed in the bottom of the glass tubes (Figure 1) and was transferred into 1 ml vials that were then tightly capped to avoid evaporation during storage in the field. These samples were analysed for isotopic composition in the laboratory in Ha Noi.

Sampling and extraction of moisture from soil and skin of coffee trees

Surface soil and skin of coffee trees are needed to determine composition of oxygen-18 or deuterium in evaporative ($\delta^{18}O_E$ or $\delta^{2}H_E$) and transpirative water ($\delta^{18}O_T$ or $\delta^{2}H_T$) which will be used to estimate F_T and F_E (Equation 2). Soil was collected at a depth of 20 cm below the surface using a metallic spoon and stored in 10 g capacity vials which were capped tightly to avoid moisture evaporation and then transported to the laboratory for moisture extraction.

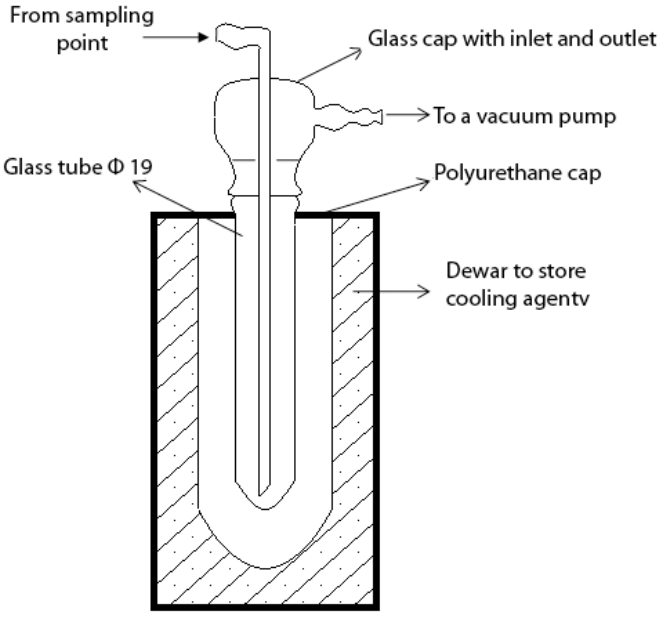


Figure 1. Diagram of a cryogenic trap used to collect atmospheric moisture along the coffee canopy.

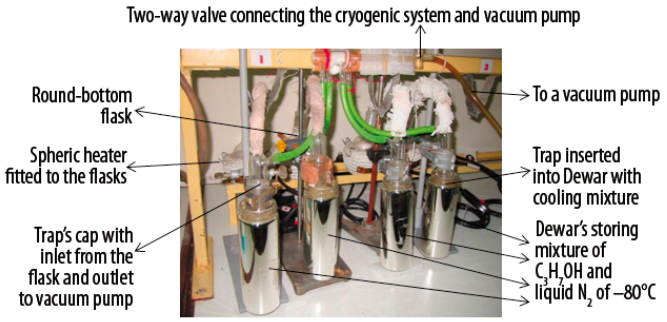


Figure 2. Cryo-distillation line used to extract moisture from soil and plant tissues before isotopic composition analysis.

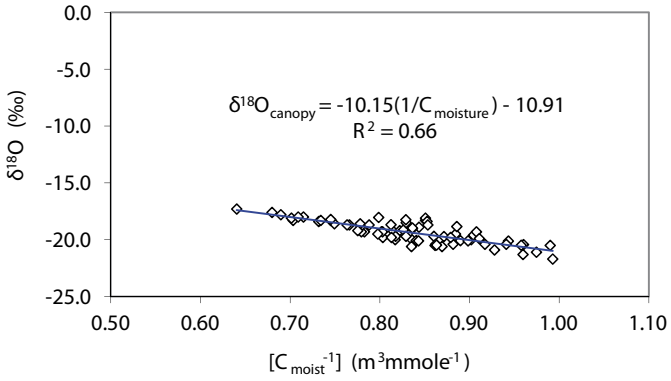


Figure 3. The Keeling Plot derived for 10-year old coffee plantation at the bean development stage with no irrigation.

Skin from secondary branches of the coffee plants was lightly scraped from dead tissue and then removed from the wooden part of the tree using a knife. The weight of the skin samples was around 3–4 g to obtain 0.5–1.0 ml of water after extraction.

Moisture was extracted using a cryogenic trapping technique and the device shown in Figure 2 which consists of a series of round bottom flasks connected to traps inserted in Dewar flasks cooled by a mixture of propane alcohol and liquid nitrogen at –80°C. This was connected to a vacuum pump to exhaust the air moisture inside before heating the flasks to 100°C. The vacuum was maintained at 25 milli bars (mb). The time needed to extract the moisture completely from soil and plant tissues was four hours and it was checked by parallel drying a part of the same samples overnight.

Analysis of isotopic composition of water samples

Water samples were analysed for their isotopic composition ($\delta^{2}H$ and $\delta^{18}O$) using an isotope ratio mass spectrometer supplied by GV Instruments (UK). The facility was equipped with an Elemental Analyzer (Eurovector, Italy) capable of pyrolysis of water into either hydrogen (1 050°C) on the nickel catalyst or carbon monoxide (CO) on glassy carbon (1 250°C), respectively. The precision of the analyses was ±2.0 and ±0.2‰ for $\delta^{2}H$ and $\delta^{18}O$, respectively. The accuracy of the analytical data was verified using Vienna Standard Mean Ocean Water (VSMOW) of the IAEA.

Table 1. Estimates of the $\delta^{18}O$ value of evapotranspiration (δ_{ET}) and the isotope values of plant transpiration (δ_T) and soil evaporation (δ_E) sources, and the corresponding T and E components (FT, FE, %) determined using Equation 2, before irrigation for the three important stages

Stage	$\delta^{18}O_{ET}$, ‰	$\delta^{18}O_{E+}$, ‰	$\delta^{18}O_T$, ‰	F_T, %	F_E, %
Mature and canopy reforming (September–November)	–11.64 (5)	–12.72 (10)	–10.43 (7)	47 (3)	53 (3)
Budding and flowering (December–February)	–9.71 (7)	–11.85 (6)	–10.52 (10)	85 (2)	15 (2)
Bean development (April–August)	–10.91 (3)	–13.85 (5)	–10.34 (5)	84 (2)	16 (2)

Figures in brackets are standard deviations in percent of mean values derived from three experiments during 2009–2011.

Table 2. Estimates of the $\delta^{18}O$ value of evapotranspiration (δ_{ET}) and the isotope values of plant transpiration (δ_T) and soil evaporation (δ_E) sources, and the corresponding T and E components (FT, FE, %) determined using Equation 2, before irrigation during the flowering stage, with and without mulch

Parameter	$\delta^{18}O_{ET}$, ‰	$\delta^{18}O_{E+}$, ‰	$\delta^{18}O_T$, ‰	F_T, %	F_E, %
With mulch	-9.42 (5)	-11.71 (7)	-9.27 (7)	94 (2)	6 (3)
Without mulch	-9.71 (7)	-11.85 (6)	-10.52 (10)	85 (2)	15 (2)

Figures in brackets are standard deviations in percent of the mean values derived from three experiments during 2009–2011.

RESULTS AND DISCUSSION

E and T components of coffee trees at different development stages

Figure 3 depicts the Keeling graph illustrating the relationship between composition of oxygen-18 ($\delta^{18}O$) in the air moisture along the coffee canopy and the reverse moisture content (m^3 $mMole^{-1}$).

The oxygen-18 composition in evapotranspirative moisture ($\delta^{18}O_{ET}$) was –10.91‰ (Figure 3), while the $\delta^{18}O$ of moisture in surface soil ($\delta^{18}O_E$) and from the skin of the plants ($\delta^{18}O_T$) were respectively –13.85 and –10.34‰. These data were used to estimate the contribution of individual T and E components (F_T and F_E) of the plant during the bean development stage (84 percent and 16 percent, respectively, Equation 2). This approach was applied for the coffee plantation at other stages and the results are summarized in Table 1.

During the mature and canopy reforming stages, T was lower (47 ± 3 percent) than E (53 ± 3 percent), while during the budding and flowering stages and bean development it was the reverse, i.e. T was higher than E (Table 1). Comparing E values during the three development stages, it is clear that in the budding and flowering stages following the bean development stage the crop needed more water than at other stages. However, the period of bean development coincides with the rainy season when the soil is saturated and the coffee can be sustained without irrigation. Hence, in order to have high yields of coffee bean, it is vital to irrigate the crop and maintain soil moisture at a level of at least 18–20 percent (Equation 4) during the flowering period.

Table 2 shows the T and E components of coffee trees in the flowering stage before irrigation (February–March) under mulch and no mulch as determined by the isotopic technique. As seen from Table 2 under mulch the T component was higher (94 ± 2 percent) than under the condition of no mulch (85 ± 2 percent), meaning that mulch improved the WUE of the coffee plants. Mulching with crop residues has been proven to be the cheapest way of reducing E since it lowers surface temperature, contributing the improvement of WUE (Hartkamp *et al.*, 2004). Reduction of E and increasing the T component by mulch can be explained by the fact that mulching materials generally reflect more solar radiation and have lower thermal conductivity than soil (Jalota and Prihar, 1998). Mulching with crop residues improves WUE of diverse crops, e.g. maize (Tolk, Howell and Evett, 1999; Deng *et al.*, 2006), wheat (Sun and Wang, 2001), rice (Xu *et al.*, 2007) and tomato (Baye, 2011).

Table 3 presents the variations in the T component of coffee plants during the flowering stage (February–March) under FI and DrIS practices. Uncertainty of the estimates was within 5–7 percent. Drip irrigation with scheduling combined with mulching (DrIS&M) increased the T component of coffee plants by around 10 percent compared with the FI and no mulch practice. However, the T in the DrIS, no mulch practice did not differ significantly, only 4 percent, from that of the traditional FI, no mulch practice (Table 3). This can be explained by the fact that the LAI of the crop was as high as 6–7 and the tree canopies covered each other making solar radiation on the soil surface to be almost the same in both cases. This is supported by the fact that soil surface temperatures in the FI and DrIS (no mulch) during the experiment were within almost the same range (26.8 ± 0.1)°C and (27 ± 0.2)°C, respectively. However, the soil sur-

Table 3. T and E components of coffee plants in the flowering stage under different irrigation practices

Irrigation practice	T (%)	E (%)
FI, no mulch	83	17
DrIS, no mulch	87	13
DrIS&M	94	6

Table 4. Coffee bean yield, amount of irrigated water and water use efficiency under different irrigation practices

Irrigation practices	Y (kg/ha)	Irrigation water, I (m^3/ha)	WUE (kg/m^3)
FI, no mulch	3550	1995	1.78
DrIS, no mulch	3630	1882	1.93
DrIS&M	3800	1784	2.13

face with FI was wetter than with DrI, suggesting that water was lost in the former mainly through runoff and/or infiltration.

Water use efficiency of coffee plants under furrow (no mulch) and drip irrigation with mulch

The data used to estimate the WUE of coffee under different irrigation practices based on Equation 5 are shown in Table 4. The WUE of coffee plants under DrIS&M was the highest (2.13 kg/m^3), and the lowest under FI, no mulch (1.78 kg/m^3). Drip irrigation without mulch improved WUE by 8.4 percent, but DrIS&M improved by 19.7 percent compared with value under FI and no mulch. The lower yield of coffee bean under furrow irrigation and the lowest WUE of the crop might be due to furrow irrigation making soil within the rooting zone too wet and thereby preventing secondary root development. This would reduce water absorption and nutrient uptake by the plants. Moreover, under wet conditions root rot disease could occur as observed for Chile pepper with *Phytophthora* (Xie *et al.,* 1999) leading to lower crop yields.

As can be seen from Table 4 that if DrIS combined with mulching with plant residues were applied to the total 290 000 ha of coffee on the Tay Nguyen Plateau, 61 million m^3 of water could be saved and local farmers could increase their coffee bean yields by 72 500 tonnes, which, in turn, would be worth US$162 million on the London coffee market ($US2 250 per tonne).

CONCLUSIONS

Over a coffee cropping season the crop needs more water during the flowering stage and therefore supplementary irrigation is required to maintain soil moisture at a level of 18–20 percent, but not higher. Compared with the traditional furrow irrigation practice, drip irrigation combined with mulching plant residues reduced evaporation by almost 10 percent and improved irrigation WUE by up to 20 percent. This allowed local farmers to gain an extra profit amounting to around US$560/ha. Drip irrigation combined with plant residues mulch is advised for all coffee plantations on the Tay Nguyen area.

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Yield and Carbon Isotope Discrimination for Wheat, Barley and Lentil under Different Crop Sequences and Water Treatments in Northern Syria

A. Wahbi[1,*], M.A. Allo[2], A.N. Aldarir[2], A. Arslan[3] and A.S. Al Shaaban[4]

ABSTRACT

Improving water use efficiency is the main challenge in areas where water is the main production-limiting factor. This research was conducted on a research station in northern Syria between 2005 and 2009. The objective was to determine water use efficiency for different crop sequence rotations and the application of different levels of supplementary irrigation. The study used bread wheat (Sham 8 variety), a local lentil variety and barley (Arabic black variety). A randomized complete block design was used, involving six different cropping sequences with three replications and two levels of supplementary irrigation (75 percent and 55 percent of 90 percent of field capacity) and rain-fed as control. Soil water content was monitored using a neutron probe, and evapotranspiration was calculated. Grain samples (for the season 2007–2008 and for the two water treatments only) were used for measurement of grain carbon isotope discrimination (CID, Δ). Results showed that water treatments had significant (at 0.1 percent level) effects with yields increasing (in terms of grain and total biomass) with increasing water use efficiency (WUE) as more water became available. Carbon isotope discrimination, as an indirect measurement of transpiration efficiency, differed for different crops, with lentil having the highest range followed by barley and wheat. This suggests that lentil has a high elasticity in terms of transpiration efficiency compared with cereals. Positive relationships between Δ and yield were obtained, but more important were the slopes of the relationship between yield and CID (kg of yield per unit of CID) for the three crop species, with highest values being obtained for wheat and lowest for lentil. A simulation model for 19 yr of growing barley and wheat showed that there is a higher tendency for shortage of soil water, reaching permanent wilting point in the months of April and May for wheat compared with barley.

1 Department of Soil and Land Reclamation, Faculty of Agriculture, University of Aleppo, Syria
2 Department of Rural Engineering Faculty of Agriculture, University of Aleppo, Syria
3 Natural Resources, General Commission for Scientific Research (GCSAR), Ministry of Agriculture and Agrarian Reform, Syria
4 Department of Field Crops, Faculty of Agriculture, University of Aleppo, Syria
* E-mail address of corresponding author: wahbi@scs-net.org

Key words: *wheat, lentil, barley, carbon isotope discrimination, supplementary irrigation, Syria.*

INTRODUCTION

Crop production in Mediterranean-type environments is invariably limited by low and erratic rainfall (200–500 mm/yr). Consequently, a major challenge is to devise cropping systems that maximize water use efficiency (WUE) (Deng *et al.,* 2005). Long-term weather data (over 30 yr) show that heavy rain showers (reaching 30–40 mm/d) followed by long periods of no rain cause soil erosion and water runoff, thereby increasing the risk of drought for crops in Syria (Wahbi, unpublished). Also, field experiments on a research station in northern Syria showed that soil evaporation varied as the season advanced and accounted for up to 70 percent of total evapotranspiration for barley despite optimum management (Wahbi, 1986).

Water productivity can be enhanced by manipulating the balance between the two components of water use, i.e. transpiration by crops and evaporation from the soil surface, through agronomic management and germplasm modification (Harris, 1994). For example, the use of different crop sequences could improve the use of nutrients and water resources. Legume crops play an important role in improving the sustainability of rain-fed dryland farming systems. Grain legumes conserve the soil, add organic matter, fix nitrogen (N), save soil N, and help in controlling cereal diseases (Diaz-Ambrona and Miniguez, 2001; Papastylianou, 1993). However, legumes are highly affected by water stress during the early and reproductive stages of growth (Leport *et al.,* 2003; Liu, Jensen and Andersen, 2003 and 2004), and replacing fallow by legumes in dryland farming systems can deplete soil water that could otherwise be available to the following cereal in the traditional wheat-fallow system. Therefore, any decision to introduce legumes or other annual crops into a rotation system either to reduce fallow or to break continuous cereal cropping must examine their potential effect on the efficiency of rain water use and system productivity. In addition, the many factors that influence WUE (Josh and Singh, 1994) must be considered. For instance, in a long-term trial in northern Syria, Pala *et al.* (2007) compared the effects of seven wheat-based rotations on soil water dynamics and WUE during both the wheat and non-wheat phases. On a system basis, the wheat-lentil or wheat-vetch systems were most efficient at using rainfall, producing 27 per cent more grain than the wheat-fallow system, while the wheat-chickpea system was as efficient as wheat-fallow system, and continuous wheat cropping was the least efficient. With N added to the cereal phase, the WUE of the

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system increased, but was still least for continuous wheat and greatest for wheat-lentil rotations. Wheat-legume rotation systems with additional N input during the wheat phase can therefore maintain sustainable production systems, but also are more efficient in utilizing the limited rainfall.

Many researchers (e.g. Condon, Richards and Farquhar, 1987; Farquhar, Ehleringer and Hubick, 1989) have suggested the use of carbon isotope discrimination (CID, $D^{13}C$ or Δ values) of leaf as an indirect measure of transpiration efficiency (TE, which is WUE at the leaf level) in water-limited environments. Ample information is available from CID analysis of plants about the relationship between TE, WUE and yield under different water regimes (e.g. Hall *et al*., 1994). However, results are inconsistent concerning the association between CID and yield for different crops and different growing conditions. (Hall *et al.,* 1994; Ngugi, Galwey and Austin, 1994 and 1996; Specht *et al*., 2001; Saranga *et al*., 2004; Monneveux *et al.,* 2007). They range from no relationship through to negative or positive relationships depending on the crop and the environment. Nevertheless, in Mediterranean environments positive relationships between the two were evident in many studies (Acevedo, 1991; Nachit, 1998; Merah *et al.,* 2001).

Genotypic variation in WUE under limited water regimes is affected more by variations in water use (WU) rather than by variations in biomass (Blum, 2005). This has also been determined for TE and stomatal conductance at the single leaf level (e.g. Juenger *et al*., 2005; Monclus *et al*., 2006; Monneveux *et al*., 2006). Hence, selection for high WUE under limited water supply tends to result in a genetic shift towards plant traits that limit crop WU (Ngugi, Galwey and Austin, 1994; Menendez and Hall, 1995). The successful and widely-cited case of dryland wheat grain yield improvement through selection for high WUE (low CID) in New South Wales, Australia (Condon *et al*., 2002) can be explained by the fact that wheat grown there mainly relied on stored soil moisture.

A major avenue for yield improvement is the control of WU during the early growing season in order to avoid the lack of soil moisture during reproduction. This was attempted by selecting for reduced root xylem diameter (Richards and Passioura, 1989), but it can also be achieved by reducing leaf area and growth duration as carried out for sorghum grown under stored soil moisture conditions (Blum, 1970 and 1972; Blum and Naveh, 1976). Such plants optimize seasonal soil moisture use and achieved high WUE for grain yield due to their relatively moderate WU and high harvest index (HI). Yet, the same genetic materials selected for high WUE were not successful in Western Australia where the rainfed wheat was not grown on stored soil moisture (Condon *et al.,* 2002).

Considering all of the above, it is not surprising that drought resistance is associated with low WUE when analysed by CID under limited water supply (e.g. Ngugi *et al*., 1994; Araus *et al*., 2003; Solomon and Labuschagne, 2004). More recently, a drought-resistant Robusta coffee (C*offea canephora*) cultivar was found to have relatively lower WUE than a drought-susceptible cultivar, due presumably to greater WU associated with its deeper roots (Pinheiro *et al.,* 2005).

Finally, crop WUE has long been known to increase with increasing water stress (e.g. Meyers *et al.,* 1984), and this has been confirmed more recently by measurements of CID (Ismail, Hall and Bray, 1994; Craufurd *et al*., 1999; Li *et al.,* 2000; Peuke, Gessler and Rennenberg, 2006). Assuming, therefore, that two different cultivars are planted side by side and exposed to drought, the cultivar with the higher WUE is likely to be relatively more stressed and at a lower plant water status than the drought susceptible one. In this regard, Blum (2009) argued that effective water use should be a major target for yield improvement in water-limited environments instead of WUE.

Supplementary irrigation is used in arid zones by applying small amounts of water during period of water stress to winter crops under rain-fed conditions (Oweis, Pala and Ryan, 1998). Deficit irrigation, i.e. irrigation with amounts less than required for full irrigation, may reduce water use by up to 50 percent of the amount used under full irrigation without reducing crop yield. Water productivity increases under deficit irrigation relative to full irrigation, as shown experimentally for many crops (Zwart and Bastiaanssen, 2004; Fereres and Soriano, 2007; Fan *et al.,* 2008). Also, water saving irrigation strategies such as regulated deficit irrigation can be used to irrigate only in drought-sensitive growing stages.

Supplementary irrigation of wheat in the Mediterranean region of Turkey increased grain yield by about 60 per cent depending on the level of rainfall and its distribution during the wheat growing season (November through May) (Sezen and Yazar, 1996). Concerning legumes, supplemental irrigation during the early and reproductive stages of their growth will reduce pod abortion and is expected to have a significant impact on final yield. The objective of this study was therefore to determine the effect of legumes on WUE in crop sequence rotations with wheat and barley by applying different levels of supplementary irrigation.

MATERIALS AND METHODS

Site

The research was conducted at the Makasem 5 Research Station in Hassakeh Province of northern Syria over four seasons started in 2005/2006. It is located in the Alkhabour basin, 8 km west of Hassakeh city, 36.5°N and 40.75°E. Long-term annual rainfall is around 272 mm during wet years and less than 150 mm in dry years. The irrigation water resource is ground water, with total salinity of about 3.5 dS/m.

Experimental design

Bread wheat (Sham 8), local lentil and barley (Arabic black) were used in the experiment. A randomized complete block design was used, with six different cropping sequences (wheat-wheat, wheat-lentil, barley-barley, barley-lentil, lentil-wheat, and lentil-barley) with three replications and two levels of supplementary irrigation (75 percent and 55 percent) of 90 percent of field capacity and rain-fed (as control treatment). There were 54 plots, the area of each irrigated plot being 164 m^2 (9 m x 18 m), whereas the rain-fed plot was 48 m^2 (6 m x 8 m).

Soil

Disturbed soil samples were taken before planting at increments of 15 cm to a depth of 105 cm. Samples were analysed to determine: pH and electrical conductivity (1:5); organic matter (%) using potassium di-chromate; Olsen-P; and available potassium (K) using wet digestion and atomic absorption spectrometry. Particle size analysis (hydrometer method) was used to determine silt, clay and sand percentages. Also the soil hydro-physical characteristics like field capacity, wilting point and total porosity were determined at the pre-planting stage.

Climate

Daily weather data minimum and maximum air temperatures, relative humidity, rainfall, and open pan evaporation were recorded throughout the experiment.

Agricultural practices

A local seeding machine was used with seeding rates of 200, 130, and 120 kg/ha for wheat, barley and lentil respectively and a row spacing of 20 cm. The amounts of fertilizer were calculated based on soil analysis before seeding.

Measurements

Soil moisture

Aluminium tubes were installed in each plot to a depth of 165 cm. A neutron probe (TROXLER-4300 type) was calibrated and used to monitor soil moisture at 15 cm increments throughout the season at about two-weekly intervals. Evapotranspiration (ET) was calculated using a standard soil water balance equation:

$$ET = AS + P + I - Dr$$

where *AS* is the change of soil water storage between the two neutron probe readings; *P* is precipitation (mm); *I* is the amount of irrigation (mm); and *Dr* is the drainage from the root zone. Since there was no drainage below the measured soil depth during the crop-growing seasons, Dr was set as zero. No surface runoff occurred throughout the seasons.

The neutron probe readings were used also to schedule irrigation of the 0–30 cm soil layer for the first crop period (from planting to tillering for wheat and barley, and to flowering for lentil), and of the 0–60 cm soil layer for the second crop period. Irrigation during the first crop period was scheduled when the volumetric soil water content reached about 23 percent and 17 percent for water treatments of 75 percent and 55 percent respectively, and about 22 percent and 16 percent for the second crop period.

Plant measurements

Grain and biological yields were determined by sampling at ground level, drying at 70°C and weighing. WUE was determined by dividing grain yield by evapotranspiration values.

Carbon isotope discrimination

Grain samples for the season 2007–2008 and for the two supplementary irrigation treatments only (since no grain yield was obtained for the rain-fed treatment) were dried and finely grounded for delta grain (ΔG) analysis at the International Atomic Energy Agency (IAEA) Laboratories, Seibersdorf.

Simulation model

Weather data from 19 years (1990–2009) were used to simulate crop production, water use (soil evaporation and plant transpiration), and the fraction of extractable soil water (FTSW) throughout the growing season. This last index is very important, since it indicates shortage of soil water when FTSW is below 0.1 and the frequency of this will indicate the need for either irrigation or a change of crop or management practice (Wahbi and Sinclair, 2007). The simulation model employed here had previously demonstrated close agreements between the measured and simulated biomass yields and evapotranspiration of barley and wheat crops with R^2 values greater than 80 percent (Wahbi and Sinclair, 2005).

Statistical analysis

Analysis of variance was done using GenStat V12 and the means compared by using least significant differences LSD at the level of five percent.

RESULTS AND DISCUSSION

Rainfall varied between seasons, being 104 mm in the 2006–2007 season and lower (less than 100 mm) during the 2007–2008 and 2008–2009 seasons; with an open class A pan evaporation reading reaching above 400 mm together with high temperatures, the crop failed on the rain-fed plot.

The soil was clayey at all depths (clay above 40 percent) except two layers (30–45 cm and 45–60 cm) which were loamy clayey (clay 34 percent, with high sand). Field capacity was 31.3–33.9 percent, electric conductivity was around 2.24 dS/m in most layers except for 0–15 cm which was 3.17 dS/m. Organic matter and Olsen-P were low, and K content was high to moderate.

Grain yield

In the rain-fed (control) treatment, yield was very low in the 2006–2007 season and in the next two seasons the crop failed. Overall, crop yield of 216, 1 342 and 2 603 kg/ha were obtained for the rain-fed, irrigation at 55 percent and at 75 percent, respectively. The influence of crop sequence was not clear, but there was a tendency for higher yields after lentil compared with continuous wheat or barley, and for lentil when barley was the preceding crop compared with wheat (data not shown).

Water use efficiency

Water use efficiency differed between seasons, but more important were the differences associated with irrigation treatments, crops, years and crop sequences, with WUE values being 1.4, 6.0 and 9.3 $kg \cdot ha^{-1} \cdot mm^{-1}$ for the rain-fed, 55 percent and 75 percent irrigation, respectively.

Crop failure occurred under rain-fed conditions during two seasons (2007–2008 and 2008–2009), and WUE was very low in 2006–2007 (no more than 1.8 $kg \cdot ha^{-1} \cdot mm^{-1}$). The WUEs associated with the 55 percent irrigation regime were 5.2, 6.2, and 8.0 $kg \cdot ha^{-1} \cdot mm^{-1}$ for the years 2006–2007, 2007–2008, and 2008–2009, respectively; with 75 percent irrigation, efficiencies were higher and progressively greater (7.6, 9.3 and 13.2 $kg \cdot ha^{-1} \cdot mm^{-1}$) between 2006–2007, 2007–2008, and 2008–2009, respectively (Table 1).

Carbon isotope discrimination (CID)

Carbon isotope discrimination values varied between 16.22‰ and 17.32‰ for barley in the irrigated blocks (55 percent treatment) and between 15.89‰ and18.02‰ under 75 percent treatment. For wheat the values varied between 16.76‰ and17.77‰ and between 17.84‰ and 18.18‰, respectively for the 55 percent and 75 percent treatments. For lentil, values were between 16.18‰ and16.87‰, and between 17.63‰ and19.28‰, respectively for the 55 percent and 75 percent treatments (Table 2). Carbon isotope discrimination values were slightly higher under the 75 percent treatments in all crops. Table 2 showed that lentil had the highest range which could mean more adaptable to irrigation conditions in this crop compared with wheat or barley.

Values for CID differed significantly between crops under 55 percent irrigation ($p < 0.01$) and also at 75 percent treatment ($p < 0.05$). Average CID values for the 55 percent treatment were 17.36‰, 16.62‰ and 16.45‰ for wheat, barley and lentil, respectively (Table 2). For the 75 percent treatment, CID values were 18.36‰, 17.98‰ and 17.29‰, respectively for wheat, lentil and barley.

TABLE 1. Water use efficiency for grain yield (kg·ha^{-1}·mm^{-1}) for three irrigation treatments and six crops sequences and three seasons (2006–2007; 2007–2008; and 2008–2009) at Makasim 5 research station

Season	Water Regimes	WUE for grain yield (kg·ha^{-1}·mm^{-1})		
		Barley	Lentil	Wheat
2006–2007	Rain-fed	1.54	3.23	0.72
	55%	5.09	7.56	2.97
	75%	8.16	10.58	3.94
2007–2008	Rain-fed	0	0	0
	55%	8.52	4.51	5.51
	75%	11.80	7.50	8.72
2008–2009	Rain-fed	0	0	0
	55%	7.96	11.13	4.84
	75%	13.75	15.54	10.25

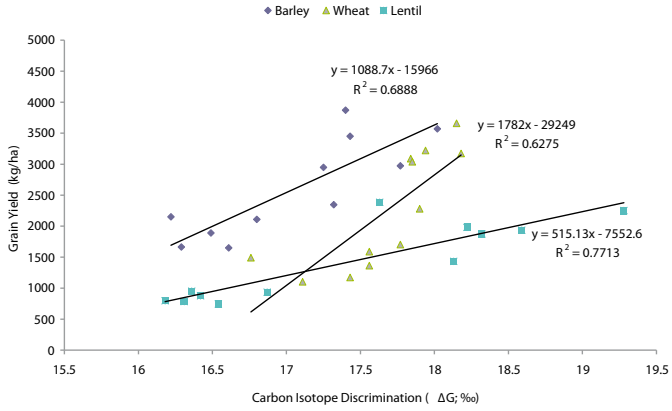


FIGURE 1. Relationships between grain yield (kg/ha) and ΔG (‰) for three crops in 2007–2008.

TABLE 2. Carbon isotope discrimination values (‰) for crops irrigated at 55 percent and 75 percent during 2007–2008

Crop	55 %	75 %
Barley	16.62	17.29
Wheat	17.37	18.36
Lentil	16.45	17.98
Fpr.	0.007 **	0.017 *
SED	0.239	0.303
LSD	0.532	0.676
CV (%)	2.5	2.9

Correlations and relationships

The relationship between grain yield and CID at 55 percent water treatment was positive but with a low R^2 value; a slightly better relationship was found at 75 percent water treatment (not shown). This indicates a higher efficiency for the increase in grain yield when CID increases at the higher water treatment (higher slope of the linear relationship) compared with the lower water treatment. However, the highest slope was found for wheat (1 782 kg/ha unit of CID), compared with 1088 kg/ha unit of CID and 515 kg/ha unit of CID for barley and lentil respectively (Figure 1).

Simulation model

Results of simulating the relationship between the fraction of transpired soil water (FTSW) and days after sowing of wheat and barley are shown in Figure 2. Wheat had the lower FTSW values indicating that barley was less exposed to drought than wheat in this area. Moreover, FTSW values for wheat were below 0.1 at about 108 d after sowing (early flowering), whereas for barley the time when FTSW values were below 0.1 was between 120 and 130 d after sowing (i.e. a period of only 10 d). Hence, application of supplementary irrigation at this time would be the most advantageous and would

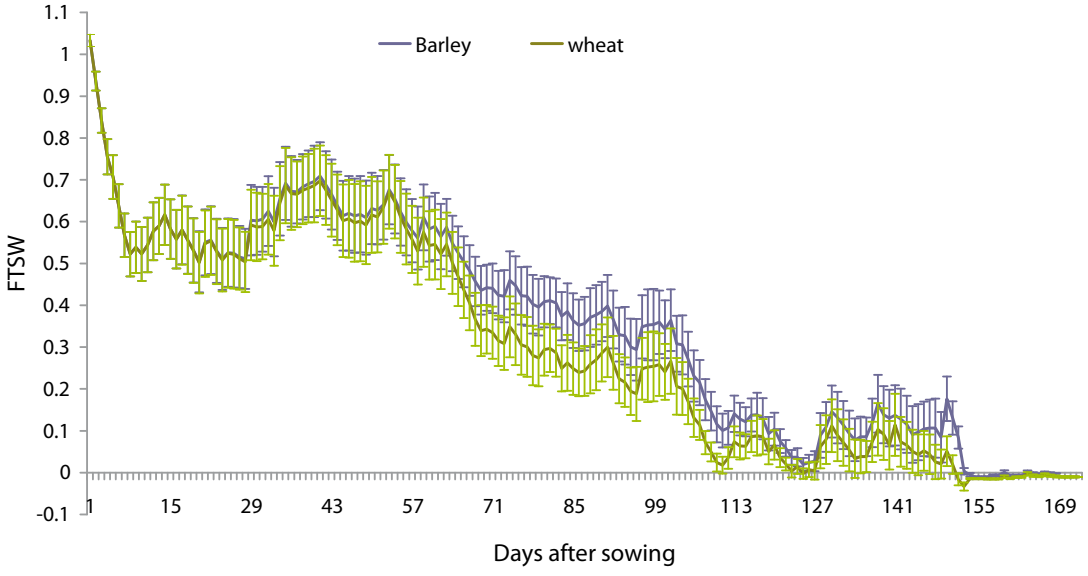


FIGURE 2. Relationships between average fraction of extractable soil water (FTSW) for barley and wheat and d after planting at Makasim 5 station. (simulation of 19 yr' data [1990-2009]; bars indicate standard error).

prevent crop death. Also, over the 19 yr of record-keeping, FTSW values fell below 0.1 on 42 and 28 d, respectively for wheat and barley. Most important, however, was the timing of the drought (below 0. 1 of FTSW, where water is not available for plants), and where barley was only severely affected from about 121–127 d after sowing whilst wheat was subjected to two severe periods of drought (at between 111 and 113 d and between 121 and 128 d after sowing) (Figure 2).

CONCLUSIONS

Irrigation had significant (at 0.1 percent level) effects on WUE and crop yields (in terms of grain and total biomass) which increased with increasing WUE. Values for CID differed between crops, with lentil having the highest range followed by barley and wheat. This suggests that lentil has a high elasticity in terms of transpiration efficiency compared with cereals. Positive relationships between Δ and yield were obtained, but more important were the slopes of the relationship between yield and CID (kg of yield per unit of CID) for the three crop species, with highest values being obtained for wheat and lowest for lentil. A simulation model can be used as a decision-support tool for crop selection under particular circumstances. Simulated results from 19 yr of growing barley and wheat showed that the tendency for soil water shortage to reach permanent wilting point during the months of April and May is higher for wheat compared with barley. This suggests using barley instead of wheat if no irrigation is available.

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Evaluating Water Stress in Wheat Using Carbon Isotope Discrimination and other Crop Physiological Indices in the Central Anatolia Region of Turkey

S. Kale[1,*], B. Sonmez[2], S. Madenoglu[2], K. Avag[2], G. Caycı[3], C. Kutuk[3] and U. Turker[4]

ABSTRACT

A study was carried out between 2009 and 2011 to evaluate the effect of different irrigation regimes on the carbon isotope discrimination ($\Delta^{13}C$) in leaf and grain samples, and the crop water stress index (CWSI), as indicators of water stress for wheat crop. The objective was to determine the relationship between CWSI and $\Delta^{13}C$ under field conditions and to calculate water-use efficiency (WUE [grain yield per unit evapotranspiration (ET)]) for wheat under different irrigation regimes (no irrigation, full irrigation, moderate and high water stress). The experiments were conducted at the Saraykoy Research Station in the Ankara-Murted Basin. Soil water content was measured with a neutron probe; while wheat canopy temperature and stomatal conductance were measured using infrared thermometer and porometer, respectively. The results suggest that water stress affects canopy temperature and stomatal resistance of the crop. Average canopy temperature was inversely correlated with stomatal conductance and can be used as indicators of stomatal closure in response to soil water deficit. The CWSI which is an index based on canopy surface temperature, is a promising tool for quantifying crop water stress. Carbon isotope discrimination values showed that grain and leaf $\Delta^{13}C$ can be used for selecting high wheat grain yield under water-limited conditions. Irrigating after-heading period is recommended to increase the WUE of wheat in the central Anatolia region of Turkey.

Key words: *carbon isotope ratio, wheat, stomatal conductance, water use efficiency (WUE), crop water stress index (CWSI).*

INTRODUCTION

In Turkey, particularly in the Central Anatolia region, lack of rainfall, drought and water scarcity are major climatic factors affecting crop production. The most important water management challenge for crop production in these arid and semi-arid regions is to use the limited water supply efficiently to maximize the yield per unit of water use. To achieve this, (i) it is necessary to know the effect of water use on the crop yield and to improve crop water productivity (to increase the marketable crop yield per unit water received by the crop), (ii) to reduce water losses from the crop root zone, and (iii) to increase soil water storage in the crop root zone by means of soil and water management practices.

For these reasons, cost effective and robust methods which can be used to reveal the effects of water stress on growth are needed during the crop growth period. The carbon isotopic technique has been shown to be useful for evaluating crop yield response to water stress and water use efficiency (Condon, Richards and Farquhar, 1987; Araus *et al.*, 1998). The ratio of the abundances of carbon-13 (^{13}C) and carbon-12 (^{12}C) or carbon isotope discrimination ($\Delta^{13}C$), can play an important role in the selection of drought-resistant crop species and breeding studies.

Similarly, canopy surface temperature measurements with infrared thermometers and other remote infrared sensors can be an important tool for detecting crop water stress. Crop water stress index (CWSI) is derived from canopy-air temperature differences versus the air vapor pressure deficit, was found to be a promising parameter for quantifying crop water stress (Jackson *et al.*, 1981; Idso and Reginato, 1982; Jackson, 1982). Gontia and Tiwari (2008) used the CWSI of wheat to schedule irrigation using infrared thermometry and Yuan *et al.* (2004) evaluated the application of three different forms of CWSI for monitoring water stress of winter wheat the North China Plain (NCP).

The response of crops to drought stress has also been assessed using stomatal conductance (Ashraf and Oleary, 1996; Kusvuran, 2012). During the early growth stage of wheat, stomatal conductance has been shown to be correlated positively with grain yield, grain numbers per spike, spike yield and spike length (Bahar, Yildirim and Baratcular, 2009).

The objective of this study is to evaluate the relationship between CWSI and $\Delta^{13}C$ for wheat grown under different water stress conditions and determine the respective WUE in the Central Anatolia Region of Turkey.

1 Department of Agricultural Structure and Irrigation, Agricultural Faculty of Suleyman Demirel University, East Campus, 32260, Isparta, Turkey
2 Soil, Fertilizer and Water Resources Research Institute of Ministry of Food and Agriculture, 5th km of Istanbul Road, 06172, Ankara, Turkey
3 Department of Soil Science, Agricultural Faculty of Ankara University, 06110, Ankara, Turkey
4 Department of Agricultural Machinery, Agricultural Faculty of Ankara University, 06110, Ankara, Turkey
* E-mail address of corresponding author: semakale@sdu.edu.tr

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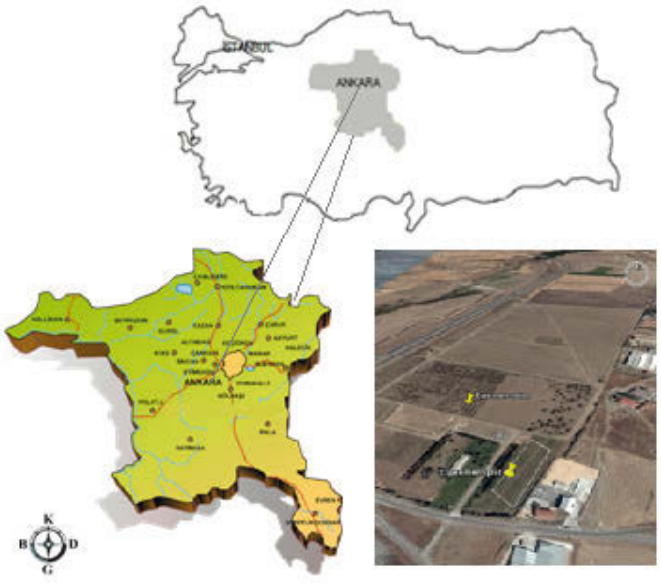


FIGURE 1. Experimental sites in the Ankara Murted Basin in the central Anatolia region of Turkey.

MATERIALS AND METHODS

Experimental site characteristics

Experimental sites were located in the Ankara Murted Basin (39° 57′ N and 32o 53′ E) of the Central Anatolia, Turkey (Figure 1). A field experiment was conducted to demonstrate the effect of water stress on yield and agronomic characteristics of wheat under different irrigation treatments during the period from October 2009 to July 2011 at the Research Farm Station of the Soil, Fertilizer and Water Resources Central Research Institute in Ankara, Turkey.

The soil of the experimental location ranges in texture from silty clay in the top 0.30 m followed by a layer of clay texture roughly 1.5 m deep. Field capacities (FC) and wilting points for different soil depths are provided in Table 1.

The climate in this region is characterized as semi-arid. In the Ankara-Murted basin, temperature differences between night and day and summer and winter are large, and rainfall is relatively infrequent. Winters are long and cold with heavy snowfall while summers are short and hot. The wettest months are November and May. Almost no effective rain falls during the summer. Annual rainfall is about 350 mm and evaporation is 1 300 mm as an average for the past 30 years.

Wheat and barley are the most important crops in the region, but yields are irregular, and crops often fail in drought years. Most of the wheat is planted in late fall, as soon as there is significant moisture for seeding. In this study, the Bayraktar wheat variety was used as the trial crop and seeds were obtained from National Seeds Research Institute.

Surface irrigation was used in the study. The electrical conductivity of irrigated water was 1.76 dS/m.

Crop management and experimental design

The experiment consisted of four irrigation regimes with four replications, giving a total of 16 plots.

I_1 — No irrigation (Rain-fed)

I_2 — Full irrigation (water content was brought to field capacity after planting, and irrigated when calculated soil water depletion was 60 mm below FC)

I_3 — Limited irrigation (two irrigations maximum) one at tillering and another at grain filling

I_4 — No irrigation after establishment until heading, after which irrigation was applied when soil water depletion was 60 mm below FC.

Plot dimensions were 3.5 m × 5 m = 17.5 m^2 for seeding and 1.2 m × 4 m = 4.8 m^2 for harvesting. According to results of soil fertility analysis between 2008 and 2012 growing seasons, commercial N fertilizers were applied in bands about 10 cm to the side of the seed row (220 kg/ha ammonium sulphate were applied before sowing and a further 350 kg/ha were applied around middle of March of each year). Sufficient phosphates were applied (175 kg/ha DAP) to ensure adequate P nutrition.

Precipitation, air temperature (maximum, minimum and average), class A pan evaporation, wind speed, relative humidity, global radiation and sunshine hours were obtained hourly from a meteorological station situated 50 m from the experimental site.

Soil water content in the treatment plots was monitored using a neutron probe (CPN) with aluminum access tubes. The measurements were taken at 0–20, 20–40, 40–60 and 60–90 cm soil depths. The neutron probe measurements were made twice weekly at all the aforementioned depths. The neutron probe was calibrated annually before plot installation.

Crop water deficit was also monitored using a Fluke 66 model infrared thermometer. The crop canopy temperature measurements were taken at least 12 times from each treatment. Measurements were taken between 13.00 and 14.00 hours (h) on cloudless days. Canopy temperatures were used to determine crop water stress index (CWSI) which was computed using the method suggested by Idso *et al.* (1981):

$$CWSI = \frac{(Tc - Ta) - (Tc - Ta)_{LL}}{(Tc - Ta)_{UL} - (Tc - Ta)_{LL}}$$

where T_c is canopy temperature (°C); T_a is air temperature (°C); $(T_c–T_a)_{LL}$ is lower limit of canopy-air temperature difference; and $(T_c–Ta)_{UL}$ is upper limit of canopy-air temperature difference. The differences $(T_c–T_a)_{LL}$ were obtained from the linear regression for the crop under no water stress and $(T_c–T_a)_{UL}$ when the crop was under maximum water stress. The non-stressed baseline, $(T_c–T_a)$ versus vapour pressure deficit (lower limit) relationship was determined using data collected from the full irrigation (I_2) treatment. On the other hand, the fully stressed baseline (upper limit) was computed according to the method provided by Idso *et al.* (1981).

TABLE 1. Some physical properties of the soil

Depth (cm)	Texture	Bulk density (g/cm)	Field capacity (%)	Wilting point (%)
0–30	SiC	1.15	34.9	17.0
30–60	C	1.21	36.3	22.1
60–90	C	1.25	37.0	21.2

TABLE 2. The dates and amount of irrigation water applied for the various treatments

Irrigation water amounts (mm)									
2009–2010					2010–2011				
Date	I_1	I_2	I_3	I_4	Date	I_1	I_2	I_3	I_4
28.10.09	—	90	—	—	25.10.10	—	85	—	—
29.04.10	—	60	60	—	25.04.11	—	—	56	—
20.05.10	—	60	—	102	12.05.11	—	60	—	60
15.06.10	—	60	111	60	14.06.11	—	60	60	60
TOTAL	0	270	171	162	TOTAL	0	205	116	120

A Decagon SC-1 leaf porometer was used to measure leaf stomatal resistance. The porometer measures stomatal aperture in terms of leaf conductance to water vapour. This is a major determinant of water loss from plant leaves and of CO_2 uptake in photosynthesis.

For isotopic measurements; 10–20 south-facing sun leaves of five marked plants per treatment were collected once at the stage of pre-anthesis (pre-emergence, second week of May). Only fully mature leaves from the latest growth period were used. Leaves were oven-dried at 60°C for 48 h and milled to a fine powder. Similarly grain samples after harvesting were collected for carbon isotopic analysis, carried out at the IAEA Seibersdorf Laboratory, Austria using isotope ratio mass spectrometer.

Carbon isotope ratios ($^{13}C/^{12}C$) of the samples ($^{13}C/^{12}C_{sample}$) and the standard ($^{13}C/^{12}C_{standard}$) values were converted into $\delta^{13}C$ (‰; per mil) using:

$$\delta^{13}C\ (‰) = \frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{standard}}{(^{13}C/^{12}C)_{standard}} \times 1000$$

Delta carbon-13 ($\delta^{13}C$) values were transformed into the C isotope discrimination/difference (Δ) using the equation developed by Farquhar *et al.* (1982):

$$\Delta(‰) = \frac{\delta^{13}C_a - \delta^{13}C_p}{1 - \delta^{13}C_p /1000}$$

where subscripts a and p represent the isotopic ratios of air and plant, respectively. In the formula, –8‰ was used for air when transforming the $\delta^{13}C$ value into Δ (Keeling, Mock and Tans, 1979).

RESULTS AND DISCUSSION

Irrigation and rainfall

Amounts of water applied to various irrigation treatments and the timing of application are given in Table 2. The total rainfall occurred in the 2009–2010 growth period was 252 mm, and 289 mm for the same growth period in 2010–2011. Due to the higher than usual rainfall, the amount of irrigation applied was much less in 2010–2011 growing season.

The total soil water content over 90 cm depth declined to near wilting point values in the periods towards crop harvest in treatment I_1 (rain-fed) in 2009–2010 but the soil water content in the same treatment remained above wilting point even though no irrigation was applied in 2010–2011 (Figure 2). Soil water in the I_4 treatment did not reach the calculated soil water depletion of 60 mm until 19 June in 2011.

Crop water consumption

Monthly and seasonal crop water consumptions are given in Table 3. They were calculated according to the soil water budget, from changes in water content in the 0–90 cm soil depth. In both seasons, the highest water consumption occurred in the fully irrigated (I_2) treatment and the lowest in the rainfed treatment (I1).

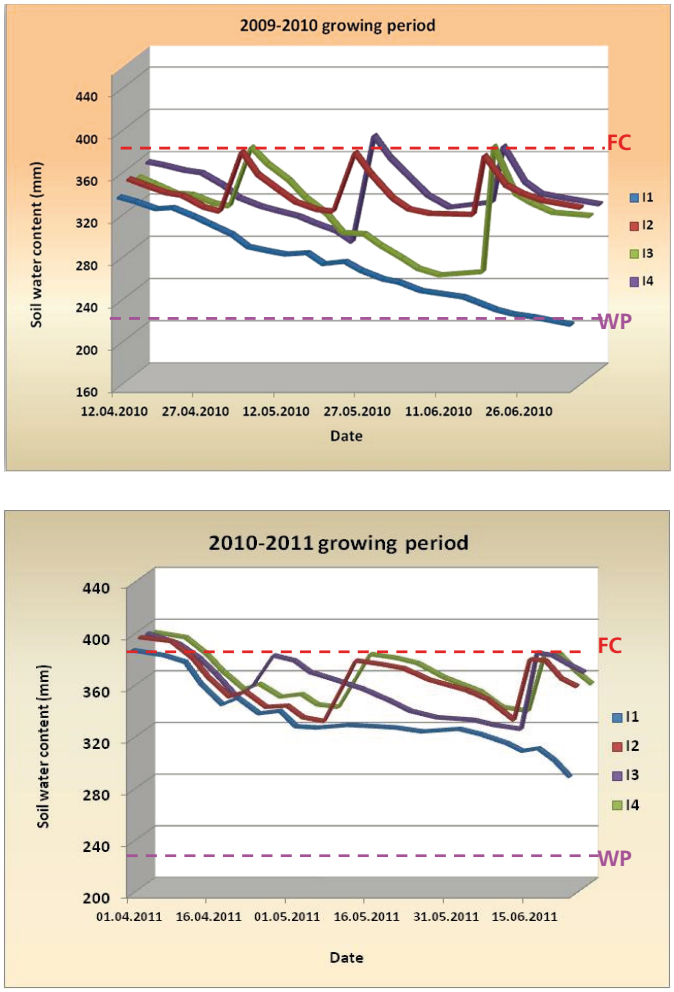


FIGURE 2. Total soil water storage to 90 cm depth during the growth periods 2009–2010 and 2010–2011.

Yield

The highest grain yield in both seasons (4.6 and 4.5 t/ha) was obtained from the fully irrigated (I_2) treatment (Table 4). Yield values for rain-fed (I_1) treatment were in general 23 percent, 15 percent and 19 percent, respectively lower than those from irrigated (I_2, I_3, I_4) treatments. However, there was no statistical significant differences ($p < 0.05$) in grain yield between I_2, I_3 and I_4 treatments I in both

TABLE 3. Monthly and seasonal wheat crop water consumption (ET) (mm) for the various treatments

Years	Treatments	ET (mm)							
		Oct.	Nov.	Dec.	Apr.	May	Jun	Jul**	Total
2009–2010	I1	33.39	61.78	112.44	60.61	58.01	70.27	25.84	422.3
	I2	49.47	77.96	112.43	125.67	120.14	95.29	27.89	608.8
	I3	37.48	49.88	110.55	122.64	119.75	112.35	21.98	574.6
	I4	33.31	57.82	106.37	68.60	92.25	107.23	20.71	486.3
2010–2011	I1	17.53	58.33	59.31	72.50	68.16	76.92	56.23	409.0
	I2	25.27	63.82	82.65	101.48	129.42	96.25	54.71	553.6
	I3	14.42	50.40	57.39	105.21	99.39	105.27	57.48	489.6
	I4	15.14	53.21	52.47	86.14	103.81	93.53	56.34	460.6

TABLE 4. Average yield, biomass and harvest index of the four irrigation treatments

Years	Treatments	Grain yields (t/ha)	Biomass (t/ha)	HI
2009–2010	I_1	3.54[b]	11.61[b]	30.5
	I_2	4.58[a]	14.90[a]	30.7
	I_3	4.15[a]	13.25[a]	31.3
	I_4	4.36[a]	13.28[a]	32.8
2010–2011	I_1	3.16[b]	11.54[b]	27.4
	I_2	4.49[a]	14.52[a]	30.9
	I_3	4.28[a]	13.68[a]	31.3
	I_4	4.25[a]	13.70[a]	31.0

TABLE 5. Water use efficiencies of four irrigation treatments

Years	Treatments	ET (mm)	Yields (t ha^{-1})	Irrigation (mm)	WUE (kg/m)
2009–2010	I_1	422	3.54	—	0.84[b]
	I_2	609	4.58	290	0.75[b]
	I_3	575	4.15	183	0.72[b]
	I_4	486	4.36	162	0.90[a]
2010–2011	I_1	409	3.16	—	0.77[b]
	I_2	554	4.49	205	0.81[b]
	I_3	490	4.28	120	0.87[b]
	I_4	461	4.25	104	0.92[a]

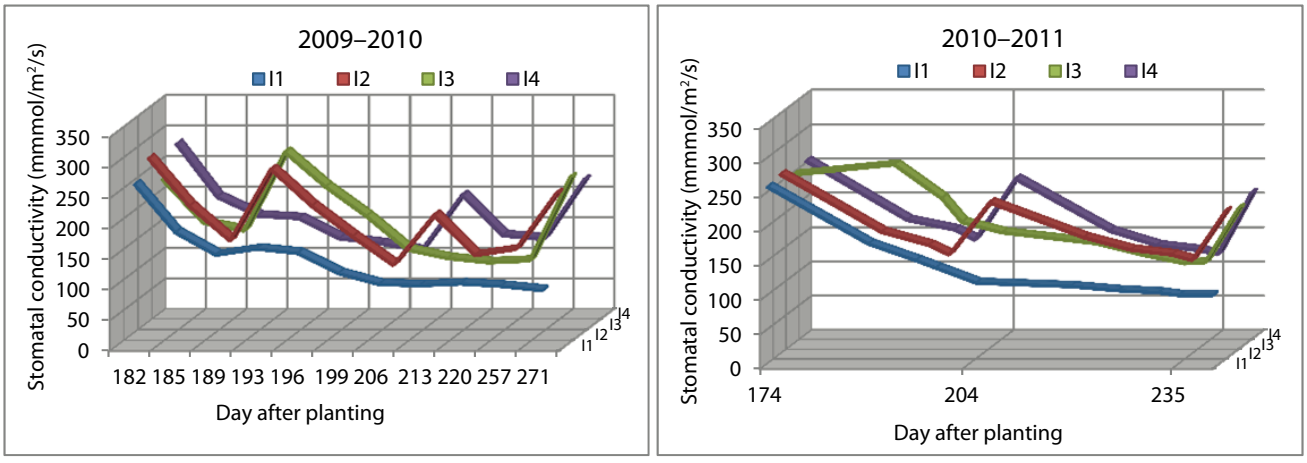


FIGURE 3. Change in stomatal conductance under different irrigation.

years. This was probably attributed to the high spring and winter precipitation in both years.

Harvest index (HI) values for the treatments were calculated using the average yield and biomass values in the respective treatments. They were 29, 31, 31 and 32 percent respectively for the four treatments. Although the highest HI was observed in treatment I_4, there was no statistical difference between all treatments (Table 4).

Water use efficiency (WUE)

Water use efficiency values calculated for both years are given in Table 5. The grain WUE ranged from 0.72 to 0.92 kg/m^3, with treatment I_4 (irrigation when the soil water deficit diminished to 60 mm during heading stage) having the highest WUE in both seasons. The results indicated the most effective water use by the winter wheat crop was obtained with treatment I_4. The above WUE is within the range obtained globally (0.40–1.83 kg/m^3) (Zwart and Bastiaans-

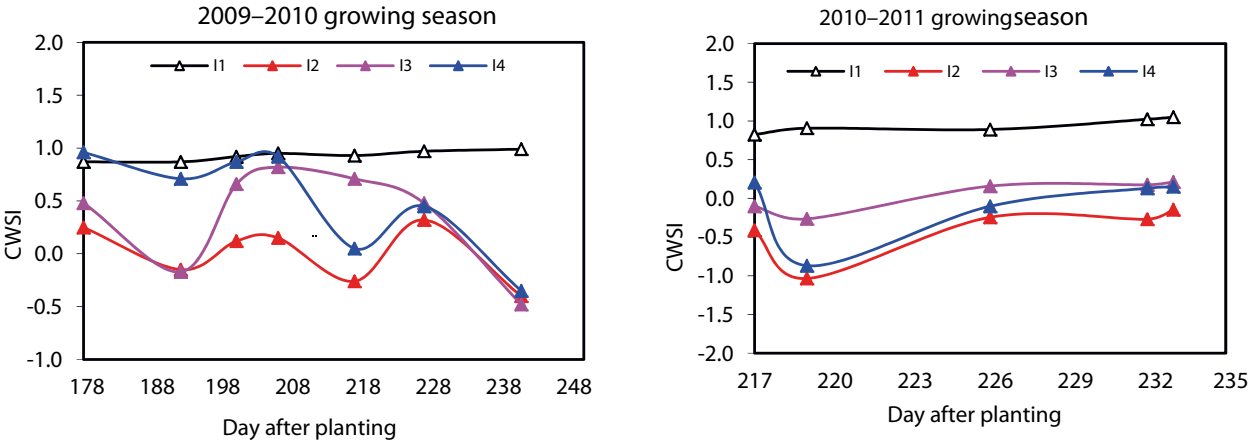


FIGURE 4. Variations in CWSI as a function of time after sowing for each irrigation treatment.

TABLE 6. Carbon isotope ratios of the leaf and grain samples

Years	Treatments	Δ ^{13}C–Leaf (‰)	Δ ^{13}C–Grain (‰)
2009–2010	I_1	19.81 [a]	17.94 [a]
	I_2	20.09 [a]	17.99 [a]
	I_3	19.85 [a]	18.47 [a]
	I_4	19.99 [a]	17.96 [a]
2010–2011	I_1	19.10 [b]	17.54 [a]
	I_2	19.97 [a]	18.04 [a]
	I_3	19.46 [ab]	17.76 [a]
	I_4	19.91 [a]	18.44 [a]

sen, 2004). In the Texas High Plains, rainfed winter wheat WUE was about 0.40 kg/m^3, while irrigated wheat WUE was 0.50–1.20 kg/m^3 with a yield of 3 000–8 000 kg/ha (Howell *et al.*, 1995).

Stomatal conductance

Figure 3 shows the changes in the stomatal conductance values taken twice per week on sunny and windless days in April (from the date irrigation was initiated), May and June (near senescence).

Stomatal conductance was the lowest for treatment without irrigation, suggesting that the pores became closed under least water available conditions. The highest stomatal conductance was obtained in fully irrigated treatment, especially after irrigation application.

Crop water stress index (CWSI)

The variations in CWSI as a function of time (day after planting) for each irrigation treatment are presented in Figure 4. In general, CWSI increases with increasing crop water stress as in rainfed treatment (I_1) and is lowest in fully irrigated treatment (I_2).

Evaluation of the carbon isotope ratios (Δ)

Carbon isotope ratio of leaves and grains of wheat are shown in Table 6. In general leaf Δ^{13}C is higher than grain Δ^{13}C for both years. Analysis of variance of Δ^{13}C for wheat leaves showed no statistical difference between the irrigation treatments in the first year; although there was a difference at the $p < 0.05$ level in the second year. Irrigation did not have a significant effect on the wheat Δ^{13}C leaf value, probably because the soil moisture did not fall low enough to cause discrimination. The analysis of variance also showed no statistical difference between the various irrigation treatments for both years with respect to grain Δ^{13}C values.

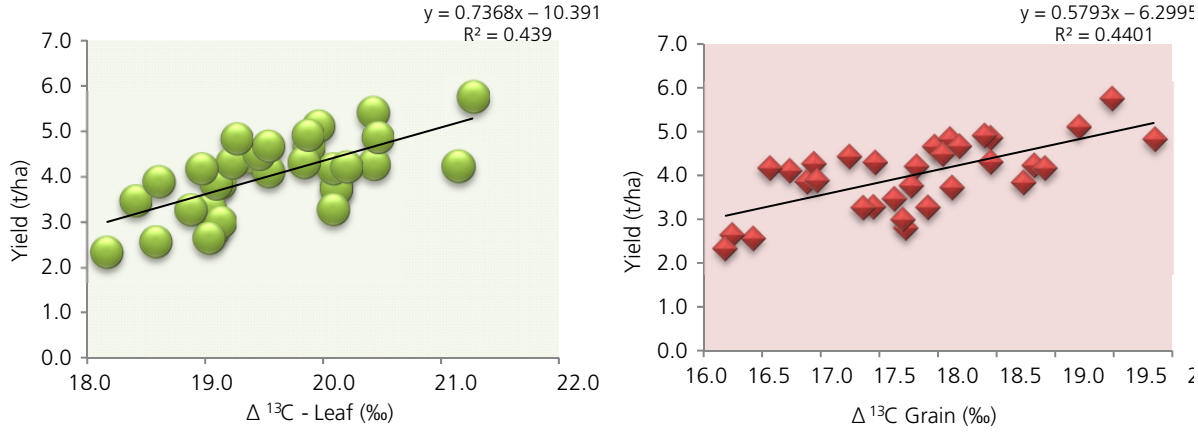


FIGURE 5. The relationships between Δ^{13}C values (in leaves and grain) and grain yield.

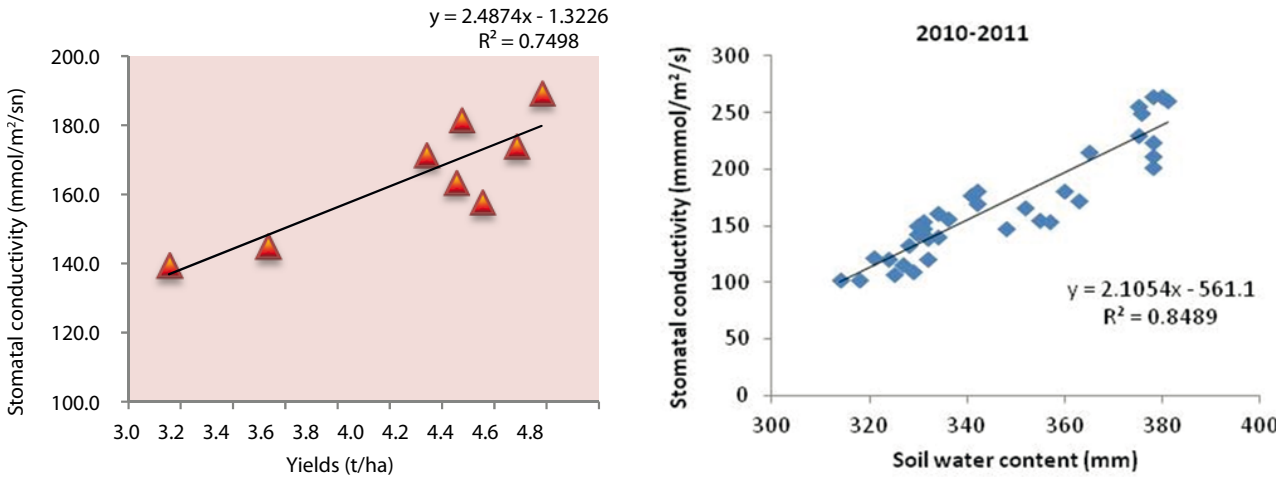


FIGURE 6. The relationship between stomatal conductance, yield (left) and soil water content (right).

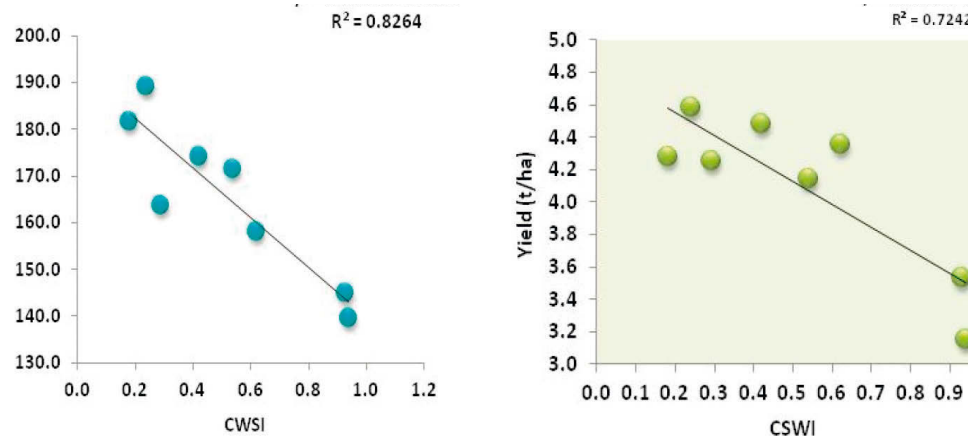


FIGURE 7. The relationship between CWSI and stomatal conductance (left) and grain yield (right).

The relationship between leaf and grain Δ values, yield and water use efficiency

There was a positive relationship between leaf and grain $\Delta^{13}C$ values and yields, as shown in Figure 5. A significant and positive relationship between leaf $\Delta^{13}C$ and grain yield was also observed for each individual treatment (data not shown). Positive relationships were often found between yield and $\Delta^{13}C$ under most climatic conditions (Sayre, Acevedo and Austin, 1995; Monneveux *et al*., 2005; Xu *et al*., 2007), especially under Mediterranean environments (Merah *et al*., 2001; IAEA, 2012).

A negative relationship was found between the WUE and $\Delta^{13}C$ values of both biomass and grain (data not shown). The relationship between the WUE and $\Delta^{13}C$ of wheat was also found to be negative by Ehdaie *et al*. (1991) and Khazaei *et al.* (2008).

The relationships between stomatal conductance, yield, CWSI, leaf Δ

A significant positive relationship was found between grain yield and stomatal conductance (Figure 6, left). The transpiration ratio increased with increased stomatal conductance which in turn was reflected in the yield. This positive relationship has also been reported (Shimshi and Ephrat, 1975; Evans, 1993; Lu *et al.,* 1998).

A positive relationship was also observed between soil water content and stomatal conductance (Figure 6, right) implying stomatal conductance is good indicator of overall moisture stress.

Similarly significant and negative relationship exists between CWSI and stomatal conductance and CWSI and grain yield for both years (Figure 7). This result agrees with many other studies (Howell, Musick and Tolk, 1986; Irmak, Dorota & Bastug, 2000), indicating that infrared thermometers can be used to quantify CWSI as a response of grain yield to water stress in wheat.

CONCLUSIONS

A study conducted during the wheat growth period for the years 2009–2010 and 2010–2011. In 2009-2010 seasons, full irrigation increased grain yield by more than 30%, however, insignificant yield difference between different irrigation inputs were observed in 2010–2011, attributed to high rainfall. The study showed a significant positive relationship between grain $\Delta^{13}C$ and grain yield, indicating that it is possible to select wheat varieties with the high yield potential using $\Delta^{13}C$.

The positive relationship between stomatal conductance and yield showed that water stress affects canopy temperature and stomatal resistance of the crop. Stomatal conductance is affected by temperatures of leaves which respond to soil water deficit. Average canopy temperature was inversely correlated with stomatal conductance.

The CWSI which is an index based on canopy surface temperature, is therefore a promising tool for quantifying crop water stress. Irrigation after-heading period may be used to increase WUE of wheat in the central Anatolia region of Turkey.

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Managing Irrigation Water to Enhance Crop Productivity Under Water-limiting Conditions: A Role for Isotopic Techniques

L.K. Heng[1,*], T.C. Hsiao[2], D. Williams[3], E. Fereres[4], P. Cepuder[5], T. Denmead[6], N. Amenzou[7], N. Dhan[8], H. Duong[9], S. Kale[10], B.G. Li[11], K. Mahmood[12], X.R. Mei[13], E. Phiri[14], S. Zingore[15] and M.L. Nguyen[1]

ABSTRACT

This paper summarizes results obtained from an FAO/IAEA Coordinated Research Project (CRP) on "Managing Irrigation Water to Enhance Crop Productivity under Water-Limiting Conditions: A Role for Isotopic Techniques", implemented from 2007 to 2012. Its objective was to identify approaches to improve crop water productivity (production per unit of water input) under water-limiting conditions using isotopic and related techniques. The CRP employed both isotopic and conventional techniques to separate soil evaporation (E) and crop transpiration (T) and to help identify factors that minimize soil evaporation losses and improve irrigation management. Field measurement of E and T were carried out on a range of crops (maize, paprika, winter wheat and coffee) under different frequencies and methods of irrigation management practices and soil fertility levels. Using both nuclear, isotopic (Keeling plot and isotopic mass balance using delta oxygen-18 ($\delta^{18}O$)), the results showed that the proportion of evapotraspiration (ET) as E was much higher in the African studies (Malawi and Zambia) due to poor vegetation cover resulting from low soil fertility and inefficient irrigation management. However, by improving soil fertility, T increased by 50 percent in the Malawi maize study. In the North China Plain, through mulching, deficit irrigation and improved irrigation scheduling, soil E losses could be reduced by 10–30 percent of the total water loss compared with full irrigation. Soil E losses were also determined for 10-year old coffee trees in Central Vietnam over various growth stages. The E component was approximately 14 percent of ET during the bean development stage. When old branches and leaves were left as mulches on the ground, the T component could be as high as 92–95 percent compared with non-covered ground where total water loss through E could be three times more. The E and T results generated were also used to validate FAO's AquaCrop model for improving irrigation scheduling and agronomic practices. In the North China Plain, long-term simulation from AquaCrop showed that in wet years, only two irrigations at the planting and jointing stages were needed for wheat while no irrigation was needed for maize. In normal years, two irrigations were needed at the planting and jointing stages of wheat and one irrigation at the planting of maize, while in dry years, three irrigations were needed at the planting, jointing and booting stages for wheat and one irrigation at planting of maize.

Key words: *irrigation, evaporation and transpiration separation, $\delta^{18}O$ isotopic techniques, Keeling plot, isotopic mass balance.*

1 International Atomic Energy Agency, Wagramerstrasse 5, PO Box 100, 1400 Vienna, Austria
2 Department of Land, Air and Water Resources, University of California, Davis, USA
3 University of Wyoming Stable Isotope Facility, University of Wyoming, Laramie, USA
4 IAS-CSIC and University of Cordoba, Cordoba, Spain
5 Department of Water-Atmosphere-Environment, Institute for Hydraulics and Rural Water Management, Vienna, Austria
6 CSIRO Land and Water, Canberra, Australia
7 National Center of Study and Energy Nuclear, CNESTEN, Rabat, Morocco
8 Institute for Nuclear Science and Technology, HaNoi, Viet Nam
9 Center for Irrigation and Water Supply Research, HaNoi, Viet Nam
10 Department of Agricultural Structure and Irrigation, Agricultural Faculty of Suleyman Demirel University, Isparta, Turkey
11 Department of Soil and Water Science, College of Resources and Environment, China Agricultural University, Beijing, P. R. China
12 Nuclear Institute for Agriculture & Biology (NIAB), Faisalabad, Pakistan
13 Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Science (CAAS), Beijing, P. R. China
14 Department of Soil Science, University of Zambia, Lusaka, Zambia
15 International Plant Nutrition Institute (IPNI), Nairobi, Kenya
* E-mail address of corresponding author: L.Heng@iaea.org

INTRODUCTION

Agriculture is the largest consumer of freshwater, accounting for about 75 percent of all withdrawals in developing countries. Water withdrawals are predicted to increase by 50 percent by 2025 in developing countries, and by 18 percent in developed countries (WWAP, 2006). There is an urgent need to improve agricultural water use efficiency (WUE) by increasing crop water productivity (CWP), i.e. the productivity of crop per unit of total water consumption (FAO, 2003). However, information on CWP and transpiration efficiency (TE), i.e. crop biomass per unit of transpired water under different irrigation technologies, and the extent and proportion of evapotranspiration

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(ET) as soil evaporation (E) and transpiration (T) under different agro-climatic and soil–plant management conditions are often unavailable.

Loss of water from the soil surface through E is often a major component of the soil water balance in agricultural systems in semi-arid regions with sparse vegetation cover due to sub-optimal or inefficient management practices such as slow germination and slow growth rate due to poor soil fertility, wide row spacing due to low planting density and inappropriate irrigation and water conservation practices (Jackson and Wallace, 1999). The ability to distinguish between E and T can help to identify management practices that cut losses and improve WUE. However, accurate assessment of soil E is challenging due to sampling difficulties, spatial-temporal variability and the multiplicity of evaporation sources (Denmead *et al.*, 1996).

Stable isotopic tracer methods have been used to separate E and T (Moreira *et al.*, 1997; Williams *et al.*, 2004). This is possible because E and T fluxes often have distinct isotope ratio values in water (delta oxygen-18 [$\delta^{18}O$] and delta hydrogen-2 [$\delta^{2}H$]). Delta (δ) values are defined as the deviation of the molar ratio of heavy (rare) to light (common) isotopes in the sample relative to that of an internationally recognized standard. Hence measurements of the isotope ratio composition in the atmospheric water vapour within the canopy boundary layer or in the residual soil water over time can provide quantitative information about the sources of and processes controlling water exchange in the soil–plant–atmosphere system (Wang and Yakir, 2000). With recent developments in laser absorption spectroscopy, real-time, *in situ* and continuous measurements of $\delta^{18}O$ and $\delta^{2}H$ in air vapour and liquid water are now possible, allowing continuous ET partitioning on a diurnal basis. Two isotopic methods: the Keeling plots (Keeling, 1961; Williams *et al.*, 2004) and isotopic mass balance (IMB; Hsieh *et al.*, 1998; Ferretti *et al.,* 2003) were used in this CRP. Results of the separation of E and T using these isotopic methods as well as those using conventional methods (e.g. using soil moisture sensors, eddy covariance and micro-lysimeters) are reported in this paper.

The FAO's AquaCrop model (Raes *et al.,* 2009; Steduto *et al.*, 2009) on crop yield response to water was used to improve irrigation scheduling and agronomic practices in several studies.

MATERIALS AND METHODS

The research network in the CRP included seven research contract holders (two from China and one each from Malawi, Morocco, Pakistan, Turkey, Vietnam and Zambia), supported by one technical contract holder (Australia) and four research agreement holders (Austria, Spain and two from the United States). The objectives of the CRP were to (i) quantify and develop means to manage soil evaporative (E) losses to maximize the beneficial use of the transpirational (T) component of evapotranspiration, (ii) quantify and develop means to improve the amount of biomass produced per unit of transpiration, and (iii) devise irrigation and related management techniques to enhance the yield component of biomass production (harvest index).

Keeling plot method

The Keeling plot method is based on the isotopic mass balance mixing relationship introduced by Keeling (1961), where the isotope mass balance of an atmospheric water vapour sample collected in the mixed boundary layer above the crop canopy is described by two equations:

$$C_a = C_b + C_{ET} \quad (1)$$

$$\delta_a C_a = \delta_b C_b + \delta_{ET} C_{ET} \quad (2)$$

where C represents H_2O concentration (e.g. mmol H_2O mol^{-1} air); δ is the isotopic composition (‰) of H or O in water; and the subscripts a, b and ET refer respectively to the air sample, background atmosphere and ET source.

An atmospheric water vapour sample taken within the mixed boundary layer above the crop represents a mixture of isotopes and concentrations between the background atmosphere and the evaporating surface of the crop. These two equations can be combined and re-arranged to yield an equation that takes the form of a linear regression (Equation 3).

$$\delta_a = C_b (\delta_b - \delta_{ET})(1/C_a) + \delta_{ET} \quad (3)$$

A plot of the isotopic composition of water samples of the air (δ_a) in the mixed boundary layer on the y-axis against the reciprocal of the water concentration of the samples ($1/C_a$) on the x-axis yields a straight line with a slope of $[C_b(\delta_b - \delta_s)]$ and an intercept of δ_{ET}, the isotopic composition of the ET flux. By knowing the isotopic signature of each of the ET sources, the direct soil evaporation and transpiration sources, the relative contributions of T and E to total ET can be separated at the field scale. This is done using a linear mixing equation:

$$T/ET = (\delta_{ET} - \delta_E)/(\delta_T - \delta_E) \quad (4)$$

where T/ET is the fractional contribution of plant T to total ET; δ_{ET} is the isotopic composition of the evapotranspiration; δ_E is the isotopic composition of soil E; and δ_T is the isotopic composition of T.

The method assumes leaf water to be at isotopic steady state. Water vapour $\delta^{2}H$, $\delta^{18}O$ isotope composition and vapour concentrations at different heights were monitored using a stable isotopic water vapour analysis system. Soil sample at different depths and plant stem samples were also collected to measure their isotopic composition.

Isotopic mass balance of soil water

The IMB of soil water is described by the following relationships:

$$m_i + m_r = m_f + m_E + m_T \quad (5)$$

$$x_i\, d_i + x_r\, d_r = x_f\, d_f + x_E\, \delta_E + x_T\, d_T \quad (6)$$

where m is mass water; x is the fraction of the total mass of water in the system; δ is the isotope ratio value; and subscripts i, r, f, E and T denote the system components: i is the initial condition, r is rainfall or irrigation input, f is the final condition, E is the water evaporated from soil and T is the transpired water.

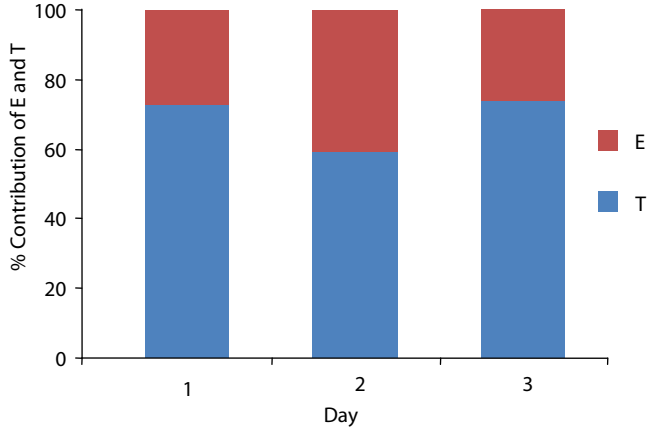
Equation 5 implies that the sum of the masses of initial water and water added as irrigation or rainfall is the same as the mass of water in the final condition plus the mass lost from E and T. The conservation of mass also applies to the isotope abundances in the system (Equation 6). The mass and isotope ratio of soil water in the initial and final conditions and in the irrigation and rainfall can be measured, and the isotope ratio of evaporated and transpired water can be calculated. The unknown quantities of mass of water transpired and evaporated are then solved. These relationships assume there is no lateral movement of water or beyond the observation zone, and that the isotopic composition of irrigation or rainfall is the same as that entering the soil water column, and roots do not fractionate soil water. The isotope composition of water evaporated from the soil (δ_E) can be calculated from the same equations shown above for estimating δ_E using the Keeling plot method, but must be integrated over the entire period of observation, from initial to final conditions.

TABLE 1. A summary of a selection of the studies carried out by the CRP participants

Country	Crop	Treatments	E & T methods	Experimental site
China (CAAS)	Winter wheat	Deficit irrigation (sprinkler) with 2 irrigations (75 and 45 mm)	Keeling plot $\delta^{18}O$, microlysimeter	Dryland farming and water-saving in Agriculture Station, Hengshui, Hebei
Morocco	Winter wheat	Flood	Keeling plot $\delta^{18}O$, AquaCrop model	Tensift catchment
Pakistan	Winter wheat	75%, 100% and 125% ETc and rainfed	Soil moisture neutron probe, AquaCrop model	NIAB Station, Faisalabad
Turkey	Winter wheat	Rainfed to full irrigation	Keeling plot $\delta^{18}O$	Saraykoy Research Station, Ankara-Mürted Basin
China (CAU)	Maize	Mulching (film and straw)	Isotopic mass balance $\delta^{18}O$	Beijing
Malawi	Maize	0, 50, 100, 150% ETc. 0, 50, 150 kg N/ha	Sap flow, isotopic mass balance $\delta^{18}O$	Southern Malawi
Vietnam	Coffee trees	Mulch vs no-mulch	Keeling plot $\delta^{18}O$	Dak Ha district, Tay Nguyen Plateau
Zambia	Paprika	50%, 75%, 100% & 125% ETc	Soil moisture using neutron probe	UNZA Field Station, Lusaka

TABLE 2. The ratio of soil evaporation to evapotranspiration (E:ET) for China CAAS studies

Crop	Sowing-Tillering	Tillering-Jointing	Jointing-Heading	Flowering-Grain filling	Grain filling-maturity	Total
Wheat	0.42	0.68	0.24	0.15	0.22	0.29



FUGURE 1. Proportion of E and T in winter wheat for three consecutive days in February 2012 in Morocco.

Other methods used such as the micro-lysimeter have been described in detail by Boast and Robertson (1982), Evett, Warrick and Matthias (1995) and Villalobos and Fereres (1990).

A selection of the studies carried out by the participants including the crop type, irrigation treatment, and E and T partitioning method is given in Table 1. Detailed methodologies and experimental designs of individual experiments are described in these proceedings. This presentation is grouped according to crop types: winter wheat, maize, coffee and paprika.

Briefly, for the winter wheat studies in the China CAAS experiment (Gong *et al.*, 2013), winter wheat was grown under deficit irrigation with two irrigations (75 mm and 45 mm) applied in spring, and E and T were partitioned using Keeling plot and micro-lysimeter methods. In the Moroccan study (Amezou *et al.*, 2013), water loss through E for winter wheat grown under flood irrigation was determined using the Keeling plot, validated using the AquaCrop model and total water loss for the growing season was determined from the AquaCrop model. In Pakistan, winter wheat was grown under different crop water requirement (ETc) conditions and E and T were separated using a soil moisture neutron probe and the AquaCrop model (Mahmood, Ishaque and Heng, 2013). In Turkey, winter wheat was grown under rain-fed to full irrigation and E and T were determined using the Keeling plot approach before and after irrigation (Kale *et al.*, 2013).

In the case of maize, this was grown under conventional as well as under mulching (filming and straw residual) in China and the isotopic mass balance (IMB) method was used to determine E and T (Li *et al.*, 2013). For Malawi, maize was grown under different crop water requirements and three nitrogen (N) fertilizer levels (0, 50 and 150 kg/ha). The IMB method was also used to partition E and T (Zingore, Fandika and Heng, 2013).

In Vietnam, E and T were determined using the Keeling plot method for a 10-year-old coffee plantation at different stages of the development cycle (mature, bean development, bean formation, flowering and bud development), as well as with and without crop residues (Dang *et al.*, 2013). The study was to investigate cultivation practices that could improve water use efficiency of coffee plants. In Zambia, E and T of the paprika crop was determined in the vegetative stage from soil moisture measured using the neutron probe (Phiri, Heng and Sinda, 2013).

RESULTS AND DISCUSSION

Winter wheat

In China, the proportion of soil E to the total water lost through ET is given in Table 2. The E/ET ratios early in the season were higher than that in the mid-season because of the smaller canopy cover. An average of 29 percent of total water was lost through soil E in this study.

In Morocco, the fractional contributions of soil E to total ET (E/ET) was measured in late spring under farmers' flood irrigation practice. Approximately 32 percent of the total ET was lost through soil E for three consecutive days in February 2012 (Figure 1). The results

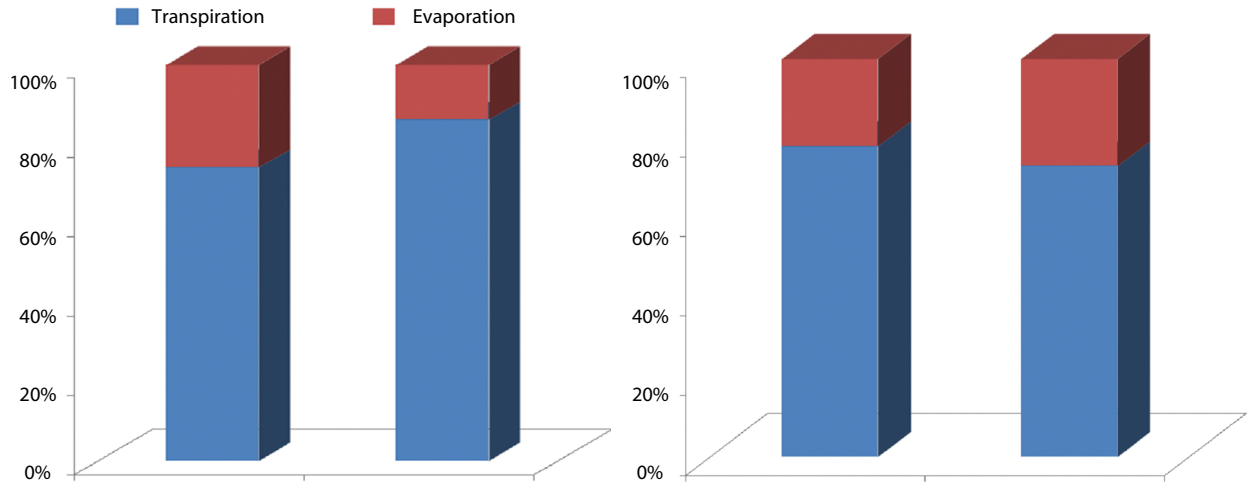


FIGURE 2. Comparison of E and T of winter wheat in Morocco for two days (22 and 24 Feb 2012) using the AquaCrop model (right) and isotopic (left) methods.

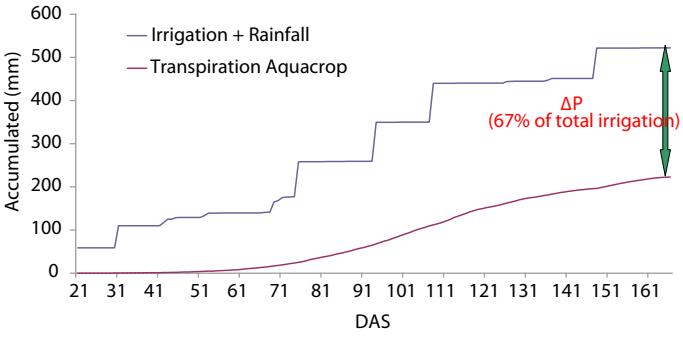
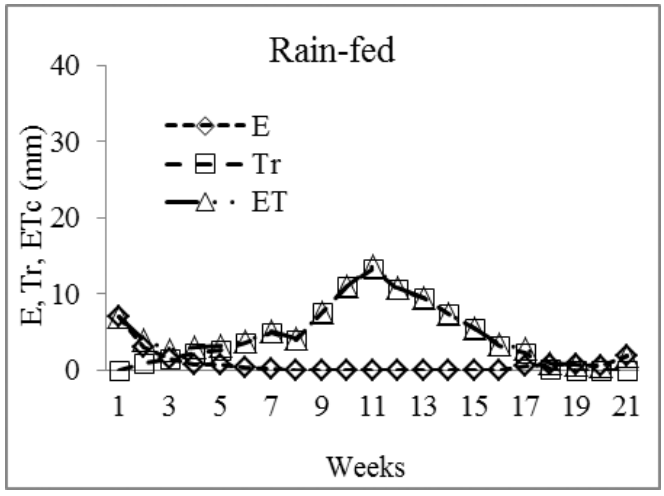


FIGURE 3. Cumulative T estimated by the Aquacrop model compared with total rainfall and irrigation in Morocco.

were compared with those simulated using the AquaCrop model (Figure 2). In order to investigate the efficiency of the irrigation practices which are representative of the practices in the region, seasonal water use was determined using the AquaCrop model. The analysis found that farmers' traditional visual observation of the need for irrigation from the physical condition of the crop is not efficient, a large quantity of the total water was lost through soil E and deep drainage ΔP (67 percent of total irrigation) (Figure 3).

Soil E was also the main component of water loss recorded in the Pakistan study under both rain-fed and irrigated treatments during the early growth stage due to the low canopy cover (Figure 4); however, in the irrigated treatment, after the fourth week, crop T increased and accounted for the majority of the total ET as canopy cover increased. Transpiration remained higher during the vegetative growth and flowering stages and started declining at grain filling. The initiation of leaf senescence during the grain filling stage resulted in a decrease in T with a corresponding increase in the E component. This was not the case in the rain-fed treatment where T remained low over most part of the season (Figure 4).

In Turkey, the proportion of T as percent of total ET was 70–96 percent before irrigation, while it was approximately 70 percent after irrigation, as determined from the Keeling plot analysis (Kale *et al.*, 2013).



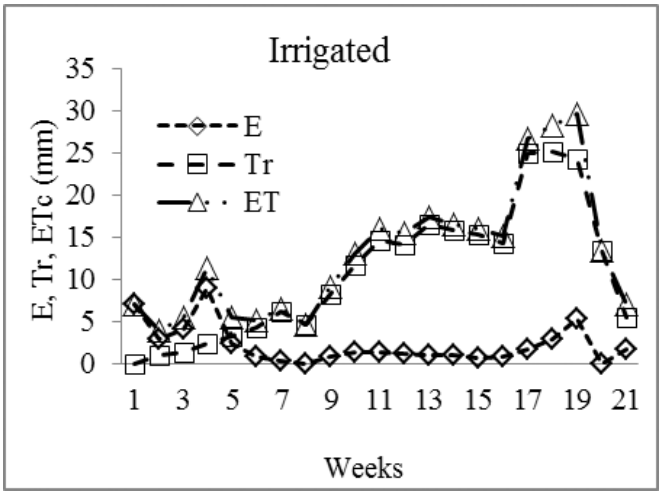


FIGURE 4. Pattern of wheat crop ET and its components under rain-fed and irrigated conditions in a Pakistan study as determined by the AquaCrop model (2010–2011).

TABLE 3. Partitioning E from ET of spring maize (values in mm) in China. Figures in brackets are percent of ET

Crop	Year	Conventional		Filming		Mulching		Bare	
		ET	E	ET	E	ET	E	ET	E
Spring maize	2010	206	63 (31)	157	22 (12)	178	43 (24)	182	65 (38)

Table 4. Partitioning the ET fluxes using the isotopic mass balance method for maize crop in Malawi

Treatment		
ET_c (%)	N (kg N/ha)	E/ET
50	50	0.92
	150	0.87
100	50	0.83
	150	0.80

Maize

In China, using the IMB method, the proportion of soil E was separated from total ET. The relatively high proportion of soil E for spring maize under the conventional method of planting (31 percent) compared with plastic filming and mulching with crop residues occurred mainly during May to June when the crop cover was lower with excessive rainfall events (Table 3). Both filming and straw mulching reduced E significantly in spring maize (by 19 percent and 7 percent, respectively) (Li *et al.,* 2013).

The proportion of T in the first 70 d of the maize crop season in Malawi constituted 43 percent for the two 100 percent ETc irrigation treatments and 36 percent for the 50 percent ETc irrigation treatment, based on the sap flow method. A simple isotope mass balance model was also used to determine the fractions of water lost through soil evaporation and crop transpiration for selected treatments. The relative contribution of evaporation (E/ET) during the first 50 d of the growing season was more than 83 percent for all treatments (Table 4).

Coffee trees

The contributions of the T and E components in the 10-year old coffee plantation showed that E was highest (53 percent) during the maturation and canopy forming stages, while during the budding and flowering stages and bean development it was the reverse, i.e. T was higher than E. However, in the budding and flowering stages following the bean development stage, which coincides with the dry season, the crop needs more water than at other stages. It is therefore vital to irrigate the crop to maintain soil moisture during the flowering period. The study showed that drip irrigation combined with mulching increased the T component of coffee plants by around 10 percent compared with the furrow and no-mulch practice.

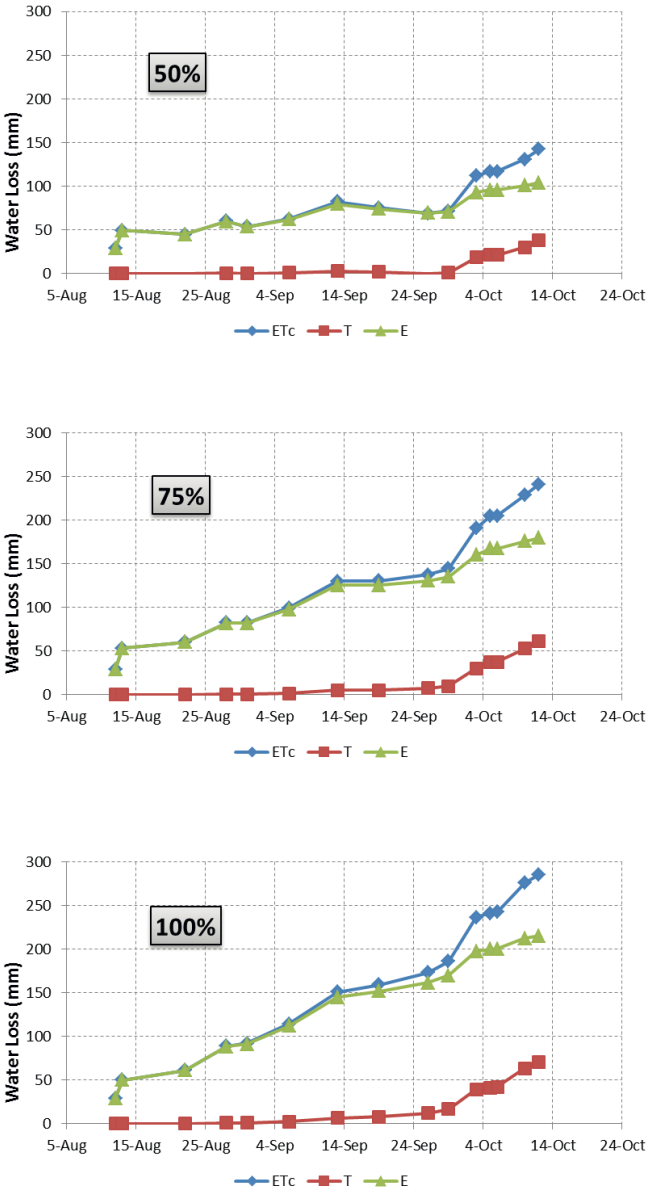


FIGURE 5. Cumulative water loss through E and T during the paprika vegetative period under 50, 75 and 100% ETc conditions.

TABLE 5. Percent of T and E of coffee plants before irrigation for the three important stages in a cropping season as determined by the isotopic technique

Stage	δ_{ET}‰	δ_E‰	δ_T‰	T%	E%
Mature and canopy reforming (September–November)	–11.6	–12.7	–10.4	47	53
Budding and flowering (December–February)	–9.7	–11.8	–10.5	85	15
Bean development (April–August)	–10.9	–13.8	–10.3	84	16

Table 6. T component of coffee plants in the flowering stage under different irrigation practices

Irrigation practice	T (%)	E (%)
Furrow, no-mulch	83	17
Drip, no-mulch	87	13
Drip with mulch	94	6

TABLE 7. Partitioning of ETc into soil E and T of paprika crop during vegetative stage. Values in brackets are percentages of E:ET

Treatment (% ETc)	ETc (mm)	E (mm)	T (mm)
50	153	114 (75%)	39 (25%)
75	241	179 (74%)	61 (26%)
100	285	215 (75%)	70 (25%)
Mean	226	169 (75%)	57 (25%)

However, the T in the drip without mulch practice did not differ significantly from that of the traditional furrow and no-mulch practice. This was due to the fact that the leaf area index of the crop was as high as 6–7, and the tree canopies covered each other making solar radiation on the soil surface to be almost the same in the both cases.

Paprika

In Zambia, paprika was grown under 50, 75 and 100 percent ETc conditions. Water loss by soil E was calculated by subtracting T values from ET calculated from the root zone soil water balance. Generally, during the vegetative stage 75 percent of ET was lost as soil E (Figure 5). As crop growth progressed, the contribution of T started to increase. While the total amount of soil E was much higher under the 100 percent ETc treatment, the proportion of E to ET was almost the same under the three treatments (Table 7). In general about 75 percent of water was lost through soil evaporation.

CONCLUSIONS

The work conducted within the framework of this CRP showed that isotopic techniques ($\delta^{18}O$ or $\delta^{2}H$) using the Keeling Plot and the IMB method were able to provide improved estimates of soil E and T components. Together with the conventional method (e.g. the eddy covariance method), the transpiration percentage of a range of crop species in different environments was estimated. The transpiration efficiency of crops was generally lower in the African studies compared with those in Asia and Europe (China, Pakistan and Turkey) due to poorer soil fertility and irrigation management. This information allows appropriate soil and water management practices to be devised and implemented. Results from China and Pakistan showed that FAO's AquaCrop model provided the means to develop deficit irrigation schedules to save water while minimizing reductions in yield through saving unnecessary soil E and improving water use efficiency.

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Evaluation of Evapotranspiration and Production of Paprika (*Capsicum Annum L.*) using the Soil Water Balance Approach under Variable Irrigation Water Applications

E. Phiri[1,*], L. Heng[2] and L.M. Sinda[1]

ABSTRACT

Precise estimation of evapotranspiration (ET) at the different growth stages is important for determining the field soil water balance and irrigation requirements. An experiment was conducted at the Research Field Station of the University of Zambia on a summer high value crop using a small-scale drip irrigation system. To maximize water use efficiency, the soil evaporation (E) and crop transpiration (T) components of the total ET were estimated for the cropping season. A randomized complete block design experiment with four replications and four irrigation water application rates based on crop water requirements (50 percent, 75 percent, 100 percent with and without plastic mulch), was carried out with paprika (*Capsicum Annum L.*) as a test crop. To determine T, soil water content was monitored with a soil moisture neutron probe to a depth of 150 cm over the season; green canopy cover was also monitored regularly using images taken with a digital camera. The results showed that T was negligible during the first 25 d after transplant (DAT) during plant establishment due to incomplete canopy cover and thereafter started to increase exponentially. The partitioning of ET into E and T using canopy cover values multiplied by ET showed that T estimates varied from 38 mm to 70 mm, depending on the treatments and resulted in an average value of 58.8 mm. Transpiration from the treatment with plastic mulch was 5 mm lower, with most of the water loss being through E during plant establishment before the plastic cover was installed. Generally, during the vegetative stage (the first 70 DAT) 75 percent of ET was in form of E while the remaining 25 percent was lost through T. The results on biomass production showed that the highest biomass production (421 kg/ha) was obtained in the 100 percent treatment while the lowest biomass (233 kg/ha) was in the 50 percent treatment; hence the higher the amount of water applied, the higher was the biomass produced. However, water use efficiency (biomass per unit ET) was highest under 50 percent ET and lowest in the 75 percent treatment (6 $kg \cdot ha^{-1} \cdot mm^{-1}$ versus 5.1 $kg \cdot ha^{-1} \cdot mm^{-1}$).

Key words: *water use efficiency, evaporation, transpiration, drip irrigation, paprika, biomass production.*

INTRODUCTION

Low-head drip irrigation systems are being promoted for the production of high value crops to mitigate the impacts of drought in Southern Africa and ensure efficient resource utilization and food security at household level. It is a low-cost system that attempts to retain the benefits of conventional irrigation systems whilst removing factors preventing their uptake by resource-poor smallholder farmers, such as purchase cost, the requirement of a pressurized supply, the associated pumping costs and the complexity of operation and maintenance. These systems are usually sold in kit form for relatively small areas of land (25 m^2) to keep the cost down and give room for incremental development not easily accomplished with normal commercial systems. Most smallholder farmers in Southern Africa and Zambia in particular rely heavily on rain-fed agriculture and are frequently faced with drought that affects their crop production. Against this background, a study was designed to evaluate the system in Lusaka as part of a concerted effort to understand water dynamics in the root zone of paprika (Capsicum annum L.) as a test crop under local conditions.

Evapotranspiration is the major component of the water balance in natural and managed ecosystems, accounting for more than 80 percent of precipitation inputs into ecosystems (Wilcox, Breshears and Seyfried, 2003). In water-limited ecosystems, partitioning of ET between plant transpiration (T) and soil evaporation (E) remain theoretical and technically challenging. In this study, the partitioning was achieved by multiplying the canopy cover values by ET to give the estimates of T. Water loss by E was then calculated by subtracting T values from ET values calculated from the root zone soil water balance. This study was part of a regional initiative to: (i) quantify and develop means to manage soil evaporative losses in ways that would maximize the beneficial use of water, (ii) quantify and develop means to improve the amount of biomass produced per unit of transpiration, and (iii) devise irrigation and related management techniques to enhance the yield component of biomass production.

Experimental design

The field experiment was conducted at the University of Zambia's Agriculture Field Station in Lusaka (lat: 15° 23″ S, long: 28° 20″ E alt: 1 262 m above sea level, asl). According to the Koeppen climate classification, the site has a warm temperate climate with dry winters and hot summers. The average daily maximum and minimum temperatures are 25°C and 15°C, respectively (Figure 1). The average rainfall varies from 800 mm to 1200 mm with an estimated precipita-

1 University of Zambia, School of Agricultural Sciences, Department of Soil Science, P. O. Box 32379, Lusaka, Zambia
2 International Atomic Energy Agency, Wagramerstrasse 5, PO Box 100, 1400 Vienna, Austria
* E-mail address of corresponding author: ephiri@unza.zm

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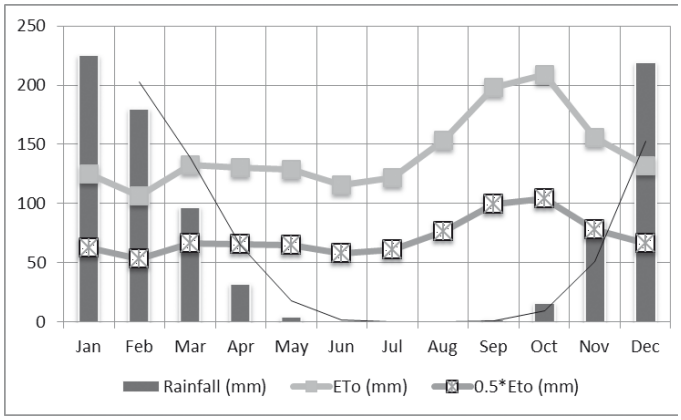


FIGURE 1. Long-term average monthly precipitation and evapotranspiration.



FIGURE 2. The drip system (left) and weather station (right) at the experimental site.

tion deficit of 647 mm per annum. The length of the rainy season is about 140 d (November–March), while the dry season is estimated at 225 d. The long season with cool temperatures at the beginning and later culminating in warmer temperatures makes it possible to grow a number of irrigated crops.

The experimental design was a complete block design with four replicates and four treatments (water application rates of 50 percent, 75 percent, and 100 percent with and without plastic mulch) Water was supplied through a metered drip irrigation system. Each plot was equipped for measurements of (i) soil water profiles using a neutron moisture probe (CPN), (ii) water application through drip irrigation, (iii) biomass, and (iv) weather data monitored with an automatic weather station (Figures 2 and 3). Evapotranspiration was calculated using a root zone soil water balance approach and partitioned into E and T using estimates of crop green canopy from digital camera pictures.

Five-week-old seedlings were transplanted at an inter-row spacing of 0.90 m and 0.75 m between plants along drip lateral lines. During the early part of the season, from transplanting date until 37 d after transplanting (DAT), a portable sprinkler irrigation system was used twice (on 3 and 14 DAT) to apply 30 mm of water to ensure plant establishment. Afterwards, irrigation was applied every two days with a drip system; with one drip line every two rows and emitters 0.30 m apart. The plants received a basal fertilizer dressing at a rate of 300 kg/ha (N–P–K: 10–20–10) and a split top dressing fertilizer application of 300 kg/ha ammonium nitrate (NH_4NO_3). One week after transplanting (37 days after sowing, DAS), plants were subjected to a two-day interval irrigation schedule using a drip irrigation system with the exception of days when rainfall was received. Above ground biomass was estimated from measured plant girth diameter which was calibrated to biomass sampled four times during the experiment on 29 and 63 d after transplanting. Green canopy cover was determined from digital canopy pictures using Green Crop Tracker software (Liu and Pattey, 2010). After at least 48 hours in a ventilated oven at 70°C, the dry weight of the samples was determined separately for leaf, stem and fruit.

Soil type

The soil was a deep, dark brown to yellowish red, well drained clay loam with a loam textured surface layer and clay textured subsurface layers derived from quartzite and classified as a Paleustalf (USDA, 1998) (Table 1). The soil has a medium to low nutrient level (Table 2), hence fertilizer application was required for stabilizing and improving crop productivity.

Irrigation scheduling

The irrigation schedule was developed based on the historical weather data for the experimental site. The crop potential evapotranspiration (ETc) was estimated from the reference evapotranspiration (ETo) using the FAO-56 Penman-Monteith equation (FAO, 1998) corrected by the crop coefficient (Kc) for pepper.

Soil water measurements

For monitoring soil water content in the root zone, PVC pipe access tubes were installed in the centre drip line for measurements with a neutron moisture meter (CPN) at depth intervals of 0.15 m up to 1.50 m. The neutron moisture meter was calibrated in situ. In addition, soil water retention functions previously developed by desorption experiments from undisturbed core soil samples using standard techniques (Klute, 1986; Klute and Dirksen, 1986) were used to transform measured soil water profiles to measured hydraulic head profiles for drainage calculations for the root zone soil water balance. The Retention Curve Program for describing the hydraulic properties of unsaturated soils (van Genuchten, 1980; van Genuchten, Leij and Yates, 1991) was used to estimate soil hydraulic parameters from the moisture retention data. In addition, the gravimetric method was applied to measure soil water content at the beginning of the experiment and during the main growth stages.

TABLE 1. Soil physical properties

Soil depth (cm)	r_b (g/cm)	Sand (%)	Silt (%)	Clay (%)	Textural class (USDA)	FC (v/v)	WP (v/v)	AWC (mm/m)
0–20	1.58	42	32	26	Loam	0.280	0.078	202
20–45	1.57	24	34	42	Clay	0.297	0.124	173
45–80	1.56	28	32	40	Clay	0.303	0.126	177
80–120	1.53	22	34	44	Clay	0.313	0.132	181

FC — field capacity, WP — wilting point, AWC — available water-holding capacity, r_b — bulk density

TABLE 2. Basic soil chemical properties in the top 20 cm soil depth

pH	Organic carbon (%)	Exch. Ca (mg·kg^{-1}·soil)	Exch. Mg (mg·kg^{-1}·soil)	Exch. K (mg·kg^{-1}·soil)	Available P (mg·kg^{-1}·soil)	Total soil nitrogen (%)
7.14	1.75	740	110	67.2	4.9	1.41

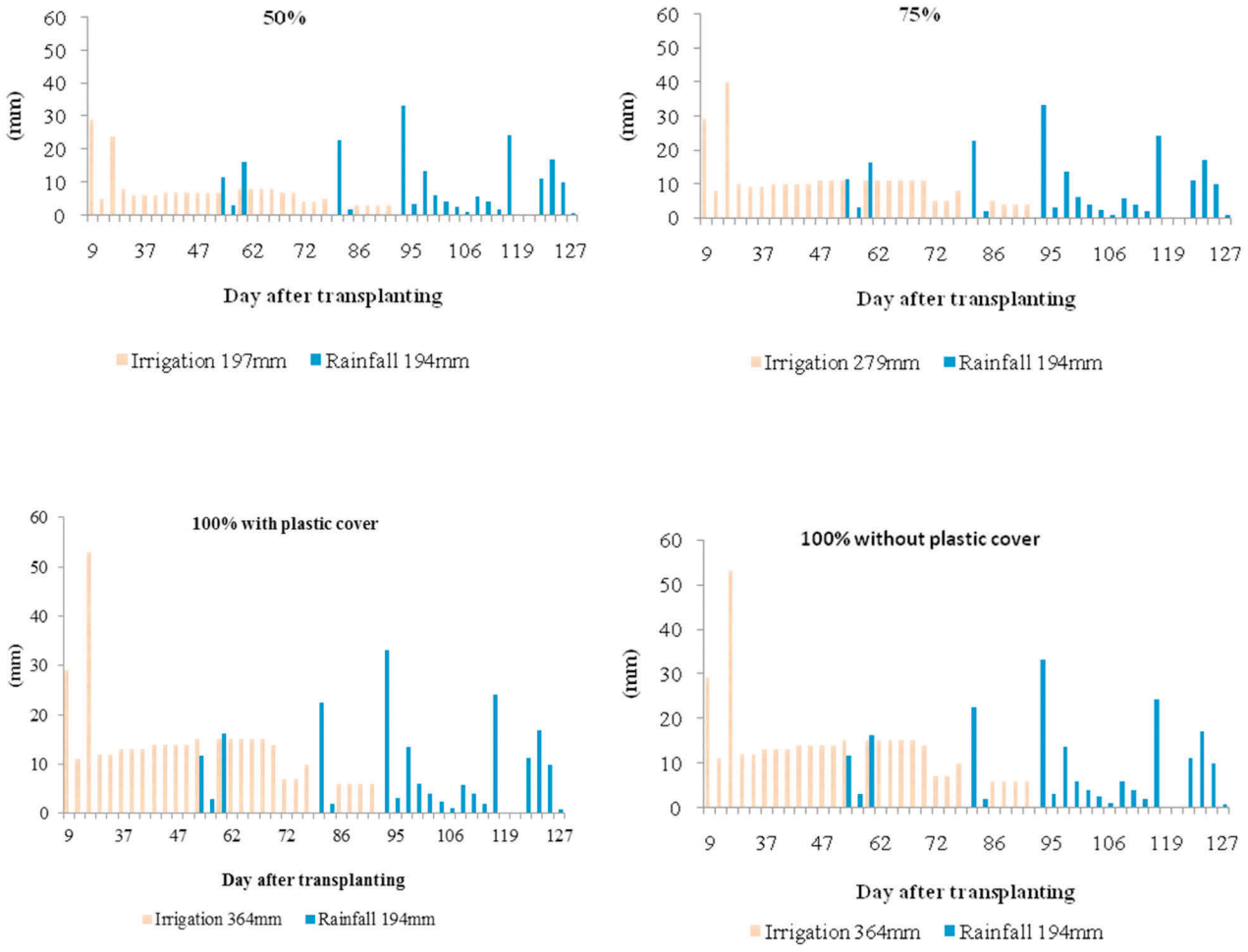


FIGURE 3. Total amount of water received through irrigation (yellow lines) and rainfall (blue lines) (2011–2012).

Root zone soil water balance

A root zone soil water balance for the paprika crop (Capsicum Annum L.), cultivar "Queen" was used to evaluate evapotranspiration. This approach to irrigation scheduling keeps track of the soil water deficit by accounting for all water additions and removals from the soil root zone. Crop water uptake or evapotranspiration accounts for the biggest (>90 percent) loss of water from the root zone while precipitation and irrigation provide the major additions. The classical water balance equation integrated over daily time periods ($\Delta t = t_{i+1} - t_i$) can be represented by:

$$(P + I) - (Q + ET) = \triangle S$$

where P — rainfall (mm); I — irrigation (mm); ET — crop evapotranspiration (mm); ΔS — soil water storage changes (mm) and Q — drainage soil water fluxes below the root-zone, during periods t_i and t_{i+1}, i being the time index.

Solving for the crop evapotranspiration, the above equation becomes:

$$ET = (P + I) - (Q + \triangle S)$$

The root zone soil water balance was evaluated to estimate the actual crop evapotranspiration (ETc). Soil water fluxes below the root zone were calculated using Darcy's equation:

$$Q = -K_{(v)} x \left.\frac{dH}{dz}\right|_z x\Delta t$$

where $K(q)$ = unsaturated hydraulic conductivity as a function of the volumetric water content; and (qv), H — hydraulic head, z — length and dH/dz — hydraulic head gradient.

The changes in root zone soil water storage (ΔS) during a given time interval ($\Delta t = t_2 - t_1$) were calculated from the integral of measured soil moisture profiles:

$$\Delta S_{(O,z)} = \int_{t_1}^{t_2} \int_{0}^{z} \theta dz$$

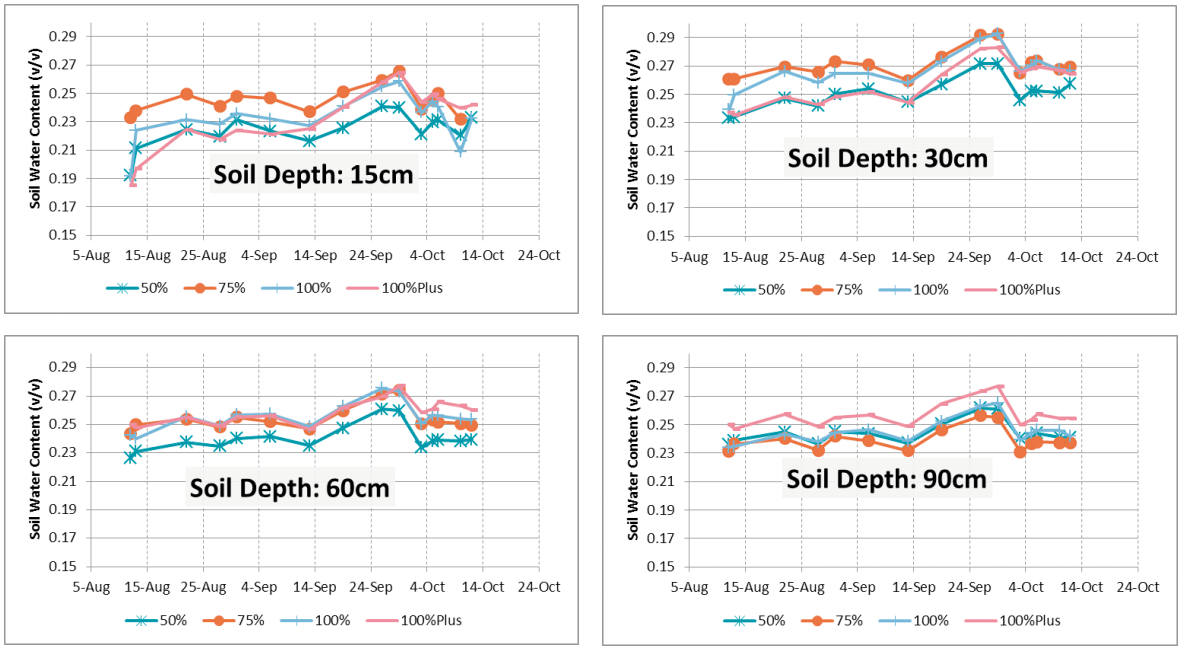


FIGURE 4. Effect of irrigation rate on soil moisture at selected soil depths.

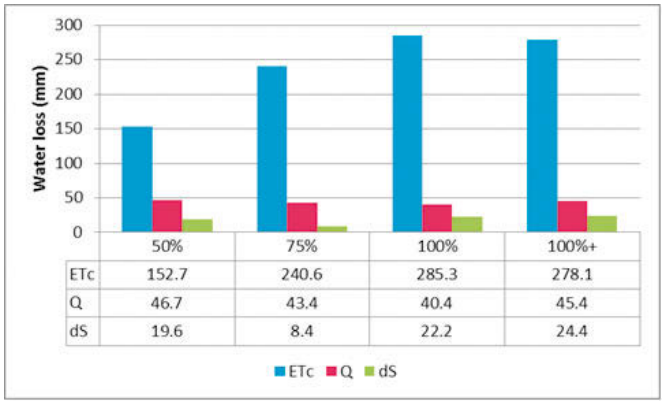


FIGURE 5. Components of the root zone water balance where ETc is crop ET (i.e. crop water uptake), Q is drainage below root zone and dS is the change in soil water storage.

Statistical analysis

The experiment was arranged in a completely randomized block design (CRBD) with four treatments and four replications. Data were analysed using GenStat and means were compared by the least square difference test (LSD) at five percent level of confidence.

RESULTS AND DISCUSSION

Weather data

The total amounts of water received through irrigation and rainfall in 2011–2012 are given in Figure 3. A total of 194 mm of rain fell during the growing period, with the majority occurring during the latter part of the cropping season.

The soil moisture at different soil depths over the cropping season affected by the rate of irrigation application is presented in Figure 4. No significant differences were recorded during the vegetative growth stage (first 70 DAT) of the crop. This may be attributed to the dominance of E during this period as the crop was becoming established. The general increase in soil water content in late September was due to the rainfall received, which to some extent cancelled treatment effects.

Generally the changes in soil water storage (DS) and drainage (Q) were positive, showing that treatments did lead to increases in soil water in the root zone (Figure 5). Results indicated that during the vegetative growth of the paprika crop, the water supplied to the root zone was partitioned into crop evapotranspiration (ETc) (78 percent), drainage (6 percent) and change in soil water storage (16 percent). No significant differences were observed between treatments and drainage and soil water storage changes except for ETc which was significantly ($p < 0.001$) lower in the 50 percent treatment compared with other treatments. The highest ETc was observed in the 100 percent treatment. The high ETc under the 100% + mulch (i.e. plastic cover) practice was predominantly E and occurred mainly during seedling establishment, i.e. before the plastic cover was placed on the soil surface. It was expected that E would be zero upon covering the surface with a plastic cover.

Crop green canopy cover and estimates of transpiration and evaporation

Digital pictures were taken fortnightly during the growing season and the data analyzed using the Green Crop Tracker software (Liu and Pattey, 2010) to estimate the percentage of green canopy cover which is an indication of the T component of crop evapotranspiration. The results presented in Figure 6 show that canopy cover was negligible during the first 25 d and thereafter increased exponentially.

Table 3 and Figure 7 show the results of the partitioning of ET into E and T during the first 70 d. The T estimates varied from 38 mm to 70 mm, with an average of 57 mm. When this is compared to the total water loss of 226 mm, T amounts to only 25 percent of total ET, implying that the majority of the added water (rainfall and irrigation) was lost through evaporation or deep drainage. The effect of mulching with plastic cover was also not significant, the T under

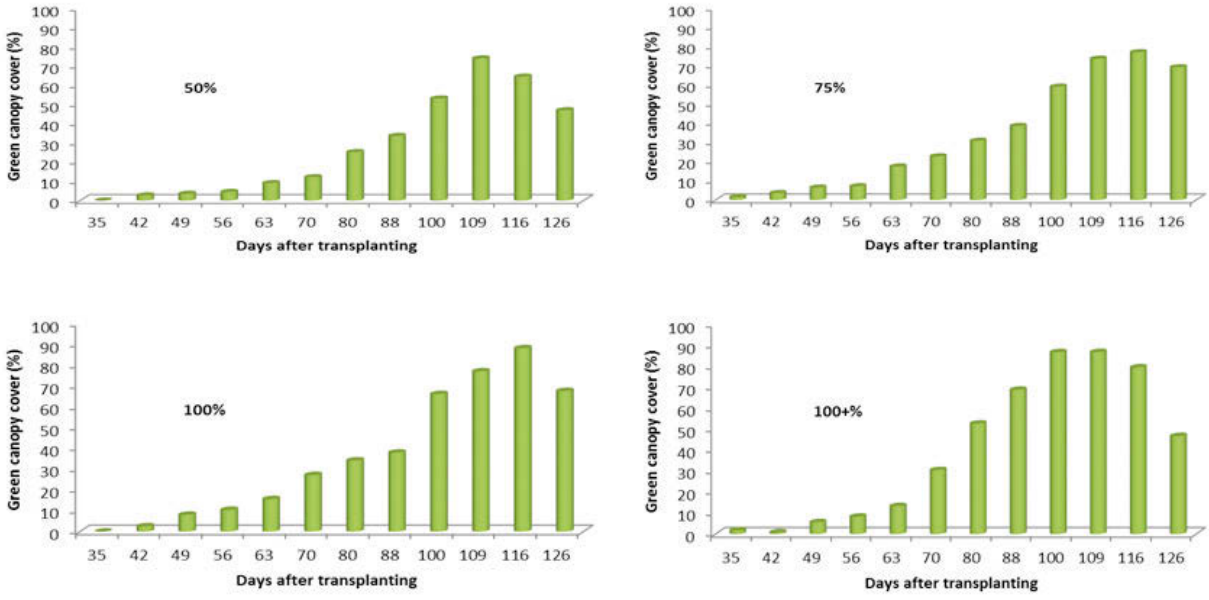


FIGURE 6. Green canopy cover of the four treatments.

TABLE 3. Partitioning of ETc (E + T), biomass production and water productivity during the vegetative stage

Treatment (% normal)	ETc (mm)	E (mm)	T (mm)	Biomass (kg/ha)	WP (ET) (kg·ha^{-1}·mm^{-1})	WP (T) (kg·ha^{-1}·mm^{-1})
50	152.7	114.1 (75%)	38.7 (25%)	232.6	1.52	6.01
75	240.6	179.3 (74%)	61.3 (26%)	310.7	1.29	5.07
100	285.3	215.0 (75%)	70.3 (25%)	421.3	1.48	5.99
Mean	226.2	169.5 (75%)	56.8 (25%)	321.5	1.43	5.69

ETc — evapotranspiration, E — evaporation, T — transpiration, WP — water productivity

plastic mulching was only 5 mm lower than without plastic as the plastic was installed after 70 DAT. The contribution of T started to increase after DAT 70 when vegetation cover increases to more than 80 percent of the total soil area.

Cumulative transpiration

Figure 8 presents the results obtained for the relationship between cumulative T and the applied water during the vegetative growth (within 70 DAT). The relationship is linear with no significant differences between the 75 percent and 100 percent treatments; however there were differences with the 50 percent treatment. The similarity between the two higher treatments may be because the plants were young and therefore unable to influence water loss through T. However, values for these levels were significantly higher than for the 50 percent treatment.

Biomass production

Results for biomass production measured during vegetative growth showed that biomass accumulated at a very low rate during the first 60 d of the growing season, and increased exponentially subsequently (Figure 9). The various irrigation treatments did not produce differences in biomass production until 60 DAP. The highest biomass was produced in the 100 percent water treatment, demonstrating the importance of water for biomass production and securing good yields.

CONCLUSIONS

This study has shown that during the vegetative growth stage of paprika, almost 78 percent of applied water was lost through ET, of which 75 percent was E and the remainder T. The water balance approach in combination with crop canopy cover analysis enabled partitioning of ET during the period studied, but whether similar levels of partitioning apply to all the growth stages requires further analysis.

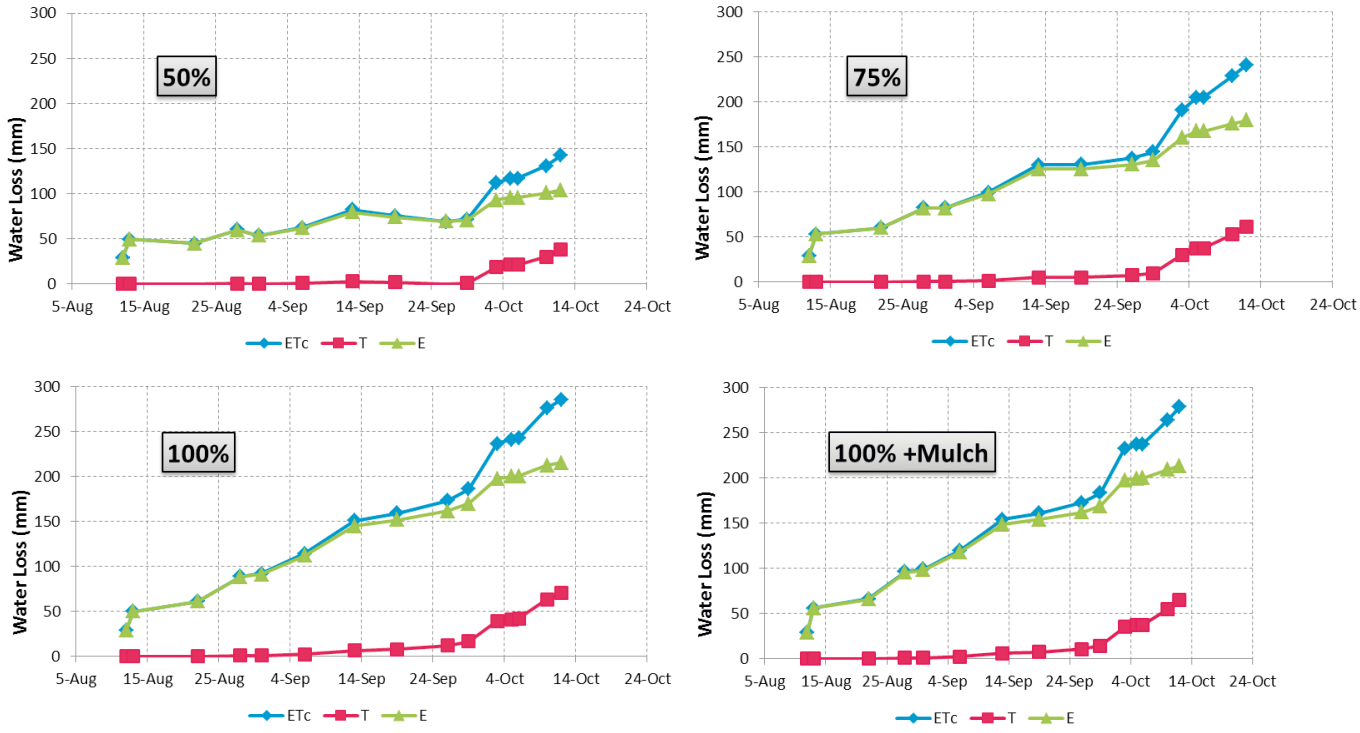


FIGURE 7. Partitioning of cumulative water loss through E and T during the vegetative period.

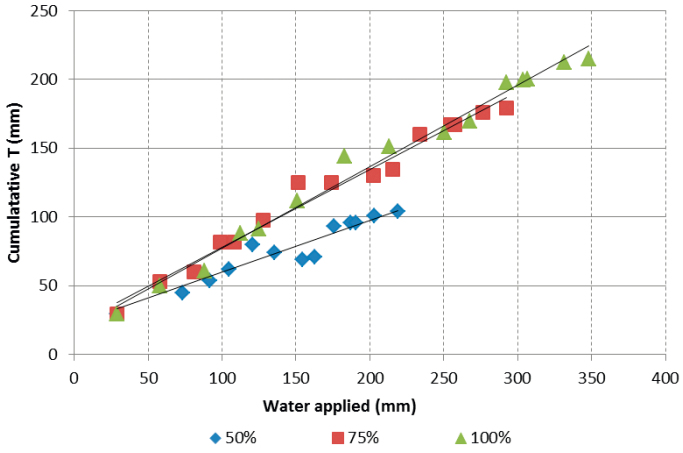


FIGURE 8. Relationship between cumulative T and applied water during vegetative growth.

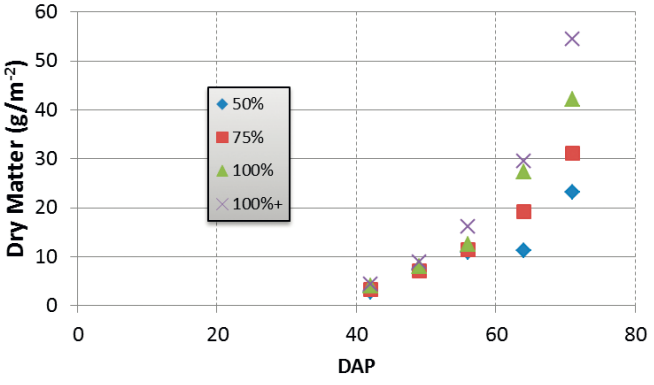


FIGURE 9. Biomass production during vegetative growth.

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SESSION 6

RECENT ADVANCES IN NUCLEAR TECHNIQUES AND INSTRUMENTATION

From Fertilizer to Food: Tracing Nitrogen Dynamics in Conventional and Organic Farming Systems Using ^{15}N Natural Abundance

P.M. Chalk[1,*], C.T. Inácio[2], and A.M.T. Magalhães[2]

ABSTRACT

Synthetic nitrogen (N) fertilizers differ markedly from organic N fertilizer sources in relative isotopic composition at natural abundance levels ($\delta^{15}N$). The objective of this paper is to provide an overview of the applications of $\delta^{15}N$ techniques to study the dynamics of synthetic fertilizers, animal excreta and composts in the soil-plant-atmosphere continuum. However, isotopic fractionation processes often complicate the interpretation of results. These fractionation processes and the factors affecting the $\delta^{15}N$ signatures of organic N fertilizers are reviewed. Published data from short-, medium- and long-term experiments with annual crop rotations and in pastures subject to organic N inputs are also examined and analyzed with respect to changes in delta nitrogen-15 ($\delta^{15}N$) signatures of the soil, the crop or pasture, the soil biota and leachates. The use of $\delta^{15}N$ to differentiate organic and conventional plant products is briefly covered. There are few data on the dynamics of N during the storage of animal excreta or the composting of agricultural wastes as shown by $\delta^{15}N$ values in the organic, inorganic or gaseous N phases. The major N loss process is ammonia (NH_3) volatilization. Reviewed data show significant relationships between bulk $\delta^{15}N$ signatures of stored manure and cumulative NH_3 loss or bulk $\delta^{15}N$ of livestock manure composts and N concentration. These significant relationships suggest that $\delta^{15}N$ may have wider applications in estimating the efficiency of N conservation during storage or composting. In addition, the combined use of bulk $\delta^{15}N$ and delta oxygen-18 ($\delta^{18}O$) signatures of nitrous oxide (N_2O) evolved during storage and composting, together with the isotopomer-derived site preference of N_2O, are emerging technologies for identifying N_2O production pathways. $\delta^{15}N$ in combination with appropriate statistical analysis is a promising diagnostic tool for differentiating organic and conventional plant products.

Key words: *animal excreta, compost, crop rotations, delta ^{15}N, isotopic fractionation, pastures.*

INTRODUCTION

In conventional farming systems the use of both synthetic and organic N fertilizers is permitted, whereas in organic systems only organic N sources may be used as fertilizer. The principal organic N fertilizer sources are animal excreta (manure and urine) and composts which may be derived from both animal wastes and crop residues. Organic fertilizer may also consist of human excreta or composts made from household or municipal wastes, including sewage sludge, but domestic- or municipally-derived organic fertilizers are not considered in this paper.

There are fundamental differences between synthetic and organic N fertilizer sources with respect to their ability to supply N for crop growth. Organic N sources must be biologically mineralized to inorganic N before they become available for plant uptake, whereas synthetic N fertilizers (ammonium and nitrate salts) are highly soluble in water and are readily available for plant uptake. Urea is the principal synthetic N fertilizer used in agriculture, and it is also quickly taken up by plants following its rapid enzymatic hydrolysis to ammonium in soil. Therefore, organic N fertilizers are often referred to as slow-release N sources, and are considered to be somewhat better synchronized with crop demand for N. However, organic fertilizers contain a mixture of both organic and mineral (NH_4^+ and NO_3^-) forms of N.

Both organic and synthetic sources are subject to several N loss processes which reduce their effectiveness to supply N for plant growth. Animal excreta must be collected and stored in intensive animal feeding operations before application to crops or pastures, and gaseous N losses (NH_3, N_2O, N_2, and other oxides of N (NO_x)) may occur during storage. Similar loss processes may occur during the composting of agricultural wastes. Nitrogen losses may occur following N fertilizer application to soil either via gaseous N emissions or nitrate leaching. Therefore it is important to estimate the N fertilizer use efficiency, and where possible to quantify the N loss pathways of synthetic and organic fertilizer sources during each phase of the continuum.

The use of the ^{15}N stable isotope is a basic tool for studying the dynamics of N in farming systems (Chalk, 1997). Both naturally-occurring differences in the relative abundance of ^{15}N in N sources, or the use of N sources artificially-enriched in ^{15}N can be used to trace the fate of fertilizer N. The literature on the application of ^{15}N to study N dynamics in animal excreta or compost-amended soils was reviewed by Dittert, Georges and Sattelmacher (1998) and Chalk, Magalhãese and Inácio (2013), respectively. The emphasis of these reviews was on quantifying post-application N use efficiency and N transformations in soil, rather than on N transformations prior to soil application i.e. during storage or composting. The objective

1 International Atomic Energy Agency, Wagramerstrasse 5, PO Box 100, 1400 Vienna, Austria
2 EMBRAPA-Soils, National Center of Soil Research, Brazilian Agricultural Research Corporation, Rua Jardim Botânico 1024, Rio de Janeiro, Brazil
* E-mail address of the corresponding author: chalkphillip@gmail.com

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TABLE 1. $\delta^{15}N$ signatures of animal diets and excreta

Animal	Diet	$\delta^{15}N$ (‰)			Reference
		Diet	Faeces	Urine	
Jersey cow	Pasture	+0.6	+2.6	–1.6	Steele and Daniel (1978)
Angus steer	Silage	+0.6	+2.1 to +2.5	–2.1 to –2.8	
Llama	Alfalfa	+0.4	+3.3	+0.1	Sponheimer *et al.* (2003)
	Bermuda grass	+5.8	+8.8	+3.7	
Swine	Not specified	+1.7 ± 1.0	+2.4 ± 0.9	–0.1 ± 1.1	Mariappan *et al.* (2009)
Dairy cow	Silage[1]	+1.8 to +8.4	+4.2 to +8.8	–0.9 to +4.1	Cheng *et al.* (2011)

[1] Nine individual silages were made from forage grasses (3 types), red clover, red clover mixed with corn or oats in different proportions

of the present paper is to provide an overview of the applications of the ^{15}N natural abundance technique to trace N transformations that occur during pre- and post-application of animal excreta and composts. The application of $\delta^{15}N$ to differentiate organic and conventional foodstuffs is only briefly covered, as this topic was comprehensively reviewed for plant products by Inácio, Chalk and Magalhães (2013).

Units of ^{15}N concentration

Stable isotopic values close to the natural abundance of the designated isotope are expressed by the notation (δ) in units of parts per thousand (per mil or ‰) relative to the international standard for that element (Chalk, 1995). Since N has only two stable isotopes (^{14}N and ^{15}N), then:

$$\delta^{15}N\ (‰) = \{[(^{15}N / {}^{14}N)_{sample} / (^{15}N / {}^{14}N)_{standard}] - 1\} \times 1\,000 \quad (1)$$

where the international standard is atmospheric N_2 ($\delta^{15}N$ = 0‰, by definition).

The $\delta^{15}N$ value can be either negative or positive depending whether it is depleted or enriched in ^{15}N relative to the standard.

Stable isotopic values of artificially-enriched samples are expressed as absolute abundance in units of atom $\%\,^{15}N$ (Chalk, 1995).

$$\text{Atom } \%\,^{15}N = (\text{number of } ^{15}N \text{ atoms / total number of } ^{15}N + {}^{14}N \text{ atoms}) \times 100 = [^{15}N / (^{14}N + {}^{15}N)] \times 100 \quad (2)$$

Thus as can be seen from Equation 2, it is incorrect to substitute atom $\%\,^{15}N$ for the $^{15}N/^{14}N$ ratio in Equation 1, as is sometimes seen in the earlier literature (e.g. Selles and Karamanos, 1986), and although a close approximation will be obtained it will nevertheless be an underestimate (Chalk, 1995).

Since the absolute ^{15}N abundance of atmospheric N_2 is 0.3663 ± 0.0004 atom % (Junk and Svec, 1958) then:

$$^{15}N \text{ enrichment (atom } \%\,^{15}N \text{ excess)} = \text{atom } \%\,^{15}N - 0.3663 \quad (3)$$

It is quite common in the literature to see atom $\%\,^{15}N$ incorrectly designated as ^{15}N enrichment. Atom % excess values are used to trace the pathways of ^{15}N-enriched fertilizers added to soil.

The relationship between the $^{15}N/^{14}N$ ratio and atom $\%\,^{15}N$ is given by:

$$^{15}N/^{14}N = \text{atom } \%\,^{15}N/(100 - \text{atom } \%\,^{15}N) \quad (4)$$

For atmospheric N_2:

$$^{15}N/^{14}N = 0.3663/(100 - 0.3663) = 0.00367647$$

$\delta^{15}N$ signatures of synthetic and organic fertilizers

Synthetic N fertilizers (ammonium salts and urea) are derived from ammonia (NH_3) produced by the Haber-Bosch process, which involves the catalytic reduction of atmospheric N_2 at high temperature and pressure by H_2 derived from methane or natural gas. Therefore the $\delta^{15}N$ signatures of synthetic fertilizers are expected to be close to that of atmospheric N_2 (0‰ by definition). A review of published data (Inácio, Chalk and Magalhães, 2013) shows that synthetic N fertilizers have slightly positive or negative $\delta^{15}N$ values within the range of –3.9 to + 5.9‰.

Organic N fertilizers are generally naturally enriched in the stable isotope ^{15}N compared with synthetic N fertilizers. A review of published data (Inácio, Chalk and Magalhães, 2013) shows that total N in manures and composts varies with $\delta^{15}N$ values in the range of +2.0 to +16.7 and +4.9 to +45.2‰, respectively. Animal excreta consist of both solid (dung) and liquid (urine) components, except for poultry where there is no urinary component. Dung and urine components often show marked differences not only in the relative amounts and N concentrations, but also $\delta^{15}N$ signatures.

Steele and Daniel (1978) reported that dairy and beef cattle urine was depleted in ^{15}N relative to the animal diet while manure was enriched (Table 1). Sponheimer *et al.* (2003) reported similar data for llamas, with faeces enriched in ^{15}N relative to both low- and high-protein diets, whereas urine was depleted in ^{15}N (–2.1‰) relative to the low-protein diet, but was not significantly different from intake $\delta^{15}N$ on the high protein diet (Table 1). Mariappan *et al.* (2009) similarly reported swine urine depleted in ^{15}N relative to diet and faeces (Table 1). More recently, Cheng *et al.* (2011) reported both positive and negative $\delta^{15}N$ values for the urine of dairy cows, while manure was always positive (Table 1). Urine was invariably depleted in $\delta^{15}N$ relative to diet, while faeces were either similar to or enriched in $\delta^{15}N$ relative to diet (Cheng *et al.*, 2011). A highly significant positive linear relationship was found between the $\delta^{15}N$ values of the feed (range of +2 to +8.5‰) and faeces (range of +4 to +9‰) (Cheng *et al.*, 2011).

Organic fertilizers contain both organic and inorganic [ammonium (NH_4^+) and nitrate (NO_3^-)] forms of N. The inorganic N concentrations are generally low compared with the total N of the manure or compost, but $\delta^{15}N$ values can be quite variable (Table 2). The inorganic N fraction is often enriched in ^{15}N compared with the total N, indicating non-uniform labelling due to isotope fractionation processes. The unusually high $\delta^{15}N$ values for inorganic N in cattle feedlot manure (Kim *et al.*, 2008; Table 2) may be indicative of substantial N losses during storage.

TABLE 2. Concentration and ^{15}N natural abundance of total and inorganic forms of N in organic N fertilizers

Material	N fraction	N concentration[1] (g/kg)	$\delta^{15}N$[1] (‰)	Reference
Cattle manure	Total	4.4–10.7[2]	+7.9[3]	Choi *et al.* (2006)
	NH_4^+	0.001–0.067[2]	+9.9[3]	
	NO_3^-	0.007–0.009[2]	+16.6[3]	
Cattle feedlot manure (sawdust bedding)	Total	9.5	+11.4	Kim *et al.* (2008)[4]
	NH_4^+	2.0	+39.8	
	NO_3^-	0.22	~+26	
Swine manure compost	Total	23.1 (1.2)	+15.3 (0.2)	Yun *et al.* (2011)
	NH_4^+	0.33 (0.014)	+12.5 (1.3)	
	NO_3^-	0.12 (0.008)	+22.6 (0.1)	

[1] Data in parentheses are standard deviations of the mean
[2] Range of values over 4 yr
[3] Mean values over 4 yr
[4] Data are time zero at the beginning of composting for 90 d

TABLE 3. Discrimination factors (ε) for some N cycle processes

Process	Discrimination factor (‰)	
	Högberg (1997)[1]	Robinson (2001)
Ammonification (organic N → NH_4^+)	≈0	0–5
Nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$)	15–35	15–35
Ammonia volatilization ($NH_4^+ \rightarrow NH_3\uparrow$)	29	40–60
Denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow N_2O\uparrow \rightarrow N_2\uparrow$)	0–33	28–33
N_2O and NO production during NH_4^+ oxidation		35–60
Biological N_2 fixation	0–2	0–6
Inorganic N assimilation by plants	0–20	0–19 (NO_3^-)
		9–18 (NH_4^+)

[1] Values given as α were converted to ε by Equation 8

Isotopic fractionation processes affecting the $\delta^{15}N$ signatures of organic N fertilizers

Units

Isotopic fractionation can occur as a result of physical (e.g. diffusion), chemical (equilibria or ion exchange) or biological (enzymatic) processes. It can be expressed by the fractionation factor (α) where $\alpha = \delta_A / \delta_B$ in an equilibrium reaction, where A is a reactant and B is a product. Isotopic fractionation can also be expressed as discrimination (Δ or ε) in units of per mil (‰).

$$\varepsilon(‰) = (\delta_s - \delta_p)\,[1 + (\delta_p\ 1\ 000)] \quad (5)$$

where δ_s is substrate and δ_p is product

An approximation of the above equation is

$$\varepsilon(‰) = \delta_s - \delta_p \quad (6)$$

The fractionation factor (α) is approximated by:

$$\alpha = (\varepsilon\ /1\ 000) + 1 \quad (7)$$

or

$$\varepsilon = (\alpha - 1) \times 1\ 000 \quad (8)$$

Thus for a fractionation factor (α) of 1.020, ε of the product = –20‰ relative to the substrate (Högberg, 1997).

Processes

Fractionation factors for all physical, chemical and microbially-mediated transformations of N in soil are significant, especially NH_3 volatilization, nitrification and dissimilatory NO_3^- reduction (biological denitrification) (Högberg, 1997; Robinson, 2001). These three processes are the major N transformations affecting the natural ^{15}N isotopic composition of the principal organic N fertilizers, animal wastes and composts. Nitrogen isotope discrimination (ε) factors for the major N cycle processes (Högberg, 1997; Robinson, 2001) are given in Table 3.

Volatilization of NH_3 involves several steps (i–iv) in which isotopic fractionation can occur (Högberg, 1997):

(i) Equilibrium effect (A ↔ B in solution)

$$^{14}NH_3 + {}^{15}NH_4^+ \leftrightarrow {}^{15}NH_3 + {}^{14}NH_4^+$$

NH_4^+ is more enriched with ^{15}N than NH_3 at equilibrium. i.e. = α = 1.020 – 1.027

(ii) Kinetic effects

1. Diffusion of NH_3 in solution to the site of volatilization (α ≈ 1 000)
2. Volatilization of NH_3 (α = 1.029)
3. Diffusion of NH_3 away from the site of volatilization (α ≈ 1 000)

The compound effect of these processes on the net fractionation can be large (Högberg, 1997) since α for equilibrium effect and for volatilization of ammonia are greater than 1.02. Ammonia volatiliza-

tion is quantitatively the most significant N loss process during storage of animal excreta, the voiding of excreta on grazed pastures and during the composting of agricultural wastes.

The first step in nitrification, the enzymatic oxidation of $NH_4^+ \rightarrow NO_2^-$ was shown to have a large fractionation factor (1.015 to 1.036) in pure cultures of *Nitrosomonas* (Högberg, 1997). Thus a decrease in NH_4^+ concentration with a concomitant increase in its $\delta^{15}N$ signature and the production of NO_3^- relatively depleted in ^{15}N all point to active nitrification (Wrage *et al*., 2004). According to Högberg (1997) the second step in nitrification ($NO_2^- \rightarrow NO_3^-$) is not normally rate-limiting and should therefore not lead to further fractionation. However, this is frequently not the case in urine patches and during the composting of animal excreta where the oxidation of nitrite is inhibited by the high pH resulting in the temporary accumulation of nitrite (e.g. Clough *et al*., 1998; Sasaki *et al*., 2006).

Denitrification can be a significant fractionation process during the storage of animal excreta and the composting of agricultural wastes. The overall process or so-called "total denitrification" ($NO_3^- \rightarrow NO_2^- \rightarrow N_2O\uparrow \rightarrow N_2\uparrow$) has a fractionation factor of 1.028 – 1.033 (Robinson, 2001) although Högberg (1997) gives a much wider range (Table 3). Robinson (2001) also shows a large discrimination factor for N_2O and NO produced during nitrification, of the same order of magnitude as for NH_3 volatilization (Table 3).

N_2O production pathways have been investigated using the dual isotope measurements of $\delta^{15}N$ and $\delta^{18}O$ of the N_2O emitted from soil (e.g. Yamulki *et al*., 2000). Several studies have reported that $\delta^{15}N$ values of soil-emitted N_2O can be as low as –56‰ and as high as +3‰, with $\delta^{18}O$ values varying between –21‰ and +57‰ (Yamulki *et al*., 2001; Bol *et al*., 2003). The $\delta^{15}N$ values of soil-emitted N_2O are thus lower than the corresponding tropospheric (+18.7‰) and stratospheric (+21.3‰) values (Yamulki *et al*., 2001; Bol *et al*., 2003). Nitrous oxide produced by nitrification or denitrification in soils is depleted in ^{15}N relative to its substrate (NH_4^+ and NO_3^-, respectively), but as noted above, the fractionation is larger for nitrification-derived N_2O. Therefore, a shift in N_2O production from nitrification to denitrification increases both $\delta^{15}N$ and $\delta^{18}O$ values up to 20–50‰ and 10–25‰, respectively (Yamulki *et al*., 2001; Bol *et al*., 2003). However, if urine is the main source of nitrification, a shift in the $\delta^{15}N$ signature (but not in the $\delta^{18}O$ signature) would be expected, because the oxygen in urea [$CO(NH_2)_2$] would be hydrolyzed to CO_2 (Yamulki *et al*., 2001).

When N_2O is further reduced to N_2 during total denitrification, $\delta^{15}N_2O$ becomes more enriched relative to the N_2 product. Thus simultaneous measurements of the $\delta^{15}N$ signatures of emitted N_2O and N_2 permits the amount of $N_2O \rightarrow N_2$ to be calculated, and hence improves estimates of the relative contribution of nitrification and denitrification to total N_2O emissions from soils (Bol *et al*., 2003).

The intramolecular distribution of N isotopes in N_2O is an emerging tool for defining the relative importance of microbial sources of this greenhouse gas (Sutka *et al*., 2006). Since N_2O has two N atoms within the asymmetric molecule (central and outer N), ^{15}N is distributed across three principal isotopomers, $^{15}N^{15}NO$, $^{15}N^{14}NO$, $^{14}N^{15}NO$. A technique developed by Sutka *et al*. (2006) enables the individual measurement of $^{15}N^{14}NO$ and $^{14}N^{15}NO$. The difference in $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ is the so-called site preference ($SP = \delta^{15}N^{\alpha} - \delta^{15}N^{\beta}$, where $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ represent the $^{15}N/^{14}N$ ratios at the centre and outer N atoms, respectively). The difference in the site preference for N_2O from hydroxylamine oxidation (~33‰) and nitrite reduction (~0‰) found in a pure culture study (Sutka *et al.,* 2006) can be used to differentiate the relative contributions of nitrification and denitrification to N_2O emissions. Yamulki *et al.* (2001) considered that site preference can provide a more fundamental and sensitive analysis of N_2O sources and production processes compared with the bulk $\delta^{15}N$ analysis of N_2O.

Factors affecting the $\delta^{15}N$ signatures of organic N fertilizers

Storage of animal excreta

Many types of storage facilities for animal excreta exist on farms worldwide. In the UK, the principal types of storage facilities for dairy, cattle and swine excreta are middens (piles or heaps with a permeable or impermeable base), slurry tanks (below or above ground) constructed of steel or concrete, and lagoons with pervious or impervious linings (Nicholson and Brewer, 1997).

Hristov *et al.* (2009) and Lee *et al.* (2011) studied the cumulative amounts and $\delta^{15}N$ signatures of NH_3 emitted from dairy manure during simulated storage under laboratory conditions. $\delta^{15}N$ values of NH_3 volatilized increased quadratically ($r^2 = 0.92$; ***$p < 0.001$) from –31‰ (1 d) to –15‰ (14 d) while the $\delta^{15}N$ of total N remaining in manure also increased quadratically ($r^2 = 0.96$) from +5.6 to +7.2‰ (Hristov *et al*., 2009). A highly significant positive linear relationship was observed between cumulative NH_3 loss and the $\delta^{15}N$ signature of the stored manure ($r^2 = 0.76$; ***$p < 0.001$) over the range of $\delta^{15}N$ in manure of 4 to 8‰ (Hristov *et al.,* 2009).

Ammonia volatilization was most significant during the first 2–3 d of storage and 90 percent of emitted NH_3 came from the urine component of the faeces as a consequence of rapid urea hydrolysis (Lee *et al*., 2011). A sigmoidal curve ($r^2 = 0.96$, ***$p < 0.001$) best described the $\delta^{15}N$ of volatilized NH_3 during incubation for 30 d (Lee *et al*., 2011). NH_3 was highly depleted in $\delta^{15}N$ at the beginning of the manure storage process, and $\delta^{15}N$ values of manure reached a plateau which coincided with the decline in NH_3 volatilization. Therefore, $\delta^{15}N$ of volatilized NH_3 is a promising tool for estimating cumulative ammonia losses during storage of animal excreta, but further testing is required with different excreta under different and more realistic conditions of storage.

The earthen anaerobic lagoon is a common method of on-farm storage of feedlot runoff and slurries from dairy and pig barns in mid-western USA. Mariappan *et al.* (2009) studied the spatial and temporal concentrations and $\delta^{15}N$ signatures of total N and NH_4^+ within 13 anaerobic lagoons of variable volumes receiving dairy (one), cattle (two) and swine (eleven) wastes. $\delta^{15}NH_4^+$ varied from +2.0 to +59.1‰, was spatially uniform within the top 1.5 m of the lagoon, and was not statistically different from the total $\delta^{15}N$ value. Based on comparisons with feed and fresh manure and urine, most ^{15}N isotopic fractionation occurred after excretion and was affected by management and environmental factors (Mariappan *et al*., 2009). $\delta^{15}NH_4^+$ enrichment increased when NH_3 volatilization increased with increasing seasonal temperatures, and lagoons that were frequently pumped out and refilled with fresh waste were not characterized by the high $\delta^{15}N$ values normally associated with animal wastes. Wastes must mature in lagoons in order to develop high levels of $\delta^{15}NH_4^+$ enrichment (> +10‰) (Mariappan *et al.,* 2009).

Composting of agricultural wastes

The $\delta^{15}N$ signature of corn silage increased by +7.9‰ during aerobic-thermophilic composting (Lynch, Voroney and Warman, 2006; Table 4). Composting created a more homogeneous bulk $\delta^{15}N$ signature compared with the feedstock, as seen by the lower sub-sample standard deviation (Table 4). Kim *et al.* (2008) similarly observed an increase in the $\delta^{15}N$ signature of cattle manure composted with sawdust bedding of +4.2‰, and of +3.4‰ for manure composted with rice hull bedding (Table 4).

TABLE 4. Natural ^{15}N abundance in agricultural wastes and derived composts

Residue	δ^{15}N (‰)	Reference
Corn silage	+0.3 ± 1.3	Lynch, Voroney and Worman (2006)
Corn silage compost	+8.2 ± 0.4	
Cattle manure	+7.6	Kim *et al.* (2008)
Cattle manure + rice hull[1] compost	+11.0	
Cattle manure	+11.4	
Cattle manure + sawdust[2] compost	+15.6	

[1] δ^{15}N of rice hull = +4.9 ± 0.1‰

[2] δ^{15}N of sawdust = +1.7 ± 0.2‰

According to Lynch *et al.* (2006), the observed compost δ^{15}N enrichment is attributable to a combination of fractionation mechanisms, including microbial isotope discrimination during N turnover, a shift to more complex N compounds, and fractionation during NH_3 volatilization, with the relative contributions being unknown. Kim *et al.* (2008) reasoned that NH_3 volatilization would be the dominant process in the early thermophilic stage of composting of cattle manure due to the fast hydrolysis of the high concentrations of urea in livestock excreta, followed by slow or insignificant increases in δ^{15}N in the latter stages. This hypothesis remains to be tested.

Kim *et al.* (2008) observed an increase in the δ^{15}N of NH_4^+ from +30.2 to +41.7‰ in manure + rice hull compost, and from +39.8 to +47.8‰ in the manure + sawdust compost, while the δ^{15}N of NO_3^- fluctuated during composting within the approximate range of +25 to +45‰. Based on both the temporal changes in the concentrations and isotopic signatures of NH_4^+ + NO_3^-, Kim *et al.* (2008) concluded that loss of NH_4^+ in the early stages of composting through NH_3 volatilization and nitrification, and loss of NO_3^- in the latter stage (after 60 d) through denitrification were the primary reasons for the increase in δ^{15}N in the composted manure.

There are presently no data available on the δ^{15}N values of NH_3 emissions during composting. However, a weak negative linear relationship was observed between N concentrations and δ^{15}N values of livestock manure composts (r^2 = 0.16; *p < 0.05) over the range of 10 to 35 g·N/kg and δ^{15}N of +9 to +22‰ (Lim *et al.*, 2010). It would be expected that treatments designed to conserve N during composting would exert a strong influence on the δ^{15}N values of volatilized NH_3 and hence compost N concentrations and δ^{15}N values, but data are not yet available.

In contrast to the lack of data for NH_3 there are a few measurements on the natural ^{15}N abundance of N_2O formed during composting. From 0.5 to 1.6 percent of total N was emitted as N_2O during the composting of livestock waste in turned and static piles, respectively, with the δ^{15}N signature of N_2O increasing markedly from –23.1 to –0.2‰ (Yoh *et al.*, 2003). The bulk δ^{15}N signature of N_2O produced during thermophilic composting of cow manure with orchard grass increased from –20 to –15‰ between day 14 and day 45 (Maeda *et al.*, 2010), but then suddenly decreased at day 45 to reach –35‰ at day 56. The isotopomer methodology was applied to identify the sources of N_2O emissions. It was found that denitrification was the main source of N_2O following the turning of the compost pile, with a concomitant reduction in the concentrations of NO_2^- and NO_3^-. An increased value in site preference indicated that nitrification, which occurred mainly in the surface of the pile, partially contributed to N_2O emissions between the turnings (Maeda *et al.*, 2010).

δ^{15}N *s*ignatures of soils, crops, soil biota and leachates under organic and conventional fertilizer regimes

Soils and plants

Experiments of short- (42 to 157 d, medium- (4 to 12 yr) and long-term (30 to 91 yr) duration have been carried out either in the glasshouse or field to compare the temporal changes in the concentrations and δ^{15}N signatures of total soil N and crop N uptake

TABLE 5. N concentration and natural ^{15}N abundance of N fertilizer sources and crop N in short-term pot and field experiments with animal excreta, composts and synthetic N fertilizer

Crop / scale/ duration	N fertilizer source				Plant material			Reference	
	Type[1]	Total N (g/kg)	N rate (kg/ha)	δ^{15}N (‰)	Part	Total N (g/kg)	N uptake (g/plant)	δ^{15}N (‰)	
Maize/ pot / 70 d	C		0				1.83	+6.3	Choi *et al.* (2002)
	U	451	150	–2.3 ± 0.2	Above ground		3.00	+6.0	
	SMC	20.6	150	+13.9 ± 0.2			3.44	+6.7	
Chinese cabbage / pot / 42 d	SMC	25.3	0	+16.2	Outer leaf[3]	24.3		+14.1	Yun and Ro (2009)
			0.5[2]			31.1		+22.2	
			1.0[2]			40.9		+24.5	
			1.5[2]			48.4		+24.4	
Maize / field / 157 d	C		0		Grain	19 ± 1		+6.3 ± 0.3	Szpak *et al.* (2012)
	AS	211 ± 1	700	–0.7 ± 0.1		16 ± 0		+5.8 ± 0.2	
	CD	24 ± 6	75	+13.9 ± 0.6		16 ± 0		+8.1 ± 1.6	
	SG	82 ± 9	200	+38.1 ± 0.6		15 ± 1		+21.2 ± 0.2	

[1] C — control; U — urea; SMC — swine manure compost; AS — ammonium sulphate; CD — camelid dung; SG — seabird guano

[2] Unit is g/kg

[3] Lsd (*p < 0.05) between N rates; Total plant N = 2.1 g/kg; δ^{15}N plant material = 1.0‰

under animal excreta, composts and synthetic N fertilizer regimes. A range of annual crops in short-term (single season) experiments or in medium- to long-term crop rotations has been used.

Short-term (single season) experiments

Choi *et al.* (2002) found large differences in the $\delta^{15}N$ signatures of urea and swine manure compost treatments applied at the same N rate in a pot experiment, which were reflected in the $\delta^{15}N$ signatures of the foliage of maize at day 30. However, these differences were short-lived and converged to the initial $\delta^{15}N$ value of total soil N of +6.9 ± 0.3‰ after 70 d, despite the increasing N uptake from control, to urea to compost treatments (Table 5).

Yun and Ro (2009) found a highly significant positive linear relationship between the N uptake of Chinese cabbage in a pot experiment and the N application rate of swine manure compost, which had a $\delta^{15}N$ value of total N of +16.2‰. There were small differences in the N concentrations between outer (older) and inner (younger) leaves, and the N concentration increased with N rate (Table 5). However, the $\delta^{15}N$ values were significantly higher for outer compared with inner leaves, which often exceeded the $\delta^{15}N$ value of the total N of the applied compost (Table 5). This apparently anomalous result was due to the non-uniform $\delta^{15}N$ label between the organic and inorganic components of the compost. The higher $\delta^{15}N$ values of the compost NH_4^+ and NO_3^- pools exerted a stronger influence than the total compost N.

In contrast to the previous short-term pot experiments, the $\delta^{15}N$ signatures of maize grain in a field experiment mirrored the $\delta^{15}N$ signatures of the organic N inputs (Szpak *et al.*, 2012). The grain in the ammonium sulphate treatment had a lower $\delta^{15}N$ value than the control, while the grain in the dung and guano treatments had much higher $\delta^{15}N$ signatures than the control (Table 5). The authors claimed that the guano had the highest $\delta^{15}N$ value (+38.1 ± 0.6‰) for an organic N fertilizer reported to date (2012), but Choi *et al.* (2007) previously reported similar values of +40.1 ± 3.4‰ and +45.2 ± 4.1‰ for two compost samples.

Medium-term crop rotations

Choi *et al.* (2006) applied variable rates of swine manure slurry, cattle manure and urea to a soil in each of four yr (Table 6). There were no significant effects on surface soil total N or its ^{15}N natural abundance compared to the control. In contrast, Zhao, Maeda and Ozaki, (2002) found the annual addition of swine manure compost for six yr increased both total soil N and its natural ^{15}N abundance compared with the control at site 1, while at site 2 annual addition of cattle manure for 12 yr resulted in increasing soil total N and its $\delta^{15}N$ signatures with increasing rates of manure (Table 6).

Long-term crop rotations

Total soil N did not differ between control and animal manure treatments after 91 yr (Bol *et al.*, 2005), whereas it was significantly higher in the manure treatment compared with the control after 42 yr (Gerzabek, Haberhauer and Kirchmann, 2001), and between livestock manure compost and the control after 30 yr (Nishida *et al.*, 2007). In addition, total N either decreased (Gerzabek, Haberhauer and Kirchmann, 2001) or remained unchanged over time in the control or synthetic N fertilizer treatments (Nishida *et al.,* 2007), whereas it increased in manure and compost treatments (Table 7). These differences are most likely due to differences in climatic and edaphic factors as well as the intrinsic differences in the characteristics and rates of addition of the organic N sources.

The $\delta^{15}N$ signatures of total N in surface soil (Table 7) did not differ significantly between control and manure treatments (Gerzabek, Haberhauer and Kirchmann, 2001) but they were higher in manure (Bol *et al.*, 2005), compost and ammonium sulphate treatments (Nishida *et al.*, 2007). The last crop of sugar beet (1985) also had higher $\delta^{15}N$ values in the tops of the manure (+6.5‰) compared with the control (+2.0‰) treatment (Bol *et al.*, 2005). Nishida *et al.* (2007) found that the $\delta^{15}N$ signatures of total N in surface soil decreased in the control and ammonium sulphate treatments over time, but increased in the compost treatment (Table 7). Therefore there were no consistent trends in the relative long-term temporal changes in total soil N and the corresponding $\delta^{15}N$ signatures (Table 7). How-

TABLE 6. N concentration and natural ^{15}N abundance of annual fertilizer amendments and total N in surface soil in medium-term field experiments (crop rotations) with animal excreta, composts and synthetic N fertilizer

Annual fertilizer amendment					Soil					
Type[1]	Time (yr)	Total N (g/kg)	N rate (kg/ha)	$\delta^{15}N$ (‰)	Depth (cm)	Total N (g/kg)		$\delta^{15}N$ (‰)		Reference
						Initial	Final	Initial	Final	
C	6		0		0–20		3.8		+6.6[2]	Zhao, Maeda and Ozaki, (2002) Site 1
SMC		56.0	800	+14.3			5.5		+9.6[2]	
CM	12	25.3	163	+19.1			4.8		+9.4[2]	Zhao, Maeda and Ozaki, (2002) Site 2
			325				4.9		+10.3[2]	
			650				5.8		+10.4[2]	
C	4		0		0–30	1.7	1.6	+4.1	+4.1	Choi *et al.* (2006)
SMS		2.6 – 4.6[3]	58–172[4]	+4.7 – + 5.6		1.6	1.6	+4.1	+4.4	
CM		4.4 – 10.7	82–397	+6.8 – + 8.5		2.0	2.2	+3.9	+4.3	
U		466	39–90	–0.6 – + 1.9		1.7	1.6	+3.9	+4.0	

[1] C — control; SMC — swine manure compost; CM — cattle manure; SMS — swine manure slurry; U — urea
[2] All values are means of 2 or 3 replicates with a standard error within ± 0.3‰
[3] Unit is g/L
[4] Yr 1–3 (no slurry was applied in year 4)

TABLE 7. N concentration and natural ^{15}N abundance of annual fertilizer amendments and total N in surface soil in long-term field experiments (crop rotations) with animal excreta, composts and synthetic N fertilizer

Annual fertilizer amendment					Soil[7]				
Type[1]	Time (yr)	Total N (g/kg)	N rate (kg/ha)	δ^{15}N (‰)	Total N (g/kg)		δ^{15}N (‰)		Reference
					Initial	Final	Initial	Final	
C	42		0		1.7	1.3 ± 0.05		+8.5[2]	Gerzabek *et al.* (2001)
AM		17.6 ± 3.0	217	+7.6[6] ± 1.4		2.2 ± 0.1		+8.7[2]	
C	91		0			1.2 ± 0.05		+4.7[3] ± 0.2	Bol *et al.* (2005)
AM			42–98			1.3 ± 0.04		+5.8[3] ± 0.2	
C	30		0		0.9	1.0	+2.6	+2.1	Nishida *et al.* (2007)
AS		212	60–192	–0.9 - –1.4[4]	1.6	1.6	+3.2	+1.4	
LMC		4.0 ± 1.6 -6.0 ± 0.9	81–127[5]	+6.4 ± 1.0 - +17.4 ± 0.7	1.0	2.6	+3.7	+6.9	

[1] C — control; AM — animal manure; AS — ammonium sulphate; LMC — livestock manure compost
[2] Values are not significantly different (*p < 0.05)
[3] The archived 1985 crop of sugarbeet (the experiment was started in 1894) had δ^{15}N values of +2.0 and +6.5‰ in the tops in control and manure treatments, respectively
[4] Values for 2002 and 2003 samples
[5] Calculated assuming the tabulated moisture content (0.703–0.811 g/g) was on a dry weight basis
[6] Six samples from individual yr in the 1980s–1990s
[7] Soil depth was 0–20 cm except for Nishida *et al.* (2007) where it was 0–15 cm

ever, Koerner *et al.* (1999) found that soil δ^{15}N signatures could be used to identify past agricultural land use where fields had reverted to forests during the past 70–100 yr. Previous garden soils with a history of manure inputs had significantly higher δ^{15}N signatures on average (+3.8‰) than ancient forests (0.0‰) or previous pastures (+1.4‰).

Soil biota

Dijkstra *et al.* (2006a) found that on average the soil microbial biomass had a consistently higher δ^{15}N value (+9.7‰) compared with the total soil N (+6.6‰) across a broad range of soil types, vegetation and climates. Dijkstra *et al.* (2006b) similarly found consistently higher δ^{15}N values of microbial N compared with total soil δ^{15}N across a cattle manure gradient extending 100 m from a reservoir in a semi-arid, high-desert grassland, and whereas the total N increased closer to the reservoir the δ^{15}N value of the total N remained relatively constant (~+10‰), while the microbial δ^{15}N increased closer to the reservoir from ~+14 to ~+18‰.

Schmidt and Ostle (1999) similarly found that soil invertebrates (earthworms) had higher δ^{15}N signatures compared with total soil N in an experiment where cattle slurry (δ^{15}N = +13.2 to +17.0‰) and NH_4NO_3 (δ^{15}N = –2.0 to +0.5‰) were individually applied to plots for three consecutive yr. Soil total N (0–10 cm) in organic- and synthetic-fertilized plots had δ^{15}N signatures of +5.9 ± 0.6‰ and +3.9 ± 0.1‰, respectively, while earthworms had δ^{15}N signatures of +6.5 ± 0.2‰ and +5.2 ± 0.3‰, respectively.

Leachates

Choi, Lee and Ro (2003) highlighted the difficulties of identifying sources of NO_3^- contamination of groundwater using δ^{15}N signatures, since the δ^{15}N value is not only a function of source but also of fractionation during formation or consumption. For example, denitrification enriches ^{15}N in nitrate, and if this process is significant it could mask predicted differences between sources such as synthetic fertilizers which are relatively more depleted in ^{15}N compared with animal excreta and composts. In a survey of wells monitored over a 3–y period within defined agricultural management systems, Choi *et al.* (2007) were able to correlate the nitrate concentrations and δ^{15}N signatures of well water with applications of compost, compost + urea fertilizer or no soil amendment.

δ^{15}N signatures have proven to be a valuable tool in identifying leakages of animal wastes stored and treated in anaerobic lagoons as well as from land application of the lagoon effluent by spray irrigation onto adjacent fields (Karr *et al.*, 2001 and Karr, Showers and Jennings, 2003; Israel *et al.*, 2005). Nitrate generated from commercial application of swine waste within a catchment was discharged to surface waters by ground water passing beneath the sprayfields and adjacent riparian buffers (Karr *et al.*, 2001). Median values of δ^{15}N of +15.4 ± 0.2‰ were measured *in situ* in the liquid total N of the waste lagoons, and in nitrate in shallow ground water beneath and adjacent to sprayfields, a stream draining sprayfields and waters up to 1.5 km downstream. Israel *et al.* (2005) pointed out that the study of Karr *et al.* (2001) was carried out on a site that had received lagoon effluent for 20 yr prior to the more stringent regulations on land application of animal waste imposed in 1993, and concluded that the riparian buffer was overwhelmed either due to the nitrate concentration in the ground water moving to the stream or to its rapid flux and shallow flow path.

Karr, Showers and Jennings (2003) further demonstrated the sensitivity of δ^{15}N monitoring where low-level export of nitrate from confined dairy farming could be detected even when stream nitrate concentrations were low and derived predominantly from natural soil sources.

δ^{15}N signatures in the soil-plant-atmosphere continuum in pastures subject to organic N inputs

Long-term (32–36 yr) measurements were made of δ^{15}N in soils and plants inside and outside enclosures in an area subject to native ungulate grazing (Frank and Evans, 1997). Across six topographically diverse sites, δ^{15}N of soil (0–20 cm) outside enclosures was 0.7‰ higher on average than inside enclosures, while plant N was 0.7‰ less on average under grazing. Soil δ^{15}N of urine and dung patches were significantly higher than control areas. Frank and Evans (1997) concluded that grazing probably led to an increase in soil δ^{15}N by promoting N losses (NH_3 volatilization, etc). In a later study, Frank,

Evans and Tracy (2004) measured $\delta^{15}N$ in soil, plants and NH_3 volatilized from simulated ungulate urine patches. $\delta^{15}N$ of acid-trapped NH_3 increased from –28‰ (day 1) to –0.3 ‰ (day 10) from urine that originally had a $\delta^{15}N$ value of +1.2‰. The isotope data showed that shoots absorbed ^{15}N-depleted NH_3 volatilized from the soil, which confirms the results obtained by Denmead *et al.* (1976) using chemical and micro-meteorological techniques.

The natural ^{15}N abundance of plants and soils in medium- to long-term experimental plots (8–50 yr) maintained under different management practices were measured in a montane grassland (Watzka, Buchgraber and Wanek, 2006). Plots differed in the types of N inputs (mineral fertilizer, cattle slurry, stable manure) and rates of application (0–200 $kg{\cdot}N{\cdot}ha^{-1}{\cdot}y^{-1}$). $\delta^{15}N$ of topsoil and plants increased with N fertilizer rate, and the $\delta^{15}N$ signatures reflected the higher values for the organic compared with the mineral inputs. N balances were calculated from N inputs (fertilizer, atmospheric deposition, biological N_2 fixation) and outputs in harvested material. Strong positive correlations were found between $\delta^{15}N$ of topsoil and N balance (range –60 to +120 $kg{\cdot}N{\cdot}ha^{-1}{\cdot}y^{-1}$). The authors concluded that the different $\delta^{15}N$ signals in topsoil were due to isotopic fractionation arising from increased N losses (mineral fertilizer induced) and to both increased N losses and preservation of the $\delta^{15}N$ input signal (organic fertilizers).

In another long-term experiment (20 yr), Krizan *et al.* (2009) measured changes in the concentration and $\delta^{15}N$ values of total soil N in a lysimeter experiment where cattle slurry ($\delta^{15}N$ = +8.9 ± 0.5‰) and calcium ammonium nitrate ($\delta^{15}N$ = –1.0 ± 0.2‰) were applied at 0 and 480 $kg{\cdot}N{\cdot}ha^{-1}{\cdot}y^{-11}$ to temperate grassland. Total N remained unchanged during the experiment and leaching losses were small. The $\delta^{15}N$ of topsoil increased on average from +1.8 ± 0.4‰ to +6.0 ± 0.4‰ and that of the plants from –1.2 ± 1.3‰ to +4.8 ± 1.2‰ with increasing N fertilizer rate, with samples from slurry plots being relatively more enriched in ^{15}N. The results suggested that part of the fertilizer $\delta^{15}N$ signal was preserved in the soil, but that isotope fractionation of up to 1.5‰ added to the $\delta^{15}N$ values in soils and plants, indicative of long-term inefficient N usage and past N management.

Yamulki *et al.* (2000) found that cattle excreta patches were an important source of atmospheric N_2O, with emissions significantly greater from urine than from dung. Flux patterns showed a marked diurnal variation with maxima in early morning or late afternoon, which were not in phase with soil temperature changes. $\delta^{15}N$ and $\delta^{18}O$ of the N_2O emitted from soil indicated that denitrification was the major loss pathway, and after a heavy rainfall the larger $\delta^{15}N$ and $\delta^{18}O$ values suggested a consumption of N_2O by reduction to N_2. In contrast, Tilsner *et al.* (2003) found no unequivocal evidence of the source of N_2O emitted in an extensively managed grassland involving an unfertilized control, a synthetic fertilizer treatment and a slurry treatment based on the ^{15}N and ^{18}O natural abundance data for emitted N_2O. They concluded that the $\delta^{15}N$ values of emitted N_2O were not only influenced by source processes but also by the microbial reduction of N_2O to N_2 and that a minimum a flux of 3.4 mmol N_2O $m^{-2}{\cdot}h^{-1}$ was required to obtain accurate isotope data.

Yamulki *et al.* (2001) showed that the temporal intra-molecular distribution of $\delta^{15}N$ and $\delta^{18}O$ in N_2O emitted from urine patches, together with the site preference values for N_2O using the isotopomer methodology, were indicative of a process shift during the measurement period. Using the same methodology, Köster *et al.* (2011) found a rapid shift from denitrification-derived N_2O to N_2O production via nitrification after three weeks following the exhaustion of labile C compounds originating from the addition of biogas fermentation residue to a grassland soil. On the other hand, Cardenas *et al.* (2007) found that the isotopomer signatures for N_2O in control and sheep slurry treatments (lucerne diet) indicated that denitrification was the main process responsible for N_2O emissions from a grassland soil. Based on the majority of the above-mentioned results, it appears that bulk $\delta^{15}N$, $\delta^{18}O$ and isotopomer signatures are promising tools for separating pathways of N_2O production in grassland soils.

TABLE 8. Range of $\delta^{15}N$ values of plant products according to production system (Inácio *et al.*, 2013)

Production system	$\delta^{15}N$ (‰)
Conventional[1]	–2.5 to +8.7
Organic[2]	+0.3 to +14.6

[1]Synthetic and organic N fertilizers permitted
[2]Synthetic N fertilizers excluded

Differentiating conventional and organic plant products using $\delta^{15}N$

Because organic and synthetic fertilizer sources often differ markedly in $\delta^{15}N$ composition, it would appear to be a promising marker to distinguish organically- and conventionally-fertilized plant products. The greater the difference between organic and synthetic fertilizer the more robust will be the differentiation. However, different crops show greater or smaller differences between $\delta^{15}N$ values of organic and conventional products, and a certain degree of overlap can occur (Table 8). For example, organic tomatoes showed greater differences in $\delta^{15}N$ values compared with conventional production (+8.1 ± 3.2‰ vs. –0.1 ± 2.1‰, respectively), whereas differences between lettuce were smaller (+7.6 ± 4.1‰ vs. +2.9 ± 4.3‰, respectively), although still statistically significant (Bateman, Keely and Woolfe, 2007). However, $\delta^{15}N$ values of organic and conventional carrots (+5.7 ± 3.5‰ vs. +4.1 ± 2.6‰) were not significantly different. Perennial crops tend to show smaller but significant differences in $\delta^{15}N$ between mode of production, such as orange fruit (+7.3 to +7.9‰ for organic vs. +5.1 to +6.1‰ for conventional) (Camin *et al.*, 2011).

Nevertheless, many production or external factors may confound product designation. e.g. (i) legume products or the use of legume cover crops on organic farms, (ii) crop species with a low N requirement, (iii) annual vs. perennial growth habit, (iv) use of organic fertilizers by conventional farmers, and (v) marketing of organic products as conventional products (Inácio *et al.*, 2013).

For animal products, $\delta^{13}C$ might be the most promising marker for mode of production in temperate regions because of its relationship to diet, i.e. differences between C4 maize grain used in intensive animal feeding operations and C3 pasture grasses and legumes available under free-range conditions. However, $\delta^{13}C$ seems unlikely to be useful in tropical regions for grazing animals due to abundant C4 pasture grasses, e.g. *Brachiaria spp.* (Inácio *et al.*, 2013).

CONCLUSIONS

The ^{15}N natural abundance of animal excreta and composts is a useful tool for following the fate of organic N sources in the soil-plant-atmosphere continuum. The alternative approach of using organic fertilizers artificially-enriched in ^{15}N is time-consuming, expensive and inefficient, with the attendant risks of non-uniform labelling and perturbation of the system under study. However, fewer studies have been conducted at the level of natural ^{15}N abundance compared with artificially-enriched materials, and δ values have generally been

used as qualitative rather than quantitative measures of N processes at the system level.

Nevertheless, recent results showing significant positive and negative linear relationships during storage of animal excreta, between the bulk δ^{15}N signature and cumulative NH_3 loss and between bulk δ^{15}N of composts and N concentration, respectively, suggest that δ^{15}N may have wider applications in estimating the efficiency of N conservation during storage or composting. The combined use of bulk δ^{15}N and δ^{18}O signatures of N_2O evolved during storage and composting, together with the isotopomer-derived site preference of N_2O, are powerful tools for identifying the processes of N_2O formation in order to enable formulation of mitigation strategies.

Finally, it has been demonstrated that δ^{15}N signatures could play a role in differentiating organic and conventional plant products provided each of the two populations for each product has been adequately described by a frequency distribution that enables a statistical analysis of a sample. The potential use of stable isotopes in differentiating conventional and organic animal products remains to be addressed. In addition, a review and analysis of the post-1998 literature on animal excreta artificially enriched in ^{15}N needs to be undertaken to complement and extend the results obtained with ^{15}N natural abundance reported here.

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Spatial and Temporal Variation in the Isotopic Signature of Transpired Water from a Conifer Forest Canopy

D.G. Williams[1,2,3,*] and B.E. Ewers[2,3]

ABSTRACT

Most conifer tree species have intrinsically low rates of leaf gas exchange and relatively high leaf water volumes. These traits establish low leaf-water turnover rates, which greatly extend the time required to achieve transpiration at isotopic steady state as environmental conditions change over diurnal periods. Canopy level variation in leaf physiology and microclimate contributes further uncertainty to the estimation of the isotopic composition of transpiration fluxes in forests dominated by conifers. Oxygen stable isotope ratios of water in needles of different age cohorts and from different canopy positions in subalpine fir *(Abies lasiocarpa)* were measured over a diurnal period during the growing season of 2008 in the Snowy Range of southeastern Wyoming, USA. Concurrent measurements of leaf gas exchange, canopy surface temperature, and the delta oxygen-18 ($\delta^{18}O$) and mixing ratio of water vapour within the canopy air allowed modelling of leaf water isotopic enrichment at sites of evaporation and the isotope ratio values of transpiration from different canopy positions. Leaf water achieved isotopic steady state with plant source water over only a brief period of the day in late afternoon, and $\delta^{18}O$ values of water from leaves lower in the canopy and from younger leaf cohorts were closer to those predicted from a steady-state model than those of older leaf cohorts and from higher in the canopy. The $\delta^{18}O$ value of transpired water differed by up to 10‰ from that of source water at midday in some parts of the canopy. Modelling the isotope composition of transpiration from conifer forest should take into account variation in leaf water turnover rates associated with differences in physiology and microclimate of needles of different age and from different canopy positions.

Key words: *gas exchange, oxygen stable isotope ratios, transpiration, conifer forest.*

INTRODUCTION

Estimates of direct soil evaporation and plant transpiration are required to understand productivity responses of vegetation to inputs of irrigation or precipitation. Direct soil evaporation is a 'nonproductive' water loss, and management strategies focus on minimizing this loss in favour of 'productive' water usage through transpiration. Measurements of the stable isotope ratios of hydrogen ($\delta^{2}H$) and oxygen ($\delta^{18}O$) in water are useful for tracing sources of evapotranspiration (ET) and partitioning transpiration (T) from direct soil evaporation (E) fluxes. Because E and T often have unique isotopic signatures, measurements of the isotope ratio composition in the atmospheric water vapour within the canopy boundary layer or in the residual soil water over time provide information on the sources of and processes controlling water exchange in the soil–plant–atmosphere system (Yakir and Sternberg, 2000; Williams *et al.,* 2004).

Several approaches employing isotope measurements have been developed to estimate fractions of evaporation and transpiration in ET and their flux rates. Each approach has advantages and disadvantages related to the type of information provided (E/T fractions versus E and T fluxes), instrumentation costs, level of technical training required, the spatial and temporal scales of inference, and the number of assumptions and parameters requiring validation and estimation. These approaches are broadly defined as: (i) steady-state mixing approaches, including the 'Keeling plot' method (Williams *et al.*, 2004), (ii) soil water isotope mass balance (Hsieh *et al.*, 1998), (iii) the isotope flux gradient approach (Wang and Yakir, 2000), and (iv) isotope mass balance calculations for canopy air (Lai *et al.*, 2006). The Keeling plot approach only provides information on the fractions of E and T in total ET, whereas the flux gradient and air mass balance approaches offer estimates of the flux rates of each of these components. Each approach involves varying levels of sophistication with respect to measurement and process-level understanding of isotopic fractionations that occur at the soil and leaf scale as water is released to the atmosphere and mixes with background air. Any of these approaches can be used in soil-plant-atmosphere transfer models to simulate and predict isotopic exchanges under varying conditions.

Using isotope measurements of atmospheric vapour to partition component fluxes requires accurate estimates of the isotope composition of plant-transpired water (δT). Spatial, temporal and species-level heterogeneity in δT makes this task challenging, and validation of leaf-level isotope fractionation models (Barbour, 2007) are required. But discerning how rigorous one needs to be in accounting for fractionation processes and variation within canopies is dependent on the context of the study. Variation in leaf temperature, humidity and leaf-water turnover rates contribute to the complexity of δT within individual plant canopies. For example, it is often assumed in studies of leaf water isotopic enrichment that leaf temperature is closely coupled to air temperature. Yet this assumption may be invalid, especially where radiation loads and boundary layer resistances are high (Helliker and Richter, 2008).

1 Department of Ecosystem Science and Management, University of Wyoming, Laramie, Wyoming, USA 82071
2 Department of Botany, University of Wyoming, Laramie, Wyoming, USA 82071
3 Program in Ecology, University of Wyoming, Laramie, Wyoming, USA 82071
* E-mail address of corresponding author: dgw@uwyo.edu

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This study addresses how heterogeneity of gas exchange properties and microclimate of different leaves within a single complex canopy influence the isotopic signature of transpired water. Low rates of leaf gas exchange in some conifer species cause low leaf-water turnover rates, which greatly extend the time required to achieve transpiration at isotopic steady-state as environmental conditions change over diurnal periods (Pendall, Williams and Leavitt, 2005; Lai *et al.*, 2006). Differences in stomatal conductance, transpiration and microclimate within the vertical profile of a conifer tree in a high-elevation environment with high radiation loads were investigated to understand the basis for variation in leaf water ^{18}O enrichment and its deviation from plant source water.

MATERIALS AND METHODS

The study was conducted at the Glacier Lakes Ecosystem Experiments Site (GLEES) (41° 22.0′ N, 106° 14.4′ W, 3190 m a.s.l.) in subalpine mixed conifer forest in southeastern Wyoming, United States (Musselman, 1994). The oxygen stable isotope ratio ($\delta^{18}O$) of water was measured in leaves of different ages and from different vertical canopy positions in a subalpine fir *(Abies lasiocarpa)* tree over a diurnal period during the growing season of 2008. Needles were collected every 3–4 h over a 24 h period to investigate the rapid changes in leaf-water isotopic enrichment. Concurrent measurements of leaf gas exchange, humidity and temperature allowed estimation of the isotope ratio of transpiration from the different canopy positions and investigation of the deviation of leaf water $\delta^{18}O$ values from isotopic steady state.

Leaf sampling, gas exchange and canopy temperature measurements

Needles were collected from an *A. lasiocarpa* tree growing adjacent to a tall (30 m) scaffold. The scaffold, used to support instrumentation for the GLEES Ameriflux measurements, allowed easy access to the canopy without major disturbance to the tree. Needles were sampled from 9.5, 13.7 and 17 m above ground surface on the approximately 20 m tall tree every 3–4 h (seven times in total) over 24 h beginning at 08:00 h on day-of-year 213 (July 31), 2008. Needles were collected separately from the current year's growth and from 1- and 3-year-old needle cohorts at each canopy position and time period. Approximately 30–40 needles were removed from twigs for each sample and sealed in screw-cap glass vials. Stems were collected during the mid-day period for plant source water isotope analysis. Small twigs were collected from each canopy position, separated from attached needles and placed also in screw-cap glass vials. Vials were covered with parafilm and stored in a freezer at –2°C until water extraction (described below).

Stomatal conductance and transpiration of needles in each cohort and canopy position were measured during the same seven time periods used for needle collection. Gas exchange measurements were made using a LiCor 6400 with a standard leaf cuvette and with all environmental conditions (cuvette temperature, light level, humidity) set to match ambient conditions. A hand-held infrared thermometer was used to monitor needle temperature at each canopy height.

Canopy air water vapour sampling

Atmospheric water vapour was collected at each of the three canopy positions used for gas exchange measurements and needle collection, employing an automated sample profiler that routed air from each height through low-absorption Bev-a-Line IV tubing to Pyrex glass traps (Helliker *et al.*, 2002) held at –80°C in an ethanol bath. The air stream from each position in the canopy was diverted frequently through an infrared gas analyzer (LI 840, LiCor, Inc.) for measurement of air water-vapour mixing ratio. The sampling system is thoroughly described by Yepez *et al.* (2003) and Williams *et al.* (2004). For the current study, water vapour was trapped in air at a flow rate of 300 mL/min for 30 min during each of the seven sampling periods at each of the three heights in the canopy.

Water extraction and stable isotope analysis

Water from needle and stem samples was retrieved using cryogenic vacuum distillation (Ehleringer and Osmond, 1989). Complete quantitative extraction was verified from weight measurements performed before and after cryogenic extraction and then again after oven drying. Extraction efficiency was better than 95 percent for all samples. The $\delta^{18}O$ values of stem, needle and atmospheric water samples were determined by the CO_2 equilibration technique using a Gas Bench II coupled to a Thermo Delta Plus XP Isotope Ratio Mass Spectrometer (Thermo Scientific Corporation, Bremen, Germany) at the University of Wyoming Stable Isotope Facility. Measured $\delta^{18}O$ values were linearly corrected to the V-SMOW international scale using two calibrated laboratory standards that were analysed with each batch of unknowns along with a QA/QC standard. Precision of repeated analysis of the laboratory QA/QC standard was better than 0.2‰.

Steady state leaf water isotope model

Measured $\delta^{18}O$ values of water extracted from needles were compared with values predicted by a Craig and Gordon steady-state model adapted for leaves (Farquhar and Lloyd, 1993):

$$\delta^{18}O_e = \delta^{18}O_s + \varepsilon^* + \varepsilon_k + (\delta^{18}O_v - \delta^{18}O_s - e_k)e_a{:}e_i \quad (1)$$

where $\delta^{18}O_s$ = the oxygen isotope ratio of plant source water; $\delta^{18}O_v$ = the oxygen isotope ratio of atmospheric water vapour; ε^*

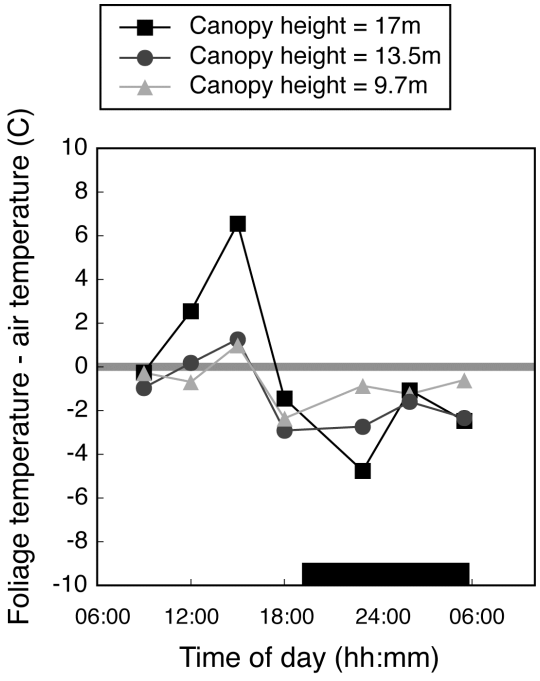


FIGURE 1. Difference between foliage (needle) temperature and surrounding air temperature in the canopy of *A. lasiocarpa*

= the temperature-dependent equilibrium fractionation factor (Bottinga and Craig, 1969); ε_k = the kinetic fractionation factor during diffusion through the stomata and boundary layer; and e_a:e_i is the ratio of ambient to intercellular vapour pressure.

The ε_k value is dependent on the proportion of diffusion resistance through the stomatal pores (r_s) and boundary layer (r_b) (Farquhar, Barbour & Henry, 1998; Cappa *et al.*, 2003) and is calculated by:

$$\varepsilon_k = (32r_s + 21r_b)/(r_s + r_b) \quad (2)$$

The $\delta^{18}O$ value of water in leaves predicted by Equation 1 is that for sites of evaporation, and not for the bulk leaf water. To estimate the $\delta^{18}O$ value of bulk leaf water at isotopic steady state ($\delta^{18}O_L$), a model was employed that accounts for the back diffusion and mixing of ^{18}O enriched water at sites of evaporation with un-enriched source water from leaf veins:

$$\delta^{18}O_L = \delta^{18}O_s + (\delta^{18}O_e - \delta^{18}O_s)(1-e^{-P})/P \quad (3)$$

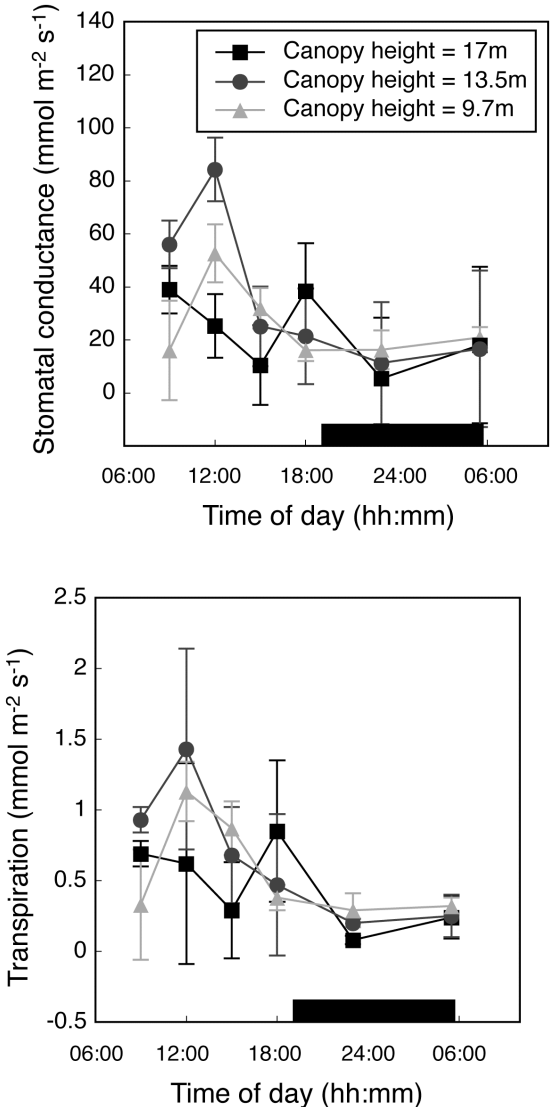


FIGURE 2. Stomatal conductance and transpiration at different canopy heights of *A. lasiocarpa* surrounding air temperature in the canopy of *A. lasiocarpa*.

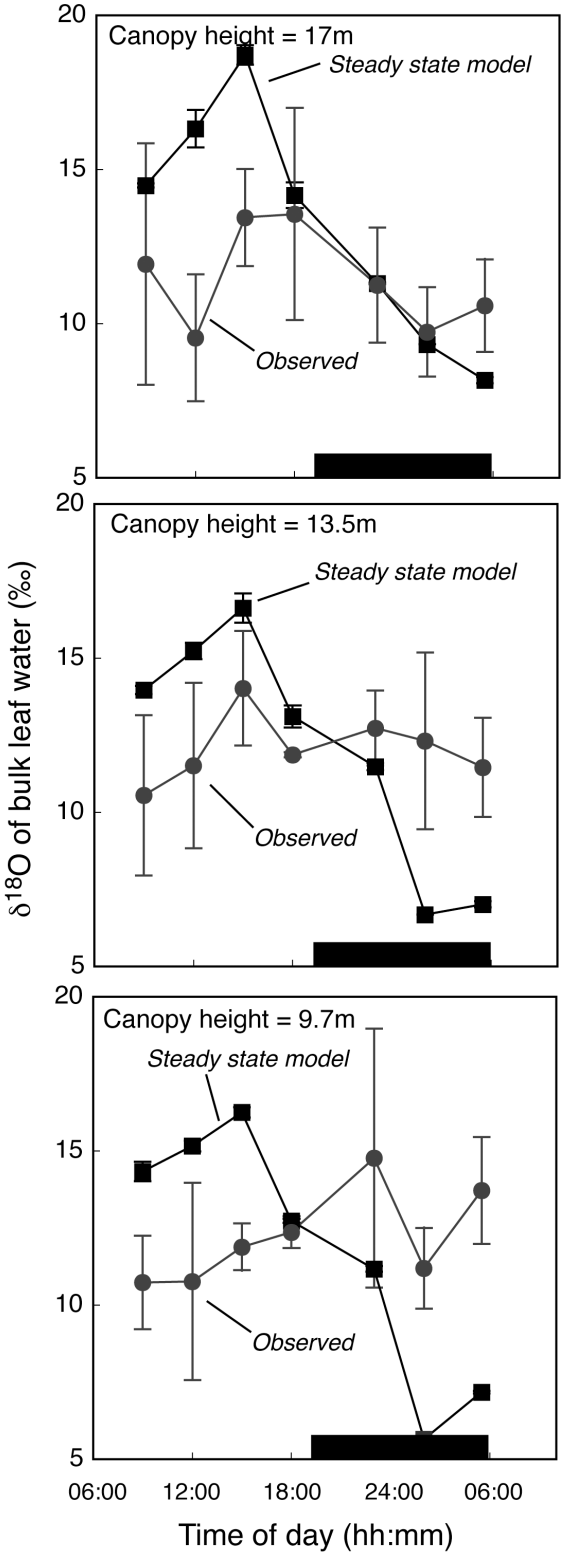


FIGURE 3. The observed and predicted $\delta^{18}O$ values of bulk leaf water at different canopy heights.

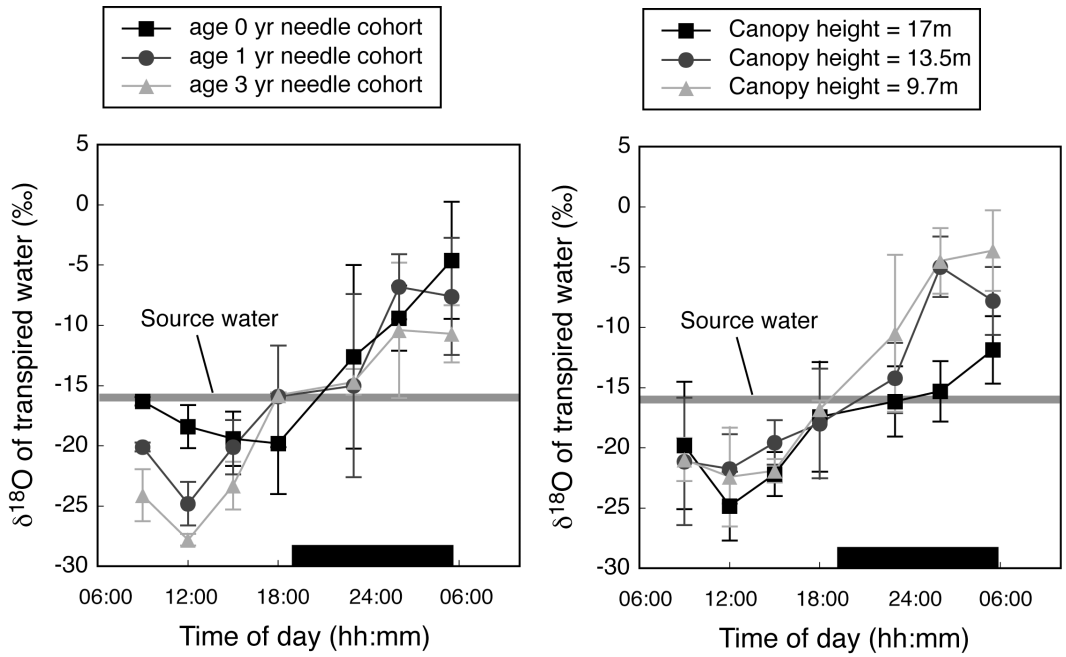


FIGURE 4. The $\delta^{18}O$ values of leaf transpired water in *A. lasiocarpa* calculated from bulk leaf $\delta^{18}O$ values.

where P is the Péclet number. P is calculated as EL/CD, where: E = transpiration rate ($mol \cdot m^{-2} \cdot s^{-1}$); L = the effective path length (taken as 0.008 m); C = the molar density of water (55.5×10^3 mol/m^3); and D = the diffusivity of $H_2{}^{18}O$ in water (2.66×10^{-9} m^2/s) (Farquhar and Lloyd, 1993).

RESULTS AND DISCUSSION

Needle temperature differed substantially from air temperature during daytime and night time periods (Figure 1). The largest difference was observed for needles in the upper canopy exposed to high radiation, where needle temperature was as much as 6.5°C higher than air temperature in mid-afternoon. Radiative cooling of the canopy during nighttime caused needle temperatures to drop below air temperature by as much as 4.5°C. Despite the high wind speeds common at this forest site, the simple assumption of close coupling of leaf and air temperature would lead to substantial error in modelling leaf water ^{18}O enrichment. For example, a 6.5°C difference in needle temperature from 15 to 21.5°C would produce a 0.6‰ difference in ε^* (Equation 1). The assumption also would introduce error in the calculation of e_a/e_i; at 15°C air temperature and 50 percent air humidity, e_a/e_i would be 0.35 assuming equal leaf and air temperature and 0.5 with a 6.5°C higher value of leaf temperature.

The hot, dry conditions in the afternoon period reduced stomatal conductance and transpiration, especially in upper parts of the tree canopy (Figure 2). Low hydraulic conductivity in the xylem of stems caused by bark beetle transmission of a fungal pathogen may have substantially limited the rates of transpiration from the upper canopy in this plant. Leaf longevity is also quite high (ca. 6–10 years) in this species and stomatal conductance and transpiration rates tend to decline as leaves age in conifers. Indeed, stomatal conductance was higher in the youngest needle cohort compared to the 1- and 3-year-old cohorts (data not shown) in the current study. Values of stomatal conductance and transpiration were used to estimate leaf water ^{18}O enrichment using Equations 1–3 for comparison against measured values. Differences in leaf gas exchange properties among the three different aged needle cohorts and at the different canopy heights were sufficient to cause substantial variations in leaf water ^{18}O enrichment within the *A. lasiocarpa* canopy that translated into variations in the $\delta^{18}O$ values of leaf-transpired water vapour.

Overall, the generally low rates of leaf-level T (and consequentially low leaf water turnover rates) in the *A. lasiocarpa* in this study did not allow $\delta^{18}O$ values of needle water to achieve steady state with environmental conditions (Figure 3). However, leaves lower in the canopy compared with those higher in the canopy were closer in their $\delta^{18}O$ values as predicted by the Craig and Gordon steady-state model.

Bulk leaf water $\delta^{18}O$ values were used to calculate leaf transpiration $\delta^{18}O$ values. These values, reported by canopy height and by leaf age cohort, were different than tree source water $\delta^{18}O$ values over much of the diurnal period (Figure 4), indicating the importance of considering isotopic non-steady state T in ET partitioning studies.

The dynamics of leaf water ^{18}O enrichment and the $\delta^{18}O$ value of transpired water in needles of subalpine fir (*A. lasiocarpa*) are poorly represented by a simple isotopic steady state model, and deviations are systematically related to canopy position and leaf age. It is concluded that modelling the isotope composition of forest T and leaf water to increase predictive understanding of ET should take into account the variation in leaf water turnover rates associated with variation in physiological properties of needles of different ages and canopy position that affect isotopic non-steady state T values. To predict short-term changes in the isotope composition of water vapour in forest canopy air (e.g. Lai *et al.*, 2006), models should account also for the potential differences between leaf and air temperature that drive equilibrium and kinetic fractionations.

ACKNOWLEDGEMENTS

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Isotope Mass Balance Method to Partition Evaporation from Soil Total Water Loss in Winter Wheat and Spring Maize Cropping Systems in North China Plain

G. Li[1,3,*], Q. Hu[2,3], X. Wang[2,3] and B. Li[1,3]

ABSTRACT

Evaporation (E) is one of main paths of soil water loss in most dryland farming systems. Since it is considered to be of no benefit to crop production, E should be minimized as much as possible. However, the partitioning of E from total water loss (TWL) via transpiration (T) and seepage (S), under field condition is not an easy task. The isotope mass balance (IMB) method is one of the few ways to do so. This study was designed to separate E from TWL in winter wheat and spring maize cropping systems in the North China Plain using the IMB method. In field experiments conducted over two years, plot surfaces were covered with plastic film, with straw mulch, left bare or left as normally in the field, and stable isotope values and amounts of soil water, rainfall, and irrigation water were measured periodically. Results showed that the proportion of E to TWL was 18 percent and 31 percent in winter wheat and spring maize, respectively in the conventional plot, 21 and 12 percent, respectively in plastic film-covered (hereafter referred to as filming) plot, and 47 and 38 percent, respectively in the bare plot. In conclusion, it was found that the IMB method is a simple way to partition average E from TWL over a growing season, and the method is sensitive enough to separate E between straw mulching, filming, conventional practice, and bare soil. The most sensitive factor in using IMB is the determination of the depth of E front within the soil. In the current experiment, isotope measurements of soil water at depths of 0–5 cm were critical to the success of IMB.

Key words: *evaporation, soil total water loss, spring maize, winter wheat, isotope mass balance, mulching.*

INTRODUCTION

Evaporation (E) is one of main paths of water loss in the winter wheat and spring maize cropping system in the North China Plain (NCP), and irrigation is managed to minimize E and seepage (S) to the deep soil layers. Assuming E represents water loss with nearly no benefit to crop production, many agronomic practices such as straw mulching and plastic filming have been used to minimize this component as much as possible. However, it is generally difficult to separate E from total water loss (TWL) under field condition. Isotope techniques may be the most reliable methods to do so. Since the pioneering work of Hsieh *et al.* (1998) to separate E and transpiration (T) using the isotope mass balance (IMB) method in natural systems and similar studies in a natural grassland system (Ferrette *et al.*, 2003) and in a manipulated closed system (Rothfuss *et al.*, 2010), there have been no reports of using the IMB method in an arable field system to separate E from TWL.

The objectives of this research were therefore to test the suitability of IMB for partitioning E from TWL in irrigated winter wheat and spring maize cropping systems in NCP, and to explore the effects of soil temperature, root water absorbing depth and depth of E on the results obtained.

MATERIALS AND METHODS

Field experiment

Four micro-plots were set up in a large area of a field with a light loam soil texture. The plots were 3 m × 3 m and located randomly within the field. The plots were set up as follows: (i) conventional practice, i.e. in the usual way to produce winter wheat or spring maize, (ii) soil left bare after sowing, and all small plants were removed from the plot just after germination, (iii) plastic filming was placed between plant rows as much as possible, and (iv) straw mulching in which maize straw was placed between plant rows as much as possible. Pictures of the field are shown in Figure 1.

Soil samples were collected by drilling soil cores at depths of 0–5, 5–10, 10–20, 20–40, 40–60 and 60–100 cm and at different times. The delta oxygen-18 ($\delta^{18}O$) and delta hydrogen-2 ($\delta^{2}H$) values of soil water were measured. Rainfall and irrigation were recorded and their $\delta^{18}O$ and $\delta^{2}H$ values were also measured.

Soil temperatures at depths of 5, 10, 15, 20 and 25 cm were measured by thermal probes and recorded using a data logger every 20 min.

Yields of winter wheat and spring maize were recorded by harvesting all plants in each plot and measuring the dry weight of grain.

1 Key Laboratory of Cropland Conservation in North China, Ministry of Agriculture
2 Department of Soil and Water Science, College of Resources and Environment
3 China Agricultural University, Beijing, 100193, China
* E-mail address of corresponding author: lgtong@cau.edu.cn

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FIGURE 1. Photos of the experimental field. Note: a, b and c represent respectively the plots of conventional cultivation, film mulching, and straw mulching in the spring maize system, and d shows the plot of film mulching in the winter wheat system.

TABLE 1. Main parameters in partitioning E from soil TWL using the IMB method

Crop	Period[1] (m/d)	Rainfall		Irrigation		Average soil T at -5 cm (°C)
		Amount (mm)	Average $\delta^{18}O$ (‰)	Amount (mm)	Average $\delta^{18}O$ (‰)	
Winter wheat	4/7 – 6/22	108	–7.33	90	–7.60	25.8
Spring maize	6/25 – 9/29	268	–7.79	0	–	22.6

1 The period for winter wheat was from tillering to harvest; for spring maize it was between elongation and harvest.

Isotope mass balance (IMB) method

The method of Hsieh *et al.* (1998) was used to calculate the ratio of E to TWL during selected periods within the whole growing seasons of the winter wheat and spring maize.

The main principle of IMB was the balance of water and its isotope during a given period.

$$x_0\delta_0 + x_r\delta_r + x_i\delta_i = x_f\delta_f + x_e\delta_e + x_{ts}\delta_{ts} \quad (1)$$

$$m_0 + m_r + m_i = m_f + m_e + m_{ts} \quad (2)$$

where m is the mass of soil water per ha within a given depth, m^3; δ is isotope value (^{18}O or ^{2}H) of water, ‰; and the subscripts 0, r, i, f, e, and ts are respectively: original state, rainfall, irrigation, final state, E, T and S, respectively.

$x_j = m_j / m_{total}$, here j = 0, r, i, f, e, and ts

$m_{total} = m_0 + m_r + m_i$

For δ_e at 20°C, the equation of Majoube (1971) was used:

$\alpha_{l\text{-}e} = (\delta_l + 1000)/(\delta_e + 1000) = 1.009563$

δ_t was assumed to be the $\delta^{18}O$ values of the weighted mixture of soil water at time 0 and of irrigation water and rainfall and:

$\delta_t = x_0\delta_0 + x_i\delta_i + x_r\delta_r$

The standard state of IMB was defined by determining the following parameters: E at a soil depth of 5 cm, average soil temperature at 5 cm depth was 19.8°C and root water absorbing depth ranging from 5 cm to 60 cm along the soil profile.

Values for the main parameters are shown in Table 1.

RESULTS

The dynamics of soil water content and the corresponding oxygen isotope values under spring maize in 2010 and winter wheat in 2012 are shown in Figures 2 and 3. For spring maize, soil water content along the upper 80 cm of the soil profile varied significantly during the whole growing season, showing that water stored in this part of the profile was influenced greatly by the water absorption capacity of maize roots, rainfall recharge and/or irrigation and E (Figure 2, a1 to d1). Thus, it was reasonable to use 80 cm as the depth of water absorption by maize roots. On the other hand, variations in isotope values of soil water ($\delta^{18}O$) caused by isotope discrimination during phase change (E), occurred only at depths above 30 cm, suggesting that E occurred mostly within this part of the soil profile (Figure 2, a2 to d2).

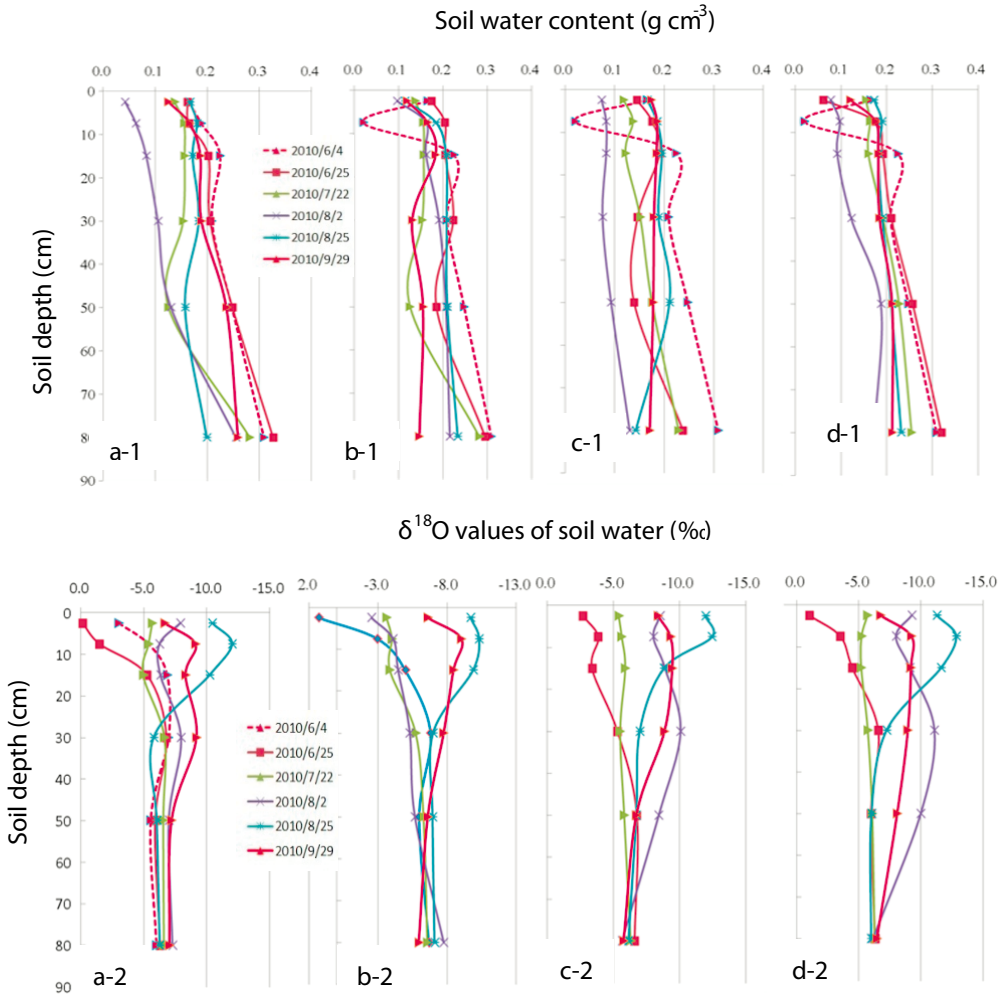


FIGURE 2. The dynamics of soil water content and the corresponding $\delta^{18}O$ values along the soil profile under spring maize. Note: a, b, c, and d represent respectively the plots of conventional cultivation, bare soil, film mulching, and straw mulching numbers 1 and 2 represent respectively the soil water content and $\delta^{18}O$ values of soil water.

TABLE 2. Depth (mm) and percent (values in brackets) of E to TWL in winter wheat and spring maize

Crop	Year	Conventional		Filming		Mulching		Bare	
		TWL	E	TWL	E	TWL	E	TWL	E
WW	2010	147	33 (22)	92	19 (21)			56	23 (41)
	2012	165	24 (15)					87	46 (53)
SM	2010	206	63 (31)	157	22 (12)	178	43 (24)	182	65 (38)

Figures in brackets are % of E to TWL; WW — winter wheat; SM — spring maize.

The depth at which the roots of spring wheat absorbed water was the same as for spring maize (about 80 cm), as shown by the great variation in soil water content along the 80 cm soil profile during the growing season (Figure 3, b). For the plot with bare soil, the soil water content decreased continuously, as no irrigation was applied to it (Figure 3, a). Again, E mostly occurred at above a soil depth of 30 cm, as reflected by the great variation of $\delta^{18}O$ values in the upper 30 cm and the relative steady values below that depth (Figure 3, c and d).

Table 2 shows the results for separating E from TWL by the IMB method and the soil water budgets at depths of 0–60 cm for winter wheat and 0–100 cm for spring maize. The IMB method enabled clear differentiation of E between the plots. For winter wheat, E was about 20 percent (range 15–22 percent) of TWL under conventional cultivation and 21 percent with filming, suggesting that filming did not reduce E significantly in winter wheat. For bare soil, E was about 50 percent of TWL (range 41–53 percent), meaning that half of water loss in bare soil was via E. On the other hand, both filming and straw mulching reduced E significantly in spring maize (by 19 percent and 7 percent, respectively).

The relatively lower E (15–22 percent) for winter wheat can be attributed to irrigation being carried out late, at the middle of May

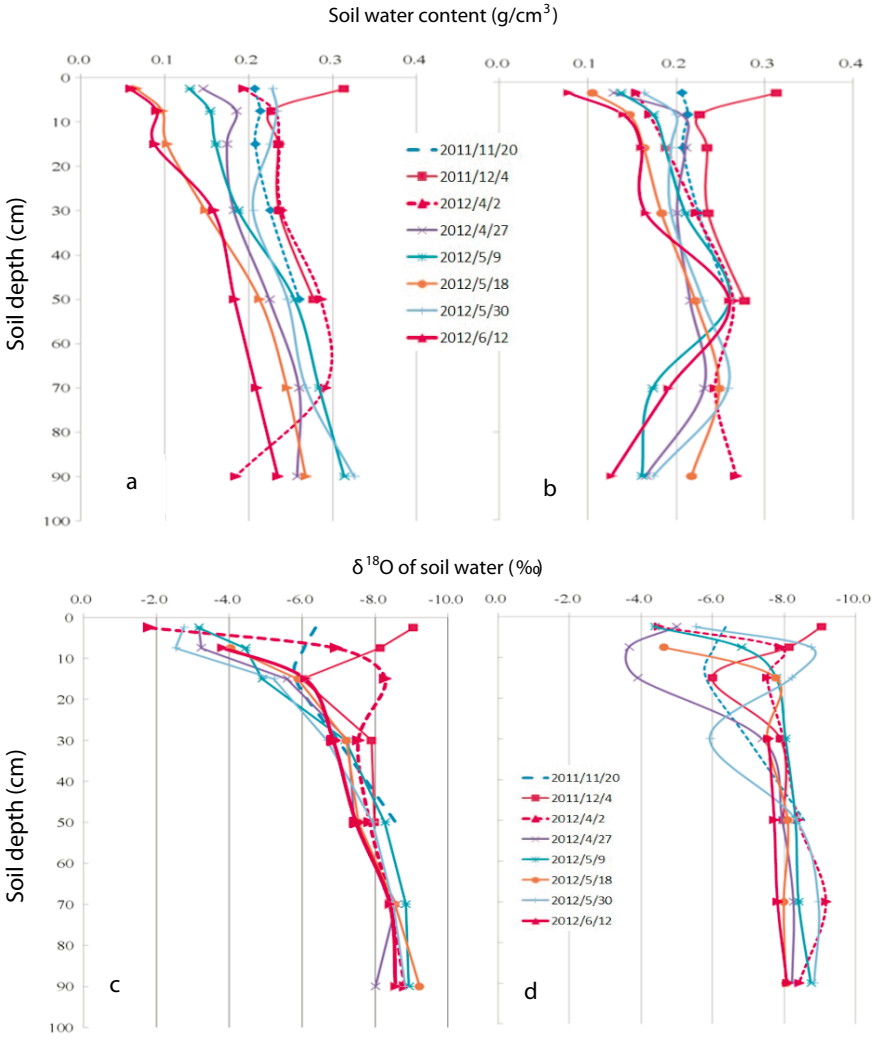


FIGURE 3. The dynamics of soil water content and corresponding $\delta^{18}O$ values along the soil profile under winter wheat. Note: a and b represent respectively the soil water contents in the plots of bare soil and conventional cultivation, and c and d represent the corresponding $\delta^{18}O$ values of soil water.

when the crop cover is nearly 100 percent. Before May, the top soil is very dry and E is inhibited.

The component of E for spring maize (31 percent) can be assumed to occur mainly during May to June when the crop cover is lower with excessive rainfall events.

DISCUSSION

In IMB, three factors affect the results of calculations, namely depth of soil E, root water absorbing depth, and soil temperature. It is difficult to determine the depth beneath soil surface at which soil water evaporates. Yet, the depth at which E takes place is critical since it determines the δ_l value of soil water to be used in the estimation of δ_e of the vapour. The present results show that with different depths of E, the ratio of E to TWL could be as much as 18 percent more or 44 percent less than the current depth of 33 mm (Table 3), indicating the importance of determining the depth to be used for calculations of E to obtain reasonable values when using the IMB method. In Table 3, a sensitivity analysis was carried out where different soil depths (0–5, 0–10 and 0–20 cm) of E were used to determine the evaporation ratio. These were then compared with that of the standard which is E occurred at soil depth of 5 cm, at a soil temperature of 19.8°C at that depth, with root water absorbing depth ranging from 5 cm to 60 cm. The aim of this exercise is to see the difference in E ratio calculated by IBM using different E depths.

Root water absorbing depth is also important in the IMB method since it determines the δ_{ts} values used in the calculations. Results obtained here show that it could vary by as much as 30 percent less than the current depth (Table 3). However, the influence of the root water absorbing depth is smaller than the depth at which E occurs.

The effect of soil temperature on E at a soil depth of 5 cm was not so significant, with only a 2–3 percent difference being recorded when the temperature changed from 20°C to 15°C, which is not likely to happen at a given location between different years.

CONCLUSIONS

IMB is a simple way to partition E from the TWL of a soil body over the whole growing season, and is especially effective for small plot field experiments. Its sensitivity is good enough to demonstrate differences in E between plot treatments of straw mulching, filming, conventional practice and bare soil.

TABLE 3. Effects of depth of E, root water absorption, and soil temperature at 5 cm depth on the evaporation ratio (%) calculated using the IMB method

State	Conventional	Filming
Standard	33	19
E occurring depth (cm)		
0–5	31 (–6%)	22.5 (+18%)
0–10	23 (–30%)	14.5 (–24%)
0–20	18.5 (–44%)	11.5 (–41%)
Root water absorption depth (cm)		
5–60	26.5 (–21%)	17.5 (–8%)
5–40	24.5 (–25%)	16.5 (–13%)
20–60	23 (–30%)	16.5 (–13%)
Soil temp. at 5cm depth (°C)		
20	24 ± 2 (S.E.)	16 ± 2 (S.E.)
15	21 ± 2 (S.E.)	14 ± 2 (S.E.)

Figures in brackets are differences between E and the corresponding current state.

The most important factor determining results using the IMB method is the depth of E within the soil. In this experiment, isotope measurement of soil water at a depth of 0–5 cm was critical to the success of the IMB technique.

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An Improved Vacuum Distillation Method for Extracting Soil Water for Stable Isotope Analyses

L. Mayr[1,*], M. Aigner[1] and L. Heng[2]

ABSTRACT

A simple, fast and portable vacuum distillation setup and methodology to extract water from soil and plant samples for water stable isotope analyses (oxygen-18 [^{18}O] and hydrogen-2 [^{2}H]) was developed. The methodology was tested on two soil types (a sandy loam and a silty clay loam) over different extraction times and soil moisture (near field capacity and permanent wilting point). Using an immersion cooler instead of liquid nitrogen or solid carbon dioxide (dry ice) as the cooling agent, the results showed that full recovery (extracting more than 98 percent of the total water) and reproducible isotopic ratios (± 0.08‰ for $\delta^{18}O$ and ± 0.83‰ for $\delta^{2}H$) can be reached after 30 min with a dry sandy soil and 120 min with a dry clay soil at 100°C distillation temperature. The methodology allows large number of samples to be prepared for analysis quickly and reliably.

Key words: *Vacuum distillation, soil water extraction, stable isotope analyses.*

INTRODUCTION

The need for a rapid, inexpensive technique for routine oxygen-18/oxygen-16 ($^{18}O/^{16}O$) extraction of water from plant and soil samples is increasing due to the greater demand for isotopic data in agro-ecological, soil water, evaporation and transpiration partitioning and hydrological studies (White *et al.*, 1985; Williams *et al.*, 2004; Van Pelt *et al.*, 2010; Soderberg *et al.*, 2011). The common sample extraction techniques include: azeotropic distillation with kerosene or toluene (Revesz and Woods, 1990), centrifugation (Edmunds and Bath, 1976), liquid/vapour equilibration in plastic bags (Wassenaar *et al.*, 2008), mechanical pressing (Patterson *et al.*, 1977; Jusserand, 1980) and vacuum distillation (Araguás-Araguás *et al.*, 1995; West, Patrickson and Ehleringer, 2006; Koeniger *et al.*, 2011), the last being the most commonly used method. Nevertheless, most of the conventional techniques are often laborious, time consuming and involve complicated setups with specially-made glass apparatus. In addition, liquid nitrogen or dry ice is needed to freeze and trap water vapour evaporated during extraction. However, both of these cooling agents can be difficult to acquire in many developing countries. With water isotope analyses becoming cheaper, easier and faster (e.g. through the development of modern laser isotope analyzers e.g. cavity ring down spectroscopy [CRDS]), the bottleneck in sample throughput is often the water extraction time instead of the isotopic analysis of water.

Here we describe a simple, fast and accurate vacuum distillation method using a commercial immersion cooler and a Dewar container filled with 2-propanol at –50°C, in place of the liquid nitrogen or dry ice for freezing water vapour. The method can be easily adopted at a relatively low cost and allows large number of samples to be extracted quickly for isotopic analyses.

MATERIALS AND METHODS

The studies were carried out using two different soil types: Ebendorf silty clay loam and Reisenberg sandy loam (Table 1), adjusted to two different moisture levels: close to field capacity (Ebendorf soil at 0.1 bar; Reisenberg soil at 0.3 bar) and near wilting point (Ebendorf soil at 12.5 bar; Reisenberg soil at 1 bar). In order to avoid fractionation of delta oxygen-18 ($\delta^{18}O$) and delta hydrogen-2 ($\delta^{2}H$) during the moisture adjustment, soil moisture at both levels was adjusted using a ceramic pressure plate extractor (Soil Moisture Equipment Corp., Santa Barbara, California, USA). The two bulk soils were initially air dried and sieved to 2 mm. About 500 g of each soil type was equilibrated with one litre of water of known $\delta^{18}O$ and $\delta^{2}H$ content ($\delta^{18}O$ = –9.28‰; $\delta^{2}H$ = –67.76‰). The slurry, made by stirring the soil-water suspension thoroughly, was left standing covered and light-protected for two days at room temperature (25°C). After the equilibration time the excess water was decanted and the wet soil poured into three metal rings (55 mm diameter, 40 mm height) for each soil type (three replicates) and placed on a ceramic pressure plate. Water was removed from the soil by applying the appropriate pressure until no more water was found flowing out of the pressure chamber.

After moisture adjustment, five subsamples of 3–5 g per soil type and per moisture level were taken from each of the metal rings using an auger (10 mm diameter) and weighed into tare 100 × 16 mm glass culture tubes, and a pre-weighed portion of dry glass wool was added on top of the soil to prevent small soil particles moving into the trapping tube during distillation. The tubes were immediately closed and stored in the deep freezer at –18°C.

The setup allowed these sample storage tubes (15 mL glass culture-tubes with GL18 screw caps) to be connected to the distillation unit without the need to transfer the sample to other containers. The tubes were connected to one end of a glass assembly with a water collection tube, and a cock valve with NTFE spindle on the other side (Figure 1).

A six-litre stainless steel Dewar was filled with four litres of 2-propanol and the immersion cooler (Peter Huber, TC50-NR) was submerged into the liquid. A temperature of –50°C was reached after about three hours (h). The cooling device was on during the whole distillation procedure.

1 Soil and Water Management and Crop Nutrition Laboratory, Seibersdorf, Austria
2 Soil and Water Management and Crop Nutrition Section, Vienna, Austria
* E-mail address of corresponding author: L.Mayr@iaea.org

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TABLE 1. Characteristics and moisture level of test soils

Soil type	Soil code	% sand	% silt	% clay	Applied pressure	% moisture
Ebendorf silty clay loam	C-wet	16	57	27	0.1 bar	33.3
	C-dry				12.5 bar	19.8
Reisenberg sandy loam	S-wet	65	28	7	0.3 bar	18.7
	S-dry				1.0 bar	12.6

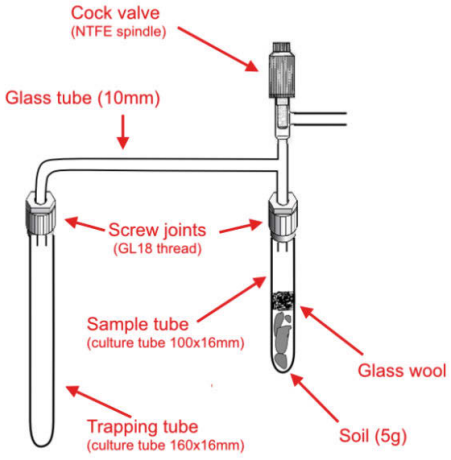


FIGURE 1. Schematic diagram of glass assembly.



FIGURE 2. An improved vacuum distillation method for extracting soil and plant water for stable isotope analyses, with heating blocks (A), Dewar filled with 2-propanol (B) and immersion cooler (C).

Prior to evacuation the sample was frozen in the Dewar connected to the immersion cooler for about three min to minimize water vapour in the gas phase and to avoid losses of water during evacuation. The assembly was attached to a vacuum pump (Edwards, E2M-15) and pumped down to a pressure of less than 1 mbar measured with a digital vacuum gauge (Vacuubrand, DVR2). The cock valve was closed to maintain the vacuum in the sample and the assembly was detached from the vacuum pump. The sample side of the unit was then put on a block heater (VWR, ANA 2BLK) set to 100°C, and the other side with the water collection tube was inserted to about one third of the total length into the cooling liquid at –50°C. Depending on the size of the block heater and the Dewar container for the immersion cooler, at least eight units can be distilled at one time. With our setup we were able to process 15 samples at once (Figure 2).

Three replicate samples per moisture level were prepared for water extraction at five different extraction times (15, 30, 60, 120 and 180 min). Fifteen samples of each soil were put on the block heater and after the corresponding extraction time three replicates each were removed, vented to air by opening and closing the stopcock valve, and left standing in a rack until the ice in the water trap had melted. Then the sample and water tubes were disconnected from the glass assembly and closed immediately with screw caps. All tubes were weighed after having reached room temperature (25°C).

Water was transferred from the trapping tubes into 2-ml autosampler glass vials (32 x 12 mm) using disposable pipettes. For water volumes of less than 0.5 ml, vials with a 0.3 ml insert were used. The samples were analysed for $\delta^{18}O$ and $\delta^{2}H$ using a cavity ring down laser spectrometer (CRDS Picarro Isotopic Water Analyzer L2130-i).

After extraction the soil dry weight was recorded and the tubes containing soil were placed on a separate block heater at 110°C and closed with silicone stoppers holding two syringe needles. One was connected to an aquarium pump blowing dry air onto the soil using a molecular sieve filter. The other needle was for release of the moist air. With this special drying procedure all remaining moisture could be removed overnight. After cooling to room temperature the soil samples were again weighed and the difference between soil weight after extraction and soil weight at "complete dryness" was determined.

MEASUREMENTS AND CALCULATIONS

The percent recovery was determined using the following equation:

$$\% \text{ water recovery} = (S_{wet}-S_{dist})/(S_{wet}-S_{dry}) \times 100$$

where S_{wet} is weight of wet soil S_{dist} is weight of soil after distillation and S_{dry} is weight of soil after oven drying (48 h at 110°C). Araguas-Araguas *et al.* (1995) suggested 98 percent recovery as the minimum for isotope analyses to obtain unfractionated water.

RESULTS AND DISCUSSION

S-wet and S-dry (sandy soil)

The results of the isotopic ($\delta^{18}O$ and $\delta^{2}H$) values for the two soil types under both wet and dry initial water levels were plotted against the extraction time (Figure 3). The isotopic value of extracted water increased with extraction time until it became essentially constant. These values were respectively 15 min for wet sandy soil and 30 min for dry sandy soil.

For Ebendorf clay soil, 30 min was required for extraction of wet soil while it took 120 min to recover 98 percent of the total water from dry soil (Figure 3; Table 2). In general, extraction of dry soil took longer to obtain unfractionated water than wet soil. The Ebendorf clay soil also took slightly longer than the Reisenberg sandy soil. Differences in soil texture and hence in pore size distribution between

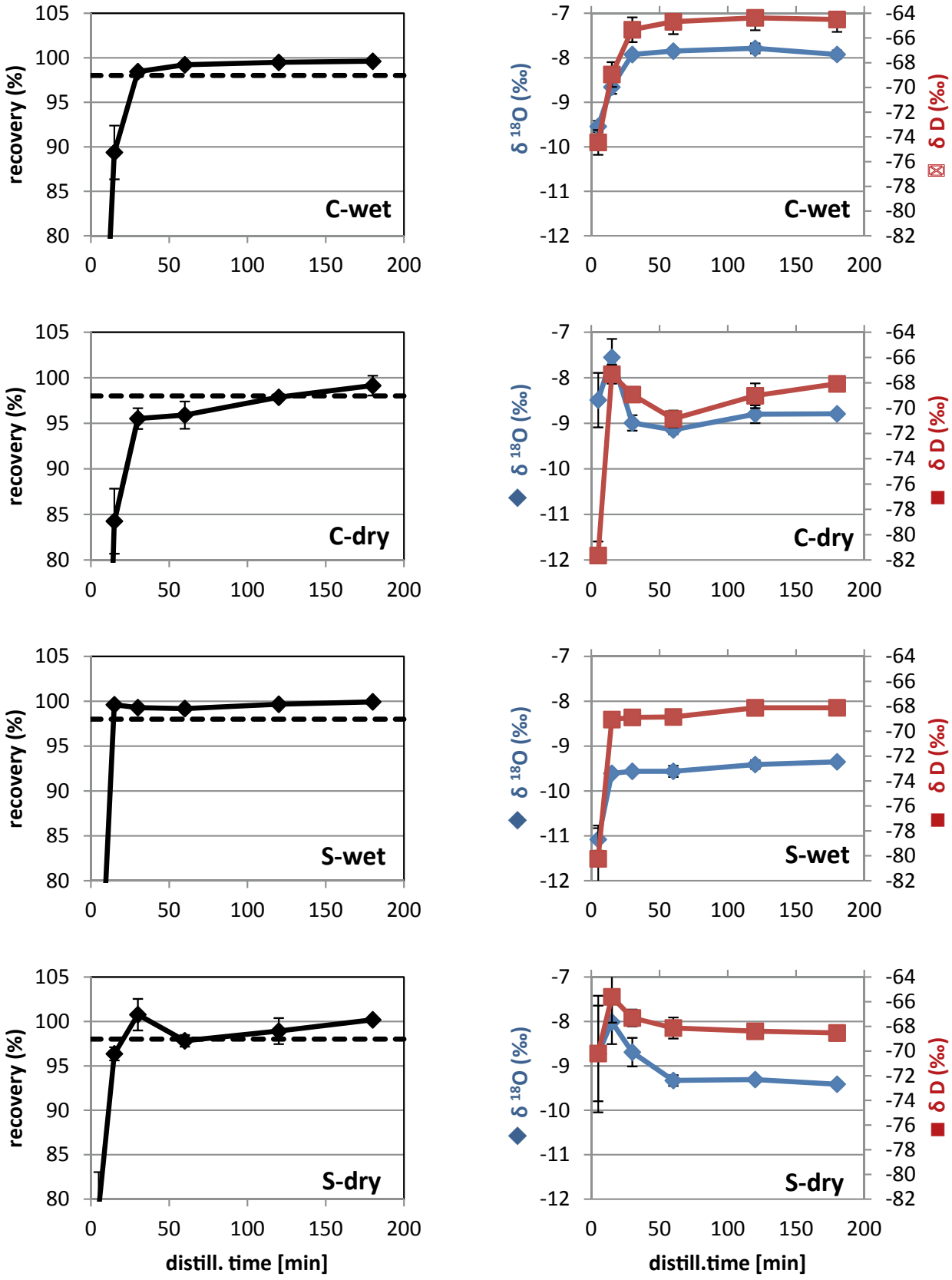


FIGURE 3. Extraction time for 98 percent recovery from Reisenberg sandy and Ebendorf clay soils under wet and dry conditions (left); corresponding $\delta^{18}O$ and $\delta^{2}H$ values under various extraction times are also shown (right).

TABLE 2. Minimum distillation time required to achieve a water recovery of > 98 percent and their corresponding $\delta^{18}O$ and $\delta^{2}H$ precisions at one standard deviation

Soil type		Time (min)	Precision (1σ SD)	
			$\delta^{18}O$ (‰)	$\delta^{2}H$ (‰)
Ebendorf clay soil	C-wet	>= 30	± 0.09	± 0.58
	C-dry	>= 120	± 0.13	± 0.83
Reisenberg sandy soil	S-wet	>= 15	± 0.11	± 0.51
	S-dry	>= 60	± 0.08	± 0.43

the soil types may influence the extraction time. West *et al.* (2006) presented water extraction times for plant and soil materials also using a vacuum distillation method. They obtained extraction times of 30 min for sandy soils and 40 min for clay soils for a 98 percent recovery of water, which is comparable with the findings from the present study.

The precision of the analytical method is shown in Table 2. Single standard deviation was calculated from all respective samples with a water recovery higher than 98 percent. Just like the findings of West, Patrickson and Ehleringer (2006), our results showed low standard deviations of extracted water for $\delta^{18}O$ and $\delta^{2}H$ in both sandy and clay soils.

CONCLUSIONS

A simple, fast, affordable and portable vacuum distillation method for extracting soil samples for isotopic analysis was developed. The extraction times were 30 min for the Reisenberg sandy soil and 120 min for the Ebendorf clay soils. The method does not require the use of liquid nitrogen or dry ice hence can be adapted easily for developing countries. The storage vials can be connected directly to minimize the transfer of sample. Other advantages of this system are the portability, the relatively low price (US$8 000 for a setup of 12 samples) and the high throughput. The fast recovery and reproducibility of the new methodology developed is valuable for quantifying the removal of water from the soil around crop roots through soil evaporation and transpiration. This information will be used to identify soil and water management practices that minimize water losses via soil evaporation.

ACKNOWLEDGEMENTS

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Dynamics of *de Novo* Formation of Amino Sugars in Soil via CSSIA

S. Bodé[1], Z. Bai[1,2], D. Huygens[1,3], X. Zhang[2] and P. Boeckx[1,*]

ABSTRACT

Amino sugars are the building blocks of microbial cell walls and have been used widely to assess microbial residues. To have a better insight in the formation dynamics of amino sugars in soil, uniformly carbon-13 (^{13}C)-labelled wheat residue of different quality (grain, leaf and root) was amended to two soils under distinct tillage managements. The isotopic composition of individual amino sugars was measured using liquid chromatography — isotope ratio mass spectrometry (LC-IRMS). Maximum formation was reached within a few days after residue addition. Glucosamine and galactosamine followed dissimilar formation kinetics. The maxima of incorporation of residue C into the amino sugar pools ranged from 1.0 percent for galactosamine to 10.6 percent for glucosamine. Formation rate constants of residue-derived amino sugars ranged from 0.11 to 0.48/d for galactosamine and glucosamine, respectively. In general, larger amounts of amino sugars were formed at a higher rate with increasing plant residue quality. The microbial community of the no-till soil was better adapted to assimilate low quality plant residues (i.e. leaf and root). All together, the formation dynamics of microbial cell wall components was component-specific and determined by residue quality and the soil microbial community.

Key words: *amino sugar, kinetics, organic residue, tillage, carbon-13, LC-IRMS.*

INTRODUCTION

Microbial decomposition of soil organic matter (SOM) releases 60 Pg of carbon dioxide and 300 Tg of methane to the atmosphere each year. Aerobic or anaerobic microbial breakdown of soil organic matter is controlled by its recalcitrance, physicochemical protection and microbial enzyme activity, and controls the potential for terrestrial ecosystems to sequester or release carbon (C) to the atmosphere (Craine, Fierer and McLauchlan, 2010). Slight changes in the rate of SOM decomposition due to human disturbances or temperature can significantly affect the concentration of carbon dioxide or methane in the atmosphere. Predicting the sensitivity of SOM decomposition to temperature change or human disturbance is therefore critical to predicting future atmospheric greenhouse gas concentrations and feedbacks to climate warming and soil degradation.

1 Isotope Bioscience Laboratory - ISOFYS, Ghent University, Gent, Belgium
2 State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, P. R. China
3 Institute of Agricultural Engineering and Soil Science, Faculty of Agricultural Sciences, Universidad Austral de Chile, Valdivia, Chile
* E-mail address of corresponding author: pascal.boeckx@ugent.be

One of the most significant impacts that microbial communities have on their environment is their ability to recycle essential elements that make up their cells (Glaser, Turrion and Alef, 2004). Therefore, there is considerable interest in understanding the biological mechanisms that regulate C exchanges between the land and atmosphere, including microbial metabolism (Allison, Wallenstein and Bradford, 2010). Amino sugars are useful microbial biomarkers to investigate the dynamics of microbial communities due to their prevalence in the cell walls of microorganisms, their insignificant content in plant residues and their recalcitrance after cell death (White, 1968; Amelung *et al.,* 2001; Glaser and Gross, 2005; He *et al.,* 2005; Liang and Balser, 2010). Over 90 percent of amino sugars are found in dead cells (Amelung *et al.,* 2001). Therefore, the amino sugar content is used to quantify microbial residues rather than a proxy for living microbial biomass and activity (van Groenigen *et al.,* 2010). Glucosamine in soil is mainly derived from chitins of fungal cell walls, though it also occurs in bacteria. Muramic acid originates exclusively from peptidoglycans of bacterial cell walls (Farkas, 1979; Amelung *et al.,* 2001 and 2008; He *et al.,* 2005). The origin of galactosamine is less clear and is typically considered to be non-specific, as actinomycetes, bacteria and fungi all likely contain considerable amounts of galactosamine (He *et al.*, 2005; Ding *et al.*, 2010).

Therefore, the first aim of this study was to elucidate residue-derived amino sugar formation kinetics following plant residue addition. Also evaluated was the use of residue C incorporation dynamics into the amino sugar pools as a tool to assess microbial physiology.

To these ends, a laboratory incubation experiment was carried out in which two soils with a distinct tillage management were incubated with carbon-13 using ^{13}C-labelled wheat residues of different quality (wheat grain, leaves and roots). The amino sugar formation dynamics were determined by measuring the evolution of the ^{13}C content of individual amino sugars via liquid chromatography — isotope ratio mass spectrometry (LC-IRMS).

MATERIALS AND METHODS

Soil description and incubation

Soil description

The soils for this study were collected from the field site located in Maulde, Belgium (50°37'N, 3°34'E). The climate of the site is characterized as temperate and humid marine with a 30-year mean precipitation of 780 mm per yr, and mean maximum and minimum temperatures of 13.5°C and 6.3°C, respectively. The soil is classified as a Luvisol (FAO, 2006). The field site has been used for cropping over 100 yr and was converted from conventional tillage (moldboard plowing to 30 cm and harrowing of the top 10 cm) to reduced tillage (harrowing of the top 10 cm) in 1995. In 2006, one third of the field was reconverted to conventional tillage, another third to "no-till"

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(no soil disturbance and direct seeding). Soils from both conventional (CT) and no-till (NT) fields were collected.

Incubation experiment

The uniformly ^{13}C labelled wheat (*Triticum aestivum*) had been grown with $^{13}CO_2$ (2 atom% excess) (Denef and Six, 2006). The grain, leaves and roots were collected, dried at 45°C and stored at room temperature until incubation. Residues were ground to a size <250 µm and mixed thoroughly with the soil. An application rate of 6 mg substrate C/g dry soil was used in six treatments: NG (NT with grain residue), NL (NT with leaf residue), NR (NT with root residue), CG (CT with grain residue), CL (CT with leaf residue), and CR (CT with root residue). There were three microcosm replicates for each treatment.

The incubation temperature was maintained at 24°C and the moisture content at 20 percent (w/w). Sampling was done after 0, 9, 24, and 45 hrs, and at 3, 5, 10 and 21 d by instantaneously freezing the microcosms in liquid nitrogen followed by lyophilization. The subsamples were stored at –20°C for subsequent analyses.

Amino sugar analysis

The amino sugar extraction procedure was based on the method described by Bodé, Denef and Boeckx (2009). Briefly, c.a. 0.2 g soil was hydrolyzed for eight hrs at 105°C using 10 mL 6M HCl. Thereafter, the soil suspension was filtered (GF/C 25mm, Whatman) using a reusable syringe filter device (Millipore, SWINNEX). Water and HCl were removed by evaporating under reduced pressure at 45°C, and the concentrated amino sugar sample was re-dissolved in MilliQ water. After purification by a cation exchange resin, the amino sugar solution was dried and re dissolved with 1.5 mL MilliQ water. Concentration and delta carbon-13 ($\delta^{13}C$) of amino sugar were determined by liquid chromatography-isotope ratio mass spectrometry (LC-IRMS) (Thermo Electron, Bremen, Germany).

The fraction of amino sugar C derived from the ^{13}C-labelled residues at a time point t was calculated as:

$$fAS_{R,t} = \left(\frac{a^{13}C_{AS,t} - a^{13}C_{AS,t_0}}{a^{13}C_R - a^{13}C_{SOM}} \right) \quad (1)$$

where $a^{13}C_R$ and $a^{13}C_{SOM}$ are ^{13}C fractional abundances ($^{13}C/(^{13}C + {}^{12}C)$) of the added residues and original SOM, respectively; and $a^{13}C_{AS,t}$ and $a^{13}C_{AS,t0}$ are the isotopic composition of the amino sugar of interest at time *t* and at the start of the incubation experiment, respectively.

It should be noted that the $^{13}C_{AS,t0}$ was not identical to the original isotopic composition of the soil amino sugar (AS_0) due the presence of ^{13}C-labelled amino sugars in the plant residues. Since plants do not produce amino sugars (Amelung *et al.,* 2008), this is likely explained by the presence of endophytic bacteria and fungi (Appuhn *et al.,* 2004, Reinhold-Hurek and Hurek, 2011) in the labelled plant material.

Bulk soil isotopic analysis

Sub-samples of air dried soil samples were ground by a planetary ball mill (PM400, Retsch, Germany) for total C and N, and ^{13}C and nitrogen-15 (^{15}N) analyses were by an elemental analyzer (EA) (ANCA-SL, SerCon, UK) coupled to an IRMS (20-20, SerCon, UK).

Statistical analysis

Statistical analysis was performed using SPSS 19.0. A three-way analysis of variance (ANOVA) procedure with Tukey's HSD (Honestly Significant Difference) *post hoc* test was used to analyse the effects of plant residue quality, amino sugar identity and tillage on amino sugar formation and C mineralization using a general linear model. When a significant interaction between factors was observed this interaction was investigated by repeating the statistical test for the different levels of the interacting factors individually. Unless otherwise stated a significant level of difference was set at $\alpha = 0.05$. Non-linear regression analysis was used to determine *k* and maxima in non-linear equation (2).

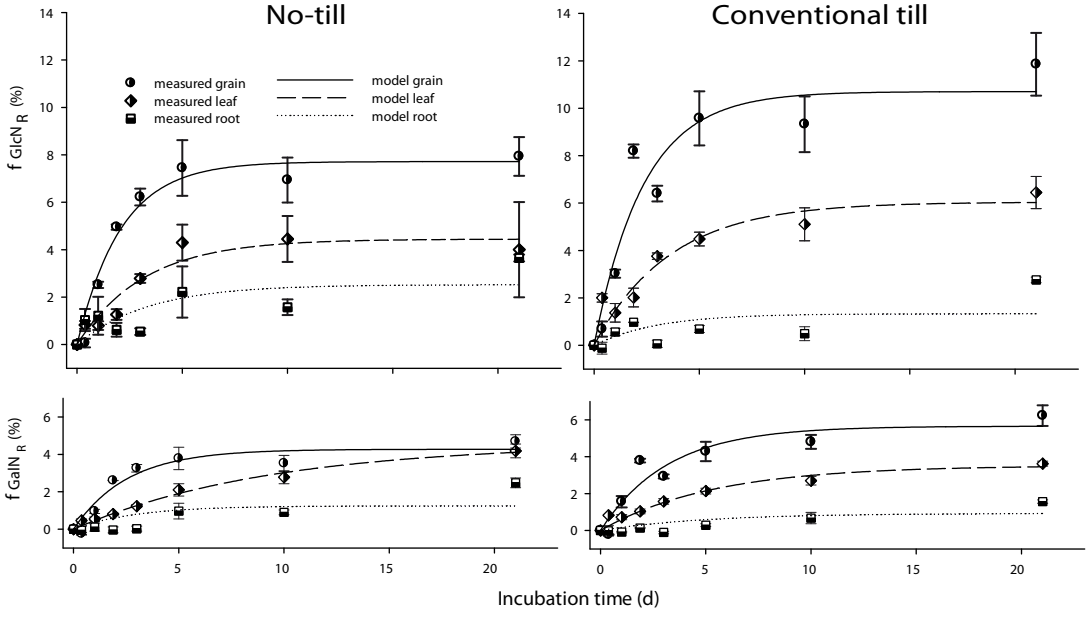


FIGURE 1. Modelled and measured contribution of residue derived C to the amino sugar pool (AS_R) as function of time. *f*$GlcN_R$ and *f*$GalN_R$ are respectively the fractions of glucosamine and galactosamine C derived from the added labelled residue. Data points are averages of three replicate experiments; error bars represent standard errors (adapted from Bai *et al.,* 2012).

TABLE 1. Parameters description for AS_R formation (adapted from Bai *et al.*, 2012)

AS	Treats	AS_0 (nmol/g dry soil)	fAS_R (%)	k (d^{-1})	R^2
GlcN	NG	3 781 ± 66	7.9 ± 0.4	0.48 ± 0.08	0.89
	NL		5.9 ± 0.6	0.20 ± 0.04	0.84
	NR		2.6 ± 0.5	0.30 ± 0.16	0.38
	CG	2 985 ± 199	10.6 ± 0.7	0.43 ± 0.08	0.86
	CL		6.0 ± 0.4	0.28 ± 0.05	0.84
	CR		1.3 ± 0.3	0.34 ± 0.24	0.35
GalN	NG	1 937 ± 102	4.5 ± 0.3	0.39 ± 0.07	0.87
	NL		4.8 ± 0.4	0.11 ± 0.02	0.93
	NR		1.6 ± 0.4	0.25 ± 0.17	0.46
	CG	1 742 ± 110	6.5 ± 0.4	0.42 ± 0.07	0.87
	CL		4.0 ± 0.2	0.22 ± 0.03	0.92
	CR		1.0 ± 0.3	0.20 ± 0.11	0.58

RESULTS

Incorporation of residue carbon in amino sugar pools

The isotopic ^{13}C composition of individual amino sugars increased exponentially during the first day of incubation, indicating a fast incorporation of the residue C into the amino sugar pools (Figure 1).

A first order kinetic model was fitted to the formation dynamics of residue-derived glucosamine and galactosamine (Figure 1) using the following equation:

$$fAS_R = fAS_{R,Max} \cdot (1 - e^{-k \cdot t}) \quad (2)$$

where $fAS_{R,Max}$ is maximum of the exponential incorporation of residue C into amino sugar pool; fAS_R is amount of residue derived amino sugar at time t; and k is the formation rate constant of the exponential formation of residue derived amino sugars.

The k-values of glucosamine and galactosamine ranged from 0.48 to 0.20/d and from 0.11 to 0.42/d, respectively (Table 1). Unfortunately, the correction for 'entophytic' (labelled) muramic acid induced a high uncertainty on the residue derived muramic acid formation, making these data unusable.

Statistical analysis of the formation kinetic parameters

Using a multi way ANOVA (Table 2), the effect of amino sugar type, residue quality and tillage history of the soil on parameters describing the dynamics of residue derived amino sugar formation ($fAS_{R,Max}$, k (Equation 1) and on $fAS_{R,Max}$ relative to the original amino sugar concentration) were investigated.

DISCUSSION

This study quantified newly formed amino sugars during peak microbial activity following plant residue addition. Noticeably, soil microorganisms prefer to feed on fresh organic residues rather than on endogenous SOM during exponential microbial activity following residue addition (Amelung *et al.,* 2008).

The incorporation of residue C into the amino sugar pool reached a maximum within ca. one week after which the residue derived amino sugars formation reached a steady state (Table 1, Figure 1). Liang *et al.* (2007) reported that amino sugar content in black soil

TABLE 2. Effect of plant residue quality (Rq), amino sugar type (AS_i) and tillage history of the soil samples (Till) on the maxima of the residue C incorporation in the amino sugar pool ($fAS_{R,Max}$) and production rate constant (k).

		$fAS_{R,Max}$	k
AS_i	rank	GlcN > GalN	GlcN > GalN
	F-value	(178) ***	(6.36)*
Rq	rank	G > L> R	G > L = R
	F-value	(355)***	(10.5)***
Till	rank	NT = CT	CT = NT
	F-value	(2.52)	(0.006)
AS_ixRq	F-value	(36.9)***	(0.006)
	AS_i on Rq	GlcN (213)***, GalN(155)***	N.A.
	Rq on AS_i	G(135)***, L(42.8)***, R(11.1) **	
Rqxtill	F-value	(5.69)*	(0.238)
	Rq on Till	G (35.9)***[a], L(2.10), R (19.3)**	N.A.
	Till on Rq	NT (123)***, CT(242)***	
AS_i xTill	F-value	32.3***	(1.24)
	AS_i on Till	GlcN (5.52)*, GalN(0.562)	N.A.
	Till on AS_i	NT (74.2)***, CT(104)***	
Rq x AS_i x Till	F-value	(3.56)	(0.133)

The levels of the factors tested are ranked in decreasing magnitude of the specific variables ($p < 0.05$) followed by the F-values (between brackets) of the factor effect, obtained from a multi way ANOVA. The F-value of the interaction of factors is followed by the F-value of one interacting factor for each individual level of the other interacting factor and an indication of the significance of the difference between the levels of the other interacting factor (adapted from Bai et al., 2012).

N.A. — not applicable; G = grain, L = leaf, and R = root; CT = conventional till and NT = no-till

Differences between factor levels were tested using Tukey's HSD ($p < 0.05$) post hoc test. *** — $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$

[a] The ranking is different from the total effect (i.e. CT > NT)

reached a maximum within three weeks upon incubation with maize residue, thereby increasing the original amino sugar content by one third. Decock *et al.* (2009) also revealed maximum ^{13}C incorporation into glucosamine and galactosamine within one week after incubation with ^{13}C-labelled wheat residues.

Glucosamine, galactosamine and muramic acid formation dynamics

As muramic acid originates exclusively from bacterial cell walls, while glucosamine and galactosamine are present in both bacterial as fungal residues (Amelung *et al.,* 2001 and 2008; Glaser and Gross, 2005; Engelking, Flessa and Joergensen, 2007), muramic acid is the preferred biomarker to differentiate bacterial and fungal activity for incorporation of residue-derived C. Unfortunately, due to the higher uncertainty concerning the muramic acid measurements and the high ^{13}C muramic acid contamination of the plant residues the formation dynamics of muramic acid could not be determined.

The $fAS_{R,Max}$ ranged from 1.3 percent 11 percent for glucosamine while for galactosamine the range was from 0.8 percent to 5.7 percent. A similar trend was also observed in other studies (Glaser and Gross, 2005; Engelking, Flessa and Joergensen, 2007; He *et al.,* 2011).

Bacteria and fungi both produce glucosamine and galactosamine (Amelung *et al.,* 2001 and 2008; Glaser and Gross, 2005). However, the strong amino sugar type effect on $fAS_{R,Max}$ and on k indicates that these amino sugars are formed through dissimilar processes. Engelking, Flessa and Joergensen (2007) reviewed the available literature on amino sugar concentrations in cultured bacteria and fungi, which revealed that the galactosamine/glucosamine ratio appeared to be on average almost three times higher in fungi compared with bacteria, making residue-derived galactosamine a 'more' fungal marker than glucosamine when considering microbial activity. The higher formation rate constant of glucosamine compared with galactosamine therefore most likely indicates that bacteria play a more important role for early stage incorporation of residue-derived C, i.e. "fast energy channel" (Rousk and Baath, 2007). The slower formation of galactosamine corroborates with the slower turnover of fungi compared with bacteria; for which fungi are involved in the slow energy channel through the soil food web (Rousk and Baath, 2007).

Effect of residue quality

The high C:N ratio and high lignin content (unpublished data) of root indicates its low quality while grain had the highest quality. This difference resulted in different $fAS_{R,Max}$ values between residues: grain > leaf > root ($p < 0.001$). The interaction between residue quality and amino sugar type revealed that the difference between the $fAS_{R,Max}$ for glucosamine and galactosamine was much more pronounced for grain than for leaf and root. Considering the higher fungal origin of galactosamine, this interaction indicates (at least during peak microbial activity) that fungi are less dependent on the quality of the residue than bacteria for *de novo* amino sugar formation. This is in accordance with expectations since it is generally believed that bacteria especially rely on easily available C compounds while the fungal community is better adapted to colonize more recalcitrant sources (Myers *et al.*, 2001; Waldrop and Firestone, 2004).

Effect of site tillage history

In general, tillage had no significant effect on $fAS_{R,Max}$ or k. However, the interaction between tillage history and residue quality (Table 2) indicated that the conventionally tilled soil was better adapted to incorporate residue C of the highest quality (grain), while the no-till soil was better adapted to incorporate residue C of the lowest quality (root). This may be explained by the microbial community differences typically found in no-till soil. Fungi, showing a great ability to decompose more recalcitrant substrates (Acosta-Martinez *et al.,* 2003; Ding *et al*., 2010; Werth and Kuzyakov, 2010), are typically more abundant in the no-till soils (Fu *et al.,* 2000; Thiet, Frey and Six, 2006; White and Rice, 2009).

Field measurements and laboratory incubations both showed that the relative change in galactosamine was significantly smaller than for glucosamine (Table 2), indicating that the more conservative response of galactosamine upon shift in tillage was (at least partially) due to a lower formation of residue derived galactosamine compared with glucosamine after receiving increased residue input in the no-till treatment.

CONCLUSIONS

A first order kinetic model could describe residue-derived amino sugar formation, which reached a maximum and steady state a few days after residue addition. During peak microbial activity *de novo* residue derived amino sugar formation was surprisingly fast, and production rate constants for glucosamine (0.20–0.48 d) were faster than those for galactosamine (0.11–0.39 d).

The faster incorporation of residue C into glucosamine underpins the role of bacteria as a "fast energy channel" as described by Rousk and Bååth (2007). In addition, the *de novo* amino sugar formation relative to the original amino sugar pool was higher for glucosamine than for galactosamine; however, this difference declined strongly with decreasing residue quality, confirming the better adaptation of fungal communities to colonize more recalcitrant C sources. Finally, the influence of tillage history on *de novo* amino sugar formation indicated a better adaptation of soil microbial community to incorporate C originating from more recalcitrant plant residues in the no-till treatment compared with conventional tillage.

ACKNOWLEDGEMENTS

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Suess Effect on Biomarkers Used to Determine Sediment Provenance from Land Use Changes

M. Gibbs[1,*], A. Swales[1] and G. Olsen[1]

ABSTRACT

A compound-specific stable isotope (CSSI) technique developed to positively identify the source of soil by landuse provides a tool to determine the provenance of sediment from watershed erosion. The CSSI technique uses the carbon-13 (^{13}C) isotopic signatures of plant-derived fatty acid biomarkers in the source soils and a mixing model to deconstruct the sediment isotopic signatures into contributing source soils by land use. While the CSSI technique provides qualitative information on where the sediment is coming from, for quantitation it requires erosion rate information from the fallout radionuclide (FRN) techniques. Together, the CSSI and FRN techniques can identify landuse practices that are exacerbating soil erosion i.e. hot spots. These tools can provide the information that allow managers to target the resources available to mitigate soil erosion. The data can also be used to map the dispersion of contemporary terrigenous sediments through an estuary and to identify land use changes through time from sediment cores. However, when the CSSI technique is used to look back in time using contemporary source libraries, the ^{13}C isotopic signatures of the biomarkers need to be corrected for the Suess effect, i.e. the isotopic depletion of the $\delta^{13}C$ signature of atmospheric carbon dioxide (CO_2) due to the admixing of isotopically depleted CO_2 from the burning of fossil fuels.

Key words: *compound-specific stable isotope, fallout radionuclides, fatty acid biomarkers, soil erosion, Suess effect.*

INTRODUCTION

Management of natural and agricultural resources in agro-ecosystems for conservation of soil and sustainable food production requires an understanding of the landuse practices exacerbating soil losses and where the 'hot spots' of erosion occur within the watershed. Information on sources is a key requirement for targeting sediment control measures (Walling, Collins and Stroud, 2008). A compound-specific stable isotope (CSSI) technique has been developed to determine the provenance by land use of soil contributing to contemporary sediment at any location in a sediment deposition zone (Gibbs, 2008). For source identification the technique uses a 'reference library' of contemporary soil bulk carbon stable isotopic ($\delta^{13}C$) data and compound specific $\delta^{13}C$ values of fatty acid biomarkers from a range of landuse types. Although only recently developed in New Zealand (Gibbs, 2008), the CSSI technique has been successfully tested in several countries including Australia, England, and Austria (Hancock and Revill, 2011; Blake *et al.*, 2012; Gibbs and Mabit, 2012), providing new information into sources and causes of erosion that could not be obtained with conventional geochemical catchment modelling techniques (Hancock and Revill, 2011).

The limitation of the CSSI technique is that it provides qualitative data on the source proportions and requires additional information on mass transport from other techniques to become quantitative. In contrast, geochemistry techniques using soil mineralogy (Foster and Lees, 2000) and fallout radionuclide (FRN) techniques using beryllium-7 (^{7}Be), caesium-137 (^{137}Cs) and lead-210 (^{210}Pb) (Walling and He, 1999; Walling, He and Blake, 1999; Wilson, Matisoff and Whiting, 2007) provide information on recent erosion but cannot positively identify the sources of the sediment within the watershed (Walling and Collins, 2008; Walling, Collins and Stroud, 2008). In combination with the CSSI technique, these techniques have the potential to provide quantitative information on soil erosion sources to enable identification of hot spots of erosion in the landscape, and which may be used to make informed decisions on the design and implementation of mitigation measures to reduce soil erosion.

Originally the CSSI technique was developed to identify contemporary sources of soil erosion by deconstructing the source soil proportional contributions in contemporary sediments (Figure 1). These data indicated that one landuse was contributing substantially more soil to the downstream environment than would be expected from the area occupied by that landuse i.e. a hot spot. On-site investigation of the landuse (production forestry) located the hot spot and identified the landuse practice (clearfell harvesting across a stream) exacerbating the erosion. With this knowledge it was possible to develop mitigation measures to reduce the erosion and protect the downstream ecosystem.

The CSSI technique has also been used to determine the sources of terrigenous soil contributing to sediment in the Bay of Islands, New Zealand, and to evaluate erosion due to changes in landuse over time (Gibbs and Olsen, 2010). This application used the present day catchment soil library of CSSI values to deconstruct the soil sources from the past contributing to depth-defined subsections from a 140 cm long core (RAN S-9) from the Veronica Channel in the Bay of Islands. Each subsection in the upper 70 cm of the core was dated using FRN techniques with ^{210}Pb providing an estimate of sediment accumulation rates. The CSSI data were aligned with the ^{210}Pb data to give relative contributions of the main landuse soil sources at different times (shadow graphs, Figure 2), and these sources of erosion were related to historical events using historical data records (Figure 2).

During this phase of the study, it was found that there were errors in the CSSI interpretations with the CSSI data indicating the presence of pasture grasses had been introduced to New Zealand before the arrival of the European settlers (Figure 2). The CSSI errors were attributed to the Suess effect (Keeling, 1979), which results in

1 National Institute of Water and Atmospheric Research (NIWA), PO Box 11-115, Hamilton 3251, New Zealand
* E-mail address of corresponding author: Max.Gibbs@niwa.co.nz

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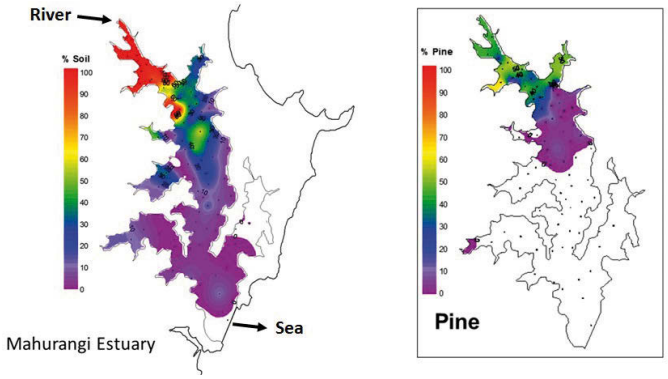


FIGURE 1. Left: Spatial distribution of terrigenous soil in the Mahurangi Estuary relative to the river inflow and the outflow to the sea. Right: the spatial distribution of the sediment component derived from pine forest. The land area occupied by pine forest in the watershed was 12 percent but the proportion of soil from pine forest in the river delta was up to 60 percent. (Contour plots produced using Surfer32 by Golden Software)

a change in the isotopic abundance of $\delta^{13}C$ in atmospheric CO_2 over time, and require correction (Verburg, 2007).

This paper defines the isotopic corrections needed to allow the CSSI technique to be used to determine changes in landuse erosion sources over time and applies these corrections to examine the spatial and temporal changes in sediment erosion by landuse in the Bay of Islands based on data from two sediment cores over a period of up to 2 500 years before present (BP).

METHODS

Study site

The Bay of Islands (Lat: –35.23°S; Long: 174.10°E) is a large coastal inlet on the east coast of the upper North Island of New Zealand (Figure 3). The area is historically important to New Zealand as it is the landing place of the discoverer, James Cook in 1796, and the signing place of the Treaty of Waitangi in 1840, the founding document for New Zealand. The bay receives the discharge from five watersheds around the bay via the Waikare, Kawakawa, Waitangi, Kerikeri and Te Punga Rivers (Figure 3). Based on hydrological modelling of these river inflows (Pritchard *et al.*, 2010), the Kawakawa, Waitangi and Kerikeri rivers are the main sources of sediment to the bay.

A 140-cm long sediment core was collected from the Veronica Channel (RAN S-9) and another shorter core (20 cm) was collected from the middle of the bay (KAH S-20) at a depth of 30 m (Figure 3). Both cores were split lengthwise to extract a 1-cm thick longitudinal slab which was X-rayed to assess layering of different density particles at different depths in the cores. The X-ray images also located shell fragments at different depths in the long core and these were removed for carbon-14 (^{14}C) dating.

Analytical

Depth-related horizontal sections 1 cm thick were taken from the remainder of the core. These were dried in an air fan oven at 50°C, then ground to a fine powder (< 100 µm mesh). An aliquot (2 g) from each dried sample was acidified with 1 M hydrochloric acid (HCl) to remove inorganic carbonates as follows.

The sediment was placed in a 50 mL plastic centrifuge tube and 2 mL of HCl was added. When effervescence stopped, a further drop of HCl was added to see whether all carbonate had been removed. This step was repeated until no further effervescence occurred.

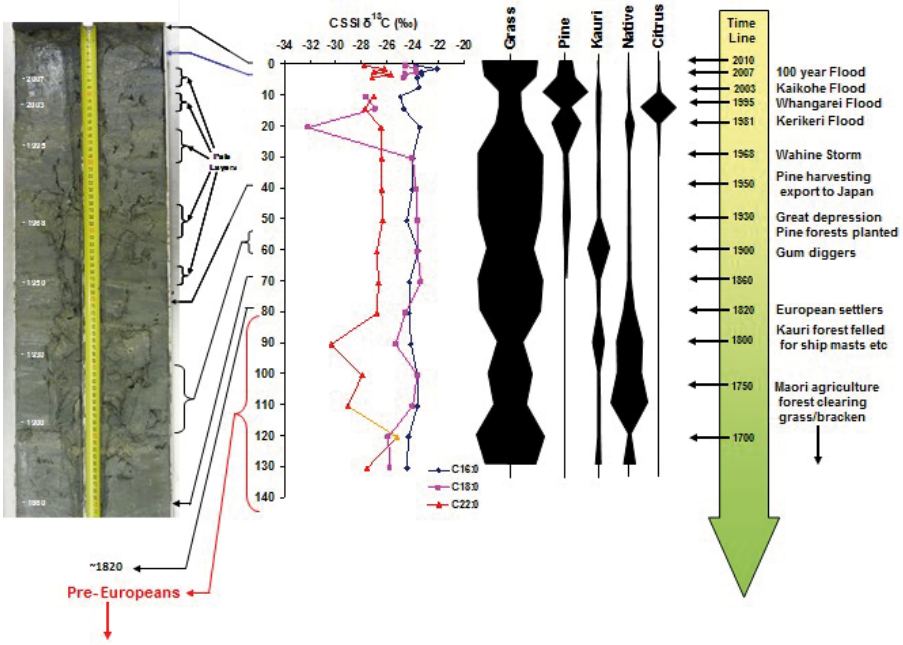


FIGURE 2. Alignment of the CSSI values for C16:0, C18.0 and C22.0 fatty acids with section depths in the top 70 cm of the sediment core RAN S-9, allowed the deconstruction of the sediment at each depth into proportional contributions by landuse at that time. The shadow graphs sum to 100 percent across the five landuse types. The timeline was constructed from historical data. (Redrawn from Gibbs and Olsen, 2010).

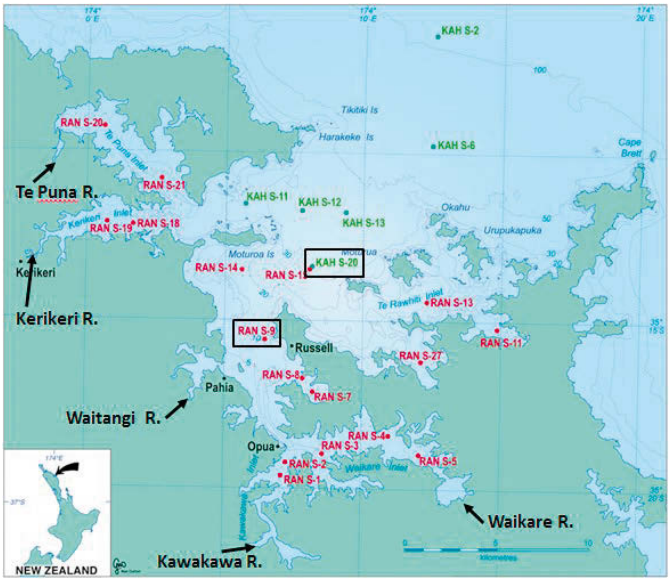


FIGURE 3. Site map of the Bay of Islands, New Zealand showing the five river inflows and the location of the two sediment cores (square boxes) used in this study. Red and green codes in the bay are sediment core locations from two visits (from Swales *et al.*, 2010).

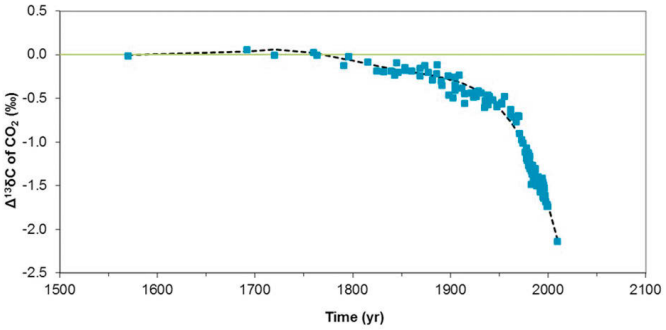


FIGURE 4. Time-series plot of the change (Δ) in $\delta^{13}C$ abundance of atmospheric CO_2 that has occurred since pre-industrial times (AD 1700), the zero line in this graph. The broken line is a 6th order polynomial curve fitted through the data. (Redrawn using data from Verburg, 2007 and papers therein)

The acid mixture in the centrifuge tube was diluted to 50 mL with distilled water before centrifuging at 3000 rpm for 10 min. The supernatant liquid was discarded and the sediment was rinsed with 10 mL of distilled water and centrifuged. The rinse water was discarded and the acidified sediment was dried at 50°C, and then reground to a fine powder before being analysed for bulk $\delta^{13}C$ and organic carbon (C) on a continuous flow, isotope ratio mass spectrometer (CF-IRMS) (Thermofinnigan Delta Plus).

The extraction and preparation of soil fatty acids used in the CSSI technique is described in detail in Gibbs (2008 and 2010). In brief, a 20 g aliquot from each dried sediment sample was extracted twice in hot (100°C) dichloromethane solvent (DCM) at high pressure (2000 psi) in an automated solvent extractor (Dionex ASE 200). After drying, the fatty acids were converted to their non-polar fatty acid methyl esters (FAME) by methylation using 5 percent BF_3 in methanol at 70°C for 20 min. The $\delta^{13}C$ isotopic signature of each FAME from each sediment section was determined by gas chromatography–combustion–isotope ratio mass spectroscopy (GC-C-IRMS).

Deconstruction of sediment into source soils

Contemporary sediment samples

The isotopic proportions of each landuse source soil contributing to a surficial sediment sample were determined using reference library samples in the mixing model "IsoSource" (Phillips and Gregg, 2003). These isotopic proportions were converted to soil proportions using the C content of the source soils in the sediment (Gibbs, 2008). Individual river delta samples were used to define landuse sources from each of the five river watersheds.

Historical sediment samples

To determine the landuse source soils contributing to the sediment sections at different depths (ages) in the sediment cores using present day reference library data, the age of the sediment section must be determined to allow the CSSI values to be corrected for the Suess effect. Dating was achieved using FRNs with excess ^{210}Pb providing date estimations for up to 150 year BP. This timeline was extended back more than 2 500 year BP to around 640 BC using ^{14}C dates from the shell fragments in the core.

Suess effect

The Suess effect refers to the isotopic depletion of atmospheric CO_2 due to the admixing of isotopically depleted (18‰) CO_2 from the burning of fossil fuels, i.e. coal. Since the beginning of the industrial revolution in the 1700s, the $\delta^{13}C$ value of atmospheric CO_2 has decreased by around 2.2‰ with the rate of that depletion increasing in recent years (Figure 4).

Since plants use atmospheric CO_2 as a major C source, the $\delta^{13}C$ values of biomarkers in present day reference plants will be up to 2.2‰ more depleted than those of the same biomarkers from the past. As the Suess effect only began around AD 1700 (Figure 4), all CSSI values from the core sections before that time were made more isotopically depleted by 2.2‰, i.e. they were corrected by –2.2‰. The CSSI values from the core sections between AD 1700 and present day were corrected by the isotopic depletion value. This value was calculated from the 6th order polynomial equation from Verburg (2007), and adding the absolute $\delta^{13}C$ value (8.55‰) of present day CO_2 (year 2012) as an offset to obtain the change (Δ) in the $\delta^{13}C$ isotopic value for the year (Y) of the core section. Between 1700 and present the CSSI values from the core were made more isotopically depleted by the correction value:

$$\text{Correction value} = 8.55 + 7.7738118 \times 10^{-16} \times Y^6 - 1.2222044 \times 10^{-11} \times Y^5 + 7.1612441 \times 10^{-8} \times Y^4 - 2.1017147 \times Y^3 + 3.3316112 \times 10^{-1} \times Y^2 - 273.715025 \times Y + 91703.261$$

Note that the polynomial coefficients in this equation have been rounded to seven decimal places. More precise coefficients to 14 decimal places can be obtained from the author of Verburg (2007).

RESULTS AND DISCUSSION

Erosion due to changes in landuse over time can be referenced to present day erosion. Contemporary sediment from soil erosion in the watershed will tend to deposit in the river delta before some is redistributed into the bay (e.g. Figure 1). Consequently, surficial sediments from the river deltas provide the present day erosion data. For changes in landuse effects on erosion, samples from different depths in sediment cores will reflect the soil sources associated with erosion events at the time that sediment layer was deposited.

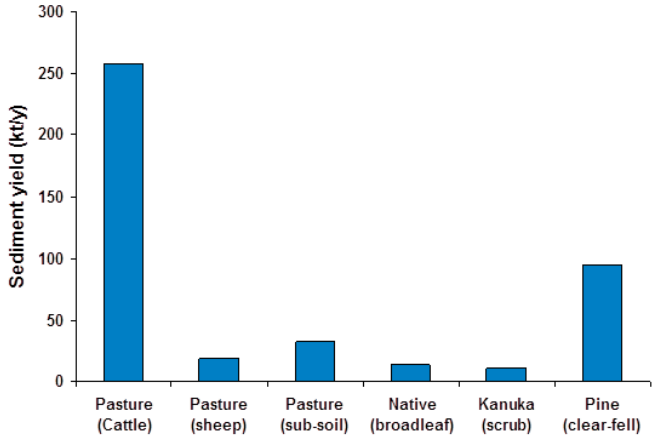


FIGURE 5. Estimated annual sediment yields (kilo tonnes, kt/yr) of sediment to the Bay of Islands from the six main soil sources identified by the CSSI technique (Gibbs and Olsen, 2010) and quantified using the flow and sediment load information from Pritchard *et al.* (2010). The pasture (cattle) includes both dry stock and dairy (redrawn from Gibbs and Olsen, 2010).

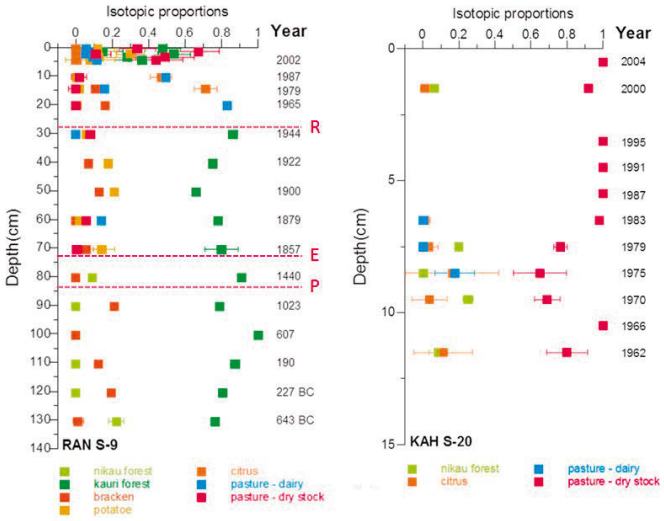


FIGURE 6. Proportional contributions of terrigenous soil in sediment cores RAN S-9 and KAH S-20 based on CSSI values corrected for the Suess effect. The markers for each landuse include the standard deviation as the error term although in most samples the error bars are within the marker point. The X-axis is in isotopic proportion with a range of 0 to 1 representing 0 percent to 100 percent. The "Year" column represents the timeline date for the depth down the core (cm). On the core RAN S-9 graph, the red broken lines represent special historical events: P — the arrival of the Polynesians around 1300 AD; E — European settlement in 1814 AD; R — returned servicemen land allocation after World War 2 in 1945.

River delta sites

Surficial sediments collected from each of the five river deltas in the Bay of Islands were deconstructed using the mixing model to obtain the present day source proportions of terrigenous soil by landuse (Gibbs and Olsen, 2010). Flow and sediment load information from Pritchard *et al*. (2010) were used to convert these estimates to total sediment yield by land use, discharging into the Bay of Island on an annual basis (Figure 5).

These results show that most of the annual sediment load comes from pasture used for beef and dairy farming (258 kt/yr) and pine forest that has been clear-felled (95 kt/yr). Other pasture landuse sources combined contribute about 50 kt/year, while native forest and kanuka scrubland contribute a total of 24 kt/yr. Overall, more than 70 percent of the present day soil deposited in the Bay of Islands came from pasture sources.

Sediment cores

The sediment core from the Veronica Channel (RAN S-9) was taken within the zone of influence of sediment inputs from the Waikare, Kawakawa and Waitangi Rivers, and a 20 cm core from a depth of 30 m in the open bay (KAH S-20) was taken within the zone of sediment accumulation for the whole bay. For core RAN S-9, the ^{210}Pb and ^{14}C data provided a historical timeline extending back 2500 years BP and for core KAH S-20, ^{210}Pb provide a timeline back to 1962 (Figure 6). After applying the correction for the Suess effect to the CSSI values from the depth-related sections from these sediment cores, it was found that the major sources of sediment before 1944 were kauri forest, nikau forest and bracken (Figure 6).

Core RAN S-9 shows that the major sources of land erosion before humans arrived in New Zealand are consistent with what is known of the region. The land was covered with native forest with broadleaf forest on the flatter land, kauri forest on the drier hillsides and nikau forest in the wetter valleys and alongside rivers. Bracken is a coarse fern which rapidly colonizes bare ground such as would be left after a landslide. With this region of New Zealand being subjected to heavy rainfall and tropical storms, it is likely that the kauri signature represents landslide events exposing subsoils to erosion and the bracken signature represents further erosion of those slip faces after the bracken had become established. The nikau forest signature is likely to reflect erosion in the valleys associated with heavy rainfall events. Broadleaf forest on relatively flat land would protect the soil from direct impact of rainfall and only minimal erosion would occur and was not detected above a threshold of 1 percent.

The arrival of the Polynesians around AD 1300 saw little change in this erosion pattern and it was not until the arrival of the European settlers in 1814 that other landuse sources of soil erosion could be detected i.e. the presence of European grasses and potatoes. A dairy signature was also detected but the potato signature was more consistent. Presumably this was because land had to be cultivated to grow potatoes exposing bare soil to erosion by the locally heavy rainfall.

The continuing presence of the kauri forest signature is likely to have been associated with the clearing of the hilly land exposing historical subsoil horizons to erosion and the draining of swamps where forest sediments had accumulated. After 1945, the kauri signature stopped. This is attributed to the allocation of land for farming to servicemen returning from World War 2. That land was cleared and planted in pasture grasses eliminating much of the subsoil erosion. The occasional presence of strong dairy signatures between 1960 and the present day are likely to correspond with the erosion of soil during flood events.

A citrus signature is consistent with the establishment kiwi fruit orchards in the Bay of Islands in the late 1960s and 1970s. Large areas of citrus orchard were removed leaving bare ground, which would carry the citrus CSSI signature, while the kiwifruit plants became established.

In core KAH S-20, the major terrigenous soil source components were from pasture associated with dry stock (beef), dairy nikau for-

est and citrus (Figure 6). Although the citrus signature was detected in the late 1960s to 1970s, the CSSI signatures suggest that most of the soil erosion from the Bay of Island watershed was from pasture used for dry stock farming. This observation is consistent with the contemporary sediment assessment from the river deltas (Figure 5) and indicates that the landuse practice causing this erosion has persisted over a period of at least 50 yr.

CONCLUSIONS

While there are similarities between the interpretation of the landuse sources of sediment with (Figure 6) and without (Figure 2) the Suess effect correction, the use of the correction has allowed identification of the causes of major soil erosion in the distant past and the effect of more recent landuse changes on erosion patterns. The use of the corrected CSSI values correctly identified the transition period between citrus and kiwifruit orchards around 1970, which was missed using the uncorrected CSSI values. The uncorrected CSSI values indicated a high proportion of sediment from production forestry harvest. Soil from this landuse was a minimal component of the total soil load identified when using the corrected CSSI values, consistent with best management practices to prevent soil entering permanent waterways during forest harvest. Using the Suess effect correction, both sediment cores showed evidence of a recent (last ~50 yr) increase in the amount of soil being eroded from pasture used for dry stock beef was much larger than previously estimated without the correction. This proportional source contribution is consistent with the proportion of the estimated annual mass load of sediment from this landuse being discharged into the Bay of Islands, as determined from the analysis of contemporary sediments from the river deltas. These results confirm the need to apply a correction to the CSSI values for the Suess effect when examining sediment source changes over time using the CSSI technique.

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Microbial Assimilation of Atmospheric CO_2 to Synthesize Organic Matter in Soils

T. Ge[1], X.J. Chen[1], H.Z. Yuan[1], S. Nie[1], H.A. Xiao[1], C.L. Tong[1], J.S. Wu[1,*] and P. Brookes[2]

ABSTRACT

There are no reports on the activity and quantitative capacity of microbial atmospheric carbon dioxide (CO_2) assimilation in terrestrial ecosystems, although such CO_2 assimilative microorganisms are numerous and widespread. Both non-phototrophic and phototrophic microbial CO_2 assimilations were investigated in two sets of microcosms of eight selected soils in a closed, continuously carbon-14 (^{14}C)-labelled CO_2 atmosphere chamber. A significant amount of ^{14}C-labelled CO_2 was incorporated into the microbial biomass, accounting for between 0.12 percent and 0.59 percent of the soil organic carbon (average 37.8 ± 19.4 mg/kg after 80 d, CV = 51.3 percent, n = 8) in the set exposed to artificial light (about 500 mmol·photons·m^{-2}·s^{-1} parabolic aluminized reflector light [PAR]), whereas none was detected in the set in the dark condition. Hence, soil phototrophic CO_2 assimilation represents a significant part of microbial activity which cannot be ignored since it is estimated to have an annual organic C assimilative capacity of 4.9–37.5 g·C/m.

Key words: *microbial assimilation, atmospheric CO_2, soil microorganisms, ^{14}C continuous labelling.*

INTRODUCTION

The atmospheric concentration of carbon dioxide (CO_2) has increased from 280 parts per million by volume (ppmv) in 1750 to 367 ppmv in 1999 and is currently increasing at the rate of 1.5 ppmv per year, primarily due to the large consumption of fossil fuels since the industrial revolution (IPCC, 2007). Terrestrial ecosystems were recognized as a major sink of global CO_2 emissions. This leads to a wide interest in carbon (C) cycling in terrestrial ecosystems and its potential to mitigate rising atmospheric CO_2 concentrations (Lal, 2004; Hill *et al.*, 2006). Autotrophic microorganisms which have the ability to assimilate atmospheric CO_2 biologically are commonly present in aquatic ecosystems (Stanley *et al.*, 2003; Savage *et al.*, 2010). Cannon *et al.* (2001) showed that the cyanobacteria and chemoautotrophs in oceans are responsible for about 40 percent of CO_2 removed from the atmosphere annually. Such microorganisms are also widespread in terrestrial ecosystems, and significant atmospheric CO_2 fixation by microorganisms has been observed in certain soil microenvironments such as in the vicinity of legume nodules (Dong and Layzell, 2001) or other niches with high hydrogen (H_2) production (Stein *et al.*, 2005). Miltner *et al.* (2004) observed a constant CO_2 fixation in soil under dark conditions. Besides the above mentioned special microenvironments, there is a critical gap in our knowledge of the microbial atmospheric CO_2 assimilation potential in soils and their contribution to global C balance. Therefore, our aim was to clarify the activity and capacity of soil microbial assimilation of atmospheric CO_2 for the better understanding of C cycling processes.

MATERIALS AND METHODS

Eight soils including four paddy soils under permanently flooded rice cultivation (referred to as P1, P2, P3 and P4), one paddy soil under flooded/drought rotation (P5), and three upland soils under vegetable (V1) or cereal cultivation (U1 and U2) were selected as representatives of the dominant types of cropping soils in the sub-tropical region of China (Table 1). The sites of the soils had a mean annual temperature about 16.8°C and an annual rainfall of about 1 400 mm. Soil cores were collected from the upper layer (Ap, 0 cm) and coarse plant residues removed. Site information and soil properties are shown in Table 1.

Prior to use, soils P1, P2, P3 and P4 were adjusted to about 100 percent of field water holding capacity (FWHC), and the others to 45 percent of FWHC with sterilized water. Two sets of microcosms of the soils with four replicates were prepared by weighing 1.0 kg fresh soil (on an oven-dried basis) in a plastic container (10 cm diameter and 22 cm height). One set, used as the control, was covered with 0.7 cm thick dark plastic foam to ban light but allow air flux through. The microcosms of the other set were perforated clear plastic sheets to eliminate duckweed growth. All soil microcosms were placed in growth chambers (China Patent No. ZL2006100197402). During the incubation period (80 d), artificial light (about 500 mmol ·photons·m^{-2}·s^{-1} parabolic aluminized reflector light [PAR]) was provided between 8:00 am and 8:00 pm each day. Temperature was set at 28–32°C in the illuminating period and 22°C in the dark period, and constant relative humidity at 80–90 percent. The ^{14}C-labelled CO_2 was generated from a ^{14}C-labelled Na_2CO_3 solution (Irvine CA, USA) containing 1.6×10^4 mg·C/mL with a radioactivity of 1.01×10^6 decays per min (DPM), by reaction with HCl (2 M) in a plastic beaker placed inside the chamber. The CO_2 concentration was controlled at 270–350 mL/L by regularly adjusting with ^{14}C-Na_2CO_3. The paddy soil pots were maintained in a continuously flooded condition, and upland soil pots kept at a moisture level

1 Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan 410125, P. R. China
2 Department of Sustainable Soils and Grassland Systems, Rothamsted Research, Harpenden, Herts. AL5 2JQ United Kingdom
* E-mail address of corresponding author: jswu@isa.ac.cn; gtd@isa.ac.cn

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TABLE 1. Characteristics of the paddy and upland soils used in this study

Soils	Cultivation systems	Soil type	Clay content (%)	CEC (c·mol/kg)	pH	SOC (g/kg)	Total N	MBC (mg/kg)
P1	Double rice	Ultisol	35	10.6	6.0	25.7	2.0	865
P2	Double rice	Fluvisol	25	6.2	5.2	15.9	1.6	1223
P3	Double rice	Fluvisol	11	7.2	4.7	14.8	1.6	638
P4	Double rice	Ultisol	12	12.2	5.1	17.9	1.7	879
P5	Rice-oil seed rotation	Ultisol	28	8.8	5.6	6.34	1.0	182
V1	Vegetable	Fluvisol	34	11.9	6.0	16.9	1.9	373
U1	Maize	Ultisol	42	10.3	4.6	9.07	1.2	152
U2	Maize-wheat	Ultisol	44	11.5	5.2	5.63	0.8	178

Note: SOC and CEC indicate soil organic carbon and cation exchange capacity, respectively.

of 45 percent of FWHC by the addition of distilled water throughout the incubation.

After labelling, samples were taken from each of the soil microcosms for analyses of ^{14}C-labelled microbial biomass C and organic C assimilated from ^{14}C-labelled CO_2. The ^{14}C-labelled soil organic C (^{14}C-SOC) was measured according to Wu and O'Donnell (1997). The total amount of extractable microbial C (MBC) was determined by the difference between K_2SO_4-extractable C in fumigated and non-fumigated soil using k factor 0.45 (Wu *et al.*, 1990). ^{14}C-organic C in the extractant was determined by liquid scintillation counting as described above. Soil organic C and total N were determined by dry combustion using an elemental analyzer (Vario MAX C/N, Elementar, Germany). Soil pH was determined in 1:2.5 (w:v) soil to H_2O ratio extracts. Soil clay content was determined using the pipette method (Müller and Höper, 2004). Cation exchange capacity (CEC) was measured using titration (Rhoades, 1982). Data were analysed with SPSS 10.5 (SPSS Inc., Chicago, IL, USA) using one-way ANOVA procedures and linear regression by the Pearson correlation method.

RESULTS AND DISCUSSION

The radioactivity in soils sampled from all of the covered microcosms was scarcely detectable, and no organic C assimilation occurred in any of the eight soils, whereas large quantities of ^{14}C-SOC were determined in the soil microcosms exposed to light for 12 h per day (Figure 1A).

The significant positive relationship ($r = 0.945$, $p = 0.0004$) between the amounts of ^{14}C-SOC and ^{14}C-MBC (Figure 2) revealed directly that the phototrophic CO_2 assimilation is a microbially-mediated process. To the best of our knowledge, this has been demonstrated for the first time. However, Miltner *et al*. (2004) found a significant transfer of ^{13}C-labelled CO_2 into soil organic matter (1.3 μmol C/g soil after 61 days) in the dark. In a ^{14}C-labelled CO_2 experiment, these authors also discovered that a great deal of ^{14}C-labelled CO_2 was fixed, corresponding to 0.05 percent of the total organic C in soils after six weeks' incubation; they suggested that this non-phototrophic CO_2 fixation process was driven mainly by aerobic heterotrophic microorganisms (Miltner *et al.*, 2005). The difference between the present and earlier findings may be explained by the extremely high (319 mg/g) SOC content in the soils and the addition of readily available substrates (such as acetic acid) used in the earlier study, both of which would stimulate the growth of aerobic heterotrophic microorganisms. Thus, the significant non-phototrophic atmospheric CO_2 assimilation observed by Miltner *et al*. (2004 and 2005) is not surprising. Obviously, based on the significant ^{14}C-CO_2 assimilated in the illuminated soils, it can be speculated that phototrophic CO_2 assimilation is the dominant microbial CO_2 assimilation process and that this was possibly and mainly driven by autotrophic microorganisms (including photo- and chemo- autotrophic microbes) instead of heterotrophic microorganisms, although further work is required to highlight the relationship between the autotrophic microbial activity and soil CO_2 assimilative rate.

The amount of ^{14}C-SOC (range 8.44–64.61 mg/kg, Figure 1A) accounted for between 0.12 percent and 0.59 percent of SOC (Figure 1C). Through further calculation (the global soil (0–1 m) organic C stock is 2 300 Pg, of which about 30 percent is present in the 0–20 cm soil layer), the capacity of soil microorganisms to synthesize atmospheric CO_2 in a period of only 80 days is equivalent globally to 0.83–4.07 Pg C. This clearly shows that autotrophic CO_2 assimilation at the topsoil potentially made a significant contribution to the C cycle. Carney *et al.* (2007) considered that C emission and absorption in the terrestrial ecosystem in response to global atmospheric change do not balance. Thus, the quantity of CO_2 emission is always larger than that from CO_2 uptake. If correct, there is a 'missing C sink' of about 2–3 Pg·C/yr at the global scale (Karim, Veizer and Barth, 2008). In this study, the calculated rate of organic C assimilation was 1.12–8.57 mg C/m per hour (12-h light exposure). It is estimated that the annual rate of microbial synthesis of organic C is 4.9–37.5 g·Cm^2, or 0.068–0.53 Pg globally, assuming a total terrestrial area of 140 000 000 km^2. It may therefore be presumed that part of the "missing C sink" is C sequestrated by soil organisms. This work provides a new research direction for attempting to account for "new and missing C sinks" in terrestrial ecosystems, although the driving forces for the observed high amounts of CO_2 assimilated at the soil surface are not clear, and require further study.

The amounts of ^{14}C-MBC ranged from 1.55 to 10.36 mg/kg (Figure 1B), and about 16.04 percent (CV = 22.6, n = 8) of assimilated ^{14}C was recovered as MBC after 80 d (data not shown). Rapid incorporation of C produced into MBC proved that "new" C derived from recent assimilates was readily utilized by microorganisms in paddy and upland soils. The ^{14}C in MBC may have had three fates: a fraction released as CO_2 through respiration, another fraction transformed to structural components of microorganisms and a third entering the SOC as microbial. However, this study provided little information about the accumulation of the "new" SOC derived from assimilation at the soil interface.

Additionally, large variations in microbial CO_2 assimilative rate were also observed across the soils tested in the present study, yet the mechanism was not clear since soil types, management practices and environmental conditions could influence the activity and popu-

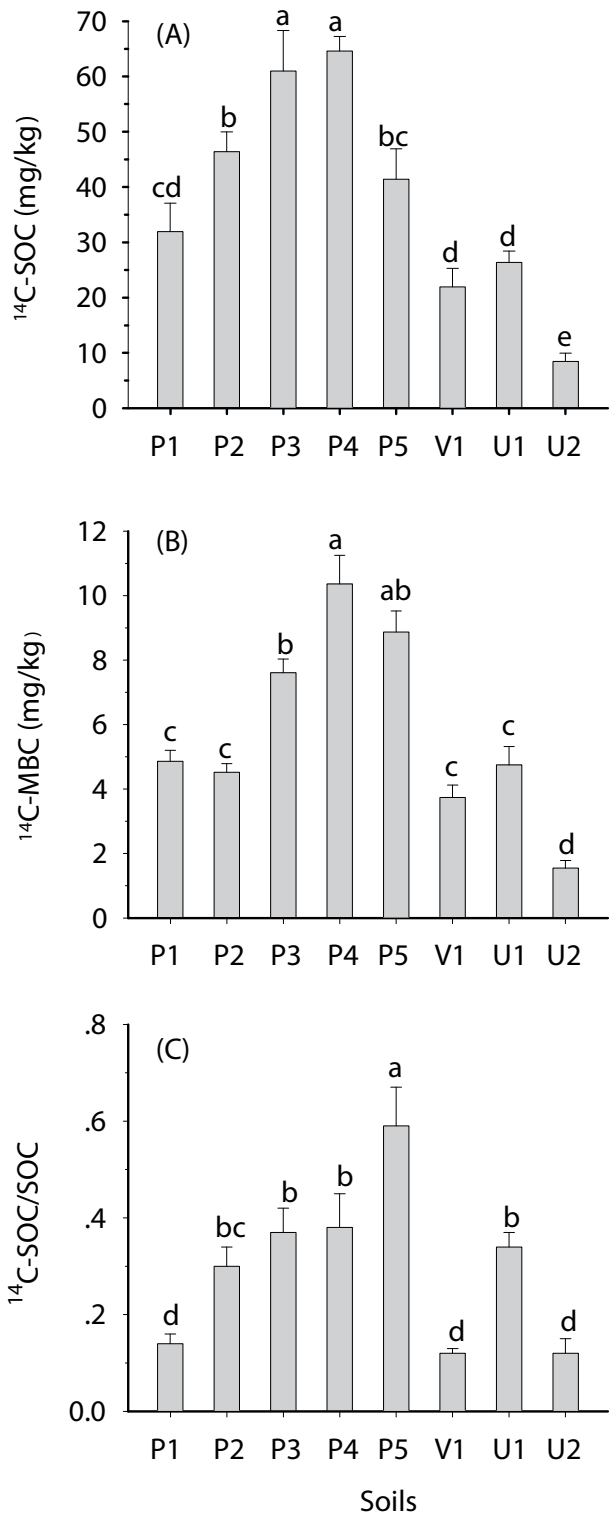


FIGURE 1. The amounts of ^{14}C-labelled CO_2 incorporated into soil organic carbon (^{14}C-SOC, A), microbial biomass (^{14}C-MBC, B) and the ratio of ^{14}C-SOC to SOC (C) in soil exposed continuously to ^{14}C-labelled CO_2 atmosphere and artificial light for 80 d. Bars indicate the standard error of the mean (n = 4). Different lowercase letters indicate significant differences among the soils at $p < 0.05$

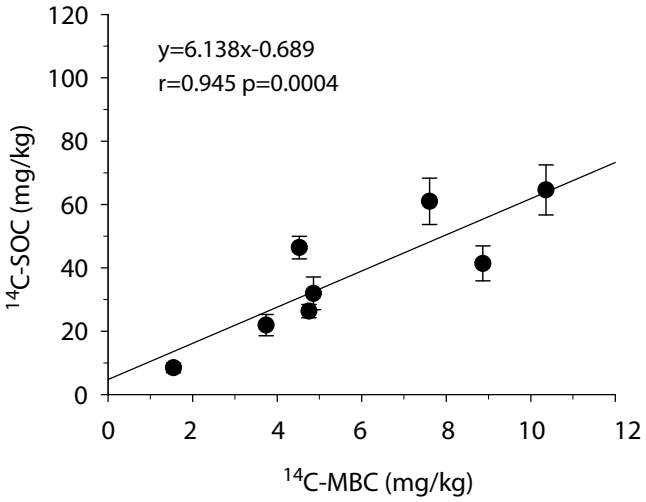


FIGURE 2. Relationship between ^{14}C-MBC and ^{14}C-SOC from eight contrasting soils during 80 d ^{14}C continuous labelling by incubation of soils under artificial light conditions. Bars represent the standard errors. For data on ^{14}C-SOC and ^{14}C-MBC, see Figure 1.

lation of microorganism associated with the C cycling process. Given the influence of climate change, further studies on the functional ecological implications of the abundance and composition of soil C assimilative microorganisms and their adaptation to local habitats and external interferences are warranted to clarify this microbial driving process.

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Cosmic-ray Soil Moisture Probe: A New Technology to Manage African Dryland Ecosystems

T.E. Franz[1,*], M. Zreda[1] and E.G. King[2]

ABSTRACT

An estimated 200 million rural smallholders practice livestock-based or mixed livestock-crop-based agriculture in sub-Saharan Africa, where levels of poverty and food insecurity are among the highest in the world. Demographic, environmental, and climate changes have led to diminishing supply of resources that is crippling dryland productivity and increasing people's vulnerability. There is a need for research to develop, monitor, and evaluate strategies to cope with diminishing resource availability and build resilient ecosystems. Reliable, long-term measurements of soil moisture are critically needed for addressing productivity and food security in African drylands. We propose that measurements of effective infiltration, plant available water and deep drainage are sufficient metrics to understand dryland ecosystem health and to assess and evaluate restoration strategies. In this work, we evaluate the advantages and disadvantages of two different measurement methods, the cosmic-ray neutron method (as implemented in the COsmic-ray Soil Moisture Observing System (COSMOS) and eddy covariance techniques, for long-term measurements of African dryland water balance. We compare estimates of ecosystem level daily evapotranspiration between the two methods over a six-month period at a site in central Kenya, and found the cosmic-ray method to be more appropriate. The eddy covariance data are smoother than the cosmic-ray measurements. However, the cosmic-ray neutron probe method provides additional information on the key variable, area-average soil moisture, allowing us to partition rainwater into infiltration, runoff, evapotranspiration, and deep drainage. In addition, cosmic-ray neutron probes are easier to operate and maintain, more robust and less expensive than eddy covariance towers, making them more appropriate for long-term deployments.

Key words: *soil moisture, evapotranspiration, cosmic-ray neutron probe, agropastoralism, Africa, drylands.*

INTRODUCTION

Sixty percent of sub-Saharan Africa is pastoral or agropastoral land (Reynolds *et al.,* 2007). These arid, semi-arid and subhumid regions are grassland to desert ecosystems and home to an estimated 200 million rural smallholders practising livestock-based or mixed livestock-crop-based agriculture (Thornton *et al*., 2002; Robinson *et al.,* 2011). These regions have some of the highest levels of poverty and food insecurity in the world (Thornton *et al*., 2002; Thomas and Twyman 2005), and they are exceptionally vulnerable to climate change. While projections of changes to mean annual precipitation vary from region to region, models agree that across all African drylands, rainfall will become less predictable, with shorter growing seasons (Thornton *et al.*, 2006), and more frequent and more severe droughts (Sheffield and Wood, 2008). This is the setting for one of Africa's greatest agricultural development challenges — dryland productivity and food security.

Of these pastoral/agropastoral areas approximately two-thirds are drylands, where annual potential evapotranspiration exceeds rainfall by 200 percent or more, and traditional smallholder agriculture relies substantially or wholly on extensive livestock husbandry (Notenbaert *et al*., 2009). This resilient production system has evolved and persisted for millennia, and represents a highly adaptive relationship between human livelihoods and the environmental stresses that typify dryland environments. Tropical drylands experience not just low amounts of rainfall, but high rainfall variability both in space and time. Systems of mobile, flexible livestock herding over extensive ranges allow smallholders to buffer themselves against the variable environmental conditions at any single location, and to access key natural resources that are distributed non unevenly across large spatial scales (Niamir-Fuller, 1998). Smallholder livestock production in drylands is not an isolated socioeconomic system, but influences the national economies of all African nations with drylands. It is a critical source of protein, nutrition, and commerce for both rural and urban populations. For example, in Kenya, livestock contributed 50 percent to the national agricultural gross domestic production in 2004, and the proportional contribution continues to increase (Hesse and MacGregor, 2006).

However the resource base for livestock production is shrinking due to four compounding effects:

(i) Population growth: there are more lives depending on each ha (Notenbaert *et al*., 2009).

(ii) Land conversion: usually to cropping, which leaves fewer ha available (Herrero *et al*., 2009), and may increase dependence on groundwater supplies to meet the increased evapotranspiration demand.

1 Hydrology and Water Resources, University of Arizona, 1133 E James E Rogers Way Rm 122, Tucson, AZ 85721, USA

2 Odum School of Ecology, University of Georgia, 140 E. Green St., Athens, GA 30602, USA

* E-mail address of corresponding author: tfranz@email.arizona.edu

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(iii) Legacy of land degradation: today the same ha produces less forage (Dregne, 2002).
(iv) Increasingly unpredictable climate: this results in fewer productive ha per year, and is expected to reduce viable pastoral areas by 20 percent in 40 years (Thornton *et al.,* 2006; Falkenmark and Rockstrom, 2008).

It is the diminishing supply of resources for livestock-based agriculture that is crippling the system's productivity and increasing people's vulnerability (Thornton *et al.*, 2006; Andersson, Brogaard and Olsson, 2011). Research is needed to proactively develop, monitor, and evaluate strategies to cope with this 'diminishing resource syndrome' in drylands.

Mitigation of the diminishing resource has typically fallen into two strategies: (i) do business as usual but search for more efficient and productive methods, or (ii) change to a new system. The first strategy falls in the sphere of range management. While managing livestock is an important issue, restoring the ecological functioning and productivity of the landscape is going to be an essential component to curtail the diminishing resource syndrome. However with increasing populations on limited lands, in many areas the demand for food is unlikely to be met by livestock management alone. The second strategy is one that many pastoralists all over Africa are now resorting to: trying to grow crops where traditionally they have only raised livestock. Depending on the context, this could increase vulnerability and food insecurity rather than reduce it. There is a need to understand these systems to find out where each of the two coping strategies or combination of the two strategies is most appropriate.

Dryland productivity is driven predominantly by landscape water balance, or the partitioning of incoming rainfall into different pathways in the ecosystem (runoff, groundwater recharge, evaporation, or transpiration). The more water that infiltrates the soil and is taken up by plants and transpired, the more plant productivity a landscape can yield. The more rainfall that is lost to runoff or evaporation directly from the soil, the smaller the proportion of soil moisture for plant growth. And importantly, the productivity of a landscape feeds back to affect the water balance in subsequent rainfall events (Ludwig *et al.,* 2005). Thus, as dryland vegetation degrades, the capacity of the landscape to capture and convert rainfall into productive growth also declines (Kefi *et al.,* 2007).

Directly improving plant water use efficiency could be a target for enhancing productivity, but in landscapes where degradation has impaired water balance, the system is still inexorably dependent on ecohydrological constraints on the supply of water available to plants. Addressing this abiotic component of the system is necessary to reinstate water–soil–vegetation feedbacks, so the system can once again sustain productivity (King and Whisenant, 2009). Increasing supply-side dynamics of water availability is the basis of the current 'Blue Revolution' in agricultural research. Here water conservation practices deliver 'more crop per drop', by enhancing water use efficiency, or the amount of productivity gained per unit of water consumed. The FAO promotes conservation agriculture with three principles: minimal soil disturbance, permanent soil cover and crop rotations (http://www.fao.org/ag/ca/; accessed on 1 August 2012). This approach has been shown to be successful in Tanzanian drylands (Owenya *et al.,* 2012).

This concept can also be applied to rangelands. Instead of targeting water use efficiency at the scale of single crop plants or agricultural fields, the approach is aimed at the scale of land tracts of a perennial ecosystem (several ha). However, at this scale additional complication is introduced, namely, spatiotemporal heterogeneity of resources. Two hallmark traits of drylands are its patchy structure and the variable, pulse-like arrival of the key limiting resource, water. Their interactions hold the key to understanding and restoring ecosystem function (Ryan, Ludwig and McAlpine, 2007; King *et al.*, 2012).

Historically, the measurement of soil water dynamics at this field scale has been notoriously difficult given the inherent limitations of direct and indirect sampling methods (Robinson *et al.*, 2008). An alternative measurement technique that uses eddy covariance towers (Stull, 1988) provides information on the water, energy, and carbon balances of ecosystems, integrated over the tower's "footprint" area, with a diameter of approximately 30 times the tower height. For example, Fluxnet is a global network of more than 400 eddy covariance towers that has operated since the 1980's (Baldocchi *et al.*, 2001), but with fewer than 20 stations in Africa, the continent is under-represented. However, flux towers provide little or no information about plant available water at the footprint scale, thus limiting our ability to fully understand the soil–atmosphere coupling (Seneviratne *et al.*, 2010) or partitioning of the water into its individual components.

The recent advent of the cosmic-ray neutron probe (Zreda *et al.,* 2008) has opened the door for measurements of near surface soil moisture at the landscape scale (Franz *et al.,* 2012). Here, the cosmic-ray moisture probe (Zreda *et al.,* 2008) is used to derive key variable needed for monitoring of agropastoral systems. We first summarize the cosmic-ray neutron probe method and COsmic-ray Soil Moisture Observing System (COSMOS) (Zreda *et al.*, 2012). Next, estimates of daily soil water flux derived from cosmic-ray neutron measurements are compared with a collocated eddy covariance tower in a central Kenyan dryland. Finally, some key points for consideration are identified for the establishment of a cosmic-ray neutron probe monitoring network in African drylands to address the critical issues of productivity and food security.

MATERIALS AND METHODS

Cosmic-ray neutron probe method

The inverse relationship between soil moisture and the intensity of cosmic-ray fast neutrons above the surface has been known for several decades (Hendrick and Edge, 1966). The removal of neutrons is dominated by neutron collisions with hydrogen atoms. Hydrogen has an extraordinarily high neutron stopping power due to a combination of its high neutron scattering cross-section and the high fractional energy loss per collision and low atomic mass (Zreda *et al.,* 2008 and 2012). Hydrogen's stopping power is an order of magnitude greater than any other element, making hydrogen the dominant factor in controlling neutron intensity (Zreda *et al.,* 2012).

Using a moderated neutron detector placed above the surface (Figure 1), Zreda *et al.* (2008) found that differences in the relative count rate of fast neutrons (~10–100 eV) in air above land surface are related to the average amount of soil water present. Desilets *et al.* (2010) found the following calibration function between soil moisture, (m^3/m), and fast neutron counts:

$$\theta(N) = \frac{0.0808}{(N/N_0) - 0.372} - 0.115 \quad (1)$$

where N is the neutron counting rate normalized to a reference atmospheric pressure and solar activity level (Zreda *et al.*, 2012), N_0 is the counting rate over dry soil under the same reference conditions, and the three coefficients were determined using a neutron particle transport code, MCNPx (Pelowitz, 2005) for pure silica sand (SiO_2). The calibration parameter N_0 can be estimated at the probe site using volumetric soil moisture around the footprint (c.f. Zreda *et al*., 2012).

Because the sensor gives an average neutron count over a circle with a radius of ~335 m (Zreda *et al.,* 2008), and the sensitivity

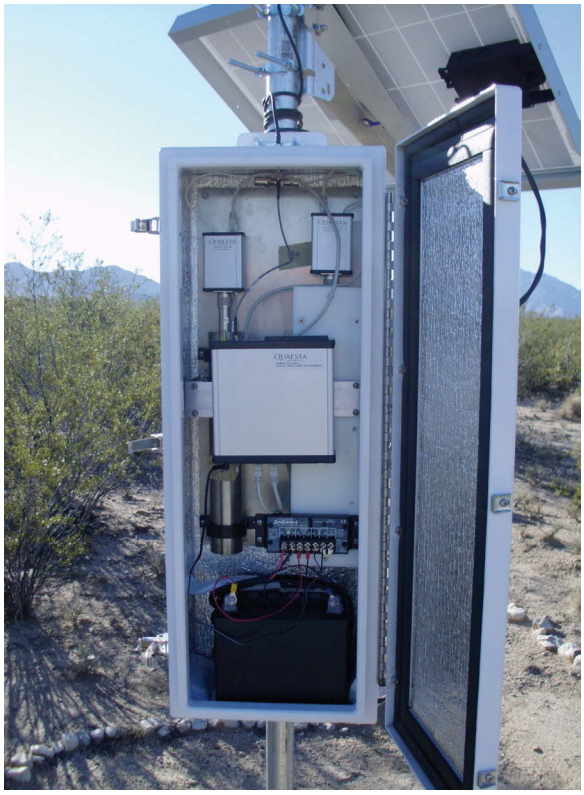


FIGURE 1. A cosmic-ray neutron probe (CRS 1000, Hydroinnova LLC, Albuquerque, NM, USA) installed at the Santa Rita Experimental Range site, located in southern Arizona, USA.

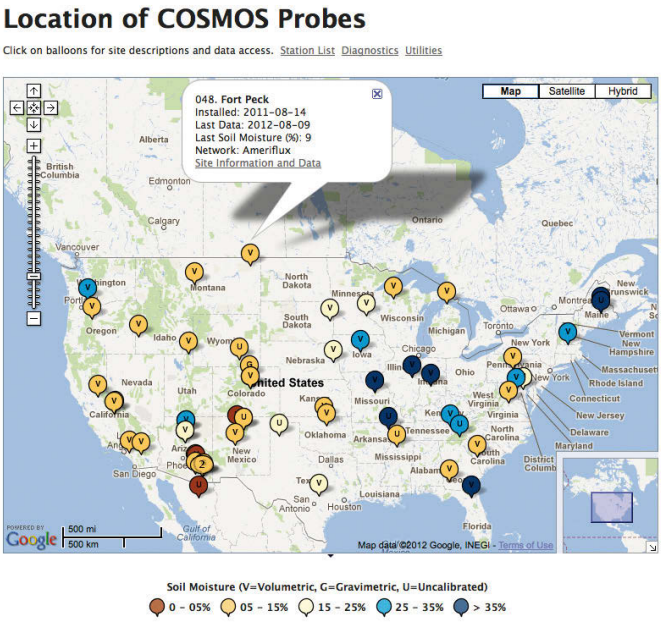


FIGURE 2. Location and status of COSMOS probes in the continental USA on 9 August 2012 (http://cosmos.hwr.arizona.edu/Probes/probemap.php). Raw and processed data from individual sites are publicly available in real time.

decreases with the distance from the probe, soil sampling at 18 locations (every 60° and at radii of 25, 75 and 200 m) gives a representative estimate of the mean water content over the footprint. Fast neutrons mix rapidly above the surface (velocities >10 km/s (Glasstone and Edlund, 1952), indicating that horizontal soil moisture heterogeneity likely plays a minor role in the average footprint neutron count. In contrast to the horizontal footprint that is independent of soil moisture content, the vertical depth of measurement of the sensor does vary with soil water content ranging between ~10 cm and 70 cm for wet and dry conditions, respectively (Zreda *et al.,* 2008). With a single or repeated calibrations at a site, Franz *et al.* (2012) found that sampling every 5 cm to a depth of 30 cm is adequate to accurately describe the average soil water content in the profile and thus estimate N_0 with an average RMSE of < 0.02 m^3/m^3.

COsmic-ray Soil Moisture Observing System

The COsmic-ray Soil Moisture Observing System (COSMOS) is a new national network in the continental USA designed for improving hydro-meteorological forecasting (Zreda *et al.* 2012, data available at http://cosmos.hwr.arizona.edu/) by providing real-time estimates of soil moisture (Figure 2). Beginning in 2009, 50 cosmic-ray neutron probes were deployed to provide hourly estimates of soil moisture. The cosmic-ray neutron probes have been designed to be rugged, energy-efficient and independently powered using solar cells, and they are equipped with a satellite data modem for reliable transmission of data from any place on the globe. A data success rate of over 90 percent has been achieved from the COSMOS probes in the continental USA and from affiliated probes in five other continents.

As part of the COSMOS project, all data are collected, processed, and checked for basic quality assurance and quality control in real-time, and then posted on the COSMOS web site. Currently, the measured neutron intensities are corrected for variations in geomagnetic latitude and local atmospheric pressure changes (Zreda *et al.,* 2012). In the near future, new corrections will be added to account for variations in lattice water, atmospheric water vapour and vegetation. Additional details about the cosmic-ray neutron probe method and the COSMOS project are in Zreda *et al.* (2012) and online (http://cosmos.hwr.arizona.edu/).

Instrumentation for water balance measurements in central Kenya

Beginning in September 2011, a cosmic-ray neutron probe was installed at a study site, referred to herein as Mpala North, in central Kenya, where a 20 m tall eddy covariance tower has been operating since 2009 (Caylor, unpublished data). The site is in the upper Ewaso Ngiro river basin of the central Kenyan highlands (36°54'E, 0°20'N). It is characterized as semi-arid woodland or shrubland, receiving 450–500 mm/yr of rainfall, typically arriving in two rainy seasons, April–May and November–December (Franz, Caylor and Nordbotten, 2010). The vegetation has 10–25 percent woody canopy cover, dominated by mixed *Acacia* species. The herbaceous layer has perennial and annual grasses, as well as a diversity of forbs and succulents, with 1–10 m bare patches with no perennial grass and sparse annual vegetation (Figure 3). Under current land use practices, average standing biomass is typically estimated to be 450–700 kg/ha (CNRIT, 2011).

The site is located on the Mpala Research Centre Conservancy (MRCC), a 20 000 ha ranch that has been used for commercial cattle production for the last century, and since 1998 has been managed as a wildlife and research conservancy while maintaining a moderate cattle herd (one tropical livestock unit (TLU) per 10 ha). Wildlife is abundant: elephant, giraffe, zebra, buffalo, impala, dik-dik, baboons, hyenas are common. The site is within three km of the Ewaso Ngiro River, which serves as the boundary between MRCC and communally-owned lands utilized and inhabited by Laikipia Maasai pastoral-

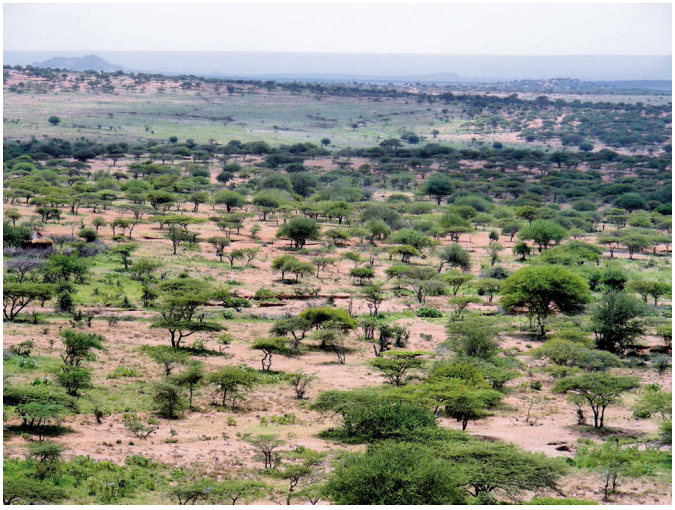


FIGURE 3. Vegetation composition and cover in central Kenya.

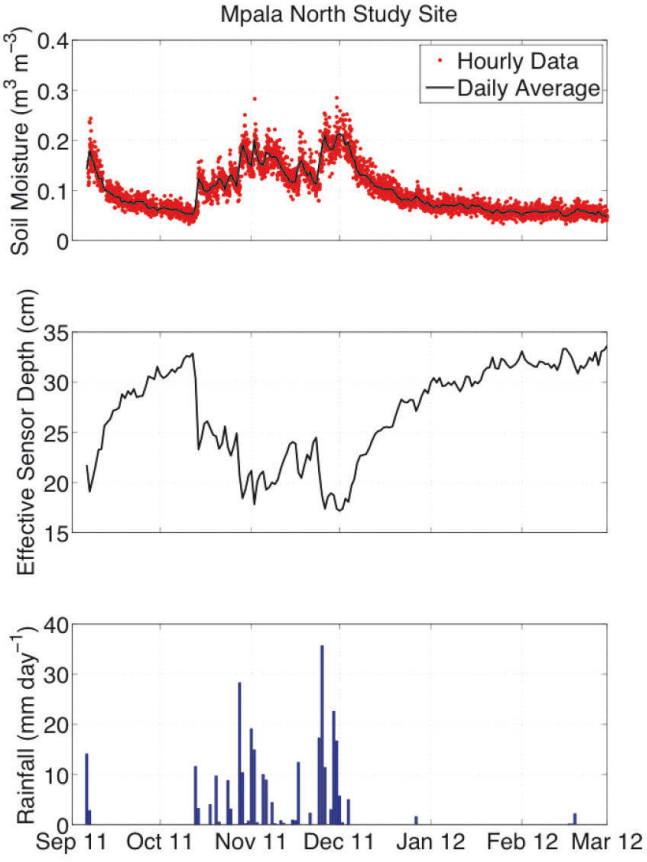


FIGURE 4. Daily soil moisture, effective sensor depth, and rainfall from the Mpala North study site in central Kenya.

ists. The communal areas have similar physiognomy and rainfall to the MRCC site, higher livestock stocking rates (one TLU per three ha), reduced wildlife densities, and decreased herbaceous vegetation cover (5–50 percent less, varying with year and season (King, unpublished data). While land use is predominantly for livestock production, a limited number of community members began small-scale maize cropping along the river in 2011. In future work, the Mpala North site and adjacent community lands will be studied to compare landscape scale water balance and ecosystem function between the land use systems.

RESULTS

The daily time series of neutron-derived soil moisture, effective sensor depth, and rainfall from the study site (Figure 4) show the response of soil moisture to rain events. Using the time series of soil moisture data, the daily flux of water into and out of the control volume were computed (Figure 5). The positive values indicate infiltration of rainwater into the soil and negative values indicate losses of water to evapotranspiration (ET) and vertical leakage (L). Small positive anomalies in the dataset not correlated to rain events are due to uncertainty in either the neutron count statistics or rainfall record. To compare different measurement techniques, the daily ET values derived from the cosmic-ray neutron probe and the daily average latent energy from the eddy covariance tower are considered (Figure 6). Despite the differences in the measurement techniques and horizontal and vertical scales of measurements, the two time series agree reasonably well over the six-month study period. Because the cosmic-ray derived ET also contains L, the values are higher than those derived from eddy covariance, which only estimate ET. Using additional information about the soil and vegetation, a soil water balance model can be used to separate the ET and L components from the integrated signal, for example using methods described in Rodriguez-Iturbe and Porporato (2004).

DISCUSSION

Comparison of the daily water fluxes indicates a general agreement between the methods, but eddy covariance derived values are smoother than those derived from the cosmic-ray neutron measurements. Given the different information obtained from each instrument, the complimentary measurements from eddy covariance and cosmic-ray instruments are preferable to understand how these ecosystems function. However, limited research budgets and unfavour-

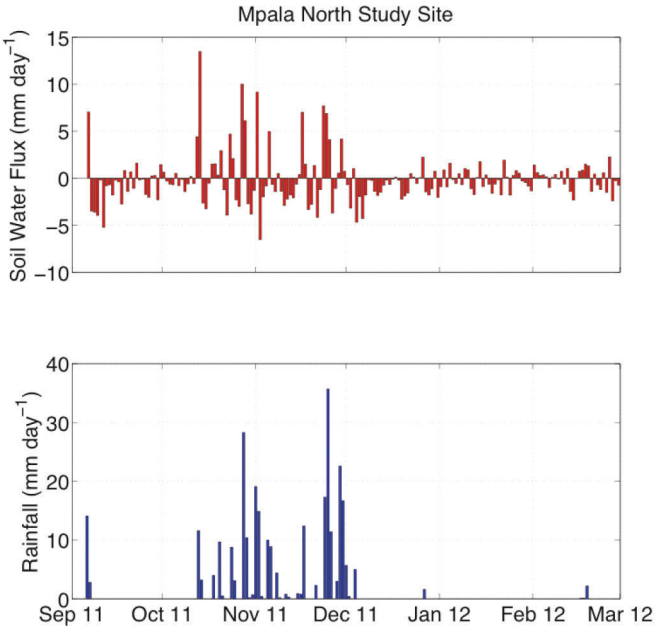


FIGURE 5. Estimates of daily soil water flux into and out of the control volume derived from the soil moisture time series.

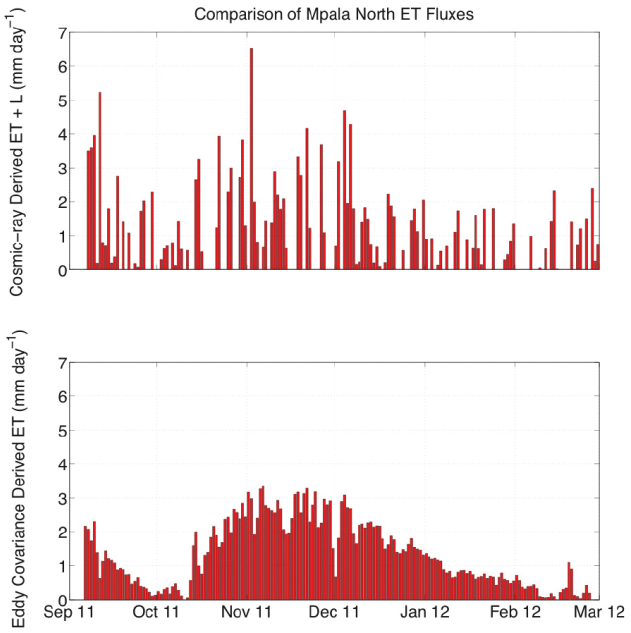


FIGURE 6. Comparison of landscape scale fluxes derived from the co-located cosmic-ray neutron probe and eddy covariance tower.

able site characteristics often restrict the total possible investment, and only one of the two instruments may be feasible. Three key differences between the measurement methods make the cosmic-ray neutron probes more suitable for monitoring and assessing the long-term ecosystem health and function of African drylands.

First, as described in the introduction, the key factor governing these dryland ecosystems is the partitioning of rainfall into run-off, infiltration, evapotranspiration, and deep drainage. Whereas the eddy covariance method provides measurements of the water, energy and carbon balances, they do not provide information on the soil water — the key variable. Knowing soil water is crucial because in order to estimate the various water fluxes in and out of the soil column, parameters and boundary conditions are needed that describe the physics of unsaturated water flow through porous media (Dingman, 2002). Point sensors are often used to measure soil moisture, but their small support volumes (~10 cm^3) result in measurements that are not representative of the footprint scale (~1 km^2) because of small-scale heterogeneities within the footprint (Robinson *et al.,* 2008). Thus, comparisons between land surface fluxes from eddy covariance towers and soil moisture dynamics based on point measurements may be unreliable. Because cosmic-ray neutrons provide area-average soil moisture (Franz *et al.,* 2012; Zreda *et al.,* 2012), effective (area-average) parameters could be derived for controlling the flow of water through porous media and thus partition rainfall into infiltration, runoff, evapotranspiration, and deep drainage at the landscape scale. For rangelands, the amount of rainfall infiltrating into the soil is the key metric to find out how the ecosystem is functioning under different land uses or to evaluate effective restoration strategies (King *et al.*, 2012). In addition, estimates of available water will be one critical piece to decide what vegetation is best suited to a particular ecosystem. Moreover, the water demand of various crops can be calculated for certain climates and compared to the estimate of available water in that ecosystem. In that regard, the amount of extra water needed from surface or ground water sources can be estimated, providing crucial information to stakeholders about the feasibility and environmental impact of the proposed land use change.

Second, from operational and maintenance standpoints the cosmic-ray neutron probes are easier to install, calibrate, and less expensive to purchase and maintain than eddy covariance towers. The cosmic-ray sensor contains a gas-filled tube, high voltage power source, data logger, 40 Ahr battery, 60 W solar panel, and satellite modem. This is simple when compared with the dozens of instruments required for an eddy covariance tower. In terms of maintenance, the major concerns are keeping the cosmic-ray probe battery charged and relative humidity inside the instrument box low. In terms of calibration, and as with most instruments, repeated calibrations are preferred and will ensure the highest quality of data, but a single calibration is adequate because of the long-term stability of the N_0 parameter (Franz *et al.*, 2012). In contrast, eddy covariance towers require yearly calibrations of the individual instruments and a full-time technician to maintain performance. Eddy covariance towers operate best with AC power but can be supported with several solar panels and battery banks. In terms of data, the cosmic-ray probe was designed to send a minimal amount data (ambient pressure, temperature, humidity, voltage, neutron count), or approximately 10 bytes of raw data, per transmission (usually every hour). Eddy covariance towers make measurements many times per sec in order to quantify the departures from the mean and accurately estimate the half-hourly averages. In addition, eddy covariance data require a significant amount of post-processing time and skilled personnel for high quality datasets (Stull, 1988).

Third, the costs of the two instruments are different. Principal investment of eddy covariance tower equipment is approximately US$100 000 with additional costs for maintenance, data transfer, data processing, and skilled onsite personnel. In contrast, cosmic-ray neutron probes cost approximately US$20 000 and require substantially less maintenance, data transmission cost and processing, and do not require permanent personnel on site.

CONCLUSIONS

Measurements of effective infiltration, plant available water, and deep drainage are sufficient metrics to understand ecosystem health and function in African drylands. This information is critical for stakeholders who wish to increase productivity and food security in their ecosystems. In addition, these data will provide useful landscape water metrics to assess and evaluate different land use and restoration strategies in drylands. Given the advantages and disadvantages of each measurement method described above, we find the cosmic-ray neutron method is more appropriate than the eddy covariance technique for environmental monitoring in African drylands. Most importantly, the cosmic-ray neutron probe method provides information on the area-average soil moisture — the key variable — allowing partitioning rainwater into infiltration, runoff, evapotranspiration, and deep drainage. Cosmic-ray neutron probes were designed to be robust, have low power consumption, low maintenance and high data reliability making them superior for long-term deployment in remote ecosystems. It is suggested that reliable long-term measurements of soil moisture constitute the critical information needed for addressing productivity and food security in African drylands.

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Large Area Soil Moisture Measurement Using Cosmic Rays Neutrons: The Australian CosmOz Network

C.J. Smith[1,*], A. Hawdon[2], R. Keen[2] and J. Wallace[2]

ABSTRACT

Field measurement of soil moisture is undertaken traditionally using point based measurement techniques such as neutron probes or time domain reflectrometry (TDR). Recently, a new technique has been developed that can be used to derive soil moisture at larger spatial scales by measuring neutrons that are generated by cosmic rays within the air and soil, and emitted back into the atmosphere. A study by Hendrick and Edge (1966) in the mid 1960s showed that the intensity of the fast neutrons above the ground varied with soil moisture content. The intensity of the neutron is mainly moderated by hydrogen ions located in the water and soil, and the density is inversely correlated with soil moisture. To soil scientists and hydrologists, this has opened up the possibility of measuring surface soil moisture automatically over an area of ~40 ha to a depth of ~0.5 m. The technique has the potential to fill the gap between point scale measurements (neutron probe or TDR) and soil moisture estimated using earth observation techniques (remote sensing). In Australia, 11 probes have been deployed across a range of agro-ecological zones to demonstrate the potential for larger scale soil moisture monitoring.

Key words: *soil water, cosmic rays neutrons, cosmOz, neutrons.*

INTRODUCTION

Ground-based soil moisture (θ_s) measurements have been used in a wide variety of applications including agriculture, hydrology, meteorology and in the calibration of satellites that can sense surface moisture remotely. Although highly valuable, most ground-based measurements of θ_s are made at a "point" (<1 dm^2) scale. The methods used vary from core samples (gravimetric or volumetric), TDR or capacitance probes or neutron probes. These measurements are at the point scale and it is therefore often difficult to obtain a sufficient number of θ_s values to capture the heterogeneity present in many landscapes. Quantifying the spatial variability in θ_s presents significant challenges and may preclude meaningful determination of temporal changes in soil water content.

Recently, Zreda *et al*. (2008) developed a technique to derive soil moisture estimates by measuring neutrons produced by cosmic rays. The method is based on the early observation by Hendrick and Edge (1966) showing the intensity of fast neutrons (energy 10 eV – 1000 eV) above the land surface was related inversely to the soil water content. Hydrologists and soil scientists have rediscovered this finding with the development of the cosmic rays technique. It opens up the possibility of measuring surface soil moisture automatically over an area of ~40 ha (Zreda *et al*., 2012). In summary, the sensor works by counting "fast" neutrons that are generated by cosmic rays as they pass through the Earth's atmosphere. At the land surface these neutrons are moderated by water molecules, and their count rate is predominantly a function of the water content of the soil.

METHOD

Details of the cosmic ray probe are given by Zreda *et al*. (2008, 2012) and Desilets, Zreda and Ferré, (2010). The theory behind the technique for measuring average soil moisture is given in Zreda *et al.* (2012). By placing the neutron detector above the ground surface, average soil water measurements can be made over horizontal footprint of hectares (ha), and to a soil depth of decimetres (dm). Figure 1a shows the typical installation of the sensor in Australia where the sensor is mounted above the soil surface. The mean free path of the fast neutrons is around 100 m, and therefore the sensor can detect neutrons from several hundred m away (Zreda *et al.*, 2008; Desilets, Zreda and Ferré, 2010). The depth to which the sensor can detect θ_s is dependent on the soil water content and is ~10 cm in wet soil and up to ~50 cm in dry soil (Franz *et al*., 2012a).

In 2010, CSIRO was the first organization in Australia to order 11 commercially available custom-designed cosmic ray soil moisture probes (CRS-1000, Hydroinnova, Albuquerque, NM, US). Together with university collaborators, these 11 sensors were deployed to form the CosmOz network (http://cosmos.hwr.arizona.edu /Probes/australia.php). It serves as a prototype Australian network of CRS-1000 probes designed to support sensor evaluation, research and the development of new sensor applications. The current network of 11 probes is deployed across the country in a range of soil types, vegetation cover and climates (Figure 1b).

RESULTS AND DISCUSSION

Experience to date has shown that the CRS-1000 probe needs to be calibrated for the local soil type to obtain accurate absolute (volume percent) values of θ_s. As the probe measures θ_s over such a large area, this is done by taking a large number (72) of gravimetric soil samples at distances up to 200 m from the probe (Franz *et al*., 2013). Once this calibration has been carried out, daily θ_s changes can be detected with an accuracy of ~0.02 percent. An example of the soil

1 CSIRO Land and Water, Christian Laboratory, Black Mountain, Canberra, ACT, Australia

2 CSIRO Land and Water, Australian Tropical Science and Innovation Precinct, James Cook University, Townsville, 4811, Queensland, Australia

* E-mail address of corresponding author: chris.j.smith @csiro.au

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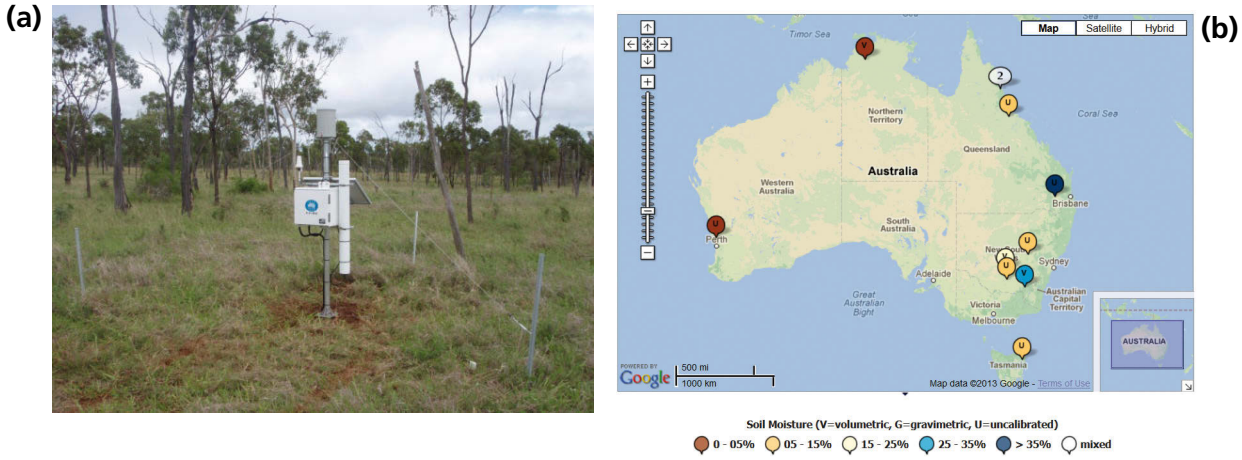


FIGURE 1. (a) A CRS-1 000 sensor installation on a grazed savannah hillslope in the dry tropics (Weany Creek, north Queensland). Picture (b) shows the locations of the current CosmOz network sites across Australia (http://cosmos.hwr.arizona.edu/Probes/australia.php).

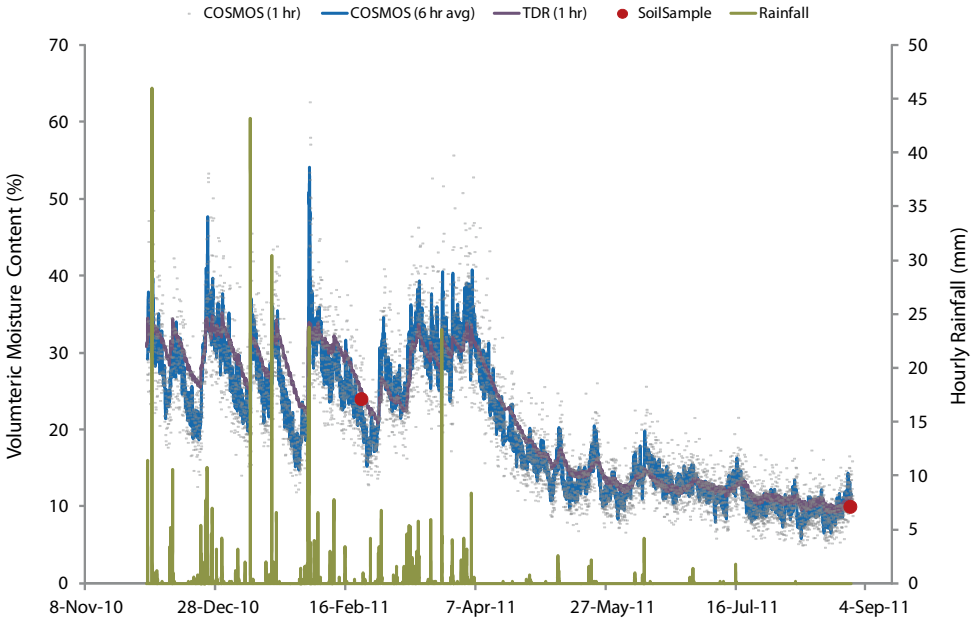


Figure 2. An example of the time series of θ_s (blue) recorded by the CosmOz probe at Weany Creek (northern Queensland). Also shown is the value of θ_s recorded using TDR probes (purple line) and rainfall (green). Two gravimetric sampling points (red) are also shown.

moisture data recorded by the CRS-1000 probe at a dry tropical site is shown in Figure 2. Over the wet season (December to April) frequent rain events raise the soil moisture content to near saturation (~40 percent) and these wetting events are followed by a period of soil drying (Figure 2). From April onwards there is little further rain and the CRS-1000 probe shows how the site dries progressively over the following months, dropping to ~10 percent by September. Figure 2 also shows the surface (0–30 cm) moisture content recorded by three conventional time-domain reflectometry (TDR) soil moisture probes. Although this is the average of only three point measurements, there is strong temporal coincidence between the TDR and CRS-1 000 time series. The CRS-1 000 soil moisture estimation equation was calibrated to the gravimetric calibration measurements made in February 2011 (Figure 2), but the second gravimetric sample in September provides an independent check of the CRS-1000 estimates.

There is significantly more variation in the soil moisture measured with the CRS-1000 probe as evidenced by the scatter in points and variation in the blue line. Less variation in water content is visible in the TDR data, because it is measuring over different depths in the soil. This is more obvious at the irrigated site (Figure 3), where there is a sharp increase in soil moisture in the surface 0.05 m shown in the TDR trace. This amplitude of the increase in θ_s is dampened in the CRS-1000 trace, showing that it is measuring the average water content over a different depth (volume) of soil, shown by the higher water content being measured with the TDR traces. These results are consistent with those reported in the literature (Franz *et al.*, 2012b).

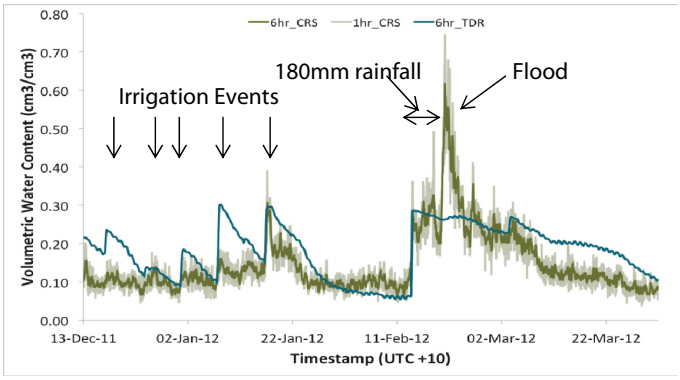


FIGURE 3. Time series of θ_s (green line) recorded by the CosmOz probe from an irrigated soil at Griffith. Also shown is the θs recorded using TDR probes (blue line).

The cosmic ray method for determining soil moisture content has some limitations. One limitation is the detection of hydrogen (H) atoms in other forms beside the soil water. For example, H can be found in the plants growing on the soil, in gypsiferous soils associated with the hydration of the calcium sulphate ($CaSO_42H_2O$) and the clay minerals that make up the soil (Schulze, 2002). Similarly, surface and/or flood water is measured as evidenced by the large increase in volumetric water content following 180 mm of rain at Griffith (Figure 3). If the hydrogen content is constant, as would be the case for clay minerals, its effect can be accounted for in the calibration and therefore largely becomes irrelevant. As with the neutron probe, if the calibration is done on pore and lattice water, the effect of the lattice water is handled in the calibration of the probe. However, the presence of lattice water must reduce the depth of measurement, and the effect will be most obvious in dry soil. If the hydrogen concentration varies with time as in the case with vegetation, it will become an unknown that may need to be determined to accurately quantify soil moisture content.

The depth of measurement depends on the water content and the amount of lattice water. Hydrogen in soil water or lattice water reduces the intensity of neutrons and the depth of measurement in the soil. That is, average soil water is measured to a greater depth in dry soils and to shallower depth in water or flooded soil. Recently, Franz *et al.* (2012a) presented the following equation to correct for soil lattice water:

$$z^* = \frac{5.8}{\rho_{bd}\tau + \theta + 0.0829}$$

where z^* is effective depth of the CRS probe (cm); ρ_{bd} is soil dry bulk density (g/cm^3); τ is weight fraction of lattice water in the mineral grains and bound water defined as the amount of water released at 1 000°C preceded by drying at 105°C (g water per g dry minerals, herein known as lattice water); and θ is volumetric pore water content (m^3/m^3). This effect is constant for any given soil and thus can be handled in the calibration.

Desilets and Zreda (2013) reported that the footprint of the CRS-1000 probe is inversely proportional to air density, and related linearly to the height of the sensor above the ground, up to a height of 125 m. There is no further impact as the height is increased. Soil moisture content has a small impact on the foot print, whereas atmospheric humidity has significant impact; reducing the foot print by 40 m for every 0.01 kg/kg increase in specific humidity. When quantifying θ_s, the effect of changes in atmospheric pressure (Rivera Villarreyes, Baroni and Oswald, 2011), incoming cosmic ray intensity (Zreda *et al.*, 2012) and atmospheric water vapour (Franz *et al.*, 2012a; Zreda *et al.*, 2012; Rosolem *et al.*, 2013) on neutron counts needs to be accounted for. Other corrections are outlined by Zreda *et al.* (2012).

The probe measures average soil moisture across a large spatial area and consequently, the site needs to be relatively uniform. Complex sites that have many different land uses would present a problem because the average water content of the different systems would be measured. Rivera Villarreyes, Baroni and Oswald, (2013) data highlights this effect as they found the calibration of the CRS-1000 probe to vary during the growing season of sunflower and winter rye. Although the neutron density was corrected for humidity, pressure, and lattice water, the CRS-1000 probe determined that the soil water content varied throughout the growing season. This probably reflects the change in the plant biomass that affects the determination of soil moisture content. The biomass of annual crops were found to change dramatically throughout the growing season and the relative water content in the above-ground vegetation ranged from 98 percent in young plant material (tillering) to 40 percent in mature plants (Teulat *et al.*, 1997). Although the relative water content was high at tillering, the mass of water (expressed on an area basis) in the biomass was low at the early stages of growth and increased significantly until maximum biomass was achieved, and then declined during maturation. For example, if there is a cereal biomass of 10 tons (t) per ha (dry weight), then it is likely to contain 15 t ha of water in the above-ground fresh material. For the purpose of comparison, a soil that has a 0.2 volumetric water content contains 200 t of water per ha in the surface 0.1 m. Thus water in the crop vegetation represents about 7 percent of the amount in the top 0.1 m of soil. Based on current knowledge and predictive capability of crop biomass using models such as the Agricultural Production Systems Simulator (APSIM), it should be possible to develop an algorithm that will correct for water in the biomass.

Because the CRS-1000 probe measures soil moisture content to different depths depending on the wetness of the soil, accurate estimates are difficult to obtain in shrink-swell soils (for example, Vertisol soils; Figure 4). When the soil is wet, the surface increases and at the same time, depth of measurement with the CRS-1000 probe would be reduced. When the soil is dry, the soil retracts (shrinks), the surface contracts and the depth of measurement with the CRS-1000 probe would increase. Although not tested, this could be solved by combining measurements with the CRS-1000 probe and the neutron probe, and using a water balance model coupled with model data fusion to integrate to a depth where there is zero change (Ringrose-Voase *et al.*, 2003).

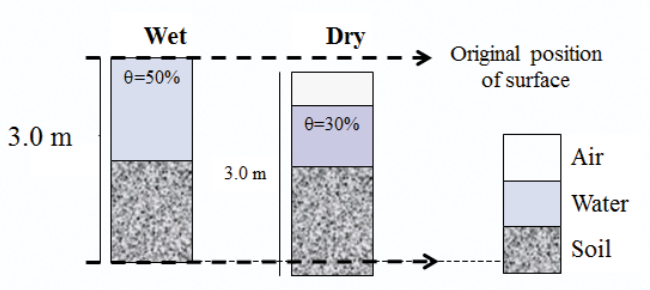


FIGURE 4. Conceptual diagram of the change in the soil surface of a Vertisol under wet and dry conditions.

Early results from the CosmOz network suggest that the CRS-1000 probe is capable of measuring near-surface soil moisture content in a range of soils and climates. The probe's large area sample creates potential uses for applications such as agricultural moisture availability monitoring and catchment scale rainfall-runoff forecasting in environments where antecedent soil moisture influences runoff generation. It may also have potential applications in weather modelling as well as short-term stream flow forecasting, where direct assimilation of ground measured soil moisture can improve forecasting. The larger scale of observation also means that the observations have applications in evaluating landscape-scale mean soil moisture estimates, for example those derived from models or from satellite remote sensing observations.

CONCLUSIONS

Results from the CosmOz network and published literature, confirm that the probes are capable of measuring near-surface soil moisture content in a large scale and in a range of soils and climates. They have the potential for use in: (i) agriculture, (ii) catchment-scale rainfall run off forecasting in environments where antecedent soil moisture influences runoff generation — however, this needs to be further tested to establish whether there are significant improvements in the predictions over the established methods, (iii) water balance assessments, and (iv) validation of soil water content obtained through remote sensing.

The large scale of observation also means that the observations have application in evaluating landscape-scale mean soil moisture estimates. The potential for data/model fusion (i.e. soil water balance coupled with vegetation and land-surface modelling) is exciting although there are some factors that need to be considered when using the probe. A key requirement is the selection of the site so that it matches the foot print of the probe (600 m). This needs to be of uniform land use and relatively uniform soils, as the probe measures average water content over this larger area. Calibration of the probe needs careful attention to include the effect of water stored in the vegetation and soil lattice water. The depth of measurement changes with water content. A cosmic ray probe has significant potential to quantify the average water content across a large area, but this needs to be validated with further research covering a range of soil types, including shrink-swell soil, differing hydrological regimes and different land-uses. Water in vegetation, especially for growing crops, needs to be quantified, and methods to correct for this effect are being developed.

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Assessing Essential and Toxic Elements in Rabbit Manure and Rock Phosphate Fertilizers Using Nuclear Techniques: A Contribution to Managing Nutrient Resources for Improved Small-Stock/Crop Integration

A.C. Avelar [1,2,*], W.M. Ferreira[1] and M.A.B.C. Menezes[2]

ABSTRACT

Industrial phosphate fertilizers are currently the major source of phosphorus for agricultural activities. Many authors maintain that the addition of organic amendments such as manure can ameliorate disturbed soils by improving some characteristics including the available phosphorus (P) in the soil. Furthermore, these products may provide macro elements such as nitrogen (N) and calcium, essential microelements and toxic contaminants. This study deals with uranium content in rabbit manure and six most common phosphates applied in Brazil. Results are compared with values from four other phosphates mined around the world. The nuclear analytical method applied was the delayed neutron technique (DNT) which is a highly precise, affordable, fast (short turnaround) instrumental technique. Three phosphates had uranium content above 145 µg/g, while rabbit manure, a high N, P and potassium (K) organic amendment contains only 2.7 µg/g of uranium. Additionally, neutron activation analysis, a sensitive multi-elemental nuclear analytical technique was used to identify essential and toxic elements in the rabbit manure and a rock phosphate, one of most inexpensive and popular fertilizers available in the Brazilian market. Results for arsenic, barium, bromine, cobalt, chromium, fluorine, iron, sodium, thallium, zinc and K are presented in order to help decision-making concerning strategies for fertilizer-soil-crop management that are both agronomically and environmentally viable. High contents of some toxic elements demonstrate the need to evaluate deposition of contaminants released by fertilizers on farmland, the wider environment and the entire food chain.

Key words: *rock phosphate, rabbit manure, delayed neutron technique, neutron activation, uranium, zinc, arsenic.*

INTRODUCTION

The UN Food and Agriculture Organization (FAO, 2006) recognizes that small farmers play a critical role in improving food security in the 21st century. If well managed, peri-urban and rural small-scale horticulture and small stock rearing and marketing could provide fresh food commodities and nutrients for a significant section of low-income populations, as well as offering a means of self-employment and income generation.

Tropical soils, a feature of some Brazilian farmland, are one impediment to realizing the full potential of small-scale farming, since they usually possess limited phosphorus (P) reserves and have a high absorbing capacity, properties that are not suitable for horticulture. Indeed, P deficiency in crops is an important constraint to plant and animal yields, especially in the hot and humid tropics where soils are predominantly acidic and often extremely P deficient with high P fixation capacities (FAO/IAEA, 2004).

The addition of organic fertilizers such as manure which is available on-farm and cost-free, can ameliorate soil deficiencies by improving their chemical and physical properties as long as the manure is high in nitrogen (N), P and potassium (K) and thereby improves fertility, increases biomass production, and enhances carbon storage in the soil (Shrestha and Lal, 2006).

Rabbit manure itself is a high quality fertilizer, in terms of N, P and K contents (FAO, 2011a and b).

Mineral phosphate fertilizers are currently the major source of P in agriculture (FAO/IAEA, 2004) Phosphates are defined as compounds which contain phosphorus-oxygen (P–O) linkages. The P–O bond has a length of 1.62 Å with bond angles of 130° at the oxygen atoms and 102° at the P atoms in the pentoxide P_2O_5, the most important oxide of P produced commercially (Sauchelli, 1965; Raschshi and Finch, 2000).

Phosphates are found naturally in rocks that form vast deposits of minerals in the strata of the earth but few phosphate sites are mined around the world. About 90 percent of world production is used to manufacture fertilizers, with the remainder being used to manufacture animal feeds, detergents and chemicals (FAO/IAEA, 2004).

Nowadays, sedimentary ore deposits provide about from 80 to 90 percent of world phosphate production (FAO/IAEA, 2004). However, igneous deposits make up 80 percent of Brazil's national reserves, the major deposits being located in the following federal states: Bahia, Ceará, Goiás, Minas Gerais, Pará, Paraíba, Pernambuco,

1 DZOO Department of Animal Sciences, Veterinary School, Universidade Federal de Minas Gerais (UFMG), Avenida Antonio Carlos, 6627 Campus UFMG, Pampulha, Belo Horizonte, Brazil
2 Nuclear Technology Development Centre, Brazilian Nuclear Energy Commission, Rua Mario Werneck, s/n, Predio 1, Campus UFMG, Pampulha, Belo Horizonte, Brazil
* E-mail address of corresponding author: avelara@ufmg.br

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Santa Catarina, Rio Grande do Norte e São Paulo. Most, however, are concentrated in the states of Minas Gerais, Goiás and São Paulo (DNPM, 2010).

In 2009, the Brazilian domestic market produced about of 5 434 022 tonnes (t) of concentrated phosphate rock, production being used for fertilizers (89 percent), animal feed (seven percent), soil amendments (one percent) and the remainder for a variety of other purposes (DNPM, 2010). Phosphate rocks may contain accessory-gangue minerals and impurities that can be hazardous to man and animal health such as arsenic, cadmium, lead, fluorine and uranium (Tokarnia *et al.*, 2000), and uranium ores in some phosphate mining sites are processed as a by-product of the fertilizer industry (USGS, 2002).

Uranium is the heaviest natural element in nature; it is hazardous to human and animal health because of both its radioactivity and chemical toxicity. In nature, uranium is more plentiful than silver (Ag) and about as abundant as molybdenum (Mo) or arsenic (As) (ASTDR, 1999). Practically every phosphate rock contains uranium, although the amounts of this and other hazardous elements vary widely among sources and even within the same deposit. Thus, mining, milling, industrializing and using phosphate products in soil and animal nutrition are anthropogenic activities increasing the potential for human exposure to uranium (Mangini *et al.,* 1979; ASTDR, 1999; FAO/IAEA, 2004).

Figure 1 provides an outline of nutrient and contaminant flows in the environment, including P flows from fertilizers and manure. Absorption of essential and toxic elements by humans and animals is mostly by oral ingestion of food, feed and water, but inhalation and dermal contact can lead to some absorption in particular cases. Workers in agriculture are more likely to be exposed to uranium, especially those applying phosphates on farmland.

The general public is also potentially exposed when eating root vegetables like potatoes grown on uranium contaminated soil. Besides the ingestion of foods, drinking uranium-contaminated potable waters is also a route for uranium to enter into the animal organism (Mangini *et al.*, 1979; ASTDR, 1999).

Studies in the 1970´s provided evidence for increasing uranium presence in rivers and ground water in regions with intensive use of fertilizers in agriculture. Uranium derived from phosphate fertilizers is likely to be adsorbed on the uppermost soil layers and its content in the water is correlated with the bicarbonate (HCO_3^-) content in the river (Mangini *et al*., 1979).

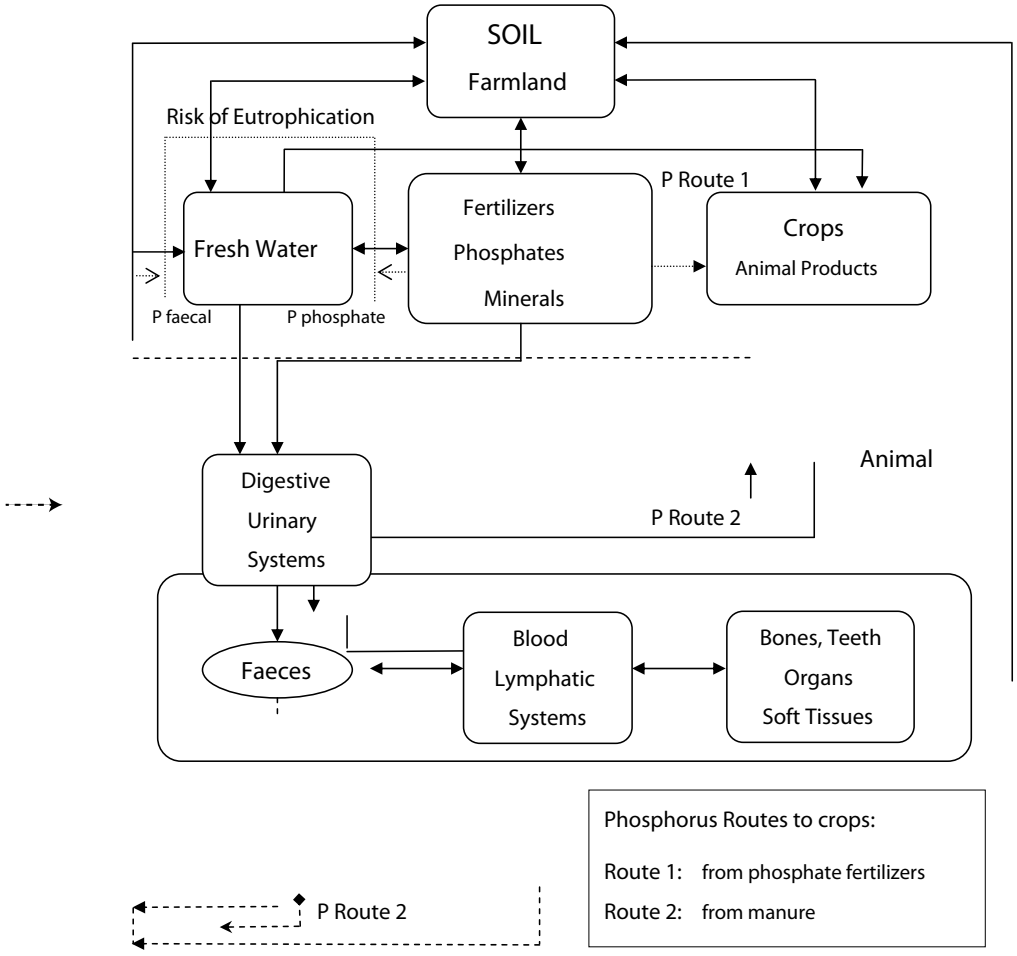


FIGURE 1. Biogeochemical flow of nutrients and contaminants in the environment, including phosphorus routes from fertilizers and manure.

Many phosphates have the ability to accumulate uranium and other toxic elements. These abilities may be used in the recovery of various useful metals from aqueous systems (El Shall *et al.,* 1993).

To face the main constraint of low inherent P in feeds, soils and plants, rock phosphates and manure are likely to be more widely applied in agriculture, raising the possibility that these sources may release contaminants from the farmland into the wider environment (Mangini *et al*., 1979; Scholten and Timmermans, 1996). Against this background, a study was made of the chemical content of selected agricultural products in Brazil.

Uranium content can be determined by some nuclear techniques including the delayed neutron technique, a precise, fast (short turnaround), sensitive, affordable and non-destructive method (DNT, 2013). The method is based on exposing the sample to neutrons in a research reactor, where fission of its uranium-235 (^{235}U) in the nucleus absorbs a neutron from the neutron flow, forming an instable, highly energetic nucleus uranium-236 (^{236}U). This unstable nucleus mostly fissions into two medium-sized nuclei emitting 2–3 neutrons as follows:

$$^{235}U_{\text{ in sample}} + \text{neutron}_{\text{ thermal, from reactor fuel}} \rightarrow {}^{236}U_{\text{ instable}} \rightarrow {}^{90}Kr + {}^{143}Ba + 3 \text{ neutrons}$$

Very quickly ($< 10^{-12}$ seconds after the fission) 99 percent of the neutrons, so-called "prompt neutrons" are emitted. The remaining neutrons are emitted from 0.0001 s up to several min later and are called "delayed-neutrons". These delayed-neutrons can be detected easily and precisely since their parameters are well known (DNT, 2013). Neutron activation analysis (NAA) was also used to measure levels of a wider range of elements. This method is based on the interaction of radiation (from the reactions occurring in the reactor) with matter (in the sample). When one natural element (present in the sample) is exposed to a neutron flux, the reaction occurs. The radionuclide formed emits gamma radiation which can be measured by suitable equipment. About 70 percent of elements have nuclides possessing properties suitable for NAA. At the Nuclear Technology Development Centre (CDTN) of the National Nuclear Energy Commission (CNEN), Belo Horizonte, Brazil, there is a TRIGA MARK I IPR-R1 nuclear reactor for application of this technique. k0- based reactor neutron activation analysis (k0-NAA), is an advanced type of NAA in which the sample is irradiated without previous chemical preparation. This specific method is based on k0 factors and some reactor radiatio n parameters. The NAA technique is more expensive than most analytical techniques and takes up to 90 d to measure long-lived elements (Avelar *et al*., 2002).

MATERIALS AND METHODS

Fertilizer samples and preparation

Phosphate fertilizers were acquired in the local market of Minas Gerais State, Brazil. Aliquots of about 100 g were taken randomly from each commercial pack and grounded to obtain a particle size of 200 Tyler mesh (75 µm) and establish 99 percent conformity of particle size of each product. An aliquot of 1.00 g ground phosphate was taken for analysis. The capsule filled with the sample was placed in a polyethylene container (vial) for the pneumatic transporting system.

Experimental animals, collection and preparation of faecal samples

Twenty-four young rabbits (30 d old) were used in the study; hard faeces samples were separated from urine and collected twice daily during the 42-d experiment, which is the average time needed for rabbits to achieve the commercial live weight of 2.0 kg.

Faeces were placed in a plastic bag (one per animal) and stored in a –20°C freezer to minimize odour and to avoid losses. A sub-sample of approximately 1 000 g from each animal was taken from the bulk manure collected during 42 d, frozen at –70°C and lyophilized. Each freeze-dried sample was powdered, homogenized and around 1.00 g was taken and sealed into polyethylene irradiation vials.

Sample analyses

Samples and standards in the vials were placed individually into the neutron flux using the pneumatic transport system of the reactor IPR-R1 at the CDTN/CNEN. The reactor was operated at 1 kW thermal power under a neutron flux of 6.6×10^{11} neutrons cm^2/s. After 60 s, samples were transported automatically out of the reactor direct to the counting room containing a measuring system consisting of a metal box filled with paraffin moderator. Six detectors (^3He filled and connected in parallel) were positioned symmetrically in order to carry out the detection. The detectors send pulses to the accompanying electronic devices, the first of which are amplified, sorted by a discriminator and the selected pulses counted in an electronic analyzer for 40 s. A decay-curve for the delayed neutrons was established as an array of 40 counts and the uranium content of samples determined by comparing their delayed-neutron intensity with those from two well-defined uranium standards from the IAEA.

For the NAA, three schemes of irradiation were undertaken: for short, medium and long half-life elements. All samples were irradiated at the CDTN/CNEN IPR-R1 reactor. After suitable decay times, gamma spectroscopy was performed in an HPGe detector with a 10 percent efficiency, connected to a multichannel analyzer. Concentrations were calculated using KayZero/Solcoy software. The only element studied with an alternative method was fluoride which was assessed by potentiometry, a well-established method (Institute of Medicine, 1997).

RESULTS

TABLE 1. Uranium content expressed as ratio of phosphorus concentration

Phosphorus Sources	Uranium Content (µg/g)
Super Simple SSP, 17% P_2O_5, Brazil	52 ± 5[a]
Dicalcium DCP, 46% P_2O_5, Brazil	181 ± 9[a]
Mono-ammonium MAP, 50% P_2O_5, Brazil	189 ± 9[a]
Triple TSP, 43% P_2O_5, Brazil	32 ± 4[a]
Phosphate Rock, 24% P_2O_5, Brazil	39 ± 4[a]
Phosphate Rock *Arad*, 31% P_2O_5, Israel	145 ± 7[a]
Phosphate Rock, 31% P_2O_5, USA	59[b]
Phosphate Rock, 33% P_2O_5, Morocco	82[b]
Phosphate Rock, 29% P_2O_5, Mali	123[b]
Phosphate Rock, 28% P_2O_5, Tanzania	390[b]
Rabbit manure (fresh content)	2.7 ± 0.5[a]

a — experimental results by DNTe, phosphates (n = 8) and rabbit manure (n = 24)

b — data from FAO/IAEA (2004)

TABLE 2. Average elemental content of rabbit manure (n = 24) and *Arad* rock phosphate (n = 8)

Elements	Rabbit Manure (µg/g)	Rock Phosphate (µg/g)
As	2.0 ± 0.5	22.5 ± 2.7
Ba	986 ± 167	17 209 ± 981
Br	1.9 ± 0.3	1.7 ± 0.3
Co	5.3 ± 0.6	14.3 ± 1.9
Cu	535 ± 48	561 ± 59
F	234 ± 112	32 500 ± 2974
Fe	3 448 ± 201	3 930 ± 208
K	5 903 ± 513	4 723 ± 455
Na	1 134 ± 101	1 100 ± 98
Th	0.54 ± 0.21	12.9 ± 1.1
Zn	244 ± 45	390 ± 52

DISCUSSION

Application of rabbit manure (Table 1) does not pose a risk to increasing the uranium concentration in farmland since the average uranium concentration in the earth's crust ranges from 2 to 4 µg/g (EPA, 1999), the same average value of the uranium content in the rabbit manure. The results also imply that from environmental standpoint, rabbit manure would be an advantageous addition to application of rock phosphates.

Concentrations of uranium were, however, quite variable in both the rock phosphates and animal manure. Differences among fertilizers (Table 1) might be due to the region of exploitation of the phosphate ores, implying different ages of mineralization, deposit types and associated accessory minerals, and by methods of industrial production.

In both rabbit manure and rock phosphates, some essential elements and some elements classified as hazards and toxic elements by the ATSDR were detected (Tables 1 and 2). These included arsenic, barium, cobalt, fluoride, thorium, uranium and zinc. Noteworthy, were the high levels of arsenic, fluoride and uranium in the rock phosphate fertilizer. The concentrations measured of these hazardous elements are at least ten times higher than those found in the rabbit manure.

Since the 1990's, Canada has placed limits on maximum acceptable cumulative metal additions to soil and maximum acceptable metal concentrations in products. For instance, the maximum acceptable zinc concentration in phosphates and manure is 1 850 mg/kg dry weight (EPA, 1999). Also, European legislation has reduced the maximum permitted level of Zn and Cu supplementation in livestock diets to minimize the environmental impact of manure disposal in the soil and waterbeds (European Commission, 2003).

CONCLUSIONS

Results indicate that the choice of fertilizers plays a key role in the control of contaminant flow in agricultural activities. In this sense, rabbit manure poses the smallest potential for being a hazard among those studied. This advantage should be even greater through time considering long-term exposure due to successive fertilizer applications over the years.

Uranium and other toxic elements in fertilizers should be investigated since these elements are normal constituents of many phosphates, and the monitoring of their presence in manure and chemical fertilizers and in applied soils should be fundamental for current and future agricultural activities in many parts of the world. Proactive measures should also be taken to avoid unnecessary exposure of humans, animals and the wider environment to these elements.

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List of abbreviations

AGRA	Alliance for a Green Revolution in Africa
ANOVA	Analysis of variance
BNF	Biological nitrogen fixation
CA	Conservation agriculture
CEC	Cation exchange capacity
CGIAR	Consultative Group on International Agricultural Research
CIAT	Centro Internacional de Agricultura Tropical
CID	Carbon isotope discrimination
COSMOS	Cosmic-ray soil moisture observing system
CR	Count ratio
CRDS	Cavity ring down spectroscopy
CRP	Coordinated research project
CSIRO	Commonwealth Scientific and Industrial Research Organization
CSSI	Compound-specific stable isotopes
CT	Conventional tillage
CWP	Crop water productivity
δ	Delta value
DAP	Days after planting
DEM	Digital elevation model
DGPS	Differential global positioning system
DI	Deficit irrigation
DOC	Dissolved organic carbon
DPM	Disintegrations per minute
DSM	Digital surface model
E	Evaporation
EA	Elemental analyzer
EC	Electrical conductivity
EPA	Environmental Protection Agency, USA
ET	Evapotranspiration
ETc	Crop evapotranspiration or actual evapotranspiration
ETo	Reference evapotranspiration
FACE	Free-air carbon dioxide enrichment
FAO	Food and Agriculture Organization
FC	Field capacity
FRN	Fallout radionuclide
FNUE	Fertilizer nitrogen use efficiency
GAP	Good agricultural practices
GC-IRMS	Gas chromatography–isotope ratio mass spectroscopy
GHG	Greenhouse gas emissions
GIS	Geographic information systems
GMWL	Global meteoric water line
GPS	Global positioning system
HI	Harvest index
IAEA	International Atomic Energy Agency
ICP-MS	Inductively coupled plasma mass spectrometry
ICT	Information and communication technology
IITA	International Institute of Tropical Agriculture
IPCC	Inter-Governmental Panel on Climate Change
IRRI	International Rice Research Institute
IT	Information technology
IMB	Isotope mass balance
ISFM	Integrated soil fertility management
KC	Crop coefficient
LADA	Land degradation assessment in drylands
LAI	Leaf area index
LMWL	Local meteoric water line
LSC	Liquid scintillation counter
LSD	Least significant difference
Ndfa	Nitrogen derived from atmosphere

Ndff	Nitrogen derived from fertilizer
Ndfs	Nitrogen derived from soil
NT	No-till
OM	Organic matter
PAE	Phosphorus acquisition efficiency
PAR	Photosynthetic available radiation
PAWC	Plant available water holding capacity
PPMV	Part per million by volume
PTFs	Pedotransfer functions
PUE	Phosphorus utilization efficiency
RH	Relative humidity
RMSE	Root mean square error
SE	Standard error
SLM	Sustainable land management
SOC	Soil organic carbon
SOM	Soil organic matter
SPAC	Soil–plant–atmosphere continuum
SSA	Sub-Saharan Africa
T	Transpiration
TDR	Time domain reflectrometry
TDS	Total dissolved solids
TE	Transpiration efficiency
TLU	Tropical livestock unit
TU	Tritium unit
USDA	United States Department of Agriculture
USLE	Universal soil loss equation
VPD	Vapour pressure deficit
VPDB	Vienna Pee Dee Belemnite
WUE	Water use efficiency

Managing Soils for Food Security and Climate Change Adaptation and Mitigation

This publication is a compilation of selected papers presented at the International Symposium on "Managing Soils for Food Security and Climate Change Adaptation and Mitigation". Six thematic topics were covered: (i) managing soils for crop production and on-farm and area-wide ecosystem service efficiency; (ii) preserving and protecting soil resources; (iii) establishing soil and water conservation zones for pollution control; (iv) managing soils for climate change adaptation and mitigation through increasing soil carbon stocks (C sequestration) and reducing greenhouse gas emissions; (v) managing agricultural water for climate change adaptation; and (vi) recent advances in nuclear techniques and applications in land management research.

It is hoped that the information presented in these Proceedings provides valuable guidance to scientists and land managers in both the public and private sectors, as well as to government and institutional policy- and decision-makers involved in addressing land management issues for climate smart agriculture and the conservation of natural resources for agricultural productivity and food security.









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