

Protocols on the use of the CSSI Technique to identify and apportion soil sources from land use

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Authors/Contributors:

Max Gibbs

For any information regarding this report please contact:

Max Gibbs
Water Quality Scientist
Freshwater Ecology
+64-7-856 1773
+64-27-604-1449
max.gibbs@niwa.co.nz

National Institute of Water & Atmospheric Research Ltd
Gate 10, Silverdale Road
Hillcrest, Hamilton 3216
PO Box 11115, Hillcrest
Hamilton 3251
New Zealand

Phone +64-7-856 7026
Fax +64-7-856 0151

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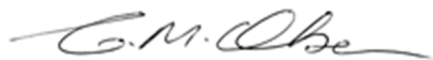
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Contents

Preface	6
1 Introduction	8
1.1 Background	8
1.2 About this handbook	9
2 Standard Operating Procedures	11
2.1 Health and safety	11
2.2 Glossary of some terms as used for the CSSI technique	12
2.3 Preparation of special reagents	13
2.4 Cleaning the equipment	14
2.5 Planning	15
2.6 Sampling	15
2.7 Processing	17
2.8 Analysis	23
3 CSSI technique overview	25
3.1 Development concepts	25
3.2 Stable isotope basics	26
3.3 CSSI	28
4 Experimental design	33
4.1 Uncertainty	33
4.2 Determining what question is being asked	33
4.3 A different question requires a different study design	34
4.4 Design strategy	35
5 Sampling	39
5.1 Sampling equipment	39
5.2 Storage	40
5.3 Representative samples	40
5.4 Appropriate samples	40
5.5 Suspended sediments	42
5.6 Soil and sediment cores	45
5.7 Sample preparation	48

5.8	Sources of error	50
5.9	Preparing test soil mixtures	51
5.10	Quality Assurance	51
6	Analysis	53
6.1	Sediment and soil characteristics	53
6.2	Organic matter and %C content	53
6.3	Bulk stable isotopes	54
6.4	Compound-specific stable isotopes	55
7	Data interpretation	67
7.1	Selecting data for the models	67
8	IsoSource.....	70
8.1	IsoSource – how it works.....	70
8.2	Using IsoSource.....	73
8.3	Soil proportions	83
8.4	Selecting isotopes and source combinations.....	84
9	SIAR	91
9.1	SIAR notes.....	92
10	Fallout Radionuclide (FRN) linking	102
10.1	FRN overview.....	102
10.2	Erosion or deposition.....	104
10.3	Dating using FRNs	107
11	Case Study.....	110
11.1	Waitetuna Study.....	110
11.2	Bay of Islands historical land-use reconstruction	115
12	Suess effect.....	119
13	Glossary.....	121
14	References.....	122

Reviewed by



G. Olsen

Approved for release by



C. Depree

Formatting checked by



Preface

Soil erosion and subsequent sedimentation are natural processes caused by water, wind and ice. Several of man's activities such as deforestation, overgrazing, changes in land use, non-sustainable farming practices and global climate change tend to accelerate soil erosion. The result is degradation of the landscape with impacts on soil fertility, crop productivity, water pollution, potential effects on global climate, and sedimentation in lakes, reservoirs and floodplains. Accordingly, determining the main sediment sources in a watershed and thus identifying the sites with critical soil erosion, is of growing importance to improve soil management and sustainable food supply.

This protocol handbook has been prepared in order to standardise sampling, sample processing, analysis, and calculations for the use of a forensic technique using compound-specific stable isotopes (CSSI) to identify and apportion soil sources from land use. This technique, linked with fallout radio nuclide (FRN) techniques, will enable quantitative assessment of source-specific rates of soil erosion and sediment mass transport within the FAO/IAEA Coordinated Research Project (CRP) number D1.20.11 "Integrated Isotopic Approaches for an Area-wide Precision Conservation to Control the Impacts of Agricultural Practices on Land Degradation and Soil Erosion". The preparation of these protocols was funded by CRP Technical Contract number 15491 "Developing protocols on the use of compound-specific stable isotopes (CSSI) to identify and apportion soil sources from land use and to integrate FRN with CSSI in establishing comprehensive soil redistribution studies." This work was coupled with the NIWA research project "Land Use Intensification: Sustainable Management of Water Quality and Quantity", Foundation for Research, Science and Technology contract number C01X0304.

The CSSI technique (Gibbs 2008) was developed to positively identify the linkages between sources of soil erosion from different land-uses within a single watershed depositing in an estuary, to allow mitigation of the sediment loads by managing the sources. While the CSSI technique can identify and apportion soil sources by land-use, the technique is not totally quantitative and requires additional mass transport information. This additional information can be in the form of measured flow-weighted sediment loads in rivers and streams, modelled sediment loads using geographical information system (GIS) and climate data, and sediment budgets or estimations of sediment erosion or accumulation rates obtained from FRN measurements.

While the forensic nature of the CSSI technique implies the use of high level technology, these protocols have been written with a focus on simplicity and low level technology wherever possible. In this way, the CSSI technique may be added on to an existing investigation to provide an indication of proportional sediment contribution from major land-use sources in a watershed, or it can be combined with FRN techniques in a detailed study designed to quantify those sediment contributions and determine the land-use practices that are exacerbating soil erosion.

Since the first release of these protocols in 2009, understanding has improved on a number of aspects of the use of this technique. There have also been improvements in the sampling and sample processing procedures prior to analysis. The update of the protocols has included the new understanding as well as a set of Standard Operating Procedures (SOP) for each step in the sampling and sample processing procedures to ensure that the CSSI

technique is applied consistently in all studies. The SOPs have been inserted at the beginning of the handbook as a quick reference guide for those who have read the handbook or who want to get on with the study and will read the detailed explanations in the text later.

A quality assurance (QA) sample has been prepared from a medium organic loam soil. The QA sample can be analysed with each batch of samples to check on sample extraction and derivatisation efficacy relative to the data supplied with the QA sample. The analytical results from the QA sample can be used as an inter-laboratory comparison reference. A time-series of analytical results of the same QA sample can also be used to assess any changes occurring in the extraction, derivatisation process and analysis in each laboratory.

Acknowledgements

While the CSSI method was developed by the author, the transition from method into a robust working technique has been made possible by the constructive criticism and discussion with the members of CRP number D1.20.11 and colleagues at NIWA including Ahmad Asfary (Syria), Moncef Benmansour (Morocco), William Blake (United Kingdom), Samuel Bode (Belgium), Pascal Boeckx (Belgium), Georg Cadisch (Germany), Sandy Elliott (New Zealand), Wojciech Froehlich (Poland), Valentin Golosov (Russian Federation), Gary Hancock (Australia), Dries Huygens (Belgium), Yong Li (China), David Lobb (Canada), Thanh Lam Nguyen (Vietnam), Greg Olsen (New Zealand), Paulina Schuller (Chile), Andrew Swales (New Zealand), Xinbao Zhang (China), Desmond Walling (United Kingdom) and encouragement from the management team including Minh-Long Nguyen and Gerd Dercon (IAEA) and Bryce Cooper and David Roper (NIWA).

1 Introduction

1.1 Background

Sediment from land erosion is the largest contaminant of water world-wide (Thrush et al. 2004). There are three main issues: 1) sediment raises the cost of potable water due to treatment requirements; 2) sediment adversely impacts on the aquatic environment affecting biodiversity; and 3) sediment from land erosion represents a threat to sustainable food production through loss of soil from arable land. The World Economic Forum (2012) suggested that 40% of soil used for agriculture around the world is classed as either degraded or seriously degraded.

Watersheds commonly support various types of land use. A watershed is defined in a simplistic terms as an area-wide of land that catches the rain and irrigation water and drains into receiving water bodies. Watersheds are host to a range of activities that affect the use and management of their natural resources, in particular, land and water quality.

Agricultural (livestock and crop production), forestry, mining, industrial and urban related activities can have negative environmental impacts in the watershed. This situation has become increasingly common during the last few decades with the intensification of agricultural activities and increasing urbanization and industrialization leading to dynamic changes and increased pressure on land and water resources. These diverse activities can also result in different forms of soil degradation, of which soil erosion and associated sedimentation is the most important on a watershed scale. Soil losses can have serious on-site impacts such as reduction in effective soil depth and water storage capacity, loss of fertility and reduction of crop productivity (World Economic Forum 2012). Moreover, sediments and associated pollutants mobilised by soil erosion and transported towards water bodies can have serious off-site or downstream impacts. These include siltation of reservoirs and water distribution channels, damage to infrastructure and property caused by sediment deposition, reduction of water quality and degradation of aquatic habitats and ecosystems. It has been estimated, that the global costs of soil loss/sediment-related environmental problems are of the order of USD \$400 billion per year (Pimentel et al. 1995).

Reducing sediment-related environmental problems represents a key requirement for sustainable land and water management. However, recent advances and existing experience shows that implementation of soil conservation measures at the scale of the individual field or farm, whilst proving effective in reducing on-site impacts, may have limited success in reducing downstream impacts, since there is a need to consider the entire watershed and to target those areas that represent important sediment sources. Investigations at the watershed level can provide the information required to understand the spatial patterns and complexity of sediment fluxes and sediment sinks and thus the efficiency of sediment delivery to the stream network and to link downstream sediment fluxes with upstream sediment sources. Soil conservation measures should target the major runoff and sediment source areas. These critical source areas may represent only a small fraction of the total surface area of a watershed and targeting these areas can result in significant cost savings when implementing soil conservation measures (OECD, 2003, 2004). Identification of key sediment source areas and sediment delivery pathways at the watershed level will enable

planners to optimise land use in accordance with both the risk and the benefits associated with the development of individual areas within a watershed.

1.2 About this handbook

Mitigation of erosion requires knowledge of the origin of the sediment; that is, the land-use or land-use practice within a watershed causing the erosion, and how much sediment is produced by that land-use practice, to enable informed management decisions. While FRN techniques and other non-isotopic techniques can quantify the total sediment erosion and sediment load leaving the watershed, the CSSI technique can identify and apportion the land-uses, within that watershed contributing to the eroded sediment.

The protocols in this handbook will enable researchers to apply the CSSI technique to a range of situations in a standardised way which will provide information on where the sediment came from within a watershed and thus whether a specific land-use practice is producing a higher than expected sediment yield on an areal basis.

This handbook is organised in the following order:

- **Standard Operating Procedures:** giving the simple step-by-step instructions required to design a study, collect samples and process them through to the analytical stage. This is a quick reference guide for those who have read the handbook or who want to get on with the study and will read the detailed explanations later. This section can be copied for use as a working reference.
- **CSSI technique overview:** including an introduction to stable isotopes, the basic concepts underpinning the CSSI technique, biomarkers, and which stable isotopes can be used.
- **Experimental design:** including acceptable levels of uncertainty, strategies to obtain the most information from the minimum number of samples, the need for a “reference library” of land-use soils, the way sediment settles and disperses in waterways, and where the CSSI technique is unlikely to work.
- **Sampling:** including what samples to collect, how to collect them, where to collect them from in a watershed, preparation of the samples for storage and analysis, and how to prepare known source soil mixtures for testing.
- **X-ray inspection:** to determine physical structure of soils and sediment cores before sectioning.
- **Analysis:** including bulk stable isotope analysis of the soils, the need for acidification, extraction of fatty acid biomarkers from the soils, derivatisation to produce fatty acid methyl esters (FAMES), the concepts of gas chromatography-combustion-mass spectrometry, and the correction of the isotopic value of each FAME for the isotopic value of the methyl group added during derivatisation.
- **Data interpretation:** including the use of mixing models (IsoSource, SIAR) to proportionally deconstruct the soil sources by land-use from a sediment mixture, and conversion of the isotopic proportions to soil proportions.

- **FRN linking:** including the use of ^7Be as an indicator of recent sediment deposition for sampling the mixed layer, ^{137}Cs as a depth date marker, ^{210}Pb as an indicator of sediment accumulation rate (SAR), the conversion of soil proportions to soil loads using FRN and other non-FRN techniques.
- **Spatial and temporal assessment:** including the use of sediment cores and FRN dating together with CSSI data to reconstruct historical land-use changes and assess spatial dispersion of sediment in estuaries.
- **Case studies:** including soil source contributions by land-use to sediment in a riverine watershed, comparison of the CSSI technique results with GIS-based catchment model sediment yields, mapping sediment deposition by land-use source across an estuary, and using land-use changes to reconstruct local history.
- **Glossary** of terms and abbreviations used in this handbook.
- **References.**

2 Standard Operating Procedures

The Standard Operating Procedures (SOP) were compiled with input from members of the RAS-5.055 team including Frank Bruhn (Australia), Yong Li (China), Md. Tarafder (Bangladesh), Sudhakar Srivastava (India), Nita Suhartini (Indonesia), Junghwan Yoon (Republic of Korea), Jalal Sharib (Malaysia), Ngu War Nwe (Myanmar), IndraBahadur Oli (Nepal), Naveed Iqbal (Pakistan), Faye Rivera (Philippines), Champa Kumari K. Dissanayake Dewage (Sri Lanka), Wanpen Wiriakitnateekul (Thailand), Prapaipit Srimawong (Thailand), Hanqing Yu (China), Zhijun Guo (China), Yingchen Li (China), Cuicui Hou (China) and Wenxiang Liu (China).

The SOP are intended as a set of protocols that will ensure that the CSSI technique is applied in a consistent way and thereby will provide reliable results that can be compared across different catchments and in different studies.

The CSSI technique is applied in a sequence of processes:

1. Planning
2. Sampling
3. Processing
4. Analysis
5. Data interpretation and
6. Reporting

The SOPs cover processes 1 to 3. Each process has several steps, most of which are non-critical, but some steps are critical and these are written in **red** and described in greater detail.

2.1 Health and safety

Field and laboratory procedures can be hazardous and personal responsibility is required for your own health and safety under all conditions. Laboratory health and safety rules for each institute should be obeyed. If no health and safety rules are available for your laboratory, they should be developed. The following general guidelines do not cover everything but may be used as common sense precautions:

- Wear appropriate clothing – lab coat, closed footwear (not open-toed sandals or high heels), safety glasses and use nitrile gloves whenever handling acids, alkali and solvents.
- Always add the acid to the water, not the water to the acid.
- Always use solvents in a fume cupboard with the extraction fan running.
- Always read all the instructions before starting a procedure.

2.2 Glossary of some terms as used for the CSSI technique

Soil	Loose upper layer of earth in which plants grow.
Land use	Classification of soil by the variety of plants and animals growing on it.
Landuse practice	How the land use is managed.
Landscape	Expanse of land visible from any viewing point.
Catchment	Expanse of land from which all surface water drains into the same waterway.
Erosion	Loss of soil from its original location caused by water or wind.
Soil degradation	Loss of soil fertility through poor landuse practice or erosion.
Sediment	Soil suspended in or deposited from water at a location.
River delta	Deposition zone at the mouth of a river entering a larger water body.
Source	Reference soil from a well-defined land use.
Mixture	Sample of sediment from a downstream location in the catchment.
Aliquot	Small portion of the main sample.
Biomarker	Naturally occurring organic compound (e.g., fatty acid) found in the sample.
%C	Percentage of organic carbon in the sample after acidification.
Acidification	Removal of inorganic carbon (carbonate) by reaction with hydrochloric acid.
Stable isotope	Naturally occurring, non-radioactive isotope of carbon (^{13}C).
Bulk $\delta^{13}\text{C}$	The isotopic value of the organic ^{13}C content in the whole sample.
Derivatisation	The conversion of a fatty acid to its methyl ester by adding a methyl group in place of the acid group. Also called methylation.
FAME	Fatty Acid Methyl Ester produced by derivatisation.
Freeze dry	The sample is frozen at -20°C before being placed in a high vacuum, which causes the ice to sublime to water vapour. The water vapour is removed by the vacuum leaving the sample dry.

2.3 Preparation of special reagents

2.3.1 10% Hydrochloric acid (10% HCl)

Actual concentration is not critical. The 10% HCl solution can be made up as required or as a bulk supply in advance and stored. Use Analytical grade HCl. **Wear gloves and glasses when preparing this reagent.**

- Using a clean 500 ml measuring cylinder, add 450 ml distilled or deionised water to a clean 500 ml polyethylene, screw-cap bottle.
- Using a clean 50 ml measuring cylinder, add 50 ml concentrated HCL to the bottle.
- Screw on the cap and shake gently to mix.
- Label the bottle and include the date.

2.3.2 Clean quartz sand

Used in the ASE extraction procedure for small sample weights. May need 20 to 30 g for each ASE cell so prepare about 500 g and store on a clean glass screw-cap bottle.

- Place a quantity of clean, 20 to 40 mesh (about 0.4 to 0.8 mm) quartz sand in a stainless steel tray and heat in a muffle furnace at 450°C for 3 hours.
- On cooling, transfer to the clean storage bottle and seal.

2.3.3 Anhydrous sodium sulphate

Used for drying the solvent extract. May need 1-2 g per extraction tube if the samples were not completely dry. Should be stored in a clean glass screw-cap bottle (Schott Durand type), preferably in a desiccator. May need to be reactivated after several weeks of opening and closing the bottle.

- Spread a quantity (250 g) of analytical grade anhydrous sodium sulphate in a small stainless steel tray and heat at 450°C for 3 hours in a muffle furnace.
- While the tray is still hot ~ 100°C + and using a clean glass powder funnel, transfer the sodium sulphate granules to the clean glass screw-cap bottle, then seal.

2.3.4 Derivatisation agent 5% Boron trifluoride (5%BF₃)

This should be prepared in a small beaker immediately before use. Only prepare enough for the number of samples being processed – allow 1 ml for each sample plus about 5 to 10 ml to allow for variation in the pipetter delivery volume. **Wear gloves and glasses when handling this reagent.** For 20 to 40 samples:

- Add 5 ml of 50% BF₃ in methanol to 45 ml HPLC grade methanol in a clean 100 ml beaker and swirl to mix.
- Use a 1 ml pipetter to transfer the 5% BF₃ reagent to the reaction tube direct from the beaker.

Dispose of any residual reagent into running water.

2.4 Cleaning the equipment

All equipment and glassware must be clean before starting laboratory procedures.

The solvent used in the extraction procedure for CSSI biomarkers must be very high purity dichloromethane (DCM). Either HPLC grade or double distilled.

1. The glassware must be ultra-clean. Recommended procedure:
 - Place flasks, test tubes and Pasteur pipettes in 5% “Decon90” solution (or equivalent laboratory grade detergent) for at least 12 hours (this includes the septa for the glassware).
 - Remove and rinse thoroughly with distilled or deionised water.
 - The septa are dried at 105°C before sealing in clean plastic bag.
 - Wrap the glassware in Aluminium foil and bake at 400°C for 3 hours, then allow to cool. Only unwrap when ready to use.
2. The Accelerated Solvent Extractor (ASE) cells must be cleaned: Routine procedure:
 - Take the ends off the cells, remove the filter pad and brush physically clean before soaking in 5% Decon90 solution for at least 12 hours.
3. Thoroughly rise with distilled or deionised water and dry at 105°C.
4. The filter pads for the ASE cells are nominally clean from the supplier but for low concentration work they should be extracted with DCM by packing them into ASE cells and running them through the extraction procedure. They must only be handled with flat-faced forceps (i.e., no gripping ridges on the tips - see Figure 1).

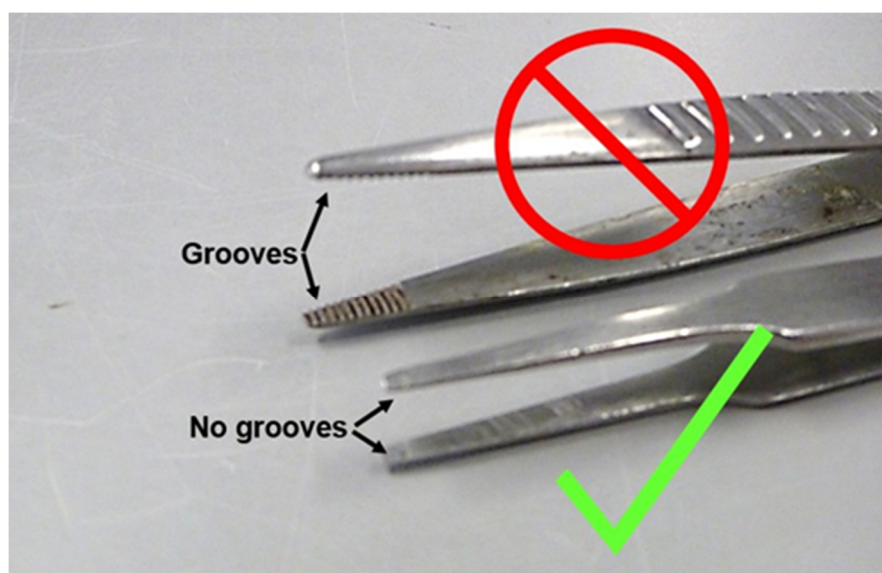


Figure 1: Forceps. The forceps for handling the filters must not have gripping grooves as these will damage the filter membrane and cause leakage of suspension into the filtrate or fine sediment into the ASE system

2.5 Planning

Planning procedures are critical

The development of a plan depends on the question to be answered.

SOP:

1. Write down the question or questions.

- Identify the mixtures to be deconstructed and list them.
- Evaluate the catchment to identify the potential landuse sources contributing soil to the mixtures and list them.

Use topographic maps, Google Earth, reports and local knowledge from a site visit (if possible) to assist finding the required mixtures and sources. In most studies there will be less than 10 sources. The number of mixtures to be deconstructed will depend on the question being asked.

2. Develop the plan and write it down

The initial plan may be modified once on site to accommodate unexpected features or unforeseen events. Revise the plan and make a note of why the change was made. The revised plan becomes the plan that will be followed.

2.6 Sampling

SOP:

At each site in the catchment specified in the plan (including both mixture and landuse sites)

1. Take 10 small samples, 2-cm deep and uniform-size, spaced across the site.
2. Remove roots, leaves stones, insects, sticks etc., as much as practical.
3. Combine the 10 small samples in a 10 or 20 l bucket.
4. Wear nitrile, non-powdered gloves. If no gloves available, first rub hands in some of the soil that has been discarded, to reduce contamination from hands.
5. Mix the sample in the bucket. It is critical that the sample is well mixed to be representative of the site.
6. Take a large handful of mixed sample (ca. 400 g) place in a zip-lock bag. Turn the top of the bag inside out to protect the zip.
7. Roll up the bag around the sample, expelling the air before sealing it.
8. Place this sample bag inside a second bag.
9. Write the label containing: site name, date of collection, location, and sample code on card or water-proof paper with water proof ink or pencil.
10. Place the label inside the second bag facing out (can be read) and seal the second bag, expelling the air.

11. Store the sample in the dark in a cool place or container for transport to the laboratory. **Do not add ice.**

Details:

Step 1: The sampler used to take the 10 small samples must be able to take the same sized sample area (not critical) and thickness (20 mm) at each sub-location in the site. This is important to avoid bias. An example of a suitable sampler is shown in Figure 2. Each small sample is taken at about 5 m from the first in a grid pattern e.g., take sample, move 5 paces, take next sample move 5 paces, turn 90 degrees move 5 paces take sample, and so on until the 10 samples have been taken. This will cover an area of about 100 m². Exceptions to 10 small samples are explained in the main text under sampling strategies.

Step 2: Remove leaf litter, roots, stones, leaves and insects by hand picking. A coarse sieve (5mm mesh) may be useful in this step but is not essential. Shake or “rumble” the soil from crop or grass roots. Fine roots cannot be removed and are not an issue.

Step 9: Labelling the sample must use a meaningful name or code plus a date and location. This information is written on a water resistant card or waterproof paper in water proof ink or pencil.

The use of waterproof marker pens on the plastic bag is not recommended as the marker pen ink does rub off and could contaminate the sample.

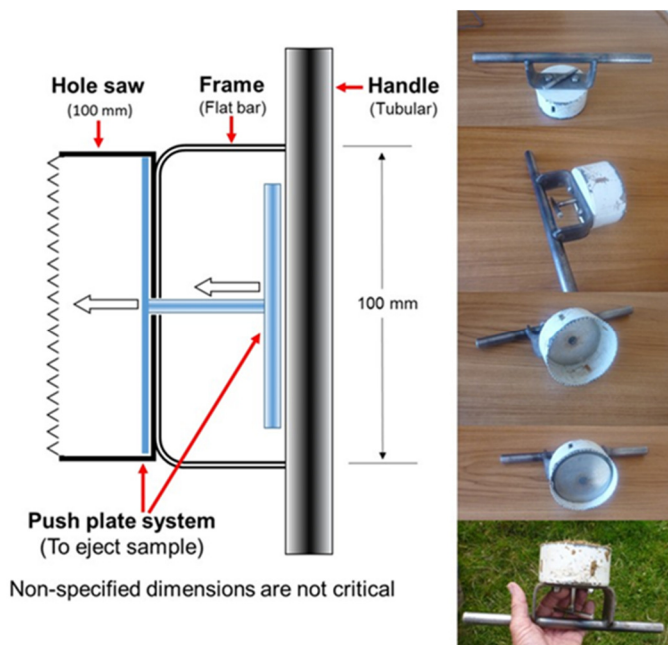


Figure 2: Schematic diagram of a hand corer suitable for taking soil and some sediment samples. The corer body is a 100 mm diameter hole-saw, which allows small roots to be cut by turning the corer as it is pushed into the soil. Turn the corer as it is pulled out of the ground to retain the soil plug in the corer body. Note that the handle frame is wide enough to allow all fingers through to grip the handle and the T-bar on the push plate system. Pushing on this T-bar extrudes the sample for trimming to 2-cm thickness and subsequently ejects the sample into the mixing bucket.

2.7 Processing

2.7.1 Drying

SOP:

1. Write the sample code on the side of a small aluminium or stainless steel tray.
2. Transfer the raw sample from the plastic bag to the tray.
3. Place the tray inside a new plastic bag leaving the end open.
4. **Oven drying:** place the sample tray in its bag in the oven at 60°C.
5. After two hours, stir the sample with a stainless steel spatula to prevent the formation of a hard cake / brick.
6. Continue heating until the sample is dry (overnight or for 24 hours, or longer as required).
7. Break up the lumps with a hammer or roller.
8. Sieve whole sample through a 2-mm mesh sieve to collect the <2 mm size fraction.
9. Briefly grind lumps >2mm in a coffee grinder or pestle and mortar (but not too fine).
10. Re-sieve and combine the <2 mm size fractions.
11. **Freeze drying:** freeze the sample to -20°C then place in the freeze drier.
12. The freeze dried sample will crumble and can be sieved directly without grinding.
13. Discard the >2 mm size fraction or grind and sieve as in step 9.
14. Store about 200 g of dry, sieved sample sealed in a properly labelled zip-lock bag at room temperature, in the dark.
15. The dry sample can be stored for years.
16. A Quality Assurance (QA) sample can be included with the batch at this point.

Details:

It is important to use a sample grain-size of <2 mm to aid extraction efficiency but do not use just the size fraction below 64 µm – use the whole size range below 2 mm.

Step 3: Placing the tray in the plastic bag stops contamination from material falling into the tray from other samples in the oven.

Step 4: Heating above 60°C may lose the volatile shorter chain-length fatty acids (C12:0, C14:0).

Step 6: Drying to constant weight is not critical, but the sample needs to be dry enough to process through the sieving. The wetter the sample, the longer it takes to dry.

Step 9: A coffee grinder can produce a very fine powder that may cause problems during extraction. Ideally the sample should have particles in the size range <2 mm and >0.1 mm. However, clays will be <0.1 mm, so all particles <2 mm are collected and used.

2.7.2 Measuring percent organic carbon (%C)

This information is required for the estimation of the amount of sample to be weighed for the determination of bulk $\delta^{13}\text{C}$ and used for the extraction of fatty acids.

SOP:

1. Weigh a small aluminium pie dish (about 5 cm in diameter) and record the weight.
2. Place an aliquot of about 5 g of dry sample in the pie dish and record the total weight.
3. Heat the pie dish and sample at 450°C for 3 hours in a muffle furnace.
4. Allow to cool, then reweigh and record the total weight.
5. Calculate the total organic matter (TOM) as the loss of weight on ignition at 450°C.
6. Estimate the total organic carbon (TOC) content as $\text{TOM} \times 0.47$.
7. Express the result as a percentage in the dry sample = %C

Details:

This loss-on-ignition (LOI) process can be replaced by analysing the sample using a TOC analyser, if one is available. If not, use this method and the calculation is as follows:

Original sample weight = (dish + sample) weight – dish weight

Final sample weight = (dish + sample) weight after heating – dish weight

$\text{TOM} = \text{original sample weight} - \text{final sample weight}$

$\% \text{TOM} = (\text{TOM} / \text{original sample weight}) \times 100$

$\% \text{C} = \% \text{TOM} \times 0.47$

Point to note: The set combustion temperature is 450°C. Above this temperature, the aluminium dish may melt as the muffle furnace temperature can fluctuate around the set temperature.

2.7.3 Preparation for bulk $\delta^{13}\text{C}$ Analysis

SOP:

Remove inorganic carbonate

1. Transfer about. 5 g aliquot of sample into a 50 ml plastic screw-cap centrifuge tube.
For the following procedure wear a face mask and gloves, and have water either running or immediately available to wash any acid splashes from skin
2. Add 2 to 5 ml 10 % HCL to the sample in the centrifuge tube, stir with plastic spatula to mix the sample with the acid. **DO NOT SCREW ON THE LID AND SHAKE!**
3. When fizzing has stopped, add another 2 ml of 10 % HCl and stir.
4. **Repeat step 3 until no fizzing occurs when the acid is added and the sample is stirred**
5. Make up the volume to 50 ml with distilled or deionized water – screw on cap and shake vigorously for 30 seconds.
6. Centrifuge at 3000 rpm for 10 min.
7. Remove the cap, decant and discard the liquid into running water. **Caution acid.**
8. Make up volume to 50 ml with distilled deionized water – screw on cap and shake vigorously for 30 seconds.
9. Centrifuge at 3000 rpm for 10 min.
10. Remove the cap, decant and discard the liquid into running water.
11. Hold the tube on its side and smear the sediment on inside of tube to aid drying.
12. Lay the tube on its side in a tray and oven-dry at 60 °C.
13. When dry, break up the sample with a spatula then screw on the lid.
14. Label the tube using as sticky paper label written in pencil.
15. Dispatch tube to the analytical laboratory to measure bulk $\delta^{13}\text{C}$ (for CSSI) and %C (for percent soil conversion). **Include the estimate of %C in the sample for the analyst.** %C = % organic C (by loss on ignition) multiplied by 0.47.

Details

Step 2: When acidifying soil to remove inorganic carbonate, **NEVER screw the cap on and shake.** If there is high carbonate in the soil, the mixture will develop pressure which will spray acid and sample from the tube.

Steps 3 and 4: These steps are critical. If there is any inorganic carbonate left in the soil, the $\delta^{13}\text{C}$ value of the organic matter will be over-whelmed by the $\delta^{13}\text{C}$ value of the inorganic carbonate and the data will be useless.

Steps 6 and 9: After centrifuging, check that the plastic centrifuge tube has not developed cracks or become distorted. Some brands may do this, in which case use a lower speed (e.g., 2500 rpm) for longer (e.g., 12 minutes). Centrifuging is not critical but the rinsing process is designed to get rid of the acid with a minimal loss of sample.

2.7.4 Preparation for $\delta^{13}\text{C}$ Analysis of fatty acids

SOP:

Extract the sample

1. Take an aliquot of dry, non-acidified sample (plus one QA sample) and use about
 - 10 to 20 g for a medium organic soil (5-10 %C)
 - 30 to 60 g for a low organic sandy soil (<1 %C)
 - 2 g for plant material (47 %C).
2. When using an ASE, the sample is placed in the sample cell in the ASE machine and the selected extraction program is run. The extraction procedure takes about 15 minutes per sample. The sample extract comes out of the ASE in a sealed bottle without any soil.
3. For Soxhlet extraction, reflux the sample with about 150 to 200 ml of DCM for 10 hours. The sample is retained in the reflux flask without any soil.
4. For Shaker (takes 24 hours) or ultrasonic (takes 6-8 hours) extraction, the sample is soaked in 100 ml DCM, in a glass 250 ml Erlenmeyer flask with a ground-glass stopper. These samples must be filtered to remove the soil.
5. Reduce the solvent to dryness in a 100 ml, round-bottom flask using a Buchi Evaporator, and retain the solvent for recycling through the distillation system.

Details:

Step 1: Re-dry the sample over night before using to eliminate moisture in the sample.

Step 2: When small samples are being extracted in the ASE, add an additional filter pad on top of the sample and fill the rest of the cell with clean quartz sand. This reduces the amount of DCM used in the extraction process.

The ASE extraction program uses two extraction cycles with the sample cell held full of DCM at 100°C and compressed to 2000 psi for 5 minutes on each cycle. If the DCM extract in the ASE bottle is cloudy, add a few grams of anhydrous sodium sulphate, to the extract directly in the ASE bottle until the DCM is clear. Then transfer the solvent only, with rinses, to the 100 ml round-bottom flask.

Step 4: Use a solvent cleaned GF/F Whatman glass fibre filter (<1µm pore size) on a clean glass vacuum filtration system to remove the sample from the solvent.

- The vacuum pressure used is about 20%.
- The sample is rinsed with a small amount of DCM to recover as much of the fatty acids as possible.

- Rinse the solvent into a 500-ml round-bottom flask for the Buchi evaporator and transfer to a 100-ml round-bottom flask for the final stage to dryness. The water bath temperature on the Buchi is 35°C and the vacuum pressure is about 50%.

2.7.5 Derivatise the fatty acids to fatty acid methyl esters (FAME)

SOP:

1. Prepare all reagents each day before starting this process.
2. Take up the dry extract from the 100 ml round-bottom flask in 2 ml of DCM.
3. Using a cleaned Pasteur pipette (Figure 3), transfer the DCM extract into a 10 ml Kimax screw-cap reaction tube with Teflon lined seal in the cap. Smaller 4 ml tubes can also be used (Figure 4A).
4. Rinse the round bottom flask with another 1 to 2 ml of DCM and use the Pasteur pipette to transfer the rinse to the Kimax tube.
5. Dry the extract in the Kimax tube by warming to 40°C in an aluminium heating block.
6. Add 1 ml of 5% BF₃ in methanol to the Kimax tube, screw on the cap, and place in a test tube rack in a fan oven at 70°C for 20 minutes.
7. Remove the test tube rack and place in a cold water bath to cool the samples.
8. To each Kimax tube add 1 ml distilled water and 1 ml of hexane / DCM (4:1) mixture, screw on the cap and mix on a Vortex mixer for 1 minute (Figure 4B).
9. Return the Kimax tube to the test tube rack and allow the solvent layers to separate (Figure 5).
10. Use a Pasteur pipette to transfer the upper (hexane) layer from the Kimax tube to a 2 ml vial (Figure 6); volume = 0.5 ml of hexane.
11. Repeat steps 8 to 9 adding 1 ml of the hexane / DCM mixture. Total volume of combined hexane is about 1ml.
12. Reduce the hexane to dryness in an aluminium heating block at 40°C under a gentle stream of dry nitrogen through a stainless steel needle positioned vertically above the open vial and blowing onto the solvent surface.
13. When dry, screw on the cap and label the vial in pencil on the frosted panel.
14. Dispatch the set of vials to the analytical laboratory to the measure $\delta^{13}\text{C}$ values of the FAMEs.
15. Remember to include a 2 ml vial containing some of the methanol used in the derivatization step to obtain the $\delta^{13}\text{C}$ value of the methyl group added.

Details:

Step 4: A standard containing a mixture of known fatty acids could be included at this stage and derivatized with the samples. This is not needed if a QA sample is included.

Step 5: The 5% BF_3 in methanol reagent is only stable for a couple of days so should be made up fresh each day. The methanol in this reagent is the source for the methyl group (CH_3) being added to the fatty acid during derivatisation. It is advised that one bottle of methanol is kept especially for this process and not used by anyone else. Send a 2 ml vial of this methanol with the FAMES to get the $\delta^{13}\text{C}$ of the methyl group added to the fatty acid (**Step 15**). This will allow correction of the isotopic value of each fame for the added methyl group, converting the isotopic signature back to the value of the fatty acid. (Section 2.8.1).

Step 9: If the mixture is difficult to separate or forms an emulsion, add 1 ml of water and mix on the Vortex mixer. Allow the mixture to stand for about 30 minutes or centrifuge to assist separation. Vigorous shaking by hand is the most likely cause of emulsion formation. The action of the vortex mixer (Figure 4B) doesn't mix air into the solution and therefore emulsions are less likely to form.



Figure 3: Pasteur pipette with rubber squeeze bulb.

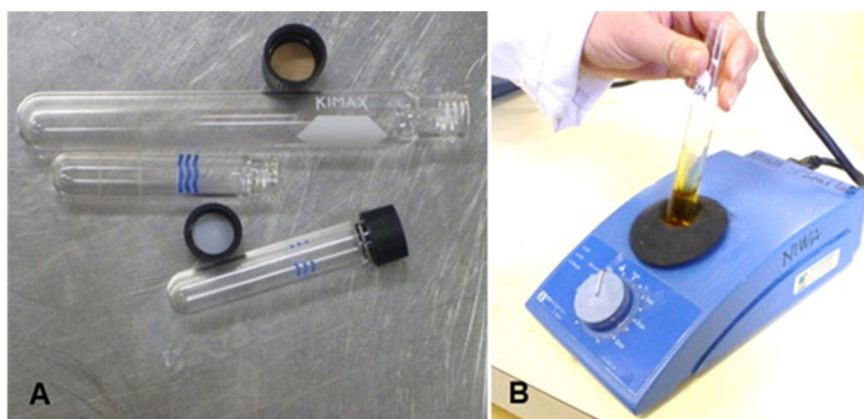


Figure 4: A) Reaction tubes and B) Vortex mixer. These 10-ml and 4 ml digestion tubes are suitable for the derivatisation process. The screw caps have Teflon liners. The vortex mixer does not produce an emulsion as can happen with vigorous shaking by hand. An emulsion will be slow to separate.

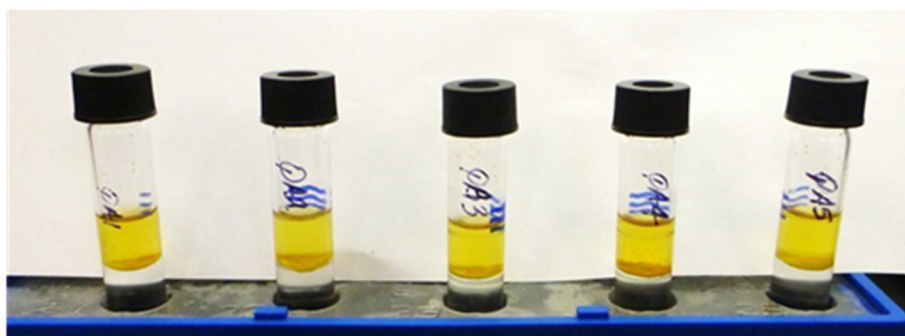


Figure 5: Separation of the hexane layer from the derivatisation process. The colour is not an issue for analysis. However, the thin wax layer between the hexane and aqueous layer should not be transferred to the final vial.



Figure 6: Screw cap 2-ml vials. The cap is fitted with a Teflon-coated silicon-rubber septum.

2.8 Analysis

Analyses should only be done by a competent analyst.

2.8.1 Correct the results for the $\delta^{13}\text{C}$ of the added methyl group

The raw analytical results will usually be supplied for the FAMES and this will include the isotopic signature of the added methyl group (CH_3) from the methanol solvent in the derivatisation step. Unless the laboratory states otherwise, the isotopic results for each FAME must be corrected for the added methyl group.

SOP:

The results from the laboratory for each sample will be:

1. $\delta^{13}\text{C}$ and %C of the bulk sample (after acidification).
2. $\delta^{13}\text{C}$ values of all fatty acids extracted from the sample and converted to FAMES.
3. $\delta^{13}\text{C}$ of the methanol used in the derivatisation step to produce the FAMES.

Because the methyl group added to the fatty acid to produce the FAME will have a different isotopic value than the fatty acid, the isotopic signature of the FAME must be corrected for that addition. The correction for the addition of one methyl group is relatively small but must be done for each fatty acid in each sample. This can be done quickly in a spreadsheet using a simple equation:

$$\delta^{13}\text{C}_{\text{FA}} = \frac{\delta^{13}\text{C}_{\text{FAME}} - (1 - X)\delta^{13}\text{C}_{\text{Methanol}}}{X}$$

Where FA is the fatty acid and X is the fractional contribution of the FA to the FAME. X can be calculated from the number of carbons in the FA molecule divided by the number of carbon atoms in the FAME derived from the FA. For example, the FA stearic acid (C18:0) has 18 carbon atoms whereas the FAME produced, methyl stearate, has 19 carbon atoms including one added carbon from the methanol and thus has an X value of 18/19 or 0.9474.

- Correct all FAMES to produce the CSSI isotopic signatures.
- Correlate the CSSI values with the bulk $\delta^{13}\text{C}$ and %C from each sample in a spreadsheet.

Use these data in the mixing model for deconstructing the mixture into soil source proportions.

2.8.2 Convert isotopic proportions to soil proportions

The outputs from the mixing model are isotopic proportions not soil proportions (Gibbs 2008). As the isotopic biomarkers are a small fraction of the total organic carbon in the soil and the total organic carbon is typically less than 10% of the whole soil, the isotopic proportions must be converted to soil proportions. That is, if the source soils were mixed together in the corrected soil proportions, the resultant mixture would have the same isotopic signatures as found in the sediment mixture.

SOP:

This conversion uses a linear correction equation based on the carbon content of each source soil:

$$\%_{\text{source}_n} = \frac{I_n / \%C_n}{\sum_n (I_n / \%C_n)} \times 100$$

where I_n is the mean feasible proportion of source n in the mixture as estimated from isotopic values of carbon by the mixing model, and $\%C_n$ is the % carbon in the source n soil.

Because this calculation only uses the %C of the source soils for scaling, the proportional contribution of each source soil is independent of any loss of total carbon or FA in the sediment mixture through biodegradation. The level of uncertainty defined by the standard deviation produced by the mixing model remains the same.

3 CSSI technique overview

3.1 Development concepts

Traditional geological techniques for determining the origin of soil rely on the elemental composition and the crystal structure to characterise the soil particles. This technique works well on a regional scale but cannot be used on a watershed scale where the soil geological characteristics may be uniform. Consequently, an alternative method of characterising the soil was required that would allow its source to be identified when working at the watershed scale.

Flora and fauna (including microbiological) in a particular habitat produce substantial amounts of organic compounds that can be stabilized to some extent in soil. Consequently some of these organic compounds can be used as biomarkers to check for the presence of particular flora and fauna in a habitat (e.g., Liang et al. 2008). One group of organic compounds often used as biomarkers are the straight-chain fatty acids (FA), especially those with a carbon chain-length from 14 to 24. Fatty acid methyl ester (FAME) analysis of a soil can provide a “fingerprint” characteristic of that soil based on a concentration profile of the FA present. That soil profile can then be compared with a library of similar profiles of soils from known geographical locations, to identify the source of that soil (Kennedy 1998; Ibekwe and Kennedy 1999). This approach has limited ability to separate soil sources in mixtures and, when used to estimate the origin of sediments in surface waters (Banowetz et al. 2006), it was found that the FA biomarkers rapidly degrade (Muri et al. 2004).

Stable isotopes are one of nature’s ecological recorders (West et al. 2006) and an advance on the FAME profile technique was to look at the stable isotopic signature of the carbon atoms in each compound i.e., the compound-specific stable isotope (CSSI) value of each FA. It was found that the CSSI values of the organic compounds present in soils were even more characteristic for flora and fauna species and habitat (Chikaraishi and Naraoka, 2003). Moreover, it was demonstrated that once formed, the CSSI value of the individual organic compound didn’t change significantly (Boyd et al. 2006), making CSSIs a valuable tool in multiple research fields such as palaeoenvironmental and archaeological dietary pattern reconstruction (e.g., Spangenberg et al. 2008; Zech & Glaser, 2008). CSSI analyses have also been used to differentiate between terrestrial and aquatic sources of sediments (e.g., Tolosa et al. 2004; Wang et al. 2008) and to estimate the contribution of C₄/C₃ plants to organic matter in the sediments (Bull et al. 1999; Chikaraishi & Naraoka, 2005).

The concepts associated with CSSI analysis of soil FAME compounds were applied to the task of identifying the source of soil erosion (Gibbs, 2008). The key factors making this possible were: 1) the CSSI values were stable when bound to soil particles and could survive hundreds of years unchanged; 2) the CSSI values were characteristic of specific flora and fauna growing on the soil; and 3) land-use is typically defined by the flora and fauna on the land. Conceptually, the CSSI values from the eroded soil represent the isotopic signatures of the soils contributing to the eroded soil mixture. By having a set of reference soils or “library” of CSSI values from known land-use soils from the same watershed, the proportion of each source soil contributing to the eroded soil mixture could be estimated using a mixing model.

The CSSI technique is being used in studies reported in the literature (e.g., Hancock & Revill 2011; Blake et al., 2012).

3.2 Stable isotope basics

Fundamental to understanding how the CSSI technique works is a basic understanding of stable isotopes, the terms used, how stable isotopes are measured, and how the measured values are interpreted.

3.2.1 Terms

Stable isotopes are not radioactive and therefore do not decay over time. A stable isotope is an element with one or more additional neutrons in the core of each atom giving it additional mass. Both the element and the heavier isotope of the element are stable isotopes. In order to distinguish between the stable isotopes of an element, the mass number is included as a super-script before the element symbol. For example, the element carbon (C) has 2 stable isotopes, the most common or abundant isotope (98.89% of all C) has a mass of 12, is written as ^{12}C , and is referred to as the light isotope. The heavier isotope of carbon (1.11% of all C) has one extra neutron giving it a mass of 13, is written as ^{13}C , and is referred to as the heavy isotope. These proportional concentrations are the “natural abundance” of the carbon isotopes.

The heavy isotope, ^{13}C has the same chemical properties as the light isotope, ^{12}C . Because of the extra neutron, the ^{13}C atoms are larger than the ^{12}C atoms causing the rate of reaction to be slower than ^{12}C . This difference allows ^{12}C to pass through a cell wall faster than ^{13}C . This results in slightly more ^{13}C remaining on the starting side and slightly more ^{12}C reaching the other. This discrimination process is called isotopic **fractionation**.

The difference in ^{12}C and ^{13}C concentrations due to fractionation through one cell wall is small at around 1 part per 1000 (‰), commonly called 1 per mil, which is written 1 ‰. In biological processes, the 1 ‰ step per transfer through a cell wall is essentially constant and is referred to as a trophic step.

By convention, the changes caused by fractionation are expressed in terms of the heavy isotope only i.e., the source pool of ^{13}C increases or becomes isotopically enriched by 1 ‰ while the destination pool decreases or becomes isotopically depleted by 1 ‰.

3.2.2 Measurement

This difference due to fractionation can be measured by mass spectrometry and results in a change in value for ^{13}C from 1.11% to 1.111% on the starting side of the cell. Because the % numbers are unwieldy, a more user-friendly system was developed for expressing this difference, focusing on the change in concentration relative to an international standard, rather than the actual change in concentration. The difference is expressed in delta notation (δ) and the δ value is calculated using the equation:

$$\delta^{13}\text{C} = \left[\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] \times 1000 \text{ ‰} \quad (\text{per mil})$$

where R is the molar ratio of the heavy to light isotope i.e., $^{13}\text{C}/^{12}\text{C}$. The international reference standard for carbon was a limestone, Pee Dee Belemnite (PDB), which had a $\delta^{13}\text{C}$ value of 0 ‰. As this primary standard has been used, secondary standards calibrated to the PDB standard are used.

Instruments commonly used to measure these $^{13}\text{C}/^{12}\text{C}$ ratios are Isotope Ratio Mass Spectrometers (IRMS) which produce the results in delta notation. They are high precision instruments. For $\delta^{13}\text{C}$, IRMS measurements typically have a precision of $\pm 0.1\text{‰}$ or better.

3.2.3 Interpretation

Carbon is introduced into the food chain by assimilation of carbon dioxide (CO_2) from the atmosphere or dissolved in water during primary production by photosynthesis. There are two main photosynthetic pathways, C3 and C4, which distinguish most cool weather grass, trees and shrubs (C3 plants) from warm weather grasses and cereal crops (C4 plants) (Figure 7).

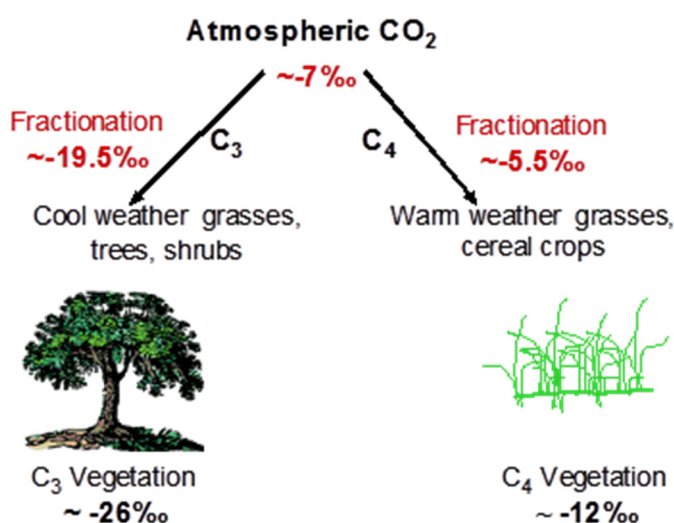


Figure 7: Schematic of the isotopic fractionation that occurs with the C3 and C4 photosynthesis pathways during assimilation of atmospheric CO_2 .

The carbon in atmospheric CO_2 has a $\delta^{13}\text{C}$ value of -7‰ . During photosynthesis multiple reactions or trophic steps occur along the C3 and C4 pathways from CO_2 to the chlorophyll molecule. The C3 pathway is longer than the C4 pathway giving the C3 plants a more depleted $\delta^{13}\text{C}$ values of around -26‰ compared with C4 plants which have $\delta^{13}\text{C}$ values of around -12‰ (Figure 7). The amount of fractionation for the C3 and C4 pathways is not absolute as different plants have slightly different assimilation pathways which alter the actual degree of fractionation that occurs. Consequently, there are range of $\delta^{13}\text{C}$ values for plants classified as C3 and C4 with the mean $\delta^{13}\text{C}$ values being -26‰ and -12‰ , respectively (Figure 8). These isotopic values are measured on whole plant material as “bulk” $\delta^{13}\text{C}$ values, which are characteristic of the individual plant.

Note that a mixture of several different plants will produce a bulk $\delta^{13}\text{C}$ value that is representative of the proportional contribution of the individual plant species in that mixture. This mixed isotopic signature can be used to discriminate between similar land uses. The range of possible plant communities associated with a specific land-use may vary from place to place giving rise to different $\delta^{13}\text{C}$ values for the same apparent land-use at different locations within the same watershed. These differences may also be caused by animals grazing on pasture such that it is possible to discriminate between pasture used for sheep, beef, dairy, deer and other stock grazing.

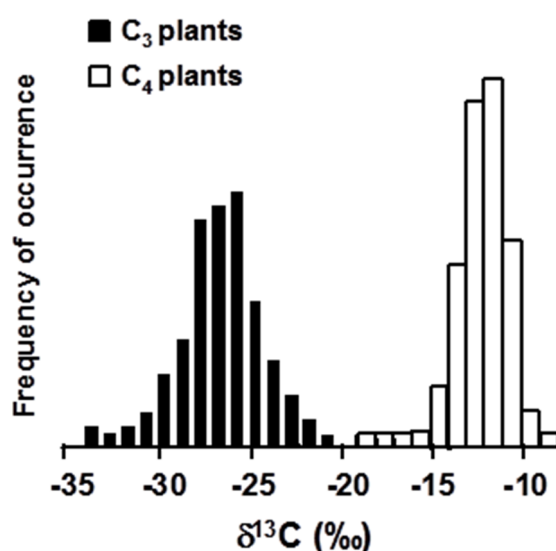


Figure 8: Relative frequency of occurrence of bulk $\delta^{13}\text{C}$ values for C_3 and C_4 plants.

3.3 CSSI

3.3.1 Plant compounds

While the plants are grouped according to their photosynthesis pathway to produce chlorophyll, plants also produce a range of other compounds (Table 1) which each have different $\delta^{13}\text{C}$ values depending on how they were synthesized by the plant. Together these compounds comprise the plant carbon and, consequently, the $\delta^{13}\text{C}$ values of these compounds make up the bulk $\delta^{13}\text{C}$ value for that plant.

Table 1: Plants produce a range of compounds in 4 main groups.

Carbohydrates	Proteins	Lipids	Nucleic acids
Cellulose	Amino Acids	Fatty Acids (Saturated & Unsaturated)	DNA, RNA
Starch	Enzymes	Sterols	Alcohols
Sugars		Waxes & Resins	Steroidal glycosides
		Fats & oils	Alkaloids
		Hormones	Aromatics

Soil without organic matter is composed of only inorganic minerals and therefore the soil has no $\delta^{13}\text{C}$ value. The compounds produced by the plants growing on the soil become incorporated into the soil from their roots and the whole plant when the plant dies and, as such, the soil acquires a bulk $\delta^{13}\text{C}$ value representative of the plants growing in/on that soil. Compounds from the most recently grown plant community will be strongest in the upper soil layer while compounds from earlier plant communities will be more dominant in the deeper layers.

3.3.2 Biomarkers

Some of the compounds produced by plants can be used as labels or “biomarkers” for a soil and, by definition, the land-use of that soil. To characterise the soil as a specific land-use, the compounds being considered as biomarkers need to be stable, long-lived, tightly bound to the soil particles, abundant, and easily measured. They also need to have a characteristic which is unique. In this case that unique characteristic is the $\delta^{13}\text{C}$ value of the compound produced by that plant.

For the CSSI technique, the biomarker compounds of choice are the fatty acids (FA), in particular, the straight-chain saturated FA with a carbon chain length of 14 to 24 atoms (C14:0 to C24:0) and an even number of carbon atoms. This group of FA are partially water soluble, due to the acid group being deprotonated at the neutral pH range of most waters, and can be carried down into the soil with infiltrating rain water where they bind to the fine soil particles, especially the clays. As biomarkers, the FA bound to the soil particles are not small pieces of plant material, they are an integral part of the soil particle.

Note that bacteria typically produce large amounts of FA with odd numbers of carbon atoms (e.g., C17:0 and C19:0) and these FA should not be used in the CSSI technique.

The mean isotopic depletion relative to the photosynthesis fractionation for bulk FA for C3 plants is $26.5 \pm 0.6 \text{ ‰}$ and for C4 plants is $10.3 \pm 0.5 \text{ ‰}$ (Ballentine et al. 1998; Hobbie & Werner 2004). There is also a range of isotopic depletions for individual FA produced by a single plant species. Once the FA has become bound to the soil particle, the isotopic signature of the FA is locked and cannot change. Degradation or breakdown (diagenesis) of the FA produces other compounds which are no longer part of the pool of that FA bound to the soil. Consequently, the isotopic signature of the FA pool in the soil does not change through diagenesis (Boyd et al. 2006), although the concentration of FA in the soil will decrease (Banowetz et al. 2006).

3.3.3 Biomarkers to land-use

Because C14:0 to C24:0 FA are produced by all plants and each plant species produces each FA with a $\delta^{13}\text{C}$ value characteristic of that plant, different plants can be distinguished by the FA-specific $\delta^{13}\text{C}$ value (i.e., the compound-specific isotopic value). When leached from the plants, FA enter the soil where they become bound to various clay particulate phases, preserving the isotopic value of the plant FA in the soil. Accordingly, the unique isotopic values of the plant FA biomarkers can be used to identify the origin/land-use of the soil. Because land-use is defined by the plant communities growing on it, e.g., pasture, crops, forestry, the land-use contributing to sediment from erosion can be identified by the FA biomarker isotopic signatures (i.e., their CSSI values). Except for monoculture crops, the plant communities growing on the land are likely to include a mixture of different plants. Consequently, the blend of FA in the soil for any land use is likely to be unique and can provide extra detail for identifying it as a soil source where erosion is occurring.

3.3.4 Deconstruction of the sediment into land-use sources

The CSSI values of the FA biomarkers in an eroded sediment mixture are derived from the FA biomarkers in the source soils contributing to that mixture. If the CSSI values of the FA biomarkers in the source soils are known, their proportional contribution in the mixture can be estimated using a mixing model. For the CSSI technique, the mixing model used during

development was IsoSource (Phillips & Gregg 2003) (Section 8). New mixing models such as SIAR (Stable Isotope Analysis in 'R') and MixSIAR can also be used (Section 9). A table of bulk stable isotope and CSSI values from representative potential source land-use soils are used as a library for the mixing model. Because the proportional contribution of each source soil is determined from the carbon content of the soil (%C), the model is run using bulk $\delta^{13}\text{C}$ as the primary isotope in the model. This allows the subsequent correction of the model output for the proportion of non-carbon material in the soil. The CSSI values of selected FA are used as the additional isotopes in the model to provide discrimination between different land-use sources from the same watershed. Increasing the number of FA used in the model enhances the power of discrimination between more similar land-uses. There is, however, a trade off in model run time and size of the memory required as the number of isotopes and sources is increased. This is less noticeable when using SIAR.

An important consideration for modelling is that the FA used in the model must be present in the sediment mixture and all the sources being tested. If a FA is not present in a particular sample then an alternative FA should be selected that is present in all samples. Alternatively, a larger amount of that sample should be extracted to ensure that FA is measured in that sample.

3.3.5 Hidden sources of biomarkers

As biomarkers, FA are primarily produced by plants. However, they are also excreted by animals grazing on those plants. The FA excreted by animals blend with the FA in the soil and allow discrimination between pasture grazed by different animals.

Some farming practices use feed-lots to feed animals, which are then allowed to free range. The feed used may be locally produced hay or silage, but more commonly, farmers are using imported (i.e., from outside the watershed) feeds, such as corn and maize, both C4 plants, and even palm oil kernel. When the animal waste is spread on the land, these exotic FA CSSI values are incorporated into the soils of the watershed, giving a different set of both CSSI and bulk $\delta^{13}\text{C}$ values for that land-use. Similarly, animal waste used as fertiliser in paddy fields will alter the bulk $\delta^{13}\text{C}$ and set of CSSI values relative to the soil isotopic values arising from rice being grown in those fields.

Each of these hidden biomarker sources provides an opportunity for higher precision in the identification of land-use sources and thus land-use practices contributing to soil erosion.

3.3.6 Limitations

- The results from the CSSI technique are feasible “best estimates” within definable limits. These limits are defined by the 95% confidence interval, which is equivalent to two standard deviations of the data.
- The CSSI technique provides qualitative proportional contributions of each source in the mixture. Additional mass transport data is required to quantify the sediment loads and to estimate fluxes.

3.3.7 Additional supporting evidence

Some land-uses include plants which may produce a unique compound, other than the suite of FA. While such compounds cannot be used in the mixing modelling because they are not

present in all samples, they can be used to confirm the presence or absence of soil from that land-use in the sediment mixture as identified from the FAs.

For example, pine trees produce a range of resin acids which are normally not present in broad-leaf plants, grasses or crops. The primary resin acid is abietic acid, which has an aromatic carbon ring structure. This and other resin acids can bind to soil in the same way as FA, however, abietic acid is sensitive to sunlight and breaks down to form dehydroabietic acid (DHAA) after a few weeks, and DHAA, in-turn, breaking down after a few months (McMartin 2003). These characteristics provide useful temporal perspectives on recent linkages to pine forest land-use. If these resin acids are present in the sediment mixture then soil from pine forests, identified and apportioned using FA, is confirmed. If abietic acid is present, then that sediment was deposited within the previous few weeks. If abietic acid is not present but DHAA is, then that sediment was deposited several months prior to sampling. If neither resin acid is present, then that sediment was deposited more than 6 months to a year prior to sampling.

3.3.8 Where the CSSI technique may not work

The CSSI technique may not work in situations where the “soil” is very low in organic matter (OM). The CSSI technique relies on the isotopic signatures of FA in the OM and FA comprise less than 1 thousandth part of the organic carbon in the OM. Consequently, in some soils such as sand and geological rock formations, the OM content may be so low that isotopic signatures cannot be obtained for FA, and possibly even the bulk carbon, when extracting practical sample sizes.

3.3.9 Interference and contamination

Older deposits of sediment may be “contaminated” with FA of new assemblages growing on the surface e.g., river bank deposits. These contaminants may provide a new unique label allowing the subsequent erosion of these bank deposits to be tracked further downstream but may interfere with or mask the original signatures of the source soils contributing to the bank deposits. However, as bank deposits are likely to have sedimented during a single large event, the sediment in the bank deposits should have been essentially homogeneous when deposited. Consequently, a sample taken from a core deep into the bank material is more likely to contain uncontaminated signatures of the original source soils.

Similar interference problems may occur on intertidal mud flats in estuaries, where local algae may coat the surface of older sediments. In this case, because the sediment is laid down in thin layers over time, coring is not an option and the algal layer may need to be removed by scraping before collecting the sediment sample. Note that the scraped material should be kept and analysed as a separate source to aid interpretation during the modelling.

Actual FA contamination of samples can occur during handling and processing of the soil. This can come from using equipment where the history of previous use is unknown and there has been inadequate cleaning. Handling raw plant material or shellfish with the sampling equipment during sampling or processing can introduce FA which may not be in the soil or sediment being sampled or processed. In general, care of the equipment includes knowing the previous use and how it was cleaned, in order to reduce the risk of contamination. As a further precaution, the bulk samples collected are large to dilute out any unexpected contaminant from equipment.

3.3.10 Theory of washing and the risk of contamination

The theory of washing assumes that after each wash a small proportion of contaminant will remain — say 1%. Starting at a ‘concentration’ of 1, the residual contaminant ‘concentration’ would be 0.01 after the first wash, 0.0001 after the second wash and 0.000001 after the third wash. If the analytical detection limit is 0.0001, only two washes are required to take the contaminant below detection level.

Conversely, the theory of contamination assumes that, as the sample is extracted, the potential for contamination from, in this case, FA on fingers remains the same — say 0.1 mg contaminant per transfer step. If the soil sample collected has a mass of 100 g, the 0.1 mg contaminant from fingers is 0.0001% of the total sample and thus negligible. However, as the extraction proceeds and the extract mass is reduced to around 2 mg in the final vial, the 0.1 mg contaminant from fingers is equivalent to 5% of the total sample. That is significant contamination that could affect the final isotopic signatures of the FA in the sample. Consequently, gloves and clean laboratory techniques must be used for the final stages of the sample preparation for compound-specific stable isotope analysis (CSIA).

4 Experimental design

4.1 Uncertainty

Experimental design is a critical part of the successful use of the CSSI technique. Decisions made at the design stage can influence the outcome of the study, especially the level of uncertainty in the results. Uncertainty can be assumed to be the sum of all error terms or variability in the procedure.

The error terms for the analytical methods and modelling should be well defined and relatively small. In contrast, the largest error terms occur during sampling and are mostly derived from poor experimental design. Variability due to sampling can be estimated using statistical techniques and thus the level of uncertainty in the sampling protocols can be managed within acceptable levels as a compromise between the number of replicate samples collected at a location (sampling design) and the available resources to analyse them (costs).

Even the best, statistically robust, sampling design, however, may be ineffective if the experimental design is poor, or wrong. The experimental design must be appropriate for the objective of the study being undertaken.

4.2 Determining what question is being asked

The question being asked should be unambiguous and the experimental design should provide the information required to answer that question unambiguously with the minimum number of samples. The question may contain information or expectations both explicit and implicit.

For example, in a CSSI development study (Gibbs 2008), the question asked was:

Does production pine forestry cause excessive sedimentation in the estuary?

Although not stated, the question recognises that there were multiple main land-uses in the watershed contributing sediment to the estuary, one of which was production pine forestry. The question also implies that there is an expectation that sedimentation from each land-use should be proportional to the area of that land-use, allowing for any land-use sediment runoff factors. With respect to runoff factors, a New Zealand study on paired watersheds found that over a 12-year period, the farmed (pasture) watershed produced four times more sediment runoff than an adjacent watershed in mature pine forest (Eyles & Fahey 2006). This sediment runoff factor gives an expectation that, per unit area of land, all pasture will produce four times more sediment than mature production pine forest, but it may not apply to young or clear-fell harvested pine forest.

The experimental design for the study was therefore required to test whether per unit area of land, pasture produced four times more sediment than pine forest land-use in the study watershed. The minimum sampling strategy was to obtain a representative sample of potentially erodible soil from each of the three major land-uses in the watershed (i.e., the reference library) to evaluate the relative contribution of each source soil to the sediment mixture in the primary deposition zone in the estuary, the river delta.

To answer the question required just four samples, one soil sample from each major land-use type and the sediment sample from the river delta at the head of the estuary.

The interpretation as to whether production pine forestry causes excessive sedimentation in the estuary relies on the areal proportion of watershed used for pine forest relative to other land-uses and the relative proportion of each of the main land-use soils contributing to the sediment in the river delta. If the ratio of pasture to pine forest relative to land-use area is less than 4 to 1, the answer is probably “Yes”.

While this is a valid result, it is not necessarily very helpful.

4.3 A different question requires a different study design

The original question could be restated as:

Where is the sediment in the estuary coming from?

This requires a more detailed evaluation of the potential sources of sediment to the estuary, including both land and sea.

Sediment from coastal erosion can be carried into the estuary on the flood tide. This may include sediment from land (terrigenous sediment) previously flushed out of the estuary on the ebb tide. Terrigenous sediment from the estuary watershed can come from multiple land-use sources, with similar land-use sources in different parts of the watershed. Each of these land-use soils would need to be sampled as would the coastal sediments outside the estuary, to provide the reference library for the modelling.

With a river inflow at one end and the ocean at the other, theoretically there will be a gradient down the axis of the estuary from land to sea in the amount of sediment from these two water sources, with 100% terrigenous sediment in the river delta progressively decreasing down the estuary to near 0% at the mouth of the estuary.

In practice, as there are buoyancy differences between fresh and marine water, the terrigenous sediment in the surface freshwater layer will be carried swiftly out of the estuary on the ebb tide, but will be dispersed around the edges of the estuary on the flood tide, especially if the estuary has fringing mangroves. If there are multiple river inflows to the estuary, this tidal redistribution raises the very real possibility that sediment from one riverine arm may be deposited in the arm of another river. This potential problem can be resolved by always including a river delta sample in the sampling strategy.

The issue is then where to collect the estuary sample. If the question is interpreted to mean “the sediment across the whole estuary”, then a spatial sampling pattern across the estuary involving many samples, is required. The watershed boundaries must include all land draining into the estuary from the confluence of the estuary with the sea. This requires a large study and the end result will provide a spatial distribution map of sediment deposition, by land-use, in the estuary.

If the question is interpreted to mean “as the sediment enters the estuary”, then only the river delta should be sampled as the point of entry of the terrigenous sediment to the estuary. In this case the watershed boundaries must only include the land draining into the river system. This requires a smaller study focussing on the sources of erosion contributing to the

sediment in the river delta and whether these are within expectations by area for that land-use.

4.4 Design strategy

As illustrated in the two previous sections, the question being asked defines the experimental design. The experimental design also depends on a number of factors including the size of the watershed being investigated, the range of land-uses within the watershed, and the presence of any point-source discharges. For example, in a large watershed with multiple tributaries, most soil erosion will be from diffuse sources. However, if one tributary is producing a high sediment load, this may be treated as a point source with respect to the whole watershed.

If the whole watershed has a single land-use such as growing wheat or pine trees, then there is unlikely to be any characteristic difference between different parts of the watershed in which case, the CSSI technique may not be applicable. It is rare, however, for watersheds to have a pure monoculture land-use and there is often an associated understorey of plants that can provide an additional CSSI biomarker label. Similarly, CSSI labels to characterise different parts of the watershed may come from a previous different land-use as part of a crop rotation scheme or land-use change. In pastured watersheds, the animals grazing the land may provide the required CSSI label. In some watersheds the source of the sediment erosion may be readily apparent without the use of the CSSI technique (Figure 9), although it may give the relative proportions of the sources.



Figure 9: A small watershed in pasture with multiple land slips. The CSSI technique is not needed to identify where the sediment is coming from as the sources are obvious. Sediment transport from this sub-catchment may be a point source for a larger watershed.

4.4.1 Break it up: subcatchments versus large watershed-scale

In a large watershed with several smaller sub-catchments, it may not be possible to evaluate the whole watershed as a single unit. A useful strategy is to break the watershed into its sub-catchment components and evaluate each of these to the level required to answer the question being asked.

A primary consideration is the amount of sediment being contributed from each sub-catchment to the river system draining the watershed. This requires a minimum of three sediment samples to be collected at the confluence of each tributary (i.e., one upstream, one downstream, and one from the tributary). Sediment from the tributary and the main river upstream of confluence become the sources for the downstream sediment sample. It is also important to collect sediment from areas of bank erosion (Figure 10) upstream of the confluence as part of the source library. Bank erosion is the re-mobilisation of sediment stored in the river channel from earlier erosion events.



Figure 10: Bank erosion contributes sediment from earlier erosion events stored in the river channel or flood plain to the river. It is only one source as the river is already carrying sediment from upstream.

4.4.2 Evaluate each sub-catchment

Each sub-catchment will have a range of land-uses that may be contributing sediment to the tributary. Evaluate the proportional contribution of each land-use in that sub-catchment to the sediment sample used as the source at the confluence with the main river. This will use the library of land-use soils collected as representative of the whole watershed. However, it may also require additional soil library samples where there is a unique feature in that sub-catchment. Often overlooked as sediment sources are roads constructed with materials transported into the watershed and sub-catchments from outside the watershed (Mukundan et al. 2010).

4.4.3 Re-combine the results

To complete the evaluation of the whole watershed, the results of the sub-catchment components must be combined. The proportional contribution of sediment from each sub-catchment has been estimated at its confluence with the main river channel. These results are given as a percentage of the sediment in the river channel below each confluence. The proportional contribution of sediment to the whole river is estimated by starting at the mouth of the river and working back upstream to the river source. At the mouth of the river, the total sediment is assumed to be 100 % of all sources in the watershed (Figure 11).

If the sediment proportion in the river at the mouth is RM % ($RM = 100$), at the confluence of the first tributary upstream from the river mouth, the total sediment content RM % will comprise TR_1 % from the tributary and RU_1 % from the upstream sub-catchments and bank erosion B_1 %. At the next tributary upstream the sediment contribution to the RU_1 % in the river channel downstream of the confluence will be TR_2 % from the tributary and RU_2 % from the upstream sub-catchments and bank erosion B_2 %. This calculation process continues upstream to the final tributary contribution TR_F %, which leaves RU_F % sediment coming from the head water catchment and this includes any upstream bank erosion (Figure 11).

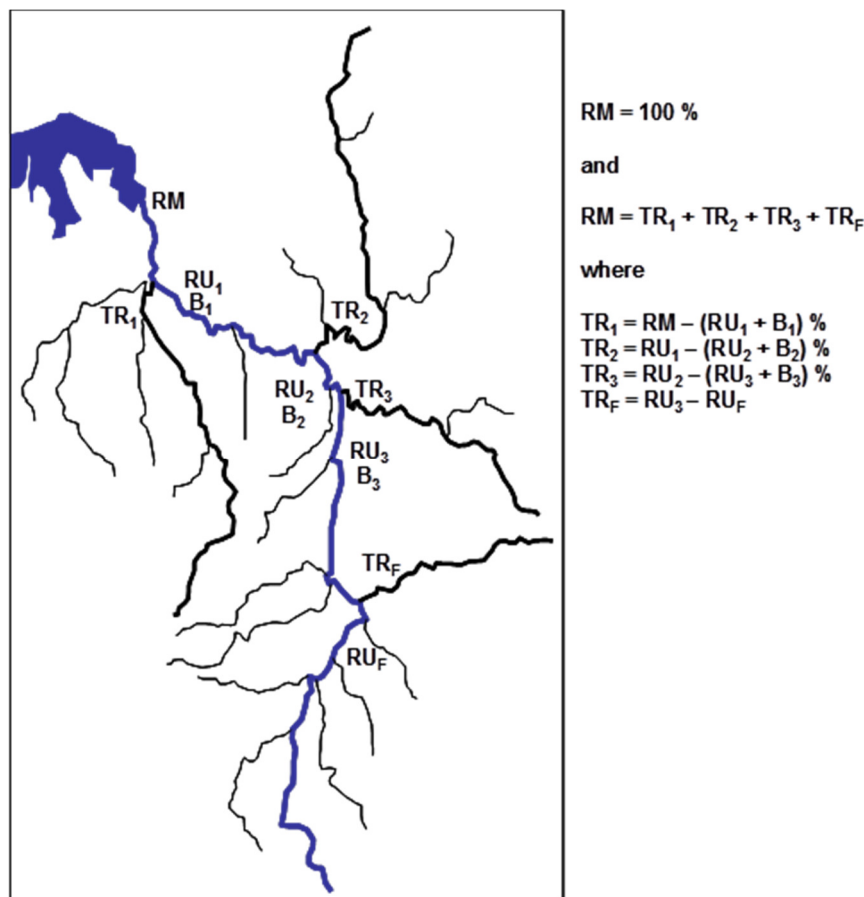


Figure 11: Schematic example of a river system flowing into an estuary. Main river channel (R) (heavy blue line) and major tributaries (TR) (medium black lines) receive sediment from bank erosion (B) in the channel and ephemeral streams (thin black lines) which may only flow during storm events. (See text).

Because the proportional contribution of each land-use in each sub-catchment has been determined at the confluence of each tributary, these proportions can be multiplied by the proportional contribution of sediment from each tributary to the main river (TR1 % to TRF % plus RUF %) and summed to provide the estimate of each land-use contribution within the whole watershed contributing to the sediment leaving the river at the mouth (Figure 11).

The bank erosion component is included in the downstream proportion of the river sediment load at each confluence and can be estimated as a separate source to the river.

Quantification requires mass transport data from FRN analyses within the watershed or a comprehensive hydrodynamic flow analysis with sediment concentrations (either measured or modelled).

5 Sampling

Sampling has two main requirements, **1)** the sample is representative of the land-use being sampled and **2)** the sample is appropriate in terms of location and depth for the study undertaken. The intention of the sampling strategy is to obtain a reference library of land-use soils that can be used in a mixing model to deconstruct the sources of soils in a downstream sediment. In general, this means looking for large, rather than subtle, differences between the source land-use soils.

For example, the investigation of a large watershed (10-100s of km²) with a few dominant land-uses (forest, pasture, crop) repeated in subcatchments throughout the watershed is unlikely to need a statistically rigorous sampling regime in each identical land-use. In this case a single composite sample from each land-use from several different subcatchments across the watershed can provide the information on variance required to assess the level of uncertainty in subsequent modelling outputs. Conversely, the investigation of a small catchment (100s of m²) with multiple different land-uses (crop-1, crop-2, crop-3, crop-4, pasture, vineyard) would benefit from a statistically rigorous sampling regime to estimate the variance of the CSSI values from each to determine whether the different land-uses are truly different or whether there is overlap between some, or whether one set of land-use CSSI signatures are a subset of another land-use, etc.

5.1 Sampling equipment

Specialised sampling equipment is not essential and a simple digging implement such as a spade, shovel, or trowel can be used. Samples can be collected using more sophisticated sampling equipment including coring devices or soil scrapers used for obtaining quantitative samples for FRN analyses. The hand corer (Figure 2) is a light-weight convenient tool for terrestrial soil sampling and for some sediments. The quantitative features of these sampling devices reduces uncertainty when collecting multiple small samples across a large area for combining into a bulk composite sample. The uniform sample size reduces bias due to more of one small sample being collected relative to another.

Because the CSSI technique uses plant FA biomarkers, the only precautions required during sampling is that there is no carry-over of soil from one land-use to another on the sampling equipment. Touching the soil with the hands should be avoided but is not critical unless those hands have been in contact with plant material or a barrier cream such as sunscreen lotions and moisturisers. Even then, the risk of contamination will usually be very slight. To reduce or eliminate the risk, rub some of the soil from the sampling site on the hands.

Sample containers can be plastic bags or plastic buckets but not paper or cardboard containers. While plastic production methods use organic lubricants, these are at very low concentrations and, because they are not FA, will not be measured during the analytical process. The large size of the sample relative to the surface area of the plastic container also reduces the possibility of any measurable contaminant when the whole sample is mixed during processing.

5.2 Storage

The samples should be sealed in the sample bags and stored in containers in a cool dark place or in a refrigerator at 4°C pending processing in the laboratory. The samples should be dried and ground as soon as practical but within a month of collection. The dry ground samples can then be sealed in plastic bags and stored at room temperature in the dark until they are analysed. Use the smallest plastic bag that will hold the sample in order to maintain a high sample-mass to bag-surface-area ratio.

5.3 Representative samples

For each land-use there will be some degree of local variability in soil composition and the plant community growing on the soil due to slope, exposure, orientation to the sun, and the availability of water. Consequently, there is likely to be some variability in the FA biomarker concentrations and potentially the CSSI value of each FA biomarker. Fortunately, because the plant community is the source of the FA biomarkers the variability in CSSI values is likely to be small for each land-use. However, to be representative of the land-use, the sampling must encompass the local variability. This is achieved by taking multiple small samples from within the boundaries of that land-use and combining them into a single composite (bulk) sample. In practice, at least 10 small sub-samples from an area of about 100 m² will provide a representative sample. The exception to this sampling strategy is where soil eroded from a large area with a single land use has accumulated at the bottom of a gully. A single soil sample from that debris accumulation will be representative of the land contributing to it from the catchment above.

Composite samples are used rather than analysing each individual sub-sample collected to give 'average' CSSI values for that land-use. Analysis of each individual sub-sample would provide information on the variability of each CSSI value from that land-use, but in most cases the costs would be prohibitive although the increased data could improve interpretation and statistics using the Bayesian mixing model, SIAR. In contrast, the composite sample captures the essential components of the land-use which should be different from any other land use within the study watershed. There may, however, be small differences between the same nominal land-use in different locations within the same watershed due to differences in understorey plant communities at each location. For example, pine forest on the slopes away from the sun are likely to have a higher proportion of ferns and mosses while those on slopes facing the sun may have an understorey dominated by broadleaf plants. Such differences can be used to discriminate between different areas of that nominal land-use within the watershed and should be sampled separately to provide that information, if that level of discrimination is required to answer the research question that is being asked.

5.4 Appropriate samples

5.4.1 Particle size

Some fallout radionuclide analysis methods for soils and sedimentary systems require the use of the finest particle size, usually clay or colloidal size material (<10 micron), because the fine substrate adsorbs and incorporates the greatest amount of the isotope from areal deposition. This means that the proportion of fine material extracted from the sample must be accurately measured in order to relate the results back to the original soil.

There are issues with this approach, which need to be considered. It is the fine clays and colloidal material that will be eroded first during light rain and the initial phase of a storm event. Consequently, the FRN study needs to address the question of the effect of the dislocation of the FRN tracer from the bulk soil when interpreting the redistribution of soil or sediment.

The alternative approach is to use a less than 2 mm particle size obtained by wet sieving the sample before drying or dry sieving after freeze drying (not oven drying). This is the approach taken in the CSSI technique. Oven drying causes the particles to aggregate so the samples need to be sieved before drying.

The incorporation of FA biomarkers into the soil is primarily from the plant roots. This makes them independent of the FRNs which are deposited from the atmosphere. The FA are polar and will bind with most soil particles. There may be a higher affinity for binding onto finer particles. However, the transfer process from plant to soil relies on water percolating through the soil which means all particles sizes have an equal opportunity to adsorb the FA. As such, separating the sample into fine clay or colloidal material could reduce the strength of the CSSI values for the soil. There is also the potential for selective binding of some FA to finer particles based on the strength of the polar bond and the type of soil particle, for example, clays versus zeolites versus organic substrates. These effects, although potentially very small, could distort the biomarker labelling of the soil in unpredictable ways.

To reduce this risk, the CSSI technique uses the whole soil or sediment that will pass through a 2-mm sieve rather than a sub-set of very fine particles.

5.4.2 Soil samples

Soil samples are primarily used for the soil library and need to include all major land-use types in the watershed. They should also include samples from the same nominal land-use where they occur as substantial areas in different parts of the watershed. With the exception of pasture or grass-type land-uses, leaf litter should be removed from the soil surface before sampling. When sampling soils from pastoral/grass areas, the soil should be shaken or rubbed from the grass roots and combined into the composite sample.

The sediment runoff from any land-use will be mainly from the exposed surface soil or the surface of the soil below the leaf litter layer. Because the FA biomarkers are from the roots of the plant community growing on the land, the highest concentrations of these and other organic compounds from the plants will be in the near-surface soil. An appropriate sample to evaluate contemporary soil erosion, and therefore suitable for CSSI analysis is a soil depth layer of between 0 and 20 mm (refer to the section on the use of berilium-7 to guide sampling layer thickness).

The recommended soil layer thickness sampled is 20 mm. This is not critical provided it is the same thickness for all source soil samples in a watershed. This will ensure that the library soil samples represent a similar time frame of soil development and land-use. A thinner layer is preferable to a thicker layer so there is less risk of intercepting an earlier land-use. FRN studies may use soil layers only 1 or 2 mm thick because of the areal source of the FRNs. The surface layers from such a sequence of samples may be suitable for the CSSI process within the depth constraints of <20 mm. However, a very thin layer may have less root

contact and therefore a lower concentration of the FA biomarker from the associated landuse plant community.

The exceptions to the surface soil sampling strategy are where land slippage has exposed a subsoil, or long term agriculture in one location has exacerbated erosion of specific parts of the land-use (e.g., Figure 12). A separate sample of each of these sub-soils should be included in the soil reference library



Figure 12: Soil erosion (lighter colour) on the brow of a hill due to repeated cropping of the same land over many years. In this case the problem was exacerbated by cultivating the land in the same direction every year and leaving the bare tilled land exposed to winter rains. The problem was subsequently remedied by recovering the eroded soil from the bottom of the slope and spreading it back on the brow of the hill before the next seasons planting. However, the tillage direction was not changed, so the erosion will continue.

5.4.3 Sediment samples

The samples representing a deposition zone for the watershed sources should be an upper 10 to 20 mm sample from the following locations: on river banks or flood plains where deposition has occurred at a recent higher water level; or in the calmer back-waters of the river; or from the surface of the river delta. Thicker layers would risk intercepting earlier deposition events which may have come from different land-uses or from the same land-uses but in different proportions. The river delta sample is representative of the sediment eroded from the whole watershed. The buoyancy of freshwater on sea water, flocculation, and the stalling action of the flood tide coupled with a predominant wind direction will cause finer sediment particles to settle along the edges of the estuary near the river inflow at high tide. This causes the characteristic muddy sediments often found near the head of an estuary. This effect is enhanced where there are plants such as mangroves that can trap sediment from the water column. In these situations, surface scrapings only should be taken to obtain contemporary sediments.

Further down the estuary (i.e., seaward) where there is significant bioturbation and/or wave action mixing, the contemporary sediment may be mixed through a layer as much as 30 cm thick. A 20-mm thick surface layer is appropriate from such zones.

5.5 Suspended sediments

Sediment in the river water (suspended sediment) is the sediment being transported at that time. It is composed of contemporary soil erosion and the winnowing of fine sediment from sediment stored in the river channel and bank erosion. Collecting suspended sediment

requires some form of sediment trap or sedimentation device. The height above the river bed will determine the range of particle sizes collected. Set at the river bed they will collect bed load material comprising larger heavier blocks, pebbles, and sands. Set at between one third and two thirds of the river depth above the river bed (i.e., the zone of fastest flow) the trap will collect an integration of the medium to small size particles and exclude the large-sized particles.

The justification for excluding the bed load materials is that those materials may have been suspended from the river channel rather than recently washed into the river from a watershed land-use. Finer materials, such as clays, are the first to be washed from the land and they are the last to settle in slow back waters. These particles represent contemporary soil erosion.

5.5.1 Conventional sediment traps

Although there are many designs of conventional sediment traps, for rivers it is best to use vertical tubes which are held in frames with the open collecting end set at selected heights above the river bed. These tubes need a length to diameter aspect ratio of at least 8:1 to ensure that suspended sediment entering the trap does not get flushed out with eddy currents (Bloesch & Burns 1980; Blomqvist & Hakanson 1981; Kozerski 1994). These sediment traps can be used at a range of water velocities from medium flows in rivers and estuaries to still or slow moving waters such as lakes or coastal waters where they are more suited. When deployed in streams and rivers they are susceptible to being washed away in flood events because of their relatively large cross sectional area. Depending on the suspended sediment concentration in the receiving water being sampled, they can take a long time to collect sufficient sediment for an FRN or CSSI analysis.

5.5.2 Velocity-reduction tubular sediment samplers

These are time-integrated fluvial suspended sediment samplers, which consist of a large volume tube, 1 m long with an internal diameter (ID) of about 100 mm, with a narrow inlet tube 4 mm ID through a cone-shaped front end and a corresponding 4 mm ID outlet tube at the other flat end (Figure 13). A detailed description is provided in Phillips et al. 2000).

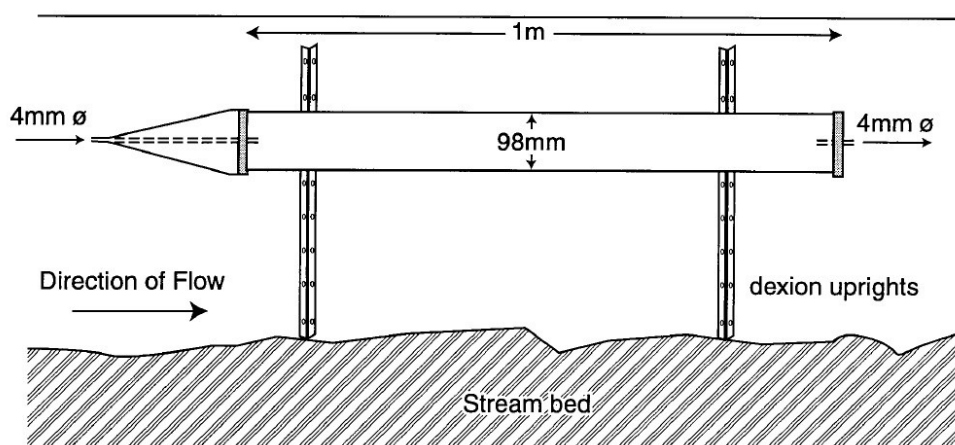


Figure 13: Cross-section of a suspended sediment sampler. From Phillips et al. (2000).

They are mounted on two or three metal support rods driven into the river bed, orientated with the nose-cone pointing upstream into the flow. The concept of operation is that the small high velocity inflow of turbid water entering the large volume (~8 L) of the main body will slow suddenly allowing the sediment to settle in the body of the tube. The sediment trap is emptied by un-screwing the flat end cap. These samplers will only work in flowing water and the rate of collection is dependent on the suspended sediment concentration and flow velocity of the receiving waters being sampled. Correctly installed, they will survive most floods and can be used in sequence to study flood events. They produce flow-weighted time-integrated samples.

5.5.3 Mat traps

These traps are a relatively recent innovation and use a sheet of artificial grass commonly known as “Astroturf” in a tray support mounted in a relatively sheltered back water of the river channel (Figure 14). They rely on the large surface area and “roughness” of the mat material to trap fine sediment. The length of fibre in the mat determines how much sediment can be caught. A useful fibre length is around 20 mm.

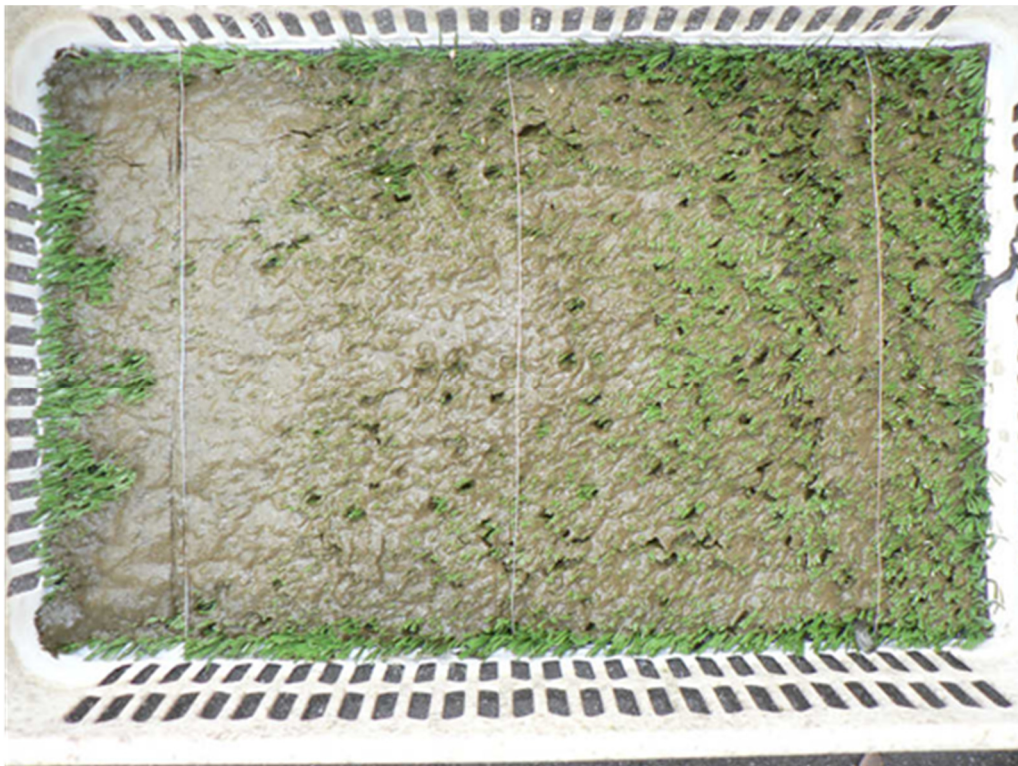


Figure 14: Mat trap showing the general construction. This mat trap has 35 g sediment collected over 2 days. Trap dimensions 0.25 x 0.4 m with a turf fibre length of 19 mm. Design by Max Gibbs.

Although not quantitative, mat traps are ideal for trapping bed-load material just above the bottom but can be used at any depth. They will work in the still waters of a lake, in estuaries, and even in flood events provided they are mounted away from the direct flow. They are mounted flat with the end facing the flow. The typical mat trap has a thin cross-sectional area and dimensions of about 0.25 m by 0.4 m, giving a trapping area of 0.1 m². They can be made by tying the mat into a plastic basket-type tray (e.g., a plastic office in-tray) with fine

stainless steel wire (e.g., welding wire; Figure 14). In bottom mounted use, a sheet of lead can be installed under the mat element to hold the trap on the bottom.

The wet sediment is recovered by removing the mat from the tray and washing it with minimal water into a plastic bucket. The sediment is sieved through a 2-mm stainless steel mesh to remove unwanted plant material, stones, and insects. The sediment is then allowed to settle and the excess water siphoned off before processing.

The rate of collection is dependent on the suspended sediment concentration in the river water, but because of their large surface area, mat traps collect more material in a shorter time than the other traps.

5.5.4 Continuous-flow centrifuges

Continuous flow centrifuges (CFC), also known as spinning-disc separators (Figure 15), are the ideal way to obtain large amounts of sediment in a relatively short time. They are based on the principle of a milk separator and produce time-integrated samples at short time intervals by continuously pumping ca. 4 L of water per minute into the separating chamber.

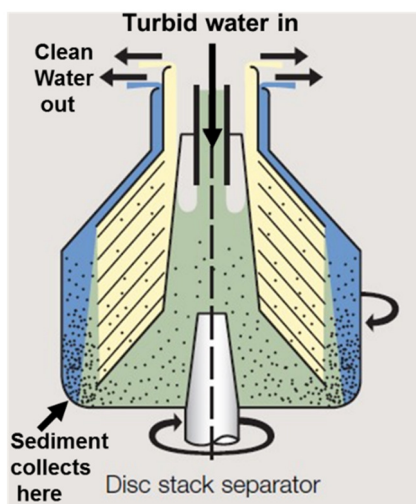


Figure 15: Cross-section through a spinning (or stacked) disc continuous-flow centrifuge.
Diagram from Alfa Laval.

The amount of sediment caught by the CFC will depend on the suspended sediment concentration in the water, the efficiency factor of the CFC (typically ca. 95%), and the length of time the CFC is run. For example, to collect 20 g of sediment from a turbid river with a sediment load of 100 g m⁻³ would take about 1 hour. At higher suspended sediment concentrations, the sampling time required would be much shorter, allowing temporal sampling of erosion during a flood event. Because the flow rate through pump is known, the CFC produces quantitative sediment samples which can indicate mass transport.

5.6 Soil and sediment cores

Sediment cores are often taken to evaluate deposition rates and use FRN techniques to date the core and determine sediment accumulation rates (SAR). They can also be used with CSSI techniques to assess historical changes in land-use by linking them with the FRN data.

5.6.1 Collection

Soil cores are taken using a variety of coring equipment ranging from stainless steel tubes driven into the ground using a sledge hammer, to sophisticated small split corers which can be driven into the ground by hand (Figure 16) or larger versions which require a portable motor powered pneumatic hammer. Sediment cores use similar shaped corers except that they are usually driven into the sediment by the weight of the corer and the speed of the descent i.e., gravity corers. Both types of coring typically use straight insertion without twisting and require lifting gear attached to tripods or winch booms to retrieve the corer with sample.

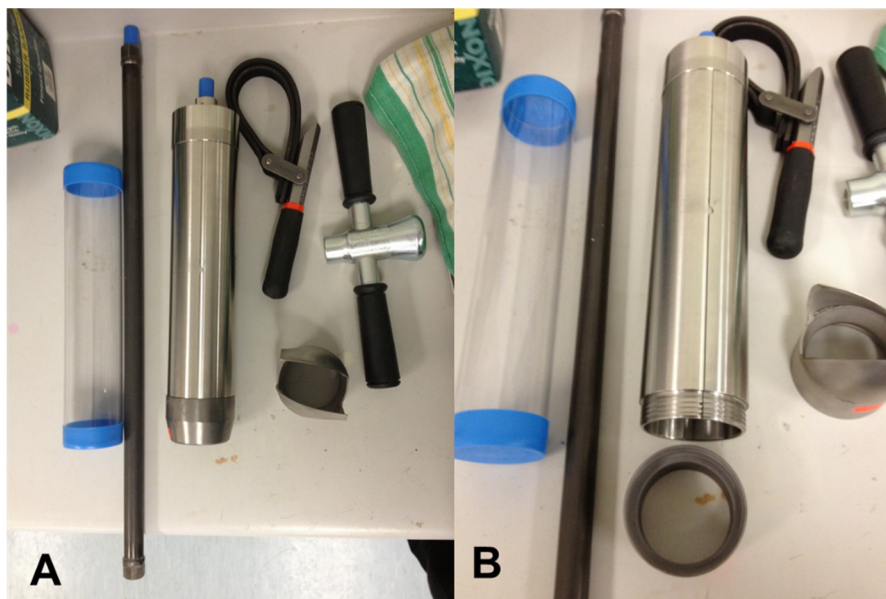


Figure 16: (A) Small hand operated split corer; (B) showing the body split with the cutting end unscrewed. The core liner (blue caps) fits inside the stainless steel core body.

Coring techniques are mostly used in FRN studies where soil or sediment depth profiles are required to determine the change in radionuclide concentration with depth. Based on the assumption of constant sedimentation – constant flux (CS:CF) and that the effective range of radionuclide concentration is around 8 half-lives, the lead-210 (^{210}Pb) radionuclide profile can be used for dating layers in the core up to 100 years before present. In addition to ^{210}Pb , the caesium-137 (^{137}Cs) radionuclide maximum depth is used as a marker for the year ca. 1954 (air testing of nuclear weapons Southern Hemisphere) and, in the Northern Hemisphere, for 1986 (i.e., Chernobyl nuclear reactor accident). These ^{137}Cs dates are also used to confirm the ^{210}Pb dating. Berilium-7 (^7Be) is also a good indicator of the depth of the surface mixed layer of a sediment.

For FRN studies the soil or sediment core is sectioned into thin layers – 1 mm to 1 cm thick. Each layer is analysed separately and a concentration versus depth profile is constructed. A smooth exponential decrease in ^{210}Pb concentration with depth is indicative of a classic constant sedimentation – constant flux curve, that is, no erosion or deposition. Discontinuities in the depth-concentration curve of ^{210}Pb indicate changes in erosion or deposition rates at those times and can be used to estimate the magnitude of past events.

Analysing selected layers from the core, or every layer if funds permit, can give information about the land-use changes over time or the land-use associated with an event identified by

the discontinuity in the depth-concentration curve. However, the sediment core is often much deeper than the workable range of the ^{210}Pb dating curve. Because the CSSI FA biomarkers are stable for several thousand years, it is possible to evaluate land-uses and land-use changes which occurred much longer than 100 years before present. Note: a correction is required for the CSSI modelling to take account of the Seuss effect (see later section).

Unless the core is sectioned at regular intervals along its length, identifying when events occurred, and thus at what depth to take a thin layer section, requires some way to “see” those event layers. The layers may be visible as a colour change or a change in grain size, but often they are visible as changes in density in an X-ray image of the core (Figure 17).

5.6.2 X-ray imaging

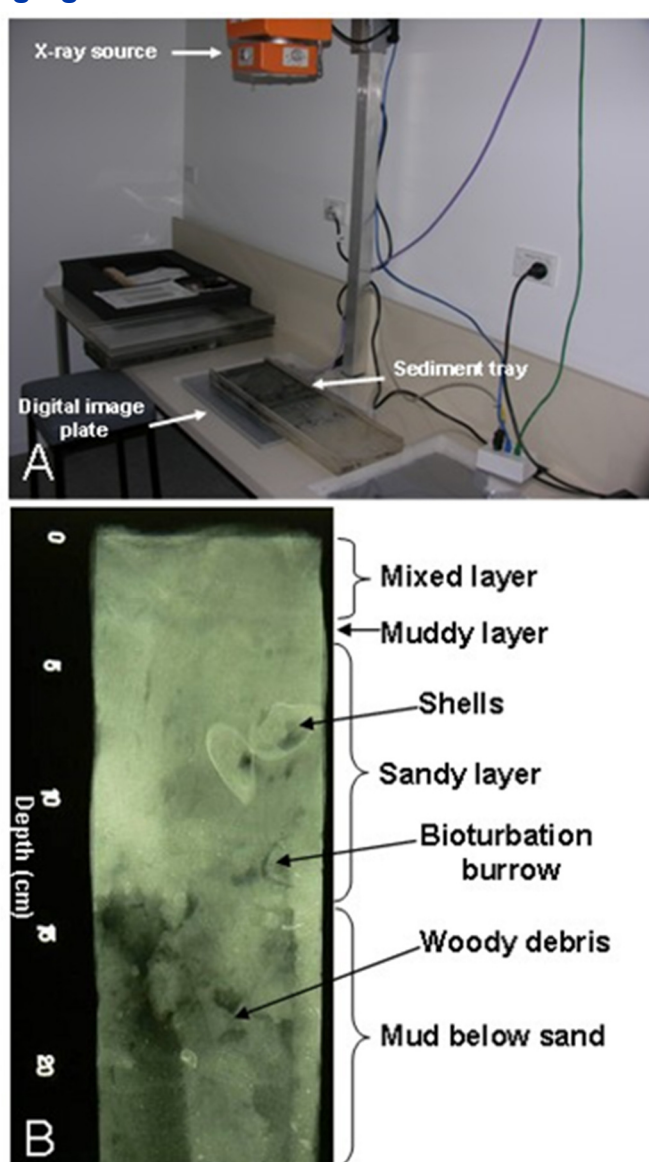


Figure 17: A) Digital X-ray set-up for analysis of sediment cores. B) Example of the upper 25 cm of a marine sediment core showing the depth of the mixed layer, the transition between sand and mud layers, shells, bioturbation burrows and woody debris as detected by X-ray imaging.

As soon as the core is collected, it is laid on its side so that the sediment doesn't compact. The ends of the core tube are plugged tightly against the sediment with high density foam plastic insert plugs to prevent the sediment spreading during transport.

In the lab, the core is split lengthwise by first cutting the plastic core tube on opposite sides using a circular saw with the blade depth set to the thickness of the core tube and then driving thin stainless steel plates through these slits. One half of the split core is laid on a sheet of plastic (cling-film or equivalent) in a wooden tray with 1 cm high sides confining the core. The remainder of the plastic core tube is removed and the top of the exposed core is sliced off with a cheese wire and transferred back into the original half core tube. The sediment slice in the tray is wrapped in cling film to prevent water loss. This technique provides a 1-cm thick layer the full length of the core. This layer is X-rayed in sections appropriate to the size of the X-ray plate (Figure 17A). An opaque depth marker must be attached to the side of the core slab to allow subsequent depth correlations.

The X-ray images show changes in sediment density as a change in grey-scale with white being very dense (opaque to X-rays) such as sand, stones and shells, whereas darker areas indicate areas of low density, for example, soft mud. Additional information that can be obtained from the X-ray image includes: changes in grain size; the thickness of the surface mixed layer; and, because bottom burrowing animals leave low density tubes through the sediment, the depth and extent of bioturbation (Figure 17B).

With long cores, the X-ray imaging also allows detection of carbonaceous material such as shell fragments or woody material which can be used to carbon date the core layers.

Following the X-ray, the three core splits can be recombined to provide enough sediment material from each depth layer being sampled for compound-specific isotope analysis (CSIA) for the CSSI technique. (See worked example in Case Study – Bay of Islands). While X-ray equipment is specialised, commercial radiographers can provide this service.

5.7 Sample preparation

It is critical that the composite sample is well mixed so that it is representative of the land-use sampled. The following process steps will ensure that the prepared sample is completely mixed, i.e., homogeneous, so that subsequent sub-sampling of smaller sediment/soil quantities (or aliquots) taken for isotopic analysis is representative of the bulk composite sample.

5.7.1 Pre-screening

The bulk composite soil sample must first be dried and ground. Before drying, the soil or sediment should be sieved through a 2-mm stainless steel mesh to remove woody debris, leaves, flowers, stones, shellfish, and invertebrates. To assist in this process, the larger non-soil material can be removed by hand picking. The sieving process is not critical but it does form part of the mixing process that ensures a completely homogenized composite soil or sediment sample. Note that fine roots are part of the soil so it is not essential to remove these.

Where the sample is difficult to pass through a 2-mm sieve, the sample may be wet-sieved by adding water to convert the sample to a slurry. The sieved slurry is allowed to settle in a cool dark place and the excess water removed by decanting or siphoning, and is discarded.

This is a useful approach when heavy clay soils are processed. The alternative approach is to use a larger sieve mesh-size to remove the larger non-soil particles and then re-sieve after drying to remove the smaller non-soil particles.

The reason for removing the leaves and woody debris is to prevent contamination of the soil by FA from plant material that may have fallen or blown onto the land-use site rather than having grown there and contributed to land-use FA biomarkers in the soil. Likewise, in sediments, invertebrates and shellfish can produce their own FA independent of the land-use or sediment site. Stones and pebbles are excluded as these could damage the grinding equipment. Although these may be large, they will have a smaller surface area to volume ratio than the smaller soil particles and thus their removal will have a minimal effect on the amount of FA in the sample.

The alternative to wet sieving is to use freeze drying but only after hand picking off the larger debris, plant material and insects.

5.7.2 Drying

Drying can be achieved either using a freeze dryer (preferred) or through heating in an oven. In either method, the wet sample is spread evenly in a pre-weighed aluminium tray. The amount of sample placed in the tray depends on how effective the mixing was during the pre-screening sieving. Good mixing means that a sub-sample from the bulk composite sample can be dried. Poor mixing may require the whole composite sample to be dried to avoid any bias due to natural variability between the sub-samples taken for the composite sample.

Freeze drying, if available, is preferable to oven drying as the dry soil or sediment retains the free-flow characteristics of the original soil and is thus easier to re-sieve and grind. There is no evidence of isotopic fractionation of the FA biomarkers during the freeze drying process.

Oven drying is a more readily accessible method for drying soil and sediment samples. Drying should be in an air fan-ventilated oven at a maximum temperature of ca. 60°C to prevent loss of the FA due to volatilisation. The main issue with oven drying is that clay soils tend to become very hard “bricks” and that affects the grinding process. To overcome this problem, the samples should be partially dried then removed from the oven. The semi-solid cake of soil should be crumbled and the returned to the oven to continue the drying process.

Drying is continued to a near constant weight and may take from 12 h to 48 h depending on the size of the sample, the particle size and the moisture content.

5.7.3 Grinding

Before grinding, the dry soil can be crumbled and very hard lumps may be placed in a plastic bag and crushed with a press or broken with a clean, flat-faced hammer. Another useful technique for breaking the lumps is to crush them with a steel roller on a steel tray (Figure 18). There are two levels of grinding, one for CSSI extractions and one for bulk $\delta^{13}\text{C}$ analyses.

For CSSI extractions, the dry soil needs to be reduced to fine particles with a grain size that will pass through a 2 mm mesh sieve with the majority of particles around 0.1 mm. While the median particle size is not critical, using this particle size enables rapid extraction of the FA

without significant numbers of very fine particles passing through the 1 μm filters in the extraction equipment. This grain size is appropriate for the 10 to 20 g of sample being extracted.



Figure 18: Lumps in the sample can be crushed with a steel roller on a steel tray to reduce the particle size to <2 mm. Crushing with a roller produces less very fine material than grinding.

For bulk $\delta^{13}\text{C}$, the grain size must be much smaller (<0.1 mm) to ensure the 5 to 30 mg of sample being analysed is representative of the whole sample and that it combusts instantly in the elemental analyser furnace. This can be achieved in a mortar and pestle or the soil can be ground in a ball mill or ring mill if these are available. Alternatively, a simple stainless steel gear-tooth coffee grinder set to 0.1 mm will provide an effective grind that can be sieved to remove particles larger than 0.1 mm. In practice, two passes through the coffee grinder produces an acceptable powder for extraction.

Cleaning of the coffee grinder is achieved by removing the 'bean' cup on top and blowing out any residual soil dust from the grinding teeth with an air jet from a dry air gas bottle. If a compressed air supply is used, the air-line needs to have an oil filter to prevent contamination of the coffee grinder with the oil vapour from the air compressor pump.

5.8 Sources of error

The largest source of error in the CSSI technique is due to sampling and the failure to obtain representative CSSI and bulk stable isotope values for the land use. The CSSI analysis requires around 10 to 20 g of dry ground sample. Collecting just the 10 to 20 g sample from one spot in the land-use raises the possibility of errors due to non-uniform labelling of the soil by the plant community or other sources of variability. The stable isotope analysis requires only around 5 to 30 mg of dry ground sample and consequently, the potential error is much

greater. The composite bulk sample (from combining multiple samples from different locations) from each land-use location from an area of at least 100 m² substantially reduces the potential errors from non-uniform sampling.

Poor mixing can introduce a similar error. Sieving the wet soil prior to grinding is an important part of the mixing of the soil sub-samples. Grinding of the dried sample completes the mixing process and produces a homogenous mixture from which the sample aliquots for analysis can be taken. The dried sample is then also available for subsequent analyses for quality control (refer to section 5.10).

The risk of cross contamination during sample processing is mitigated by using relatively large sample sizes of sediment/soil. As such, even if there was dust left in the coffee grinder from a previous sample, the amount would be so small relative to the large sample size that the effect on isotopic values would not be measurable. That being said, it is still important (and certainly 'best practise') to clean equipment as much as possible between samples to keep any carry over to an absolute minimum. Do not use a solvent to clean the grinder between samples because this can release contaminants from the bearings and seals around the gear shaft. Clean all equipment thoroughly when finished and store clean.

5.9 Preparing test soil mixtures

With a set of dry samples from potential land-use sources in a watershed it is possible to construct artificial soil mixtures that incorporate a range of different source soil proportions for testing the CSSI technique. Dry ground soil samples should be weighed into a large sealable plastic bag in the correct mass proportions required for the test mixture. The plastic bag should be partially inflated before sealing to leave a large air gap in the bag to enable the soil mixture to be mixed thoroughly via shaking. This mixing process can be augmented by passing the test mixture through the 100 µm sieve. Do not discard any residue caught by the sieve but re-incorporate that material into the sample and re-mix in the plastic bag.

The resultant mixture can then be treated as another sample in the analytical process.

5.10 Quality Assurance

Quality assurance (QA) is important. To enable CSSI values from different sampling areas to be compared or used in a reference library, there must be assurance that the analyses have that same accuracy and precision in each analytical run. A shift in isotopic signature due to instrument drift or any other factor can be corrected if the extent of that shift is known. To achieve this, QA samples should be analysed together with the standards that would normally be included in an analytical run. These QA samples should be taken through every phase of the analytical procedure and the values obtained should be recorded to enable post correction of the analytical run and to monitor any trends or changes in the isotopic values that could indicate an analytical error such as using a new bottle of methanol, for the derivatisation step, which has a different isotopic signature.

An aliquot of a previously analysed soil can be included in an analytical run as a QA sample. Alternatively, a certified QA standard soil can be used and compared between laboratories. A single QA sample can be used to confirm the isotopic signature of the added methyl group in the derivatisation step. Three aliquots of the same QA sample would test the precision of the

analytical procedures. Three different QA samples with CSSI values across a broad range could provide a standard curve for extraction efficiency.

Analysing a specified amount of a certified QA standard soil with each batch of samples will test the extraction and derivatisation efficacy. Collating a series of the certified QA standard soil results will provide an indication whether changes are occurring in the analytical procedures.

6 Analysis

Analysis of each soil or sediment sample consists of measuring the sediment physical characteristics (i.e., density and porosity), measuring the organic content (i.e., OM), measuring the bulk stable isotopic composition of the sample, and measuring the CSSI values of the FA (via FAME derivatisation) in that sample.

6.1 Sediment and soil characteristics

6.1.1 Density

The wet density (d) of a soil/sediment sample is determined as:

$$d = \frac{\text{Volume of wet sediment sample}}{\text{Wet weight of sediment sample}}$$

The sample is transferred to a finely-graduated glass measuring cylinder in which 25% of the volume is filled with water. The weight and the volume of the sample is determined as the difference in volume and weight before and after putting the sample in the measuring cylinder. The presence of water in the measuring cylinder ensures that the added sediment is below the surface when reading the volume. A suitable sized measuring cylinder is 100 ml. In practice the measuring cylinder must have a volume of approximately two-times the volume of sample added in order to obtain sufficient accuracy. Density is determined for all soil and sediment samples and is used to determine mass proportions of eroded soil or deposited sediment when quantification is required.

6.1.2 Porosity

The porosity of the soil/sediment sample is determined via:

$$\text{Porosity} = \frac{ww - dw}{vol}$$

where:

ww = wet weight of sample

dw = dry weight of sample

d = density of the sample

vol = volume of the wet sample (= ww/d)

Dry weight is determined after drying the sample at 105°C until constant weight. This temperature for drying is more severe than should be used for the sample to be extracted for FA and may lose trace levels of the more volatile compounds.

6.2 Organic matter and %C content

The organic matter (OM) content of the sample is measured by the loss on ignition (LOI) technique. A small (5 g) aliquot of the dried (60°C) ground sample is weighed into a pre-weighed vessel (porcelain or aluminium) and heated to 450°C for 3 hours in air, in an electric muffle furnace. This temperature and time will ensure that all OM is removed from the

sample. It is, however, on the threshold for losing water of crystallisation from inorganic salts and may cause a slight but non-critical overestimation of the OM content.

Once cooled, the sample is re-weighed and the loss of weight is calculated as a percentage of the original sample and recorded as the percent OM (%OM) content. As an approximation, the percent organic carbon content (%C) is estimated by multiplying the %OM content by 0.47 (i.e., %C is about 47% of the total OM). The %C content of the sample is used to estimate the amount of sample required for the measurement of the bulk stable isotopic composition of the sample. For example, a sample with 20% OM will have about 10% organic carbon (OC) and will require a sample weight of about 0.2 mg to provide the 0.02 mg of C required for the stable isotope analysis. In contrast, a sample with 0.2% OM content will have about 0.1% OC and therefore a sample weight of about 20 mg is required to yield the 0.02 mg of C required for the stable isotope analysis.

Determining the required sample size for analysis is important as too much C will swamp the IRMS and too little C will result in a higher level of uncertainty in the analytical results. Repeat analyses are required in both cases, increasing the costs.

6.3 Bulk stable isotopes

Bulk stable isotope analysis by IRMS can produce both carbon and nitrogen isotopes i.e., ^{12}C , ^{13}C , ^{14}N , and ^{15}N . The nitrogen isotopes in fresh sediment are useful for distinguishing between terrigenous and marine samples, as well as providing information on specific land-use practices such as organically grown food, and animal farming versus crops. However, while they are also a key component of food-web studies, they cannot be used in the CSSI technique because nitrogen is rapidly assimilated in the aquatic environment resulting in unknown levels of isotopic fractionation that cannot be linked back to the source. Fortunately, this is not an issue with carbon, especially the way it is used in the CSSI technique.

6.3.1 Removal of inorganic carbonates

Note, if the $\delta^{15}\text{N}$ and %N values are required, the non-acidified sample must be used.

Bulk $\delta^{13}\text{C}$ values of soil and sediment need to be of the organic carbon component only. This means that the inorganic component (i.e., the inorganic carbonates) must be removed by acidification (see standard operating procedures). This is achieved by stirring 2 to 3 g of the finely ground dry sample with 2 to 5 ml of 1N hydrochloric acid (HCl) in a 50 ml plastic centrifuge tube. The mixture should be left until the effervescence has ceased. Then add another 1 ml of acid, stir and leave until the effervescence (if any) has ceased. If there is no further effervescence upon addition of HCl, make the volume up to 50 ml with deionised water, shake to mix then centrifuged at 3000 rpm for 10 minutes. The liquid phase is decanted and discarded (**caution: acidic residues should be discarded into running water**). The sample is then rinsed by making the volume to 50 ml, with deionised water, shaking and centrifuging again. The liquid phase is again decanted and discarded.

The acidified sample should be dried at 60°C in an air fan-ventilated oven before grinding to a fine powder in a mortar and pestle. Alternatively, freeze dry the well-rinsed samples and they will not require grinding. Aliquots of the dried acidified sample are then weighed into pure tin (Sn) cups which are then pressed into pellets ready for the IRMS.

Because the %C content of the sediment/soil samples is large relative to the FA content, the risk of contamination of the bulk C isotopic signature is small and standard procedures for handling the sediment/soil samples are appropriate.

6.3.2 Stable isotope measurement

The IRMS requires the carbon from the sample to be converted into gaseous form. This is achieved by combusting the sample in the tin pellet at $>1000^{\circ}\text{C}$ with pure oxygen in a helium gas stream in an elemental analyser (EA). This converts the carbon to CO_2 and the $\delta^{13}\text{C}$ of the CO_2 is measured by the IRMS relative to an international reference gas standardized against the international standard, Pee Dee Belemnite (PDB). The IRMS output includes the percentage of carbon (%C) in the sample. These two values, $\delta^{13}\text{C}$ and %C, are precision measurements and are used in the mixing model and for the conversion of isotopic proportions to soil proportions respectively.

6.4 Compound-specific stable isotopes

Analysing compound specific stable isotopes uses the non-acidified material that has passed through a 2 mm sieve rather than the finely ground acidified powder used for the bulk stable isotope analyses. Fatty acids must first be extracted from the sample, then derivatized to their methyl esters (i.e., fatty acid methyl ester = FAME) so that they can be separated into individual FA by gas chromatography (GC) before combustion and analysis by IRMS. The sample is not acidified because the FA from plants can bind to the inorganic carbonates and acidification would remove these.

6.4.1 Equipment options

The extraction procedures described in these protocols focus on preferred equipment. This equipment was used during the development of the CSSI technique and will produce good results. It is recognised, however, that this equipment may not be available in all laboratories and thus alternative extraction procedures are also described. The alternative extraction procedures were used prior to the invention of the newer equipment and will also produce good results. Those methods will take longer to complete the extraction and it is recommended that those methods are tested to determine optimum extraction times for the soils/sediments being extracted. This is where a certified QA standard can be useful for checking the extraction method.

The preferred method of FA extraction from soil/sediment samples is with a Dionex ASE 200 accelerated solvent extractor (Figure 19) and the subsequent newer versions of this equipment. This instrument has a stainless steel sample cell (Figure 20) to contain the sample, which is subjected to hot (100°C) solvent extraction at high pressure (2000 psi). It is an automatic sequential extraction instrument with the samples being loaded into a carousel. The solvent extract is collected in glass screw-top vials by injection through a silicon rubber septa in the vial cap. This method is preferred because of the rapid extraction time (minutes), the minimal use of solvent per sample extract and the large number of samples that can be processed in a day.

Alternative extraction procedures include traditional Soxhlet reflux systems, continuous shaking with solvent and the use of ultrasonic techniques (See Standard Operating Procedures). These methods take a long time (hours) to complete an extraction and the

number of samples that can be processed in a batch is determined by the number of complete Soxhlet systems or shaking flasks available or the size of the ultrasonic bath.

After extraction by the chosen method, the extract is processed in exactly the same way using the same glassware and solvents.

6.4.2 Avoiding contamination

In contrast to the bulk stable isotopes, the OC content and the extract volumes are small. Consequently, the risk of contamination is high and special care is required to prevent losses through spills or contamination from external sources with high concentrations of FA. The main sources of contamination are the processing glassware and extraction equipment, which may have been used in another project where FA concentrations were high e.g., lipid extraction for bulk stable isotopic analysis of food web tissues. Consequently, all equipment must be thoroughly cleaned before use.

6.4.3 Solvents

The first priority is to prepare ultra-clean solvents. Four solvents are used in the CSSI technique - dichloromethane (DCM), acetone, hexane, and methanol. These solvents can be purchased as high pressure liquid chromatography (HPLC) grade solvents. However, because of the large volumes of DCM and acetone used for cleaning, these two solvents are purchased as reagent grade in 20 litre drums and then doubly distilled in the laboratory. Note that 'waste' or washing and rinsing, solvent recovered from the cleaning steps can be recycled by double distillation. All solvent distillation should be done in a fume cupboard.

All solvents are stored in bulk 2.5 litre glass bottles designed for solvents. Working quantities of each solvent are transferred to 250 ml or 500 ml conical glass flasks with ground-glass stoppers. Excess working solvent is never returned to the bulk solvent bottles, to prevent contamination of the bulk supply. The bulk and working solvents should be stored in designated dangerous goods cupboards when not in use. The working area should be well ventilated when using these solvents.

6.4.4 Cleaning

Glassware, including extraction vials and vial caps, should be soaked in working strength laboratory glassware cleaner (e.g., 5% Decon90) for at least 12 hours. The glassware is then rinsed thoroughly with distilled water then sets of glassware are loosely wrapped in aluminium foil and the package heated in an oven at 400°C for 3 hours. The clean glassware package is only opened in the clean environment of the extraction laboratory. Septa for the extraction vials are rinsed with distilled water and ultrasonically cleaned in distilled acetone before being sealed in a clean plastic bag.

Before use for CSSI extractions, the ASE extraction cells are disassembled and ultrasonically cleaned three times in solvent; once with distilled acetone and twice with doubly distilled DCM. The cells are then dried at 104°C. This level of cell cleaning is not required between samples for CSSI as the previous analysis will have removed any FA from the cell. The filter pads used in the extraction cells are 'clean' from the supplier, and must be handled with forceps (Figure 1). For low concentration work it is recommended that the filter pads are placed in an ASE cell and subjected to the same extraction procedure as used on the samples.

6.4.5 Extraction

The preferred technique for extraction of FA from soils or biological samples uses DCM in an accelerated solvent extractor (e.g., Dionex ASE 200 or later models). However, other extraction techniques such as Soxhlet and ultrasonic techniques can also be used, but will take longer to achieve complete extraction (see below).

The following protocols are for the use of a Dionex ASE 200 accelerated solvent extractor (Figure 19). ASE parts descriptions will vary between different models.



Figure 19: Dionex ASE 200 accelerated solvent extractor with load (sample) cells in the upper carousel and the extract collection vials in the lower carousel.

The ASE load or sample cell (Figure 20) consists of a stainless steel tubular extraction cell body fitted with a pair of screw on stainless steel end caps. The standard cell can hold up to 20 grams of dry soil, and larger cells are available. The cell body has a mark engraved towards one end to indicate which way is up. The cell body and two end caps should be marked as a set to ensure consistency in the extraction procedure and to aid tracing errors or contamination, should these occur.

Each end cap is identical and has a small central Teflon O-ring seal on the outside of an inlet/outlet port through which the extraction solvent enters (when used as a top) or the solvent extract leaves (when used as a bottom). A metal frit on the inside of each end cap protects the inlet/outlet port from the sample and is used to support a filter pad when that end cap is used as a bottom. A hard polyether ether ketone (PEEK) plastic washer in each end cap provides a seal with the tubular body when assembled. This essentially inert material will withstand high pressure/temperature solvent extractions without deforming.



Figure 20: Two sample cells, one with the upper end cap off and showing the PEEK seal inside the end cap. The O-ring seal is located in the centre of the outside of each end cap and is visible in the top of the fitted end cap. The dark patch on the tubular body indicates the top. The filter pads (in plastic bag) fit tightly inside the cell body and need to be pushed down to the bottom of the cell with the assembly rod (black). Use smooth-tipped forceps to handle the filters.

It is critical that the Teflon O-ring seals on the inlet/outlet ports on the cell end caps are clean and undamaged. These need to withstand hot DCM solvent at high pressure; consequently any leak will result in sample extract (i.e., FA) losses. Although damage is uncommon, grit or other contaminants can lodge in the conical depression holding the O-ring seal. To prevent this, the work bench surface where the cells are assembled and loaded should be clean and preferably covered with a sheet of white bench cover paper. Inspect each seal and clean before assembly. If necessary, replace the Teflon O-ring seal if it is suspect (e.g., scratched) or the hole in the O-ring is severely deformed such that it could restrict flow.

The cleaned sample cells are assembled to firm hand tightness with the tops left off. Two filter pads are inserted and pressed down to the bottom of the cell with the assembly rod, one at a time. Two filters are used to prevent very fine sediment particles leaking into the ASE instrument where they could jam or damage the high pressure valves. Weigh or tare the empty cell.

With the open cell placed upright on a clean facial tissue, load the dry ground sediment into the cell using a wide neck powder funnel to avoid spills. The tissue is to catch the spills and fine dust. Note that this dust has electrostatic properties in very dry conditions and will coat the surface of glassware and plastics if it is released into the air by pouring too fast. At this stage, it is more of a nuisance rather than a serious contaminant, as the amount released is very small relative to the sample size (mg versus g) and will be below detection level.

The cell should be filled to about 90% of its volume and the weight of sample recorded. It is important not to vibrate or tap the cell to get more sample in as this will cause the sediment to pack tighter and potentially reduces the extraction efficiency. Screw on the upper end cap and firmly hand tighten. Discard the facial tissue if there was a spill or a dust release.

Where plant material is being extracted, the sample size may be small (1 to 2 g). To reduce the amount of solvent used, the small sample is covered with another filter pad and the cell body is filled with clean coarse sand (See Standard Operating Procedures for details of sand preparation). After the extraction, the sand can be recovered for reuse on other small samples.

The loaded cell is installed in the upper carousel of the ASE and the location number recorded. Sample carousel positions are correlated with extract vial positions. The extract vials are 60 ml flat-bottomed glass tubes which have a screw-on lid. The lid carries a silicon rubber septum with a Teflon surface facing into the vial. This orientation reduces the risk of hot DCM attacking the silicon rubber of the septum. The septum is punctured by two large-bore needles during the extraction process. Septa can be cleaned and re-used several times but should be discarded and replaced if they become damaged by the needles e.g., silicon plugs cut out and the Teflon torn.

The ASE is primed by running a flushing cycle to remove air from the solvent lines. In run mode, the auto-seal arms (top and bottom) form a seal with the end cap Teflon O-rings on the sample cell and transfer the sample cell to the ASE oven where it is heated to 100°C. The auto-seal arms carry the solvent lines. The sample cell is filled with doubly distilled DCM through the tubing in the auto-seal arms. The solvent pressure is increased to 2000 psi and held at that pressure for 5 minutes. The pressure is reduced and the solvent is flushed from the cell through the bottom auto-seal arm tubing into the collection vial. This extraction program is run twice and then the sample cell is returned to the carousel. The ASE flushes all transfer lines between samples to reduce any possibility of solvent carry over between samples.

6.4.6 Alternative extraction techniques

In the **Soxhlet technique**, the sample (10-30 g) is held in a compressed cellulose filter thimble exposed to hot solvent vapour and in the path of the condensing solvent. The solvent used is either DCM or a mixture of methanol (MeOH) and DCM. Because the extraction thimble is made of plant material it must be pre-extracted to remove the possibility of contamination by FA from the thimble itself. The extracted thimbles can be reused after each soil/sediment extraction because the extraction procedure should have removed all FA. A typical FA extraction involves refluxing for 10 hours with DCM to recover all FA from the soil/sediment sample. The volume of solvent used will depend on the size of the reflux flask but is likely to be in the order of 150 to 200 ml.

In the **ultrasonic extraction** technique the sample (10-30g) is placed in a conical, flat-bottomed glass flask and is then suspended in at least 5 times the sample volume of extraction solvent (DCM or MeOH/DCM), typically about 100 ml. The flask is stoppered and placed in an ultrasonic water bath for 6 to 8 hours. **Shaking or tumbling** methods can use smaller volumes but take 24 hours (Figure 21). In both cases, the solvent is decanted through a pre-cleaned GF/C filter to remove any sample material from the solvent.

Note that the solvent mixture MeOH/DCM cannot easily be recycled through double distillation.

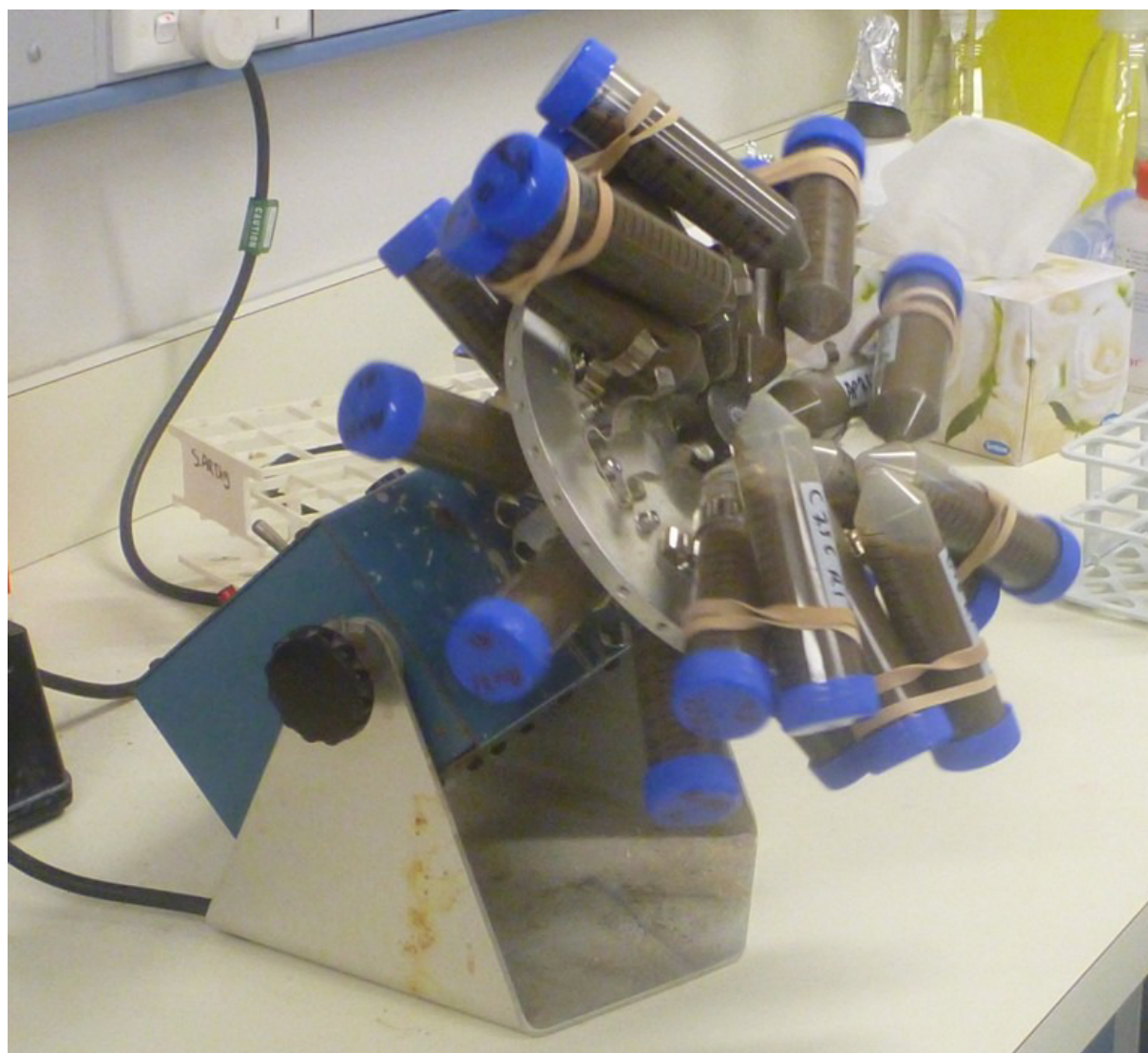


Figure 21: Example of a rotating tumbling extraction system. This unit uses 50 ml centrifuge tubes as the extraction vessels. Larger units can extract more sample mass each time.

These techniques use large volumes of solvent and take considerable time to effect a complete recovery of all FA. To confirm the efficiency of the extraction, one or two samples should be re-extracted or a certified QA standard soil should be extracted. Depending on the results the solvent volume and extraction time may need to be adjusted.

In contrast, the ASE is more conservative with respect to solvent use, faster to complete the extraction, automated, and gives more consistent and reproducible stable isotope data. The speed and automation of the ASE instrument enables between 24 and 48 samples to be extracted in a 24 hour period. In addition, the sample cell can be re-extracted to confirm the complete recovery of all FA from the sample.

6.4.7 Time-saving tip

The ASE has 24 cell slots in the carousel and will extract a sample every 15 minutes i.e., a full carousel will be extracted in about 6 hours. If there are a large number of soil samples to be extracted, these can be run on consecutive daily runs at up to 120 samples per week. While the limiting step would appear to be the cleaning of the sample cells between extraction runs, this level of cleaning is not necessary provided only soil extractions of similar origin are being run. Simply remove the end caps from the sample cell and transfer the extracted soil into a plastic bag (to prevent the spread of dust), and then reassemble the cell with new filters and refill the cell with the next sample. To remove as much extracted soil as practical, tap the sides of the cell to dislodge any material caught inside the cell but do not scrape the inside of the cell. A residue of the previously extracted soil is not an issue as it will not contain any extractable FA.

The logic behind this concept is that the sample cell was cleaned before the first sample was extracted because the previous history of use of that cell was unknown. After being used for extraction of FA from soil samples, the history is known. There will be no FA left in the cell because they have already been removed by the extraction process. Consequently, the sample cell is as clean as it was before the first sample, with respect to FA.

Caveat: The samples need to be of similar type. If low FA content samples are to be extracted after samples that had high fatty acid content, the sample cells should be disassembled and cleaned thoroughly or the order of extraction should be revised to analyse the low FA content samples first.

Day and night sequential runs are possible but the time constraints associated with cell cooling, emptying, and reloading, may make it difficult to achieve.

6.4.8 Drying the extracts

The solvent extracts are often highly coloured with the colour intensity reflecting the amount of organic material in the extract (Figure 22). A lack of colour does not mean a lack of FA as FA are colourless. Dry soil is hygroscopic and cloudy samples indicate the presence of water (e.g., Figure 22; “pasture [steep]” and “pasture [alluvial]”). Water is removed with anhydrous sodium sulfate. Note, oven drying the soil sample at 60°C overnight before extraction will eliminate the water problem and circumvent the need for adding sodium sulfate. Anhydrous sodium sulfate is prepared by heating analytical grade sodium sulfate in aluminium trays to 450°C in a muffle furnace for 3 hours and then storing the combusted dry powder in a solvent-cleaned sealable Schott Duran glass bottle, in a desiccator. Using a spatula, add sufficient powder so that, when shaken, the solvent becomes clear. Usually 1 to 2 g is sufficient. Add more if required to remove the water. The sodium sulfate does not affect the FA CSSI values.

The DCM is decanted into a 100 ml round-bottomed flask. The collection vial is rinsed twice with a small volume (~5 ml) of DCM and the rinse solvent is combined with the extract in the round bottomed flask. Do not transfer any sodium sulphate crystals if these have been used to dry the solvent. The solvent volume is reduced to near dryness on a Buchi rotary evaporator at about 400 mm Hg vacuum (half atmosphere) in a water bath at 35°C. The cooling water in the condenser should be chilled to 4°C to assist condensation of the DCM. This DCM can be re-used after being distilled twice.

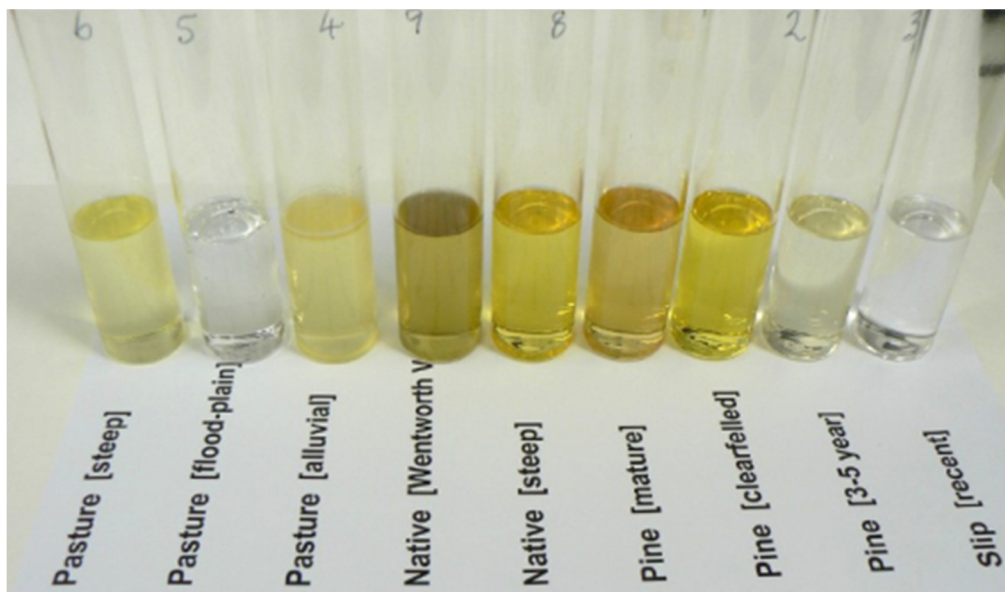


Figure 22: A range of land-use soil extracts from one watershed, showing the variability in colour intensity of the DCM extract. The “Pasture [steep]” and “Pasture [alluvial]” extracts are cloudy due to water.

The small volume of concentrated extract in the round-bottomed flask is transferred to a 10-ml (Kimax® brand or equivalent) screw-cap digestion tube using a clean glass Pasteur pipette fitted with a rubber squeeze bulb (Figure 3). Smaller 4 ml screw cap tubes can also be used (Figure 4A). The caps must be fitted with Teflon liners.

The round-bottomed flask is rinsed twice with about 0.5 to 1.0 ml of DCM and then transferred to the digestion. The DCM extract is reduced to dryness in the digestion tube at 40°C under a slow stream of nitrogen gas from an oxygen-free dry nitrogen gas bottle. The digestion tube is then sealed and placed in the dark prior to derivatisation.

6.4.9 Derivatisation

Because FA are acids, they are polar and therefore will bind to any ionic surface such as soils. They will also bind to the packing material in gas chromatograph columns. To overcome this problem, the acid group on the FA is replaced with a methyl group (CH_3) in a process called derivatisation. Because a methyl group is being used, the process is also known as methylation and the end product is the methyl ester of the FA i.e., fatty acid methyl ester or FAME.

The methyl group is obtained from methanol (MeOH) and the process requires a catalyst to proceed. The catalyst can be strong hydrochloric acid (HCl), strong alkali such as potassium hydroxide (KOH) (Chowdhury & Dick, 2012) or a metal halide salt. The catalyst of choice is boron trifluoride (BF_3) because the reaction can be carefully controlled, is highly reproducible, and does not produce unexpected by-products. As with any fluoride, BF_3 is hazardous and should be handled with caution. Use disposable gloves, wear suitable eye protection and

avoid skin contact. In the event of an accident, wash off skin with copious quantities of water immediately.

Reagent preparation

The BF_3 is used as a 5% solution in MeOH. The 5% BF_3 solution is prepared by diluting 5 ml of 50% BF_3 solution in MeOH to 50 ml with HPLC grade MeOH in a volumetric flask. This reagent is best prepared daily or before each batch run.

Because the methyl group comes from that MeOH, a small sample of that MeOH should be put into an actinic 2-ml screw-cap GC vial (Figure 6) and sealed ready for analysis to determine the $\delta^{13}\text{C}$ value of the carbon in the methyl group added to the FA. Once this value is obtained, it should be noted on the MeOH bottle and that bottle should be held exclusively for all methylation procedures. This $\delta^{13}\text{C}$ value is used to correct the CSSI $\delta^{13}\text{C}$ value of each FAME for the isotopic influence of the methanol derived methyl group added to the FA. This correction then gives the original CSSI $\delta^{13}\text{C}$ value for the FA in the soil/sediment sample.

The other reagent used in the derivatisation process is the partitioning solvent mixture. This is a mixture of hexane and DCM in the ratio of 4:1 by volume e.g., 40 ml of hexane plus 10 ml of DCM gives 50 ml of the partitioning solvent. This mixture is stored in a glass flask with a ground-glass stopper.

Time constraints

The time between drying the extract in the digestion tube and derivatisation should be kept as short as possible to prevent loss of FA due to oxidation and/or photolysis. Ideally the derivatisation should be done the same day. If this is not possible, take up the dry extract in 1 ml DCM to stabilise the FA and store in a cool dark place. Reduce the extract to dryness shortly before derivatization.

Stepwise procedure

1. Take the digestion tube with the dried extract, add 1 ml of 5% BF_3/MeOH reagent and cap tightly.
2. Stand the digestion tubes in a rack in a fan-ventilated oven at 70°C for 20 minutes.
3. Remove the rack from the oven and cool the tubes in water.
4. Add 1 ml of distilled water and 1 ml of the hexane/DCM (4:1) mixture and cap tightly.
5. Shake the tubes vigorously for 1 minute using a vortex mixer (Figure 4B).
6. Allow the layers to separate (Figure 5) and transfer the upper organic (hexane) layer to an actinic 2-ml GC vial using a glass Pasteur pipette.
7. Repeat the extraction with another 1 ml of the hexane/DCM mixture.
8. Combine the hexane layers in the 2-ml GC vial.

9. Reduce the solvent to dryness by warming in a heating block/bath at 40°C. Solvent removal can be assisted with a gentle stream of nitrogen gas.

As soon as possible, the GC vials are sent to a laboratory specialising in the measurement of CSSI values of individual FA by GC-combustion-IRMS. Include a vial of the MeOH to obtain the $\delta^{13}\text{C}$ value, if this has not been done.

If the FAME samples for CSSI analysis cannot be sent to the analytical facility for some time (months), add 0.5 ml of DCM to the GC vial, cap tightly and store in a refrigerator at 4°C. This will slow the degradation rate of the otherwise volatile FAMES. Reduce the solvent to dryness before sending the samples to the analytical facility.

GC-combustion-IRMS

The basic concept of a GC-combustion-IRMS system for CSSI analysis of FA is that the GC separates the mixture of FAME compounds into a predetermined order defined by the column used in the GC. As the individual FAME elutes from the GC column, it passes through a micro oven at high temperature (>600°C) in the presence of oxygen where the FAME is converted into CO₂. The excess oxygen is removed by passing over hot copper and the $\delta^{13}\text{C}$ value of the C in the liberated CO₂ is measured in the IRMS.

The IRMS produces a time-series trace similar to a GC trace (Figure 23). Because the FAMES are separated by the GC column in a known order and at an exact time interval after sample injection (retention time), the $\delta^{13}\text{C}$ values can be assigned to the individual FAMES in the extract based on the retention time in the IRMS trace.

Details of the GC-combustion-IRMS run conditions are as follows:

Stable isotope ratios of FAMES were analysed using a Trace GC (Thermo Finnigan, Milan, Italy) coupled to a DeltaplusXP IRMS (Thermo Finnigan, Bremen, Germany). Samples were injected into a split/split less injector at 300°C and separated using a BP225 GC column (25m, 0.25 mm i.d., 0.25 µm film; SGE, Melbourne, Australia). The GC oven was held at 50°C for 5 minutes before being ramped to 230°C at 7°C/min, where it was held for 10 minutes. The carrier gas was helium at a flow rate of 1.8 mL/min).

Pulses of working standard CO₂ gas were injected at the beginning and end of each sample to correct for intra-sample drift (Figure 23A). A mixture of standardised FAMES were analysed every 6 samples and used to correct for instrumental drift during batch analysis and to standardize FAMES to the PDB scale.

The DB225 column used in the GC-combustion-IRMS will separate the resin and fatty acid methyl esters adequately for the CSSI technique. Note: If the GC column is changed to a DB5 column, it is possible to analyse the same extracts for the 14 n-alkanes in the chain length range of 17 to 31 carbon atoms which might also be useful in discriminating between soil sources where resin and FA are indistinct.

Newer instruments may use different GC columns and run conditions.

6.4.10 Standards

The identification of the individual FAME peaks in the GC trace is typically done using a GC-mass spec. This instrument does not give the isotopic values of the FAMES but identifies

them by their mass and structure. The alternative and preferred approach is to include a sequence of known FA standards as one of the 'samples' run through the derivatisation process and then analysed by GC-combustion-IRMS (Figure 23B). Some analysts at a known amount of a less common FA to each sample as an internal standard.

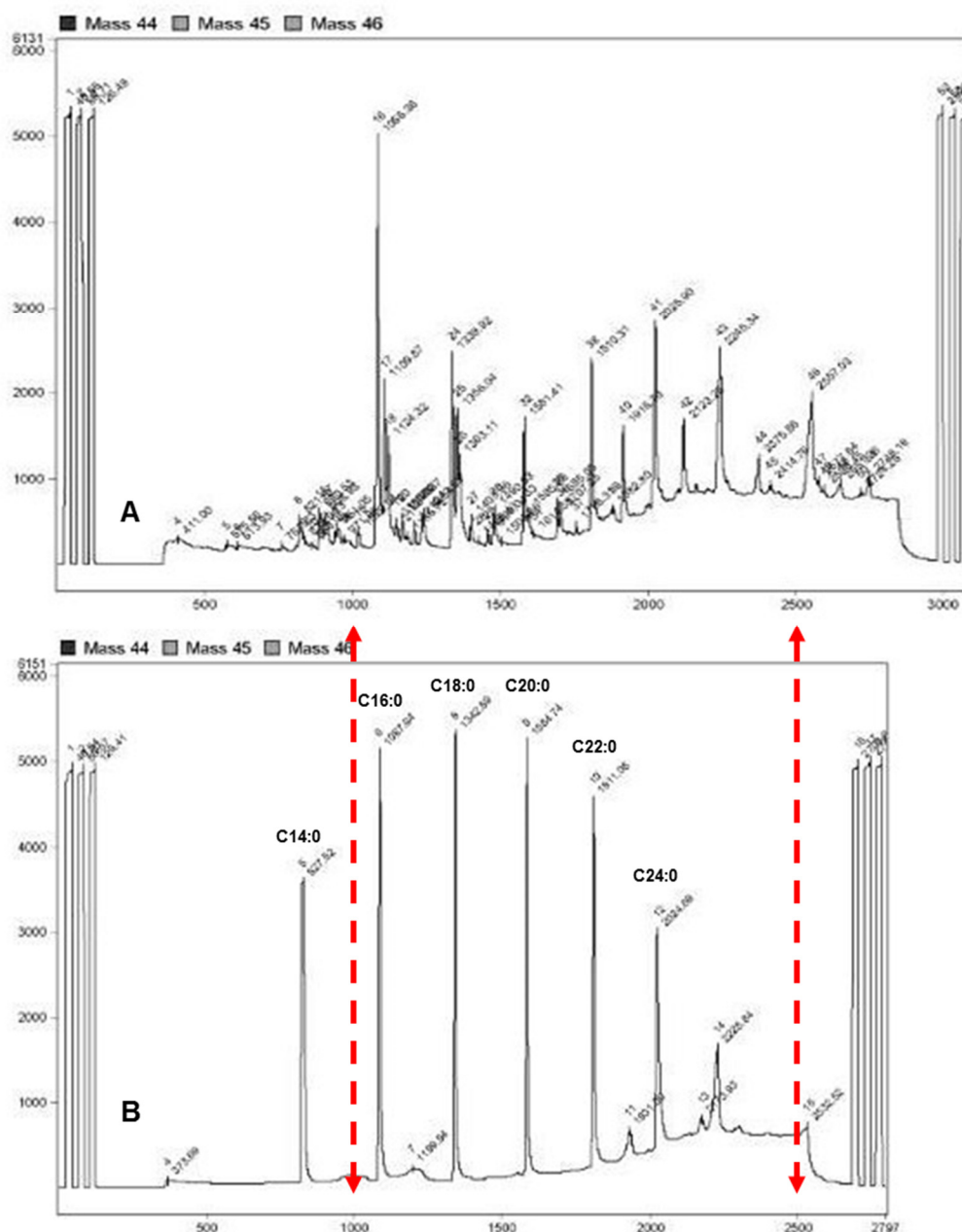


Figure 23: CG-combustion-IRMS time-series traces for A) a soil used for growing maize and B) a set of 6 known FA which were derivatized and analysed at the same time. Red broken arrows indicate the points of trace alignment based on retention time. The FA chain length is noted above each standard in trace B. Additional peaks are unknown until identified by GC-mass spec but are most likely to be C26:0, C28:0 and C30:0 which may also be useful in the CSSI technique.

The sequence of straight chain FA used in the standard (Figure 23B) were myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0), and lignoceric acid (C24:0). These standards cover almost the whole range of FA that are routinely used in the CSSI technique.

Standard preparation

The standard was prepared by dissolving pure FA standards in DCM to give an absolute concentration of 2000 mg/L for each standard in a stock solution. For the GC-combustion-IRMS analysis, there needs to be around 10 nmol C on the column for each FA. This represents around 120 ng C. A 1 µL injection of extract is used in the analysis so the 120 ng C is in 1 µL. This is the same as 120 µg C / ml. Because the final extract is made up to 1 ml in the GC vial prior to injection, this means that the GC vial must have around 120 µg C of each FA.

The 2000 mg / L stock solution can be considered to be a 2000 µg / mL solution of the FA standards. The average C content of the 6 FA in the stock solution is around 1500 µg / ml. Consequently, an aliquot of 0.1 mL will contain 150 µg C for each FA and this is close enough to ensure the standards can be measured by the GC-combustion-IRMS.

In simple terms, put 0.1 ml of the 2000 mg / L stock solution in the digestion tube, dry it, and derivatize it when the soil extracts are being derivatized.

6.4.11 Correction of the CSSI value for the added methyl group.

The methyl group added to the FA to form the FAME will have a different $\delta^{13}\text{C}$ value than the $\delta^{13}\text{C}$ value of the FA. Typically, methanol produced from natural gas may have a $\delta^{13}\text{C}$ value between -30 ‰ and -70 ‰. This will affect the estimation of the actual $\delta^{13}\text{C}$ value of the FA. Provided the $\delta^{13}\text{C}$ value of the methanol used in the derivatization process is known, this effect can be corrected using a simple equation:

$$\delta^{13}\text{C}_{\text{FA}} = \frac{\delta^{13}\text{C}_{\text{FAME}} - (1 - X)\delta^{13}\text{C}_{\text{Methanol}}}{X}$$

Where X is the fractional contribution of the FA to the methyl ester. This can be calculated from the number of carbons in the FA divided by the number of carbon atoms in the FAME derived from the FA. For example the FAME, methyl stearate, has one added carbon and thus an X value of 18/19 or 0.9474.

The correction for the addition of one methyl group is relatively small but must be done for each FA in each sample. This can be done quickly in a spreadsheet but may have already been done by the analytical laboratory.

Confirm that the data have been corrected before interpretation.

7 Data interpretation

Data interpretation requires the use of mixing models to deconstruct sediment mixtures into their component sources. There are several possible models to use including **IsoSource** (Phillips & Greg 2003), which is a simple “brute force” approach, and **SIAR** (Parnell et al. 2008, 2010, 2013) which uses a Bayesian approach. The IsoSource model is freely available from the USEPA website (www.epa.gov/wed/pages/models/stableisotopes/isotopes.htm).

A full description and manual for SIAR is also freely available and can be downloaded from the internet at website (<http://cran.r-project.org/web/packages/siar/siar.pdf>). These documents include a very useful Ecologist's Guide (Inger et al. 2012).

Both models produce essentially the same results for the same data inputs except that the SIAR model results can include statistical estimates on the uncertainty associated with fractionation. This is especially useful in food web studies where the fractionation step is uncertain or variable between species. However, a basic premise in the CSSI technique is that fractionation does not occur. Consequently, in these protocols the simpler IsoSource mixing model is described first, as many of the features of SIAR are not required or are not readily applicable to the CSSI technique, unless there have been multiple analyses of the same landuse sources soils to assess variability.

Both mixing models can be used.

The output from either mixing model does not give the proportions of each source soil in the sediment mixture. The model outputs give the proportion of each source soil required to obtain an isotopic balance with the sediment sample based on the ^{13}C isotopic signatures of the bulk carbon and the FA biomarkers in the source soils and the sediment. These are **isotopic proportions**.

The mixing model will attempt to produce an isotopic balance with the data provided as potential soil sources for the sediment being deconstructed. If a source is missing, the output may have high levels of uncertainty. High levels of uncertainty will also occur if a potential source, that could not be present in the sediment mixture due to geographical constraints, is included in the modelling.

Note that the **isotopic proportions** must be converted to **soil proportions** before the data can be interpreted in terms of erosion or deposition in the watershed.

Important

Do not include concentration data in the same model run with stable isotope data because the parameters are not compatible. While the stable isotopic values for a particular source soil will remain constant through sediment diagenesis, the concentrations will decrease and the relationship between the source soils and those same soils in the sediment mixture will change.

7.1 Selecting data for the models

With conventional linear models often used in food web studies, the data used comprises the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data for each whole organism (or part thereof) i.e., two isotopes. This allows for three sources to be partitioned. To increase the number of sources that can be partitioned

in the linear model, the number of isotopes must be increased. This is achieved by analysing the isotopes of sulphur (^{34}S), oxygen (^{18}O) and hydrogen (^2H or ^2D) if that is possible.

For the CSSI technique, the IsoSource and SIAR mixing models can cope with more sources than the $n+1$ isotopes and the isotopes used are the $\delta^{13}\text{C}$ of the bulk soil and the $\delta^{13}\text{C}$ values of the FAs. This raises the questions “Which fatty acids should be used in the mixing model?” and “Is it essential to have the bulk soil $\delta^{13}\text{C}$ as one of the isotopes in the model?”

Answering the second question first, “No, if the soils are all from C4 or C3 land uses. The bulk soil $\delta^{13}\text{C}$ value must be used if there is a mixture of C4 and C3 plants in the landscape (Figure 24).” The response might also be “Why wouldn’t you use the bulk soil $\delta^{13}\text{C}$ value in the model? It is present in every sample and it is the largest carbon component in the sample.

Which fatty acids should be used in the mixing model can be resolved by examining the CSSI data received from the analyst. There may be as many as 52 FAs identified in the extract (Table 2). The choice of FAs can be simplified by considering the role of the FA as a biomarker.

Table 2: List of Fatty Acids that might be present in a soil extract analysed for CSSIs.

Green highlights are mostly straight-chain saturated FAs that have been tested in the method development (Gibbs, 2008). Yellow highlights may also be suitable but have not been tested in soil mixtures.

c13:0	c14:1	c14:0	br15:1	i15:0
a15:0	c15:1w6c	i14:0	c15:1	c15:0
c14:0 2-OH	br16:1	c14:0 3-OH	i16:0	c16:1w9c/7c
c16:1w9t	c16:1w5c	c16:0	10Me16:0	12Me16:0
i17:0	cy17:0/17:1w6c	a17:0	c17:0	2-OH 16:1
c16:0 2-OH	10Me17:0	c18:3w6/3w3	c18:2w6c/6t	c18:1w9t/7c
c18:1w9c	c18:1w7t/5c	c18:0	br19:1a	10Me18:0
c19:1	cy19:0	c19:0	c20:4w6	c20:3w6
c20:5w3	c20:3w3	c20:2	c20:1w9	c20:0
c21:0	c22:6w3	c22:1w9/22:2	c22:0	c23:0
c24:1w9	c24:0	C26:0	C28:0	C30:0

To be a useful biomarker, the FA must occur in both the source soils and the mixture. Often there will be FAs missing in one or more of the sources or the mixtures. Do not use any FAs which do not occur in the mixtures.

The inclusion of C12:0 as an internal standard during analysis excludes the C12:0 FA or another FA used as an internal standard.

The concentration of the FA biomarkers present must be sufficient to produce reliable $\delta^{13}\text{C}$ values. FAs with low concentrations should not be used or they should be flagged in the spreadsheet table as potentially unreliable so that they are only use with caution.

A key assumption of the CSSI method is that the FA biomarkers used have been produced by the plant community growing on the soil so that they bind to and thereby label that soil source. FAs with an odd number of carbons (C17, C19, C21) are mostly produced by bacteria, not plants so they shouldn't be used.

Many of the FAs reported (Table 2) are present in small quantities and are polyunsaturated FAs (PUFAs). These are mostly produced by soil organisms including soil fungi, which may also be present in the sediment mixture. That would confuse the mixing model, especially if they were different to those produced in the soil sources. PUFAs are also unstable in the derivatisation process resulting in poor reproducibility of methylation (can vary by up to 2 per mil), and there is a lack of good standards for analysis. The recommendation is do not use them.

The original method development used the saturated FAs (green highlights Table 2) as these are mostly present in all plants in high concentrations. It is recommended that these FAs are used as the first choice to select from. Principal Component Analysis (PCA) or Linear Discriminant Analysis (LDA) can be used to assist selection to get the best discrimination possible in the mixing model.

If all else fails, use the fall back position using a combination of C16:0, C18:0 and C18:1 as described in the original method (Gibbs, 2008).

The original protocols are set out in section 8.4 Selecting isotopes and source combinations.



Figure 24: A New Zealand farming landscape adjacent to urban development in the Waikato region. The land uses include pasture, mixed exotic tree plantations (C3 plants), maize (C4 plant), intensive dairy farming and urban gardening. The land has previously been fallow pasture used as a hay crop and subsequently used for grazing by dairy cows. [Photo montage by Max Gibbs]

8 IsoSource

The IsoSource model was designed for the situation where there are too many sources. Although it was originally intended for the study of food webs, it is ideally suited to the identification and apportionment of sources in a sediment mixture. While the model can cope with too many sources, to run the model requires a minimum of 3 sources. The model also requires a minimum of 2 isotopes, one of which should be the ^{13}C of the bulk soil carbon.

To use this model, the data from the potential source soils and the sediment from the deposition zone need to be collated into a table including the bulk $\delta^{13}\text{C}$ values and the corrected CSSI $\delta^{13}\text{C}$ values of the FAs (e.g., Table 3). A constraint with the use of IsoSource is that there must be a $\delta^{13}\text{C}$ value for every FA from every source soil as well as the sediment mixture. To meet this requirement a sub-set of parameters should be produced as a working database. A useful starting point is to take the $\delta^{13}\text{C}$ isotopic value of the bulk soil carbon and the two fatty acids, oleic and palmitic acids, to test the level of discrimination. This trio of isotope values are referred to as the COP isotopes (i.e., carbon, oleic and Palmitic; Gibbs 2008) but a third FA isotope can be added if needed (e.g., myristic acid). An example of these isotope values are presented in Table 2.

Table 3: The $\delta^{13}\text{C}$ values of the bulk carbon and three selected FA from six different land-use sources.

Land-use / Sources	Bulk carbon	CSSI (Fatty acids)		
	$\delta^{13}\text{C}$	Myristic	Palmitic	Oleic
	(‰)	(C14:0) (‰)	(C16:0) (‰)	(C18:1) (‰)
Pasture	-22.2	-27.0	-24.0	-21.6
Native Forest (Nikau)	-27.7	-34.9	-30.4	-28.2
Native Forest (Kauri)	-25.1	-28.9	-25.6	-27.8
Pine forest (Mature)	-26.2	-40.7	-32.4	-29.5
Pine forest (Clear-felled)	-26.5	-32.7	-28.7	-28.2
Seagrass (estuary)	-8.0	-11.7	-10.9	-16.9

Because the bulk $\delta^{13}\text{C}$ value represents the integration of all OC in the soil or sediment, it must be included as the first parameter in the IsoSource modelling. The data in Table 3 show that while the bulk soil carbon $\delta^{13}\text{C}$ values may be similar between some land uses, the CSSI $\delta^{13}\text{C}$ values of the three FA have sufficient differences to allow separation of these sources by the model.

Note that the FA component of OM in the soil is about 1 thousandth of the bulk OC content but their CSSI $\delta^{13}\text{C}$ values are not affected by this concentration difference.

8.1 IsoSource – how it works

The IsoSource mixing model apportions the sources as feasible by making a scratch-pad table using all possible combinations of the isotopic values from the potential sources, and then selecting only those combinations that match the isotopic values of the sediment mixture, within a selected tolerance (‰). This is a brute-force approach rather than a true model. The number of parameters used and the number of sources tested directly affect the speed of processing. When these numbers are small (<5) IsoSource will run relatively

quickly. When the numbers are larger, IsoSource will run slower. If the scratch-pad table is larger than the available memory space, IsoSource will stop. The number of matches found for the mixture depends on the tolerance. If this is set too large, a large number of matches will be found, but set too small and no matches may be found. A stepwise reduction in the tolerance will define the lower limit for each sediment/source combination. The brute force approach means that if a match is not found, it is not possible to obtain an isotopic balance with that specific combination of sources and that combination is not feasible. Conversely, the matches found are feasible combinations (solutions). The number of times each feasible solution occurs is summed to give a distribution histogram (Figure 25).

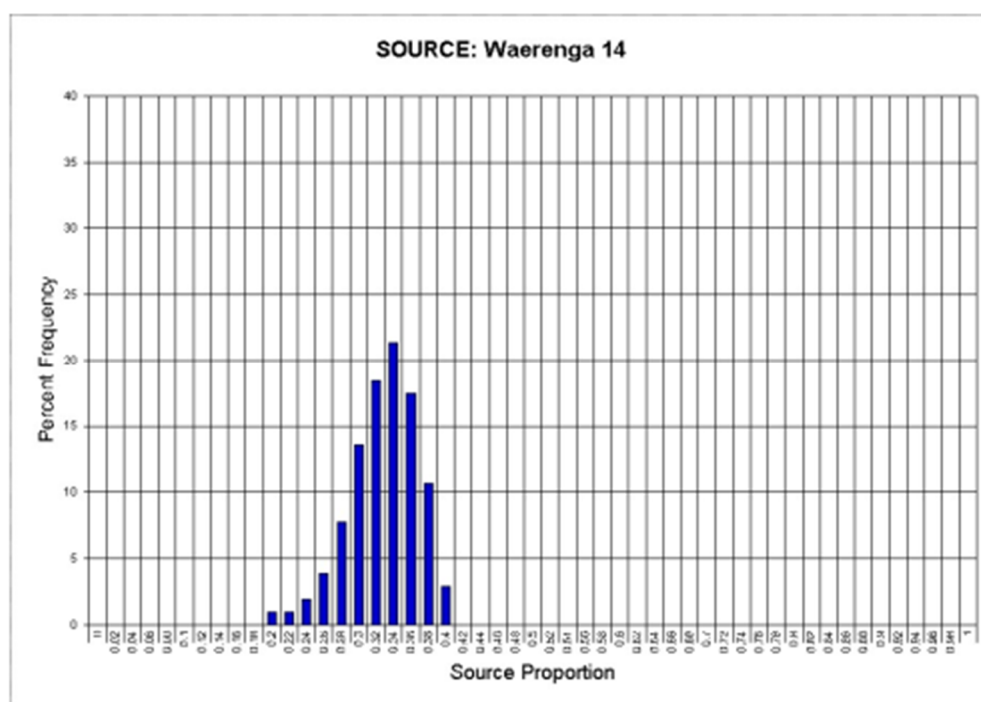


Figure 25: An example of the histogram of feasible solutions for a specific source relative to the sediment mixture being tested. This output confirms that this source is present in the mixture at between 20% and 40%. The corollary is that this source is not present at greater or lesser proportions. The geometric mean value in this example is 34% which is the most likely source proportion.

Any value within that distribution may be valid but the more times (percent frequency) that value occurs, the higher the probability that that is the correct proportion. To simplify the data presentation, the mean feasible solution (Source Proportion) value can be used provided it is treated as the best estimate with a defined level of uncertainty rather than an absolute value. Because there is no isotopic fractionation of the FA once formed and the sources are given as mean $\delta^{13}\text{C}$ values for each land use, the uncertainty is limited to the proportional relationship between the sources. The level of uncertainty is defined by the standard deviation (SD) about the mean expressed as a percentage of the whole.

A similar histogram of feasible solutions is produced for each source used in the model. In combination, these histograms give the feasible proportions of each source in the sediment mixture being examined (Figure 26).

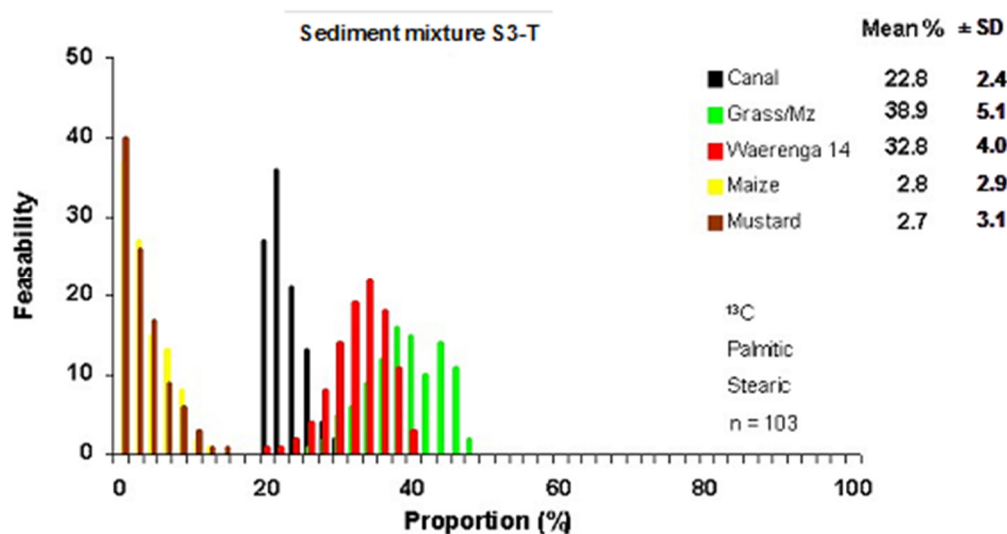


Figure 26: Histograms of feasible contributions of all source soils contributing to the sediment mixture S3-T. Mean and standard deviation (SD) values are given in the IsoSource output. The value of 'n' shows that there were only 103 possible combinations of these sources contributing to the sediment mixture. The low mean % values with a similar SD value indicates that these sources are minor and may not be present in the mixture..

Each feasible solution is expressed as the isotopic proportional contribution (%) of that source soil to the sediment mixture required to produce an isotopic balance. Isotopic proportions that extend from 0% to a finite value are less definitive than those with a full distribution curve as it is possible that those sources may not be present (e.g., Figure 26) while those with a full distribution curve (e.g., Figure 25) are present within the range of the distribution curve.

For example (Figure 26), a potential source with a feasible isotopic proportion estimate of 22.8% and a standard deviation of $\pm 2.4\%$ indicates that that source is present and contributes between 20.4% and 25.5% of the sediment in that mixture. Conversely, a potential source with an isotopic proportion estimate of 2.8% and a standard deviation of $\pm 2.9\%$ indicates that that source may be present at up to 5.7% but it is just as likely that it is not present in that sample. Such high levels of uncertainty typically occur at the less than 5% contribution and such sources are considered to be minor.

The total number of feasible solutions (n) found by IsoSource provides a level of confidence in the solutions. The confidence level increases as n decreases towards 1, which is a unique solution.

8.2 Using IsoSource

The IsoSource mixing model (Phillips & Gregg 2003) is freely available from the USEPA website www.epa.gov/wed/pages/models/stableisotopes/isotopes.htm. The model must be installed on the computer, usually as the folder /IsoSource in the programme directory. This folder contains 3 sub-folders: /data, /help, and /output.

The /help sub-folder contains information on the use of IsoSource with examples. The /data sub-folder is used to save the parameter data used in the modelling. These data files are small at 1KB each. It is recommended that individual working folders are set up in the /data and /output sub-folders for each different project/study to keep track of the results. The /output sub-folder is used by the model to save the successful scratch pad results – as a filename.OUT file – and the result file – as a filename.TOT file. The .OUT files are large and list the feasible combinations of the sources. The .TOT files are always small at <10KB each. The .OUT and .TOT files are written in a comma delimited format, similar to a .CSV file, which can be read as a text file or can be imported directly into a spreadsheet such as Microsoft Excel.

The use of the IsoSource model is simple and is demonstrated in the following sections.

8.2.1 Loading the data parameters

The IsoSource model has an interactive screen (Figure 27) which allows data to be entered or modified, and saved. It allows the source proportions to be calculated and the histograms of feasible solutions to be displayed. The data is entered by moving the cursor to the field where data is to be entered, 'clicking' the left mouse button to activate that field (background turns red), and then typing in the data or text required (Figure 28).

		ISOTOPES				
Mixtures						
SOURCES						

Figure 27: IsoSource programme interactive screen.

The **Title** field should contain a simple but meaningful name.

The **Increment** field can be set to 5% meaning the programme will calculate and report the results at 5% step intervals from 0% to 100%.

The **Tolerance** is the range of variability of the source isotopic values that are acceptable for a match. The tolerance is in ‰ and should be set at 1 for the first run.

The **Isotopes** fields (dark blue) contain the names or abbreviations of the isotopes being used in the model run. The data are entered in subsequent fields as defined by these column headers. For convenience, the first column should be ^{13}C representing the bulk $\delta^{13}\text{C}$ values.

The **Mixture** fields (pale blue) are the isotopic values of the selected isotopes for the sediment mixture being tested.

The **Sources** field (green) lists the names of the sources being tested by the model and collates the isotopic values for each source under the column header for that isotope.

When data entry is complete (Figure 28) it is saved by left clicking on the **File** tab in the screen header. This brings up a new screen that requires a file name. This should be meaningful and should have a run or version number (Figure 29).

The screenshot shows the IsoSource software window. The title bar reads 'IsoSource'. The menu bar includes 'File', 'Calc', 'Print', 'Clear', 'View', 'Graph', 'Exit', 'About', and 'Help'. The 'Title' field contains 'Sediment mixture S3-T'. The 'Increment' field is set to '2' with a '%' symbol. The 'Tolerance' field is set to '1'. The main data table has a header row for 'ISOTOPES' with columns for '13C', 'Palm', 'Stear', and two empty columns. Below this is a 'Mixtures' row with values -27.1, -31.6, -30.6, and two empty cells. The 'SOURCES' section (indicated by a vertical green bar on the left) lists five sources: 'Canal', 'Grass/Mz', 'Waerenga 14', 'Maize', and 'Mustard'. Each source has corresponding isotopic values in the columns: Canal (-22.2, -33.8, -28.6), Grass/Mz (-26.0, -27.4, -30.7), Waerenga 14 (-29.2, -33.4, -34.5), Maize (-23.7, -24.8, -29.7), and Mustard (-27.6, -33.1, -34.6). There are also five empty rows below the 'Mustard' source.

		ISOTOPES				
		13C	Palm	Stear		
Mixtures		-27.1	-31.6	-30.6		
SOURCES	Canal	-22.2	-33.8	-28.6		
	Grass/Mz	-26.0	-27.4	-30.7		
	Waerenga 14	-29.2	-33.4	-34.5		
	Maize	-23.7	-24.8	-29.7		
	Mustard	-27.6	-33.1	-34.6		

Figure 28: The interactive screen with the data entered.

Entering the File menu also allows an existing file to be loaded by clicking on the filename in the list displayed. This feature is useful when there are multiple iterations of a modelling session. Each change in the data or settings should be saved with a separate identifier (e.g., run number) before the model is run, as a safeguard - in case the model stalls. The program automatically returns to the interactive screen after saving or loading a file.

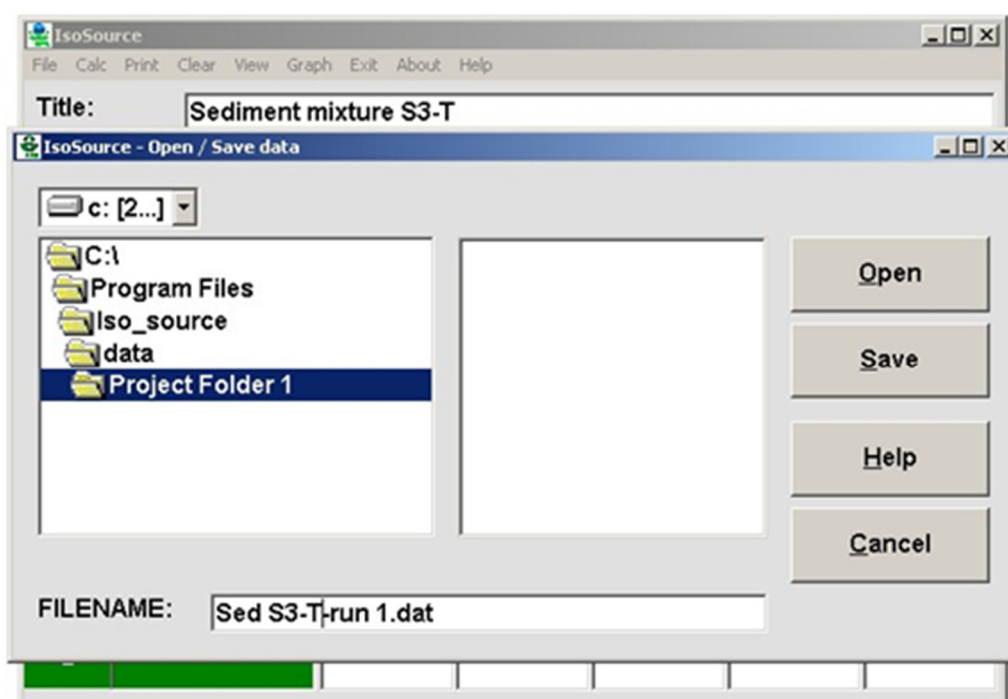


Figure 29: The File menu allows the data just entered to be saved or data previously entered to be retrieved and loaded for use in the model. Type in the name and click [Save] to complete saving the data.

8.2.2 Running IsoSource

For test runs always set the **Increment** to 5 as this will run faster and can be reset to 1 and rerun when the smallest **Tolerance** value is found. With the data saved, run the model by clicking the **Calc** tab in the screen header. The model runs displaying a Calculation progress bar in the centre of the screen (Figure 30).

The progress bar, and the interactive screen behind, provide information about the data and the settings. Steady progress across the Calculation complete bar indicates that the model is working. Very slow progress indicates that the model is working and there may be too many parameters, or the settings are too broad. No apparent progress indicates that there may be too many parameters or the settings are too broad, and that there is not enough memory available for the scratch pad file.

Note that when IsoSource is running very slowly, the red highlighting in the field last used on the interactive screen will 'turn off' if the model is capable of running. However, this red highlighting will remain 'turned on' if the program has stalled. To stop the run, click on the [X] in the Calculation progress bar. This will close down IsoSource completely and all unsaved data lost.

Restart IsoSource and use the File menu to reload the last data and setting used. These should be modified either by reducing the number of parameters (isotopes) or the number of sources, or by adjusting the tolerance setting down or the Increment setting up. Save the changes in the same file name as before. This will over-write the data that produced the stall so that that combination does not get used again by accident. Similarly, run the model using the same output filename and this will also be over-written.

At the end of the model run, the program replaces the Calculation progress bar with a statement of failure (Figure 31) or success (Figure 32).

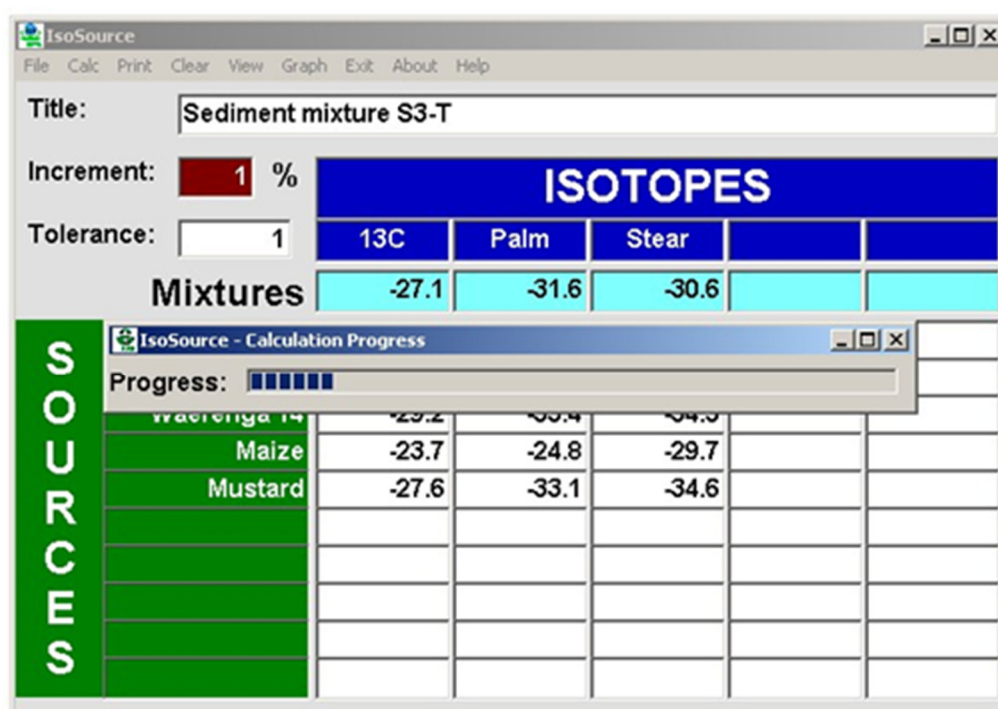


Figure 30: The IsoSource model calculates the combinations of all source isotopes and shows a progress bar across the centre of the screen.

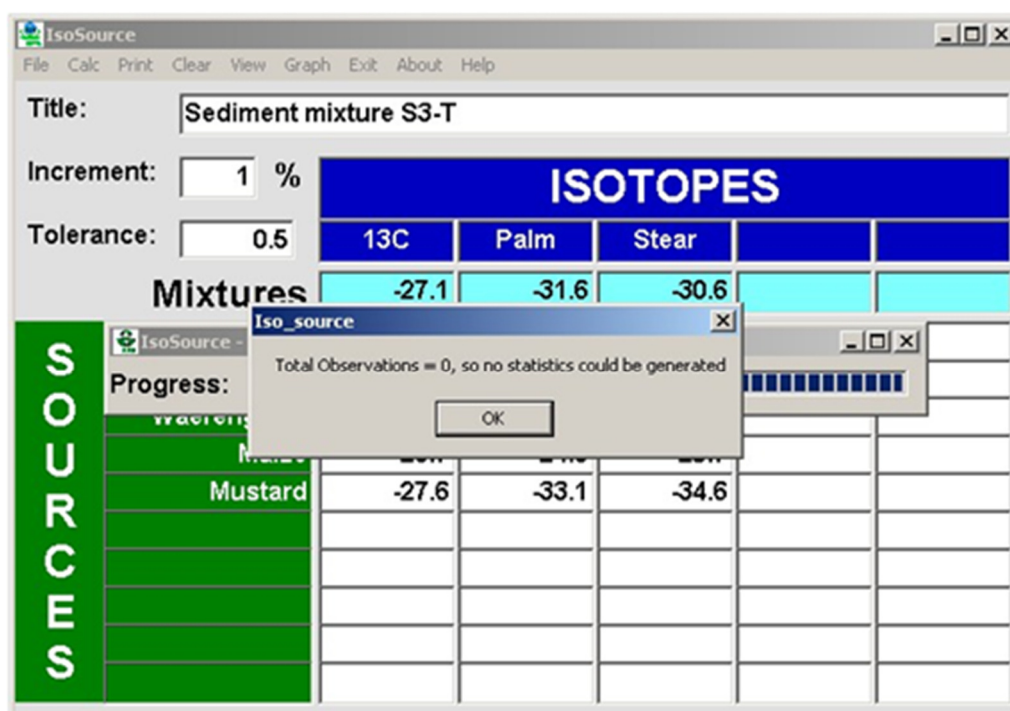


Figure 31: Notice of run failure. No observations were found so no statistics could be generated.

The failure message means that the tolerance was set too small and that there were no feasible solutions in the scratch-pad file inside the range of that tolerance. Remedy: Increase the tolerance range and rerun the model using the same filenames. The outcome of re-running the model on the same data set with an increased tolerance (1 as opposed to 0.5) is shown in Figure 32

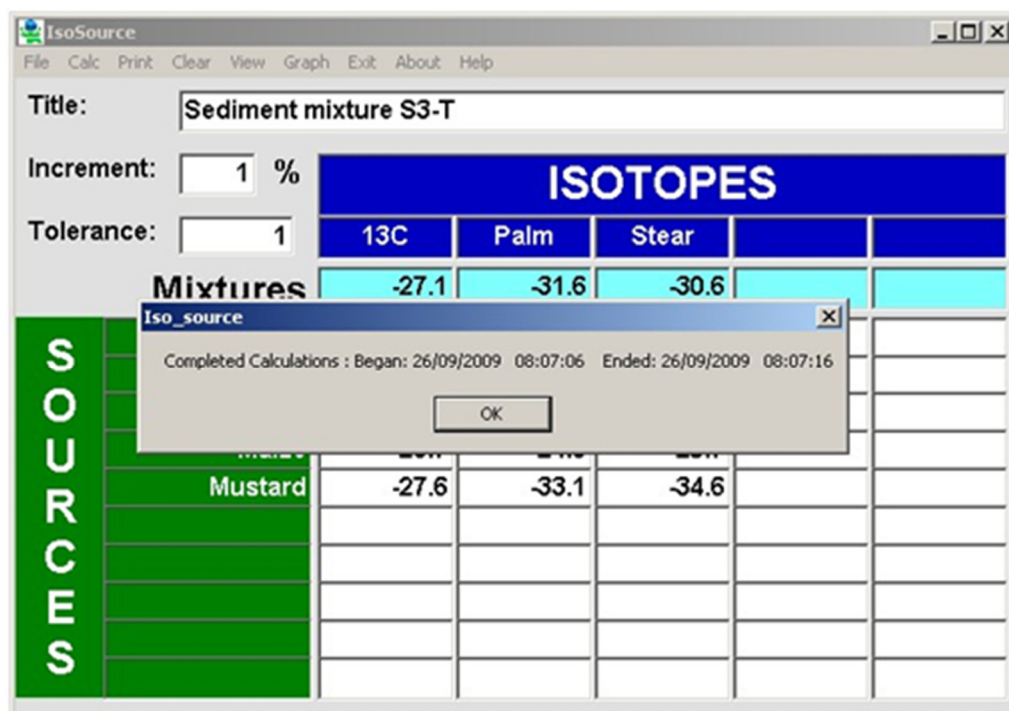


Figure 32: A successful model run. This shows the time taken for the model to run, in this case 10 seconds. Note that data is the same as for Figure 31 but the tolerance range has been increased from 0.5 ‰ to 1 ‰.

8.2.3 Viewing the results

To assess the results, click the **Graph** tab in the screen header. This will open a new window with a screen header in the upper right hand side. Click the File tab to display the output files (filename.out) and select the appropriate file to display. The results will be displayed as a histogram of each source on a single page (e.g., Figure 25). Step through each source histogram sheet using the Next or Previous tabs. Leave the Graph window by clicking the Exit tab.

The numerical results can be viewed in the **View** window. Click the View tab in the screen header and the View window opens. Before the View window opens, the program displays the same warning about the use and interpretation of the data, as is presented on the IsoSource web-page (Figure 33). This caution is important and the suggestions should be heeded. The mean values provided are not unique solutions and the whole range are equally valid. However, as the CSSI technique requires a single number for the conversion of isotopic proportions to soil proportions, the mean value is used and the standard deviation ($\pm$ 95% SD) is used as the uncertainty term. Click on the .TOT filename and it will be displayed.

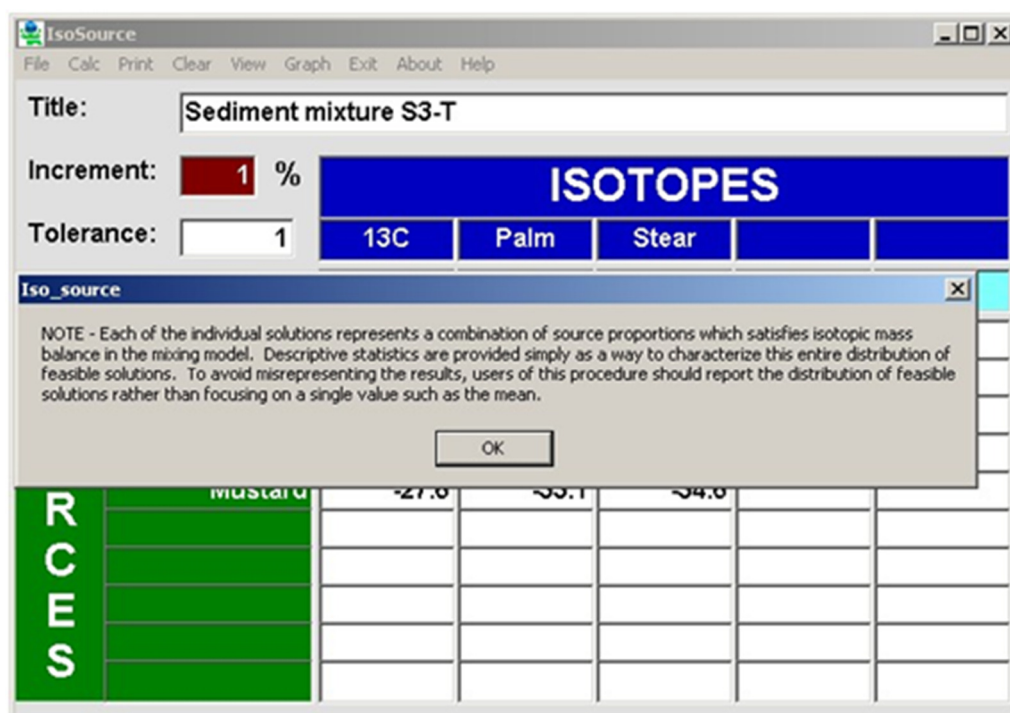


Figure 33: Warning notice displayed before the data can be viewed.

Click [OK] and the View window opens. This is a blank spreadsheet. Click the File tab in the screen header to select the output file (Figure 34). The output file required is the **filename.tot** file. This displays the data (Figure 35) as it was entered in the interactive screen. Scrolling down, the data generated for the histograms are displayed in a table and below that are the statistics. The statistics include:

n the number of solutions,

Mean the mean of the histogram increment range between 0.0 and 1.0 for each source,

Standard Deviation the standard deviation about the mean, and the

Minimum, Maximum, 1 percentile, 50 percentile, and 99 percentile

for the histogram range for each source.

While the data in the view screen cannot be manipulated, the **n**, **mean** and **standard deviation** data can be manually extracted or copied into a working folder to convert isotopic proportions into soil proportions.

To work further with this data, the **filename.tot** file can be retrieved from the IsoSource/output/project1 folder and imported into a spreadsheet (e.g., Excel, Matlab, etc.,) as a comma delimited file. In the spreadsheet, the HISTOGRAM_INC column can be multiplied by 100 and the data displayed graphically as the isotopic proportional % contribution of each source in the sediment mixture.

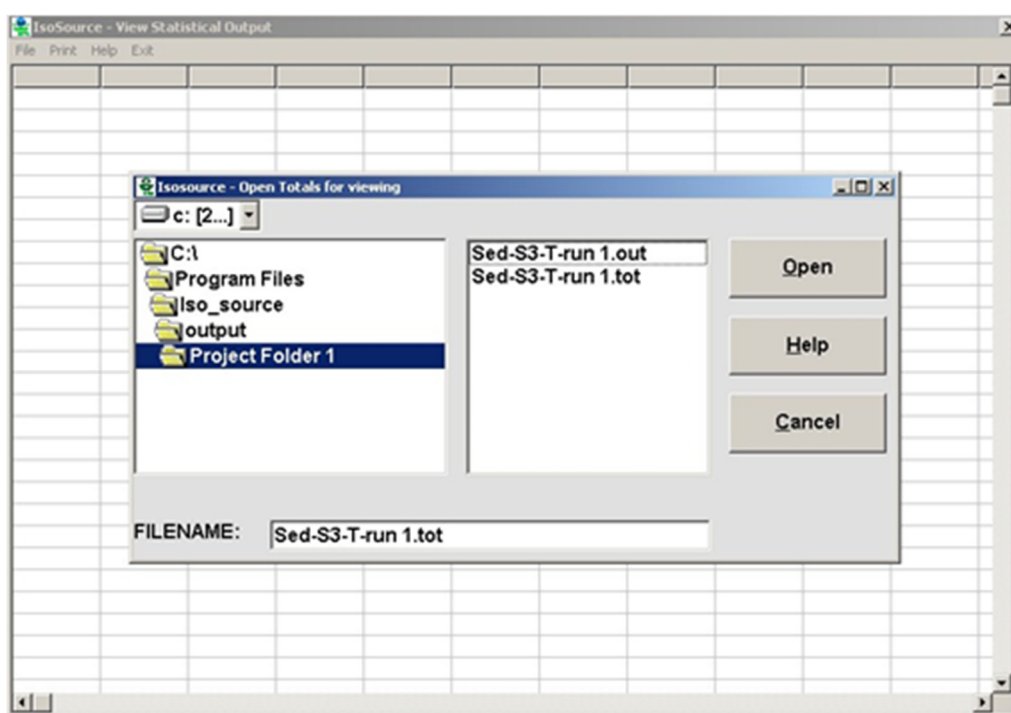


Figure 34: Clicking the File tab on the spreadsheet opens the file menu. Click on a '.tot' filename and that file will automatically open as a passive display (i.e., cannot be manipulated).

The screenshot shows the 'IsoSource - View Statistical Output' window with a detailed data table. The table is organized into sections. The first section contains parameters and settings. The second section is a histogram showing the distribution of data for different sources.

Parameter	Value
Date:	27/09/2009
Time:	08:15:54
Title:	Sediment m
Increment:	1%
Tolerance:	1
Isotopes:	13C Palm Stear
Midures:	-27.1 -31.6 -30.6
Sources:	
Canal	-22.2 -33.8 -28.6
Grass/Mz	-26.0 -27.4 -30.7
Waerenga 1	-29.2 -33.4 -34.5
Maize	-23.7 -24.8 -29.7
Mustard	-27.6 -33.1 -34.6

HISTOGRAM	Canal	Grass/Mz	Waerenga 1	Maize	Mustard
INC					
0	0	0	0	267	287
0.01	0	0	0	230	238
0.02	0	0	0	189	196
0.03	0	0	0	154	158
0.04	0	0	0	129	127
0.05	0	0	0	94	97
0.06	0	0	0	81	74
0.07	0	0	0	59	54
0.08	0	0	0	44	39
0.09	0	0	0	25	20

Figure 35: Example screen shot. The data displayed shows the parameters and settings used to generate the file as well as the output data and the data statistics related to the histogram increment used. The scroll bars side and bottom allow the whole file to be viewed.

8.2.4 Fine tuning the model

Run-time problems

If the run-time is slow with an increment of 1%, increase the increment to 5%. For a final more precise run, set the increment back to 1%. The increment affects the number of feasible solutions given by **n** in the results and thus the number of iterations the model needs to do when writing and matching data in the scratch-pad.

The tolerance is the range of variability of the source isotopic values that are acceptable for a match. The tolerance is in % and is valid from 0.05 upwards, although values above 5 may indicate problems. For the initial test run, the tolerance should be set at 1. If a set of solutions is not found, then it should be increased stepwise by 1 and retested until a solution is found. Values much above 1 may indicate a potential problem with the source data including the possibility of a missing source. When feasible solutions are found, the tolerance should be decreased incrementally and retested until a solution is not found. Set the tolerance to the last value that produced feasible solutions.

If the increment was set to 5 during the testing, set it to 1 and rerun the model.

Remember to save the data before each test. Saving to the same filename overwrites the current parameters to that file. Saving the output file to the same filename overwrites the current output results to that file. Note: If the run is a failure and no observations were found (Figure 31), the file will contain no results. An earlier iteration of the run parameters must be rerun using a different combination of settings that will give a successful output (e.g., Figure 32) to obtain a set of results for that sediment.

Broad histogram peaks

A successful model run does not mean that the results are “good” or even meaningful. They are simply valid calculated solutions based on the parameters loaded into the interactive screen. For example, a result which spreads across the whole range from 0.0 to 1.0 (Figure 36) simply means that almost all the solutions include that source. This result is valid but has very low certainty of the proportional contribution, so it is not very helpful. This result can be interpreted in several ways:

- there may be a missing source, or
- there may be several sources with similar isotopic signatures, or
- the isotopes selected are not appropriate for the sources being evaluated, or
- the run settings are too broad.

There may be other possibilities, but these are the main causes of broad flat histograms.

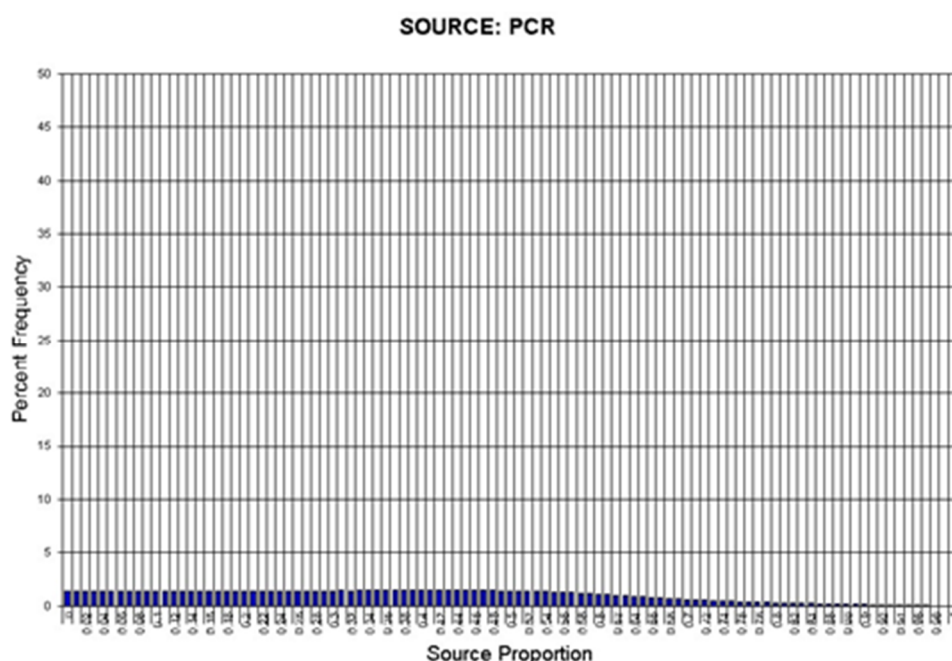


Figure 36: Example of a broad distribution histogram indicating that that source may be included in almost all feasible solutions.

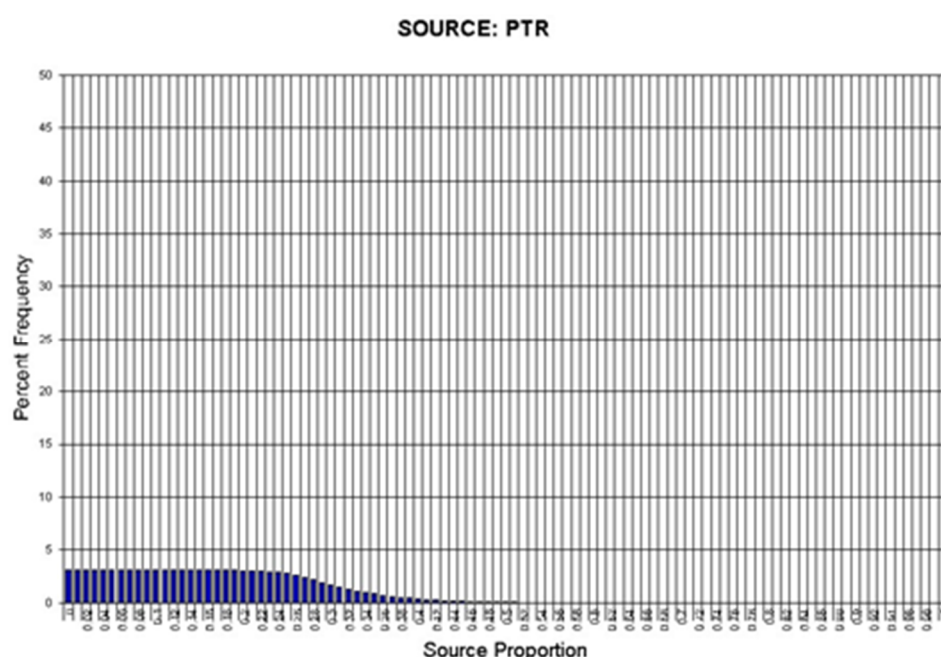


Figure 37: More commonly, broad histogram peaks cover only part of the range, in this case from 0.0 to 0.5.

It is more common for broad peaks to spread over part of the range in which case, adjusting run settings or the isotope selected may be sufficient to resolve the problem. For example the pasture soil, PTR (Figure 37), gave a broad histogram range from 0.0 to 0.5. After a series of test runs adjusting settings and the isotopes used in the model, a more meaningful result was obtained (Figure 38).

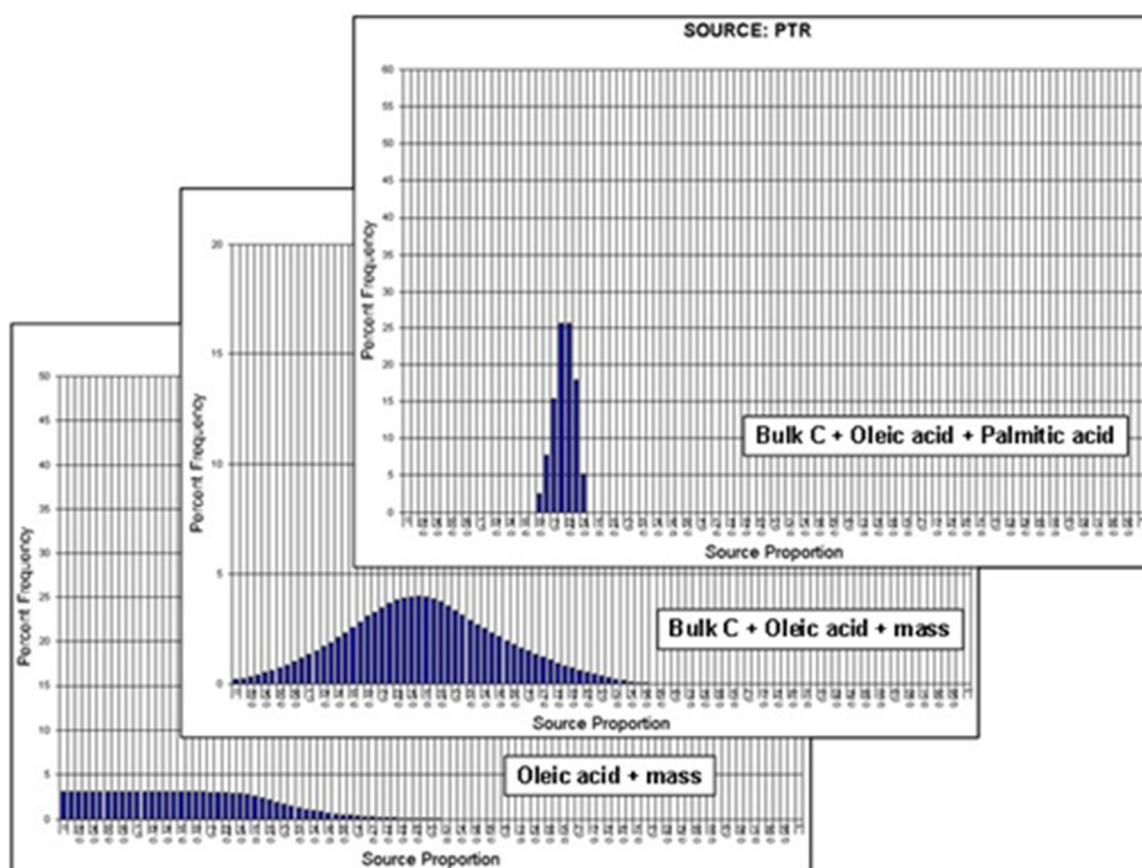


Figure 38: Sequential improvement in model discrimination obtained by changing the isotope range used in the model. Note that mass is not a valid element for use in the mixing model.

The PTR source was one of six sources in the model run. The initial run used the CSSI values for oleic acid and the mass data of oleic acid (note, the use of mass is discussed below) for each source in the sediment mixture.

Adding the bulk carbon isotopic data produced an improvement in the form of a bell-shaped curve with a well-defined maximum but still having low certainty, because of the breadth of the histogram peak.

Adding the third isotope, the CSSI values for palmitic acid, and removing the mass data dramatically reduced the uncertainty, as indicated by a narrow histogram range. This combination of isotopes, ^{13}C (C), oleic acid (O), and palmitic acid (P) or 'COP' are the recommended basic elements used in the modelling in the CSSI technique (Gibbs 2008).

Using mass data

The reason for testing sources using the mass data in the IsoSource mixing model was an attempt to allow for the different concentrations of FA and carbon in the source soils. However, prolonged exposure of the soil sources to water enhances biodegradation of the bulk carbon and the FA components (Branowetz et al. 2006), and the concentrations change in the sediment mixture. That change causes the uncertainty observed. Because the CSSI values do not change, more robust source apportionment solutions are modelled using only isotopic parameters, without mass. Concentration issues can be addressed subsequent to the modelling.

8.3 Soil proportions

The outputs from IsoSource, as it is used in the CSSI technique, are based on isotopic values of carbon and are given as carbon **isotopic proportions** not **soil proportions**. As the isotopic biomarkers are a small fraction of the total OC in the soil and the total OC is typically less than 10% of the whole soil, the isotopic proportions must be converted to soil proportions. That is, if the source soils were mixed together in the corrected soil proportions, the resultant mixture would have the same isotopic signatures as found in the sediment mixture.

This conversion is done using a linear correction equation based on the carbon content of each source soil (Gibbs 2008):

$$\%source_n = \frac{I_n / \%C_n}{\sum_n (I_n / \%C_n)} \times 100$$

where I_n is the mean feasible proportion of source n in the mixture as estimated from isotopic values of carbon by IsoSource, and $\%C_n$ is the % carbon in the source n soil.

Because this calculation only uses the %C of the source soils for scaling, the proportional contribution of each source soil is independent of any loss of total carbon or FA in the sediment mixture through biodegradation. The level of uncertainty defined by the standard deviation remains the same.

The use of the conversion equation can be illustrated by considering a solution with a mixture of three different sodium salts – sodium chloride (NaCl; mw 58.45), sodium nitrate (NaNO₃; mw 85.0), and sodium sulfate (Na₂SO₄; mw 142.0) – and each salt contributes 33.33% of the sodium to the solution (Table 4). What is the proportion of each salt in the mixture?

The percent salt in the mixture is inversely proportional to the %Na in the salt. Consequently, less NaCl is needed than NaNO₃ or Na₂SO₄ to have a solution with the same %Na in the mixture from that source.

Table 4: Illustration of use of the conversion equation and the need for its use. The %Na in the salt is calculated from the atomic weight of sodium (23) divided by the molecular weight of each salt. The %Na in the mixture was given. The equation is the numerator in the conversion equation. The sum is the denominator in the conversion equation. .

Salt	% Na in salt	% Na in mixture	<u>% Na in mixture</u> <u>% Na in salt</u>	% Salt in mixture
NaCl	39.35	33.33	0.85	20.48
NaNO ₃	27.06	33.33	1.23	29.78
Na ₂ SO ₄	16.2	33.33	2.06	49.75
Sum =			4.14	

Consider now that the salts are equivalent to different source soils and the %Na is equivalent to the %C in each soil. The %Na in the mixture is equivalent to the %C in the mixture as determined from the isotopic signatures by IsoSource. The % Salt in the mixture column becomes the % soil in the mixture (Table 5).

Table 5: Applying the conversion equation to the isotopic proportions from Figure 26. The %C in soil is analytical data. The IsoS-% in mixture is the isotopic proportion estimated by IsoSource.

Source	%C in soil	IsoS-% in mixture	<u>IsoS-% in mixture</u> <u>%C in soil</u>	% Soil in mixture
Canal	9.77	22.8	2.33	7.50
Grass/Mz	2.94	38.8	13.20	42.42
Waerenga 14	2.22	32.8	14.77	47.49
Maize	4.80	2.8	0.58	1.87
Mustard	12.15	2.7	0.22	0.71
Sum =			31.11	

It is apparent that the concentration of carbon in the sediment (or the concentration of the salt in the solution) is not a factor in the conversion provided the %C in the soil (%Na in the salt) is known. In the CSSI technique the %C is measured as part of the bulk stable isotopic composition of the soil. This means that the apportionment of soil by land use in a sediment mixture is independent of any degradation or diagenesis that may have occurred in the sediment mixture since that soil was deposited. Note that these results are expressed as percentages as opposed to soil mass.

8.4 Selecting isotopes and source combinations

This expands from section 7.1. "Selecting data for the models"

8.4.1 Isotopes

The selection of isotopes for use in the CSSI technique modelling is based on data obtained during method development (Gibbs 2008). The recommended isotopes to use are bulk ^{13}C , Oleic acid, and Palmitic acid – C, O, P. While any of the FA could be used (e.g., stearic acid instead of oleic acid), the $\delta^{13}\text{C}$ values of the bulk soil are always used.

This protocol is a convention that was based on the reasoning that, for a valid isotopic balance, the $\delta^{13}\text{C}$ value of the dominant form of carbon in the soil must always be met. However, while a soil carbon content at 5% represents the bulk soil C, the isotopic balance for a FA (e.g., palmitic acid) content of 50 mg/kg or 0.005% must also be met. Within the model, like is modelled with like, so the 1000-fold difference in concentration between bulk soil carbon and the FA does not bias the result towards the bulk carbon data. This also means that any isotope can be used to provide discrimination between sources relative to a sediment, provided that isotope is present in all sources and the sediment. The FAs from C14:0 to C20:0 appear to have larger differences across different land-uses and are therefore potentially more useful than the FA from C20:0 to C26:0. Oleic (C18:1) and palmitic (C16:0) acids seem to work best. Myristic acid (C14:0) has good discriminatory powers but is often missing from older sediments because it is more volatile than the other FAs.

8.4.2 Sources

The selection of potential sources has the caveat that it must be physically possible for soil from a specific source to reach the sediment site in the deposition zone. This geographical constraint is obvious in a unidirectional flow path i.e., the downstream sample cannot

influence the upstream site, and can be a guide as to the source soils that should not be used in the modelling. However, consideration should be given to factors that can overcome this constraint.

An example encountered in an estuarine study involving multiple river inflows with different dominant land-uses, found that silt in the freshwater from one river inflow floating on the saline water of the estuary was carried further up the estuary on the rising tide and deposited near the delta of a different river with a watershed that did not have that land-use source. Accordingly, it is useful to have a basic understanding of the physical processes that influence mixing and dispersion in the environment being studied.

During the modelling phase it will become apparent when a source is not a major contributor to the sediment site i.e., <5%. That source can be assigned a proportion of 5% or less and can be removed from the modelling. This will both speed up the modelling runs and will improve the discrimination between sources.

8.4.3 Worked example

The following sequence is a worked example of the effect of different isotopes on the identification and apportionment of source contributions to a receiving environment sediment and the effect of removing a potential source. In each iteration, the model has been fine tuned to give the best result with the selected combination of sources and isotopes. The source data are taken from Table 3. The sediment data are taken from a river delta sample at the head of the estuary.

All sources: adding isotopes

The sequence, Figures 27 to 29, looks at the effect of adding isotopes to the discrimination between 6 sources. The sequence, Figures 30 and 31, look at the effect of removing minor sources or combining similar sources while using 4 isotopes. The settings remain the same for each run with an increment of 1 and a tolerance of 0.2‰. The data were extracted into Excel spreadsheets and the histograms graphed as a group.

Using two isotopes gives some separation and apportionment of the sources, but the discrimination is poor (Figure 39). Pasture is a definite source and probably the Nikau native forest. Clear-felled pine (Pine-CF), mature pine (Pine-M), and the Kauri native forest all start high at 0% and decline in frequency of occurrence through to around 50%. These sources may be present but there is an equally valid possibility that they are not. The seagrass proportions are all less than 10% and are highest at 0%. The extremely large n of 279,083 indicates that these results have very high uncertainty.

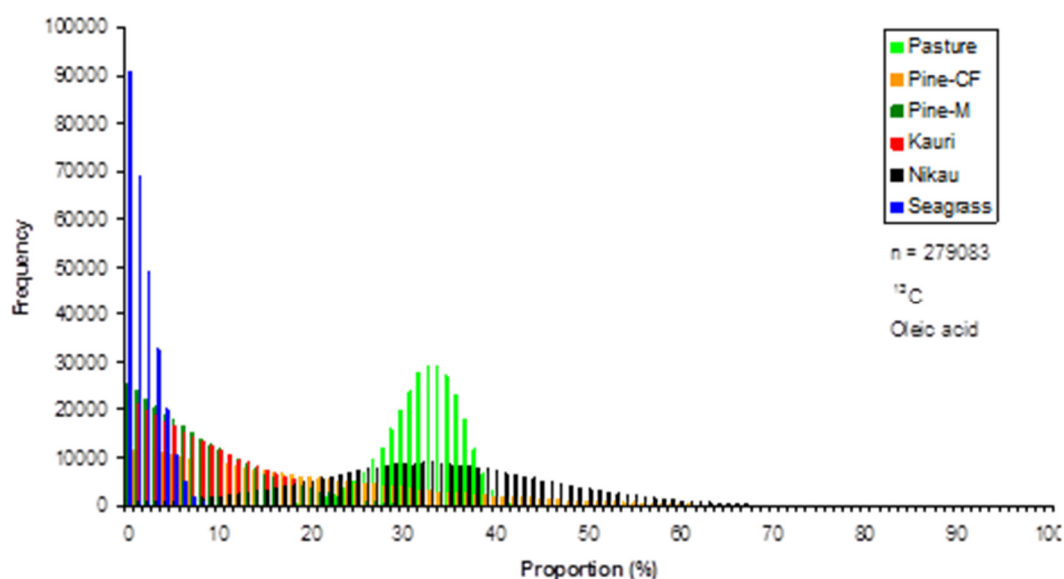


Figure 39: Two isotopes – bulk carbon and oleic acid. The $n = 279,083$ indicates low certainty.

Adding another isotope (Figure 40) improves the discrimination between sources as well as the apportionment of the source contributions to the mixture. The Kauri source has resolved into a bell-shaped peak and the uncertainty has decreased substantially (i.e., two orders of magnitude) with a new n of 2286

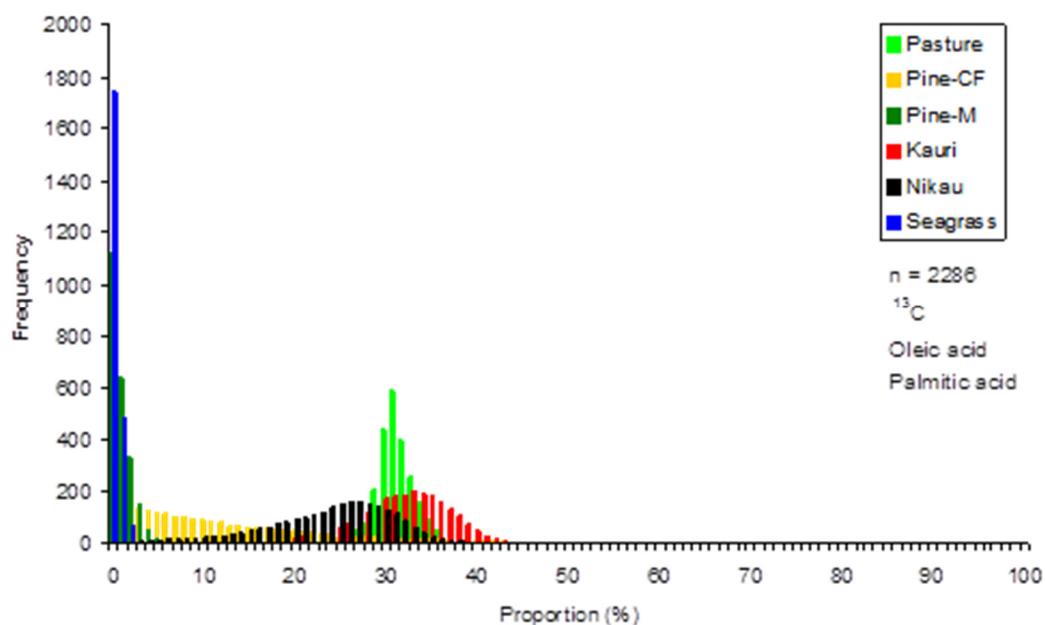


Figure 40: Three isotopes – bulk carbon, oleic acid, and palmitic acid.

This combination of isotopes is the basic COP set selected as the optimum group of isotopes to use in the CSSI technique (Gibbs 2008). Both the mature pine and the seagrass sources, at less than 5%, are unlikely to be major sources. The Nikau forest broad peak has reduced in width but the clear-felled pine is still represented by a wedge of feasible proportions declining from 0% to 30%.

Try adding another isotope (e.g., myristic acid) to improve discrimination (Figure 41).

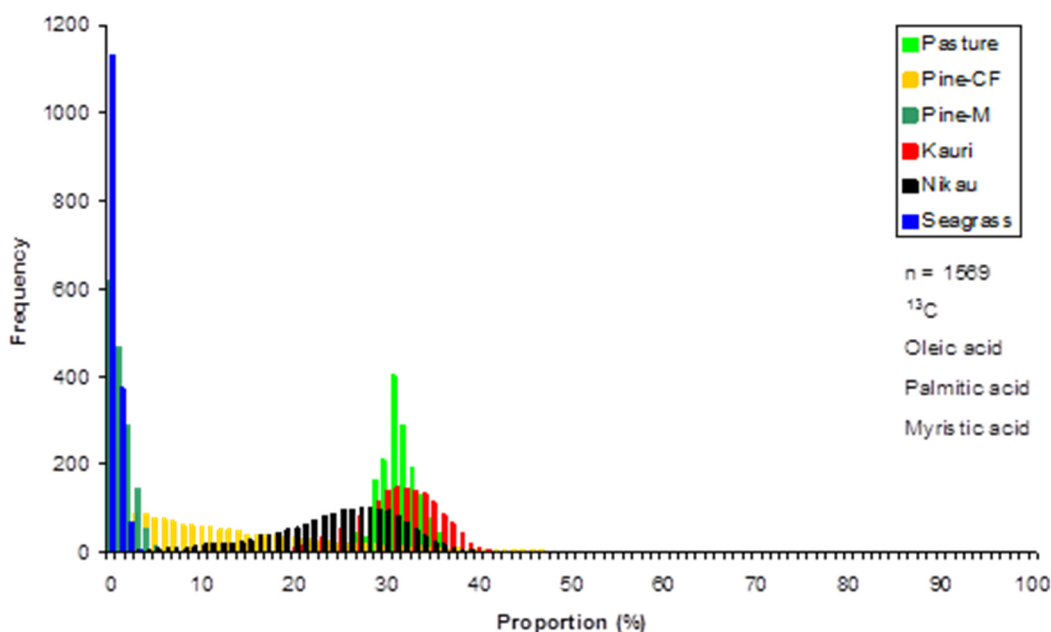


Figure 41: Four isotopes – bulk carbon, oleic acid, palmitic acid, and myristic acid.

The addition of the fourth isotope to the model increased the run time but made only a small improvement in the certainty with $n = 1569$. There is little difference in the source proportions.

All isotopes: removing sources

Broad unresolved histogram peaks can be caused by a source that shouldn't be in the potential source library for the site being evaluated. The next step, therefore, is to systematically start removing these minor or suspect sources from subsequent model runs – in this example, seagrass was removed.

Removal of the seagrass acknowledges that this source was unlikely to be a major source. It also recognises that, as a plant community habitat in the lower reaches of the estuary, it was unlikely to influence the upstream river delta site and, therefore, it is reasonable to exclude it from the modelling.

Although the value of n decreased from 1569 to 1122, the resultant proportions for the clear-felled pine were not substantially improved (Figure 42).

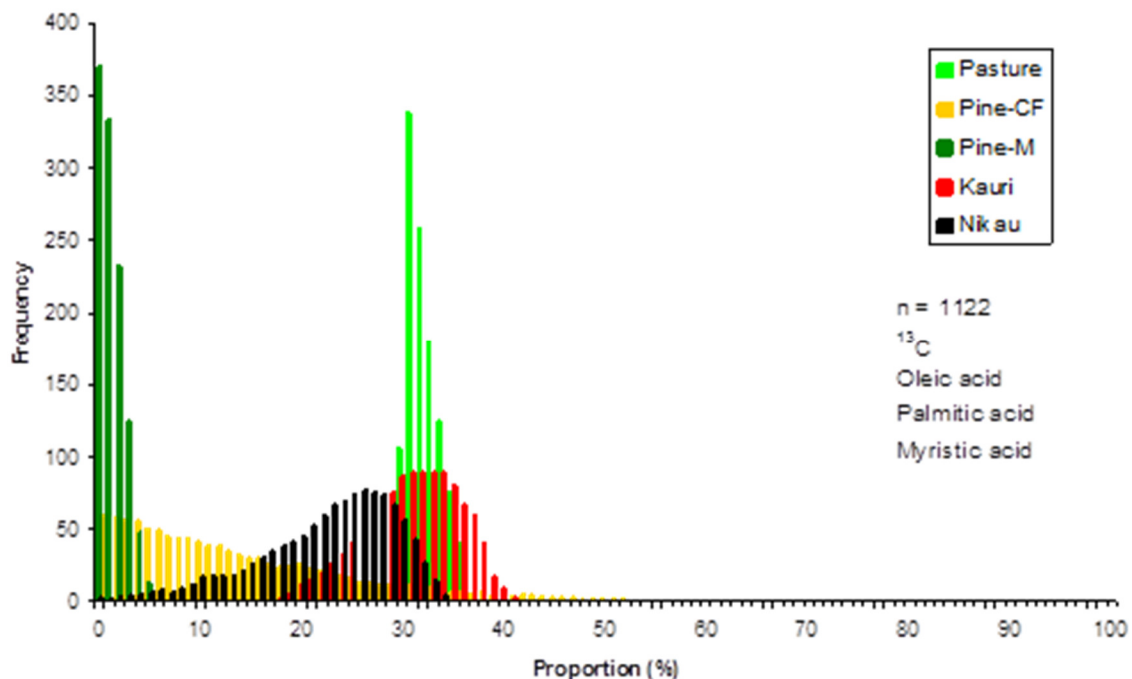


Figure 42: Model run with the seagrass source removed reduced n from 1569 to 1122 but did not resolve the broad pine-CF histogram wedge into a finite peak.

While this appears to be an acceptable result for these data, the lack of resolution of the clear-felled pine source into a well-defined peak indicates that this is the area where the model is ‘failing’. The cause of this may be that there are two very similar sources for native forest soil, Nikau and Kauri, which IsoSource has assessed as having similar feasible proportions in the output (Figure 42).

Closer examination of the geographic locations of these sources showed that the Kauri forest was confined to the steep gullies and ridge lines above the pine forest while the Nikau forest was a small fringe of trees along the side of the estuary below the river delta sediment sampling point. Consequently, the Nikau forest is unlikely to be a major source to the estuary and soil from the Nikau forest may not reach the river delta to contribute to that site. For these reasons, the Nikau forest source was removed and the model was re-run (Figure 43). This allowed the remaining four land-use sources to be resolved into discrete peaks, with a very high level of certainty. With $n = 3$, these are almost unique solutions. In this case, the indeterminate clear-felled pine histogram range changed producing a peak at around 0.5, and the Kauri forest proportions reduced from around 0.3 to around 0.2. The inclusion of a non-valid source caused a distortion of the source proportions as IsoSource tried to find matches which included the isotopic signatures of that source.

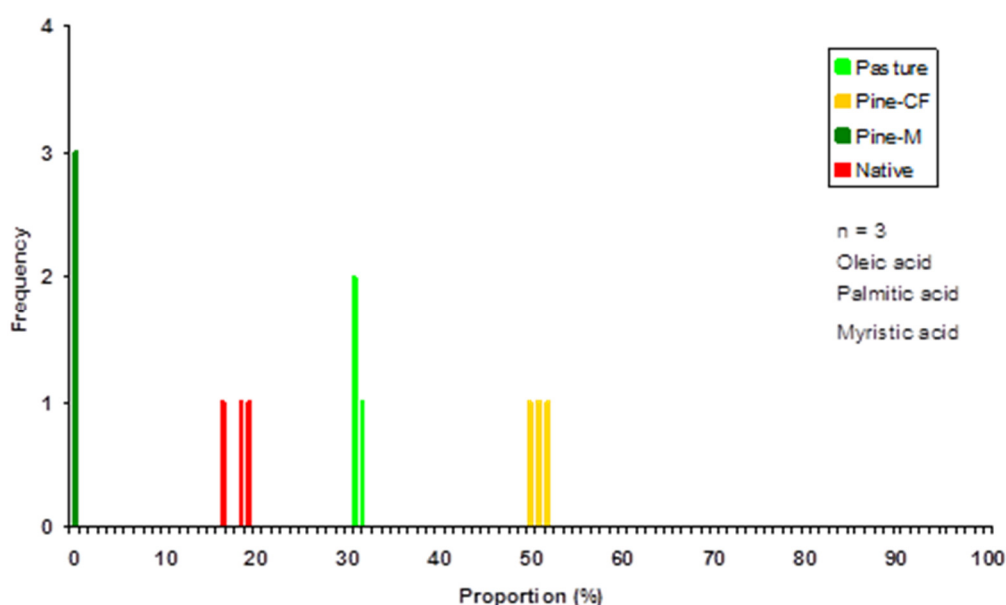


Figure 43: Model run with 4 isotopes and sources:- Pasture, Clear-felled Pine, Mature Pine, and native Kauri forest.

This example was intended to illustrate how isotopes might be selected and the effects on source proportions of having sources that were not from the watershed contributing to the sediment at the site sampled. In practice, the geographic constraint on the selection of sources must be checked before the modelling begins. The recommended starting point for isotope selection parameters should be bulk carbon, oleic acid, and palmitic acid.

Interpreting these results

After conversion from isotopic proportions to soil proportions, the native Kauri forest land-use was found to be contributing about 14% of the soil to the sediment deposition site from 16% of the watershed land area. This is within expectations given that the Kauri forest was on steep hill sides and ridges. Although the total pine forested land-use area was 12%, mature pine land-use was contributing very little soil. This is lower than might be expected but is generally consistent with observations of 4-fold lower sediment yields from mature pine than pasture (Eyles & Fahey 2006). In contrast, the clear-felled pine forest was estimated to be contributing about 50% of the total sediment from the watershed. This was 60% more than the 32% sediment yield from pasture land-use, which occupied 72% of the total watershed area.

This is a disproportionately high sediment yield given that the clear-felled pine forest was only a small proportion of the total pine forest land-use and less than 4% of the total watershed land area. In this case, clear-felling of the production pine forest for timber (Figure 44) left large areas of bare land on steep (>20°) slopes exposed to heavy rain. This exacerbated the soil erosion until the replanting of trees and the joining of the leaf canopy once more protected the soil from severe erosion.



Figure 44: Different views of clear-fell pine forestry showing the slope and area of bare ground left after tree harvesting (upper), and the potential for channelling of water and thus enhanced sediment erosion down the steep hillside where logs have been hauled up across a stream bed (lower).

9 SIAR

SIAR is a package designed to solve mixing models for stable isotope data within a Bayesian framework. The name of the mixing model SIAR is an acronym for Stable Isotope Analysis in R, where R is a computer programming language. SIAR was developed by Andrew Parnell, and others (Parnell et al., 2008) and it was presented to the IsoEcol conference in Hawaii in 2008. It was developed in response to concerns about the inability of the IsoSource model to provide information on uncertainty in food web analyses and the growing tendency for researchers to ignore the caution provided in IsoSource (Figure 33) and the accompanying documentation, that all feasible solutions were equally valid and not to use just the mean values.

Although no expertise is required in the use of R, the developers assume that the user has a sound working knowledge of stable isotopic mixing models, and the assumptions and potential pitfalls associated with these models. They warn that

SIAR will always try to fit a model even if the data are nonsensical

Failure to take account of the assumptions could lead to erroneous results (e.g., Fry, 2013).

In contrast to IsoSource, which is a number crunching process, SIAR is a true model in that it calculates the results from equations. Instead of counting the number of occurrences of each feasible solution as in IsoSource, SIAR runs the model up to 200,000 times using all combination and calculates the statistics from these results. In SIAR there is an opportunity to include estimates of variability due to fractionation, which allows SIAR to produce estimates of uncertainty for the sources contributing to the mixtures. This is a big improvement on IsoSource when it is used in food web studies. However, when used in the CSSI technique, the isotopes bound to the soil and sediment particles do not experience fractionation so there is no modelling advantage in using SIAR, except processing speed. When tested on the same input data, SIAR produced an output essentially the same as obtained from IsoSource. The only difference being that if the analytical variability was included in the SIAR model, it produced an estimate of uncertainty based on those variables.

Consequently, either mixing model can be used to deconstruct sediment mixtures in the CSSI technique. IsoSource is easier to start with than SIAR, but SIAR is likely to be easier to use in the long term once the programming has been learnt. The caveats and limitations to the data as discussed in section 8 apply to the data whether they are used in IsoSource or SIAR.

The main differences between SIAR and IsoSource is that SIAR does not use an interactive screen to load and manipulate data (it uses files), and iterative fine tuning, as demonstrated for IsoSource in section 8.4.3, would be more difficult in SIAR. Also, the value 'n', as a guide to the confidence in the results, is presently not produced by SIAR. The graphical packages in SIAR produce the same histograms as IsoSource, which helps assess the validity of results.

On the positive side, the data is only entered once in a **source** and a **mixture** file for SIAR, and SIAR can produce multiple runs if there are several sets of data in these files. It runs very fast and can produce a range of graphed outputs as well as data output files.

Further information on the SIAR package is available from:

<http://cran.r-project.org/web/packages/sair/sair.pdf>

R can be downloaded from <http://www.r-project.org/> and then installed and run by following the instructions on the R website.

It is recommended that an IT service person installs R and the SIAR package on the user's computer. Because the filenames for data include the absolute address in the computer, it is recommended that SIAR is installed in a 'Desk top' folder which is easy to access and that the data files are loaded into the same folder.

It is also recommended that the user reads the suggested literature.

A particularly good document to read is 'SIAR for Ecologists' written by Richard Inger, Andrew Jackson, Andrew Parnell and Stuart Bearhop (2010). This available free on the internet at

http://www.tcd.ie/Zoology/research/research/theoretical/siar/SIAR_For_Ecologists.pdf

This document provides step by step examples of how to load and run the SIAR package.

9.1 SIAR notes

The following notes were made during a training session on SIAR in March 2013. Problems / bugs encountered at that time have been fixed in the latest updates of the software. Use the latest versions of R and SIAR.

9.1.1 Start R

Start R and select either R 2.15.0 (32 bit) or RGui (64 bit). The 32 bit version runs with less errors than the 64 bit version (Now fixed).

Screen shows:

```
R version 2.15.0 (2011-10-31)
Copyright (C) 2011 The R Foundation for Statistical Computing
ISBN 3-900051-07-0
Platform: i386-pc-mingw32/i386 (32-bit)
```

```
R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions.
Type 'license()' or 'licence()' for distribution details.
```

```
Natural language support but running in an English locale
```

```
R is a collaborative project with many contributors.
Type 'contributors()' for more information and
'citation()' on how to cite R or R packages in publications.
```

```
Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.
```

```
>
```

The red > is the command prompt where the next command should be loaded. Commands may be copied and pasted from a separate text file or typed in directly. There can be issues

with copying and pasting text from some files downloaded from the internet for use as command strings. Sometimes extra hidden characters are included. If the result is not what was expected, try typing in the command string directly. If this works, save a copy of your command string in a working text file (Word or notebook) for later use.

Load the library with the command typed in at the **>** prompt:

```
library('siar')
```

Screen shows:

```
Loading required package: hrcde
Loading required package: locfit
Loading required package: akima
Loading required package: lattice
locfit 1.5-6    2010-01-20
Loading required package: ash
Loading required package: ks
Loading required package: KernSmooth
KernSmooth 2.23 loaded
Copyright M. P. Wand 1997-2009
Loading required package: mvtnorm
Loading required package: rgl
Loading required package: misc3d
hrcde 2.15 loaded
Loading required package: coda
Loading required package: MASS
Loading required package: bayesm
Loading required package: mnormt
Loading required package: spatstat
Loading required package: mgcv
This is mgcv 1.7-9. For overview type 'help("mgcv-package")'.
Loading required package: deldir
deldir 0.0-16
```

Please note: The process for determining duplicated points
has changed from that used in version 0.0-9 (and previously).

```
spatstat 1.24-2
Type 'help(spatstat)' for an overview of spatstat
'latest.news()' for news on latest version
'licence.polygons()' for licence information on polygon calculations
```

```
Attaching package: 'siar'
```

The following object(s) are masked from 'package:spatstat':

```
convexhull
```

```
Warning message:
package 'siar' was built under R version 2.14.2
```

```
>
```

9.1.2 Data files

Set up the data files to be read into the programme and modelled. There are three files and these should be created as **TAB DELIMITED** text files in notepad.

The first is the isotopic data for the '**mixture**' being deconstructed. It should look like this:

File name: `mixture.txt`

	d13C	c16	c18	c20	c22	c24
HA2	-27.53	-30.79	-30.70	-34.71	-34.64	-31.71
HA2a	-25.61	-33.68	-30.15	-33.06	-37.53	-34.58
HA2b	-24.84	-25.54	-26.69	-27.60	-35.19	-31.31
HA3	-26.38	-31.46	-33.06	-34.35	-39.59	-39.25
HA5	-26.77	-31.11	-30.24	-33.76	-34.87	-36.30

The first column heading is left blank so that the programme ignores the data names.

Because this is a matrix of mixtures and the results for each mixture are required separately, the data could be written in the format:

File name: `mixture-code.txt`

code	d13C	c16	c18	c20	c22	c24
1	-27.53	-30.79	-30.70	-34.71	-34.64	-31.71
2	-25.61	-33.68	-30.15	-33.06	-37.53	-34.58
3	-24.84	-25.54	-26.69	-27.60	-35.19	-31.31
4	-26.38	-31.46	-33.06	-34.35	-39.59	-39.25
5	-26.77	-31.11	-30.24	-33.76	-34.87	-36.30

The change allows SIAR to write a separate output for each mixture. If the same code number is assigned to two or more rows of data, SIAR assumes that these are from the same mixture and it will combine rows with the same code number into a single mixture and use the variability in these data to calculate statistics and estimates of uncertainty.

The next file is the isotopic data for the '**sources**' that contribute to the mixture. These require the standard deviation (std) as well as the isotopic values and should look like this:

File name: `sources.txt`

Sample	d13C	std	C16	std	C18	std	C20	std	C22	std	C24	std
PASTURE	-25.26	0.2	-24.21	0.2	-31.00	0.2	-25.04	0.2	-31.10	0.2	-30.33	0.2
TOTARA	-27.53	0.2	-31.15	0.2	-32.23	0.2	-37.70	0.2	-32.83	0.2	-34.16	0.2
NATIVE	-26.99	0.2	-30.66	0.2	-27.57	0.2	-30.54	0.2	-31.71	0.2	-31.71	0.2

Unless there have been multiple samples analysed to provide a mean and standard deviation for each isotope from each source, the std value used should be the analytical standard deviation, which is typically 0.2. A std value must be included.

The third file is the isotopic fractionation data or Trophic Enrichment Factor (TEF) for the sources. As there is no fractionation in the soil mixtures, the TEF data can be set to zero or the TEF file can be excluded from the model run (preferred).

9.1.3 Running the model

The command strings to load the data and run the models are as follows:

```
data<-read.table("C:/users/gibbs/documents/R/win-library/2.14/siar/data/mixture-  
code.txt",header=TRUE)  
sources<-read.table("C:/users/gibbs/documents/R/win-  
library/2.14/siar/data/sources.txt",header=TRUE)
```

The words `data<-read.table(...)` assign the data in the text file "mixture-code.txt" to the command name "data"

The words `sources<-read.table(...)` assign the data in the text file "sources.txt" to the command name "sources".

The mixture and sources .txt files can have any names provided the names are entered into the appropriate command string after `data/`

The section `C:/users/gibbs/documents/R/win-library/2.14/siar/data/` is the directory path of the data files are on the computer. To reduce the length of this string, the R program and the data files could be installed and loaded in C: drive. It is best that the data files are in the same directory as the program R.

Note that the forward slash `/` is used in the command string, not the back slash `\` as is used in the computer directories and filenames.

The command to run the model specifies the model name `model1` (it could be `model2`, etc.,) which will hold the output using the data in the files assigned to "`data`" and "`sources`" in the loading command strings.

There are two ways to run the model.

For a matrix with multiple sets of each mixture to be analysed separately, the run command used is either

```
model1<-siarmcmcdirichletv4(data,sources)
```

or

```
model1<-siarmcmcdirichletv4(data,sources,concdep=0,500000,50000)
```

The second version specifies the number of iterations the model will perform to deconstruct the mixture.

The screen shows:

Stable Isotope Analysis in R
An MCMC for Normally distributed data with a dirichlet mixture mean

This is the multi-group version with the following parameters:

Number of groups: 5

Number of iterations: 200000

Burn in: 50000

Thinning by: 15

Number of isotopes: 6

Number of sources: 3

Running group number 1 of 5...

200000

190000

180000

170000
160000
150000
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0

Job completed successfully.

Duration: 2.4 seconds.

Running group number 2 of 5...

200000

190000

.....(and so on for each mixture to the end of the model run)

30000

20000

10000

0

Job completed successfully.

Duration: 2.4 seconds.

>

Each data point in the mixture is run separately to give individual results

For a single data point for the mixture, the run command is either

`model1<-siarsolomcmc4(data,sources)`

or

`model1<-siarsolomcmc4(data,sources,concdep=0,500000,50000)`

The screen shows:

Stable Isotope Analysis in R

An MCMC for Normally distributed data with a dirichlet mixture mean

Solo version: no more than one target per group.

This is the single group version with the following parameters:

Number of iterations: 200000

Burn in: 50000

Thinning by: 15

Number of isotopes: 3

Number of sources: 5

200000

190000

```
180000
170000
160000
150000
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
```

Job completed successfully.

Duration: 0.7 seconds.

>

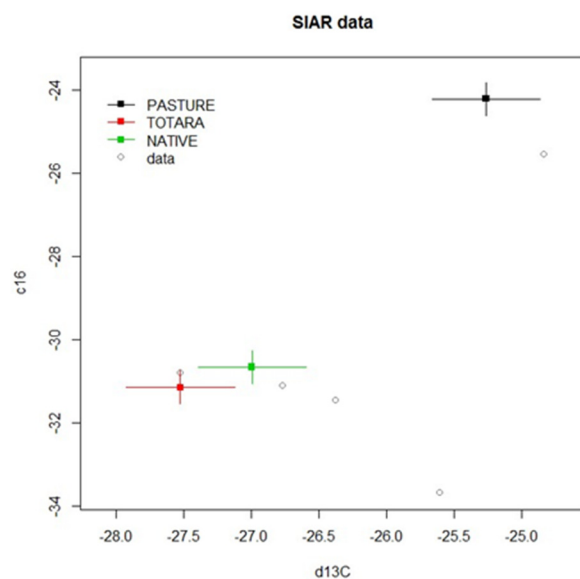
The difference between the matrix and solo versions is that the matrix version attempts to incorporate the standard deviation data into the output whereas the solo version ignores the standard deviation in the output.

9.1.4 Data graphical output

The data output can be plotted in several ways using the following command strings:

Scatter plot:

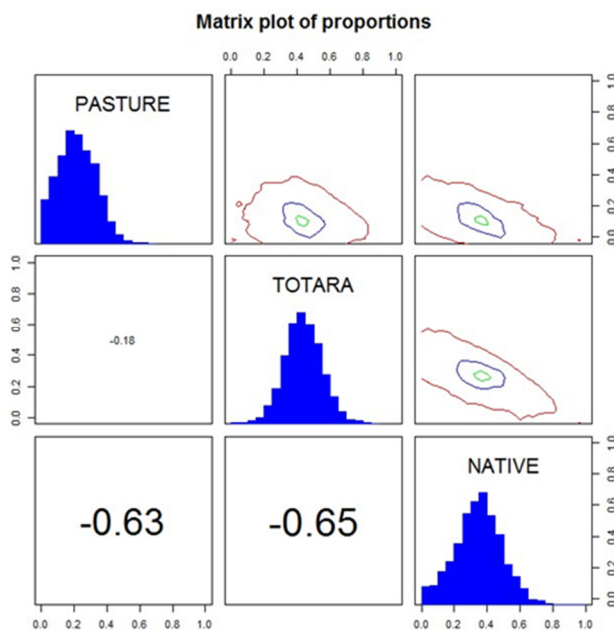
`siarplotdata(model1)`



Each combination of isotopes will be plotted. The plot just shows the data. Clicking on the plot will place the key at the location clicked. Then move to the next combination of X-Y plots. Close the graph to move to the next command.

Matrix plot:

`siarmatrixplot(model1)`



Histogram plot

`siarhistograms(model1)`

Screen shows:

Plots of single groups proportions.

Do you require each plot on a separate graph or all on the same one?

1: Each on a separate graph

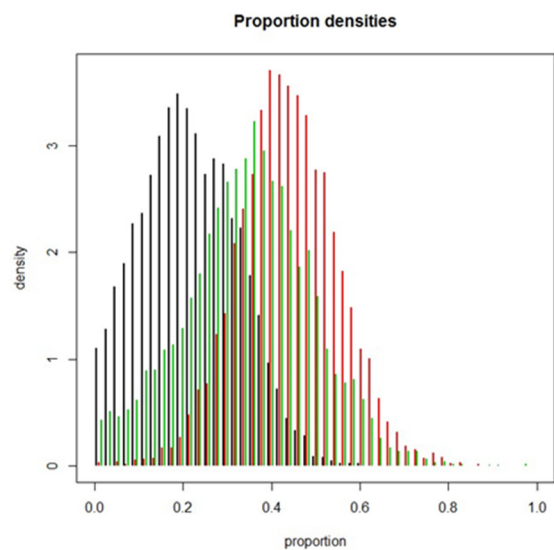
2: All together on one graph

Selection: 2

Producing plot.....

>

The program waits for either a 1 (individual histogram) or 2 (multiple histograms). These are equivalent to the IsoSource histograms but the vertical axis is labelled "Density" rather than "Frequency".



Box plot (whisker diagram)

`siarproportionbygroupplot(model1)`

Screen shows:

Plot of proportions by group

Producing plot.....

Enter the group number you wish to plot

The choices are:

1: 1

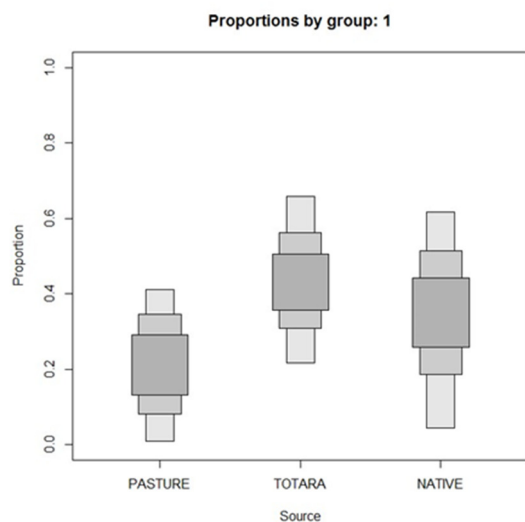
Selection: 1

Please maximise this graph before saving or printing.

Press <Enter> to continue

>

The program waits for the selection before plotting. If there were more than one mixture in the data file, the results for each mixture could be plotted separately.



9.1.5 Data output file

The program generates and plots the data, but the data are not saved in a form that can be used in other software packages. As with the IsoSource model, the results are isotopic proportions and need to be converted to soil proportions using the CSSI equation (section 8.3).

To achieve this the isotopic proportion data can be extracted from the program using the command:

```
siarhdrs(model1)
```

The screen shows:

Summary information for the output file ...

	Low 95% hdr	High 95% hdr	mode	mean
PASTURE	0.008684684	0.4108690	0.2000441	0.2167042
TOTARA	0.217407897	0.6581594	0.4134816	0.4371981
NATIVE	0.043790084	0.6171158	0.3583478	0.3460977
SD 1	0.530470314	3.1128642	1.0734136	1.5799794
SD 2	1.455351719	8.0994548	2.9183954	4.1624498
SD 3	1.087185539	5.6469882	2.1873665	2.9547658
SD 4	1.415801388	7.6126419	2.8321760	3.9690919
SD 5	2.433466147	12.8234383	4.8001855	6.6254587
SD 6	1.871402059	9.7057504	3.6573789	5.1249937

===== READ THIS =====

There may be some problems with this data.
Some of the standard deviations seem especially large.
Please check to see whether the target data lie outside
the convex hull implied by the sources.

SIAR rates the problem with this data set as:

Mild - but still may affect results.

=====

Press <Enter> to continue...

Running convergence diagnostics on output.

Output parameters need to have been loaded in or created.

Worst parameters are ...

SD4	SD3	SD1	SD2	PASTURE	SD5	TOTARA	SD6	NATIVE
0.04582935	0.10283549	0.15280214	0.17214999	0.28627247	0.34417014	0.38631380		
0.40434923	0.43510860							

If lots of the p-values are very small, try a longer run of the MCMC.

>

The output includes a summary of isotopic proportions for the sources and stats (95% (2 std), mode mean) and residuals for the isotopic tracers.

The lines SD1 through to SD6 are meaningless for the CSSI technique and can be ignored.

They are the consequence of using the **matrix** command line

```
model1<-siarmcmcdichletv4(data,sources,concdp=0,500000,50000)
```

instead of the solo command line

```
model1<-siarsolomcmc4(data,sources,concdep=0,500000,50000)
```

which would have ignored the standard deviation in the output.

The data output needed is contained in the lines

	Low 95% hdr	High 95% hdr	mode	mean
PASTURE.	0.008684684	0.4108690	0.2000441	0.2167042
TOTARA	0.217407897	0.6581594	0.4134816	0.4371981
NATIVE	0.043790084	0.6171158	0.3583478	0.3460977

The isotopic proportions used to obtain soil proportions are the values for means and these are used in the equation (Section 8.3).

10 Fallout Radionuclide (FRN) linking

Source identification and apportionment from the CSSI technique are not quantitative. To convert these source soil proportions to soil loads requires mass transport data for the sediment as a whole. This can be obtained by using FRN or other non-FRN techniques including geographical information system (GIS) data, catchment modelling or direct measurements from time series photographs or surveys. To meet the requirements of an area-wide precision conservation of soil, the rates of loss or denudation of the soil from individual land-uses must be calculated and compared with the area of that land-use and the expected loss of soil from that land-use.

Where FRN data can produce a quantitative sediment budget for a watershed measured in mass/unit-time at a location, the denudation rate of soil from a specific land-use can be calculated as the mass/unit-time from the watershed at that location multiplied by the proportional contribution of that land-use soil to the sediment at that location, as determined from the CSSI technique:

$$\text{Denudation}_{(\text{land-use})} \text{ rate} = (\text{sediment_mass}_{(\text{watershed})} \times \text{soil_proportion}_{(\text{land-use})}) / \text{time}$$

This simple approach can be applied to sediment transport rates derived from other techniques including hydrological measurements and GIS based watershed modelling. These rates may be event-based, based on ^7Be data, or estimated for longer time periods using ^{210}Pb data as required.

The CSSI technique soil proportions are percentages of each source soil in the sediment at the location sampled. To change from denudation rates of mass/unit-time to mm/yr, the CSSI soil proportions need to be corrected for the dry bulk density of the source soil and divided by the area of that land-use.

The following sections provide a brief over view of the parts of the FRN technique that can be used with the CSSI technique to improve sampling strategies and to provide quantification of sediment yields from specific land-use practices.

10.1 FRN overview

The FRNs, ^7Be , ^{137}Cs and ^{210}Pb , are short lived radioactive isotopes which emit gamma radiation as they decay. This radiation activity is measured in low background gamma spectrometers capable of simultaneous isotope detection and is reported in Becquerels per kilogram of soil (Bq kg^{-1}). These FRNs are, in their own right, unique environmental indicators that can be used for tracking the movement of sediment at a range of scales from the field to the watershed. The measurement units (Bq kg^{-1}) allow quantification of the mass of soil that has moved.

10.1.1 Berilium-7

Berilium-7, with a half-life of 53.44 days, is a natural radionuclide that is formed in the atmosphere by the interaction of cosmic rays with nitrogen and oxygen. It is a very reactive element and attaches instantaneously to particulate material. Because of this, it is confined to the surface layer of soil and can be used to evaluate recent erosion events, such as sediment movement across fields, and to distinguish recent (months) sediment from older sediment in deposition zones. Because the highest measured activity corresponds to the

greatest sediment accumulation rate, the distribution of ^7Be in the top 1 cm of lake or estuarine sediment defines the sediment deposition centres.

10.1.2 Cesium-137

Cesium-137, with a half-life of 30.23 years, is a thermonuclear by-product of anthropogenic origin. Its presence is directly related to the atmospheric testing of nuclear devices during the 1950's and early 1960's. With the exception of the Chernobyl failure in 1986, and the Indian/Pakistani tests, there has been no ^{137}Cs released to the atmosphere since the cessation of atmospheric nuclear testing in 1998. In Northern Hemisphere countries, ^{137}Cs provides a date marker in the sediments for the 1950s and for 1986. The 1986 Chernobyl ^{137}Cs marker is not found in Southern Hemisphere countries although the presence of ^{137}Cs from 1952 tests in Australia and Bikini Atoll in the Pacific Ocean is still detectable.

10.1.3 Lead-210

Lead-210, with a half-life of 22.3 years, is a natural radionuclide that falls from the sky and, like ^7Be , is rapidly adsorbed to or incorporated on particulate material.

A member of the uranium-238 series, ^{210}Pb is subject to disequilibria with its distant relative radium-226 (^{226}Ra) due to the physio-chemical activity of the intermediate gaseous progenitor radon-222 (^{222}Rn). Radioactive disequilibrium arises when the gaseous ^{222}Rn escapes from the soil into the atmosphere. With a half-life of 3.8 days, the ^{222}Rn decays through a series of very short half-life isotopes to form ^{210}Pb in the atmosphere and subsequently the hydrosphere. This production process is essentially constant and precipitation of this material produces a near constant flux of ^{210}Pb on the soil surface where it adds to the ^{210}Pb . The ^{210}Pb already in the soil or sediment is in equilibrium with ambient ^{226}Ra and is known as "supported ^{210}Pb ". The new ^{210}Pb is unsupported by ^{226}Ra and is called excess ^{210}Pb ($^{210}\text{Pb}_{\text{ex}}$). The amount of $^{210}\text{Pb}_{\text{ex}}$ is determined by subtracting the ^{226}Ra activity (= supported ^{210}Pb) from the total ^{210}Pb activity (Figure 45). The decay of the $^{210}\text{Pb}_{\text{ex}}$ provides the mechanism for age and depositional assessment.

The exponential decay curve (Figure 45) is derived from the half-life of ^{210}Pb . Ideally, the decay curve can span about 7 half-lives or about 150 years. However, in practice the variability in $^{210}\text{Pb}_{\text{ex}}$ at low levels prevent reliable estimates of age beyond 100 years. Consequently, ^{210}Pb is ideal for most ecosystem studies, where changes have occurred within the last century.

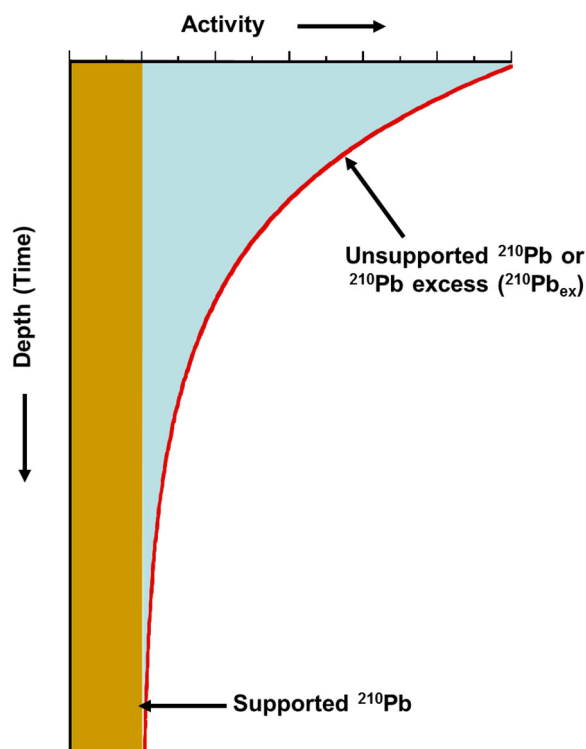


Figure 45: Stylised ideal lead-210 (^{210}Pb) accumulation. The brown area represents ambient or supported ^{210}Pb derived from ^{222}Rn in the sediment itself. The blue area represents the excess ^{210}Pb content accumulating via precipitation and deposition. The graph depicts constant sedimentation – constant flux (CS:CF) showing a decline in excess ^{210}Pb with depth as a function of time (i.e., constant deposition offset by radioactive decay). The ^{210}Pb decay curve (red line) represents 7 half-lives (150 years) but the limit for useful dating is about 100 years.

10.2 Erosion or deposition

While all FRNs have application in assessing soil erosion and sedimentation, each has its advantages and limitations (Mabit et al. 2008a). It is important to select the most appropriate FRN for the study e.g., use ^7Be for very short term investigations (single events) but for longer term studies use ^{137}Cs or $^{210}\text{Pb}_{\text{ex}}$ or a combination of these.

To determine whether erosion or deposition is occurring, the background flux of FRN deposition or reference inventory needs to be known. Typically this is obtained from a measurement of the surface concentration of FRN at a reference site with a known history of no disturbance and which does not experience erosion or deposition. The reference inventory is compared with concentration measurements from other sites to determine whether it is an erosion or deposition site. Erosion sites will have lower concentrations of FRN than the background flux as the most recent and highest activity FRN will have been washed away. Conversely, deposition zones will have higher concentrations of FRN as more of the most recent and highest activity FRN will have accumulated than can be accounted for by the background flux.

The spatial distribution of an FRN is obtained by converting the concentration data from Bq kg^{-1} to an areal flux inventory as Bq m^{-2} (e.g., Mabit et al. 2008b, 2009) and converting these into soil redistribution rates ($\text{t ha}^{-1} \text{yr}^{-1}$) using the Mass Balance Model 2 (Walling et al. 2002).

10.2.1 CSSI linkage to erosion and deposition

FRN data can estimate the amount of soil that has accumulated in a deposition zone, and thus the average erosion rate from the whole upstream watershed. Similar quantitative estimates may be obtained from GIS models as a reality check, if the data is available.

Quantification

The CSSI technique can discriminate between and apportion different land-use sources contributing to the sediment in the deposition zone (e.g., Figure 46), but has no means of quantification by itself. In combination with the FRN derived mass balance data, the CSSI technique can give quantitative estimates of the soil erosion from each land-use (e.g., Figure 47). These estimates can then be compared with the corresponding land-use area to identify land-use practices that are exacerbating soil erosion and producing excessive amounts of soil. The data in the examples (Figure 46, Figure 47) are from the Bay of Islands, New Zealand (website <http://www.os2020.org.nz/>).

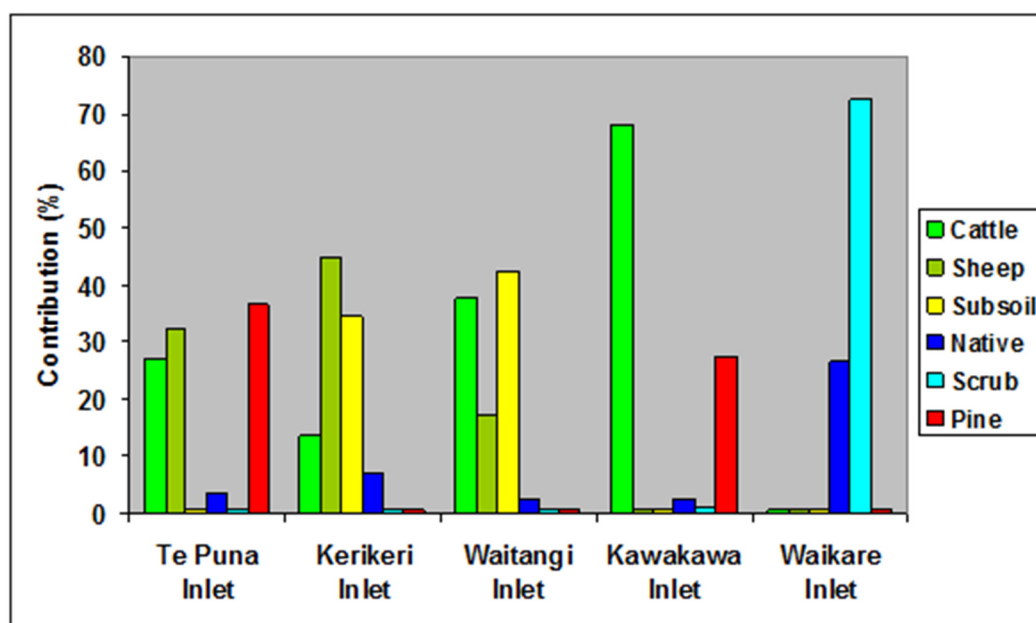


Figure 46: Proportional contributions of six major land-use soils in the sediment deltas of five rivers discharging into a large harbour. (Cattle include dairy and beef).

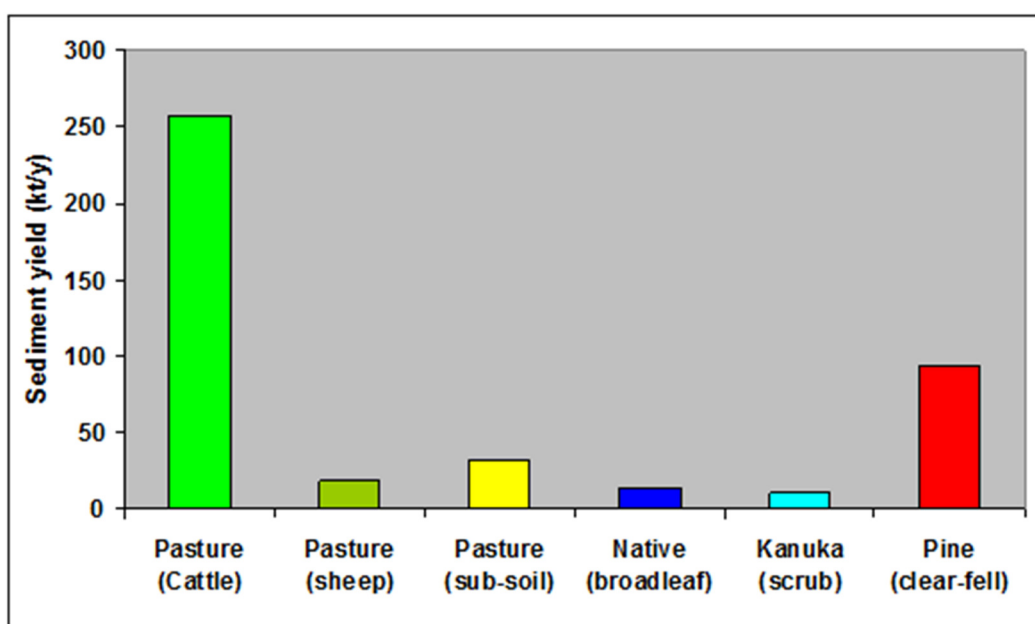


Figure 47: Quantitative estimates of the annual sediment yield by land-use after conversion of the proportional contribution data from the river deltas (Figure 46).

In this example, although there were three different types of pasture across four of the sub-catchments, more than 80% of the pasture sediment came from land used for cattle (dairy and beef dry-stock) farming. The other major source of sediment in this watershed was clear-fell production pine forestry, which occupied less than 5% of the total watershed area.

Dispersion

The spatial distribution pattern produced from the FRN data can show how much sediment has been deposited at different locations in a lake or estuary but cannot identify where that sediment came from or whether it has been redistributed or dispersed after the initial deposition. If the CSSI data were collected using the ^{7}Be profile data to ensure that only the most recently deposited sediment was used in the analysis, the spatial distribution pattern of a single FA biomarker may provide an initial guide as to where the sediment came from (i.e., which river) and how far it dispersed (Figure 48). These data are also from the Bay of Islands, New Zealand (website <http://www.os2020.org.nz/>)

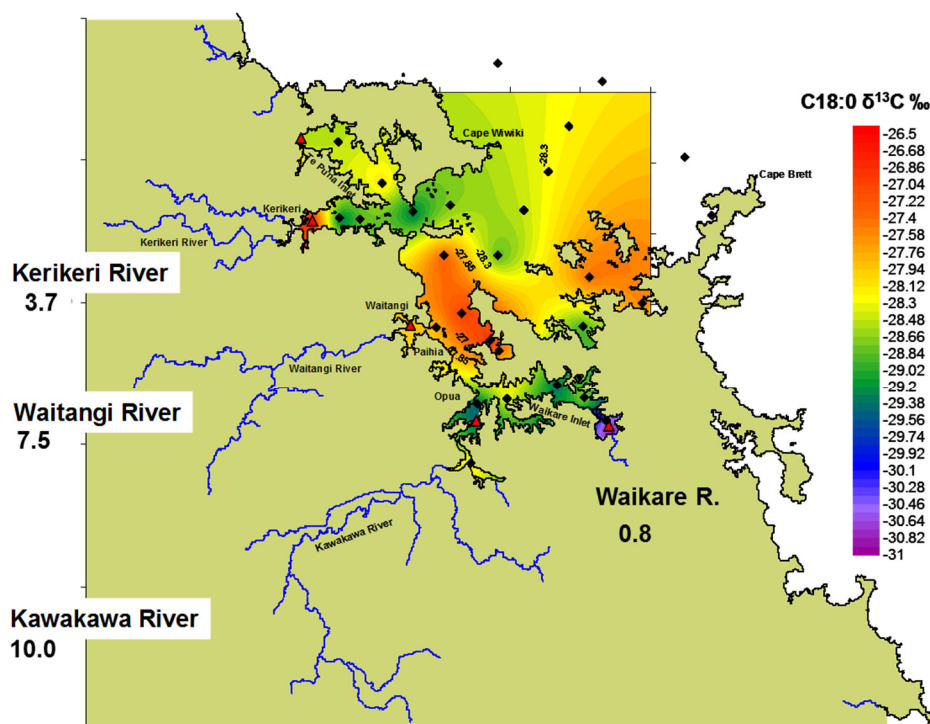


Figure 48: Spatial distribution pattern of the $\delta^{13}\text{C}$ values of the C18:0 FA biomarker in the surface sediments across the study harbour. These data indicate that the northern river (Kerikeri) is unlikely to mix with sediment from the two southern rivers (Waitangi and Kawakawa) until well out into the harbour. Axis tick marks are 10 km; values beneath the river names are mean annual flows ($\text{m}^3 \text{s}^{-1}$).

In this example, the majority of the sediment came from three of the five river inflows. The northern inflow was separated from the other two major inflows by an island, which appears to have guided that input across the northern half of the harbour away from the other two inflows. The two southern inflows merged and then appeared to have moved around the southern headlands and dispersed across the southern harbour.

10.3 Dating using FRNs

10.3.1 Half-life calculation

Because FRNs are short lived isotopes they have characteristics which make them ideal for establishing chronologies based on the use of their half-life. A half-life is defined as the time it takes for half a given number of atoms in a sample to decay to another element. The age of sediment is calculated by comparing the original FRN concentration with the percent of that FRN remaining in the sample. The criteria for a radionuclide to be a candidate for dating are:

1. The chemistry of the isotope (element) is known.
2. The half-life of the isotope is known.
3. The initial amount of the isotope per unit substrate is known or accurately estimated.

4. The substrate adsorbs and incorporates an adequate amount of the isotope (in sedimentary systems, this is the finest, usually clay or colloidal size material).
5. Once the isotope is attached to the substrate, the only change in concentration is due to radioactive decay.
6. In order to be useful, it is relatively easy to measure.
7. The isotope has an effective range for the scale of time investigated (about 8 half-lives).

With these criteria met, the age of a substance can be calculated by the following formula:

$$\text{Age} = \ln(A_0 / A_s) \times 1/k$$

where A_0 is the isotopic activity at time zero (the present) and A_s is the activity of the unknown, k is the decay constant for the isotope. For ^{210}Pb , $k = 0.03114$ per year.

CSSI analysis of layers extracted from a soil or sediment core at different times before present can give estimates of the most likely land-use at those times/ages (section 10.2).

10.3.2 Sediment accumulation rates.

An alternative way to use FRNs for dating of sediment cores is to re-plot the radionuclide activity decay curve on a natural log scale ($\log_e$) (Figure 49) to estimate sediment accumulation rates (SAR). The slope of a linear regression through the radionuclide activity data points gives the SAR in mm per year. This rate can be checked using the ^{137}Cs data to estimate the total sedimentation that has occurred since 1952 (Southern Hemisphere).

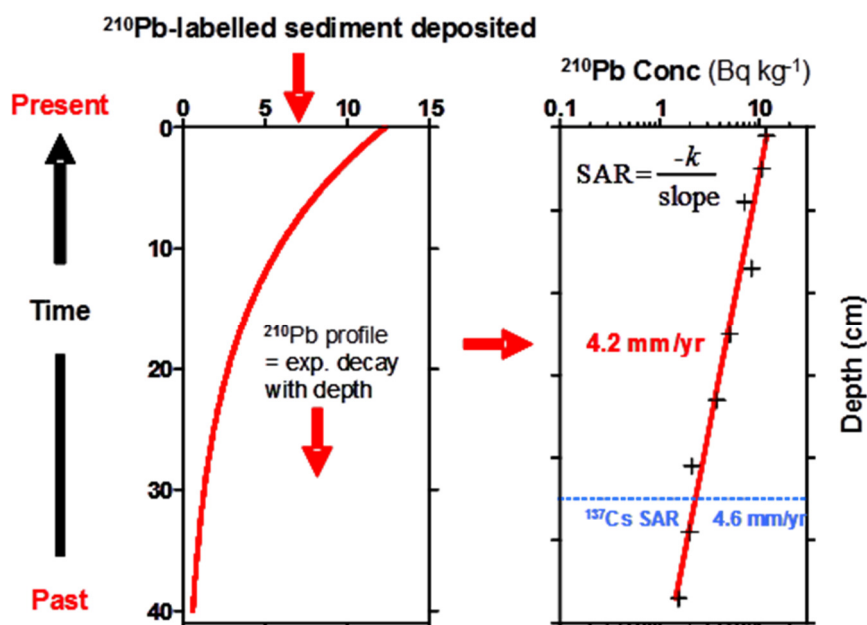


Figure 49: The ^{210}Pb depth profile decay curve (left) X-axis re-plotted on a $\log_e$ scale (right) allows the calculation of sediment accumulation rates (SAR) in mm/yr. This rate can be checked using the ^{137}Cs data – broken line is ^{137}Cs maximum depth. (k is the decay constant for $^{210}\text{Pb} = 0.03114/\text{yr}$).

For mass balance estimates, these data should be converted to mass accumulation rates (MAR) before they are used with the CSSI data. A simple way to convert the SAR to a MAR is to multiply the SAR by the bulk density of the sediment and the area of the deposition zone. This gives a net mass value and is integrated over the time-frame of the SAR. It also provides the mass/unit-time value required to calculate the denudation rate.

Slope calculation

Because the decay curves for FRNs are exponential, they are linearized by taking the natural log (i.e., log to the base $e = \text{Log}_e$) of the FRN energy in Bq kg^{-1} . The graphs produced from the sediment core (Figure 49) have been rotated to put Depth on the vertical axis. This rotation of the graph is for the users' convenience and does not change the fact that depth is equivalent to time, which is on the X-axis. The regression analysis has depth as the X-axis and $\text{Log}_e(\text{Bq kg}^{-1})$ as the Y-axis to produce the slope used to calculate the SAR. An example (Figure 50) shows the procedure using an Excel spreadsheet. The slope value is obtained from a trend line through the graphed data (Figure 50) or by regression analysis.

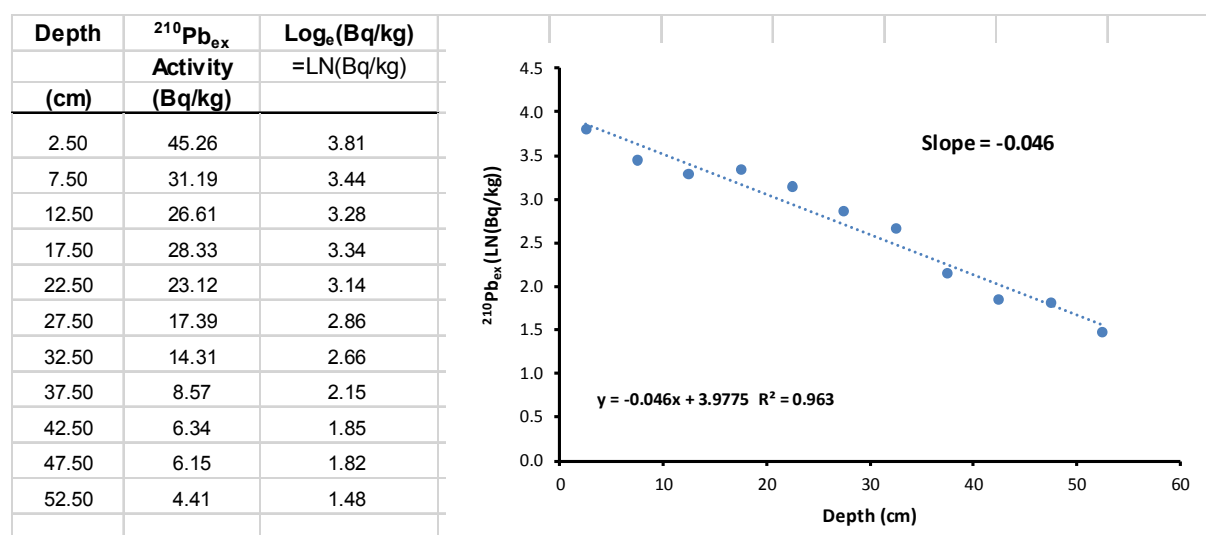


Figure 50: Example procedure for analysing the FRN data to obtain the slope for the SAR calculation. Broken line is a trend line fitted through the data such that $Y = -0.046X + 3.9775$.

11 Case Study

11.1 Waitetuna Study

11.1.1 Whole watershed sediment tracking

The design concepts presented in section 4.4 and illustrated in Figure 11 were based on an actual study on the Waitetuna River catchment near Hamilton, New Zealand. Figure 11 is a line drawing of the actual river system and Figure 9 is an aerial photo of a small sub catchment on tributary TR3 (Figure 11), which is the Mangakirikiri sub-catchment (Figure 51). One objective of this study was to quantitatively determine where sediment, which deposited in the estuary at the mouth of the river during a summer storm, came from in the watershed.

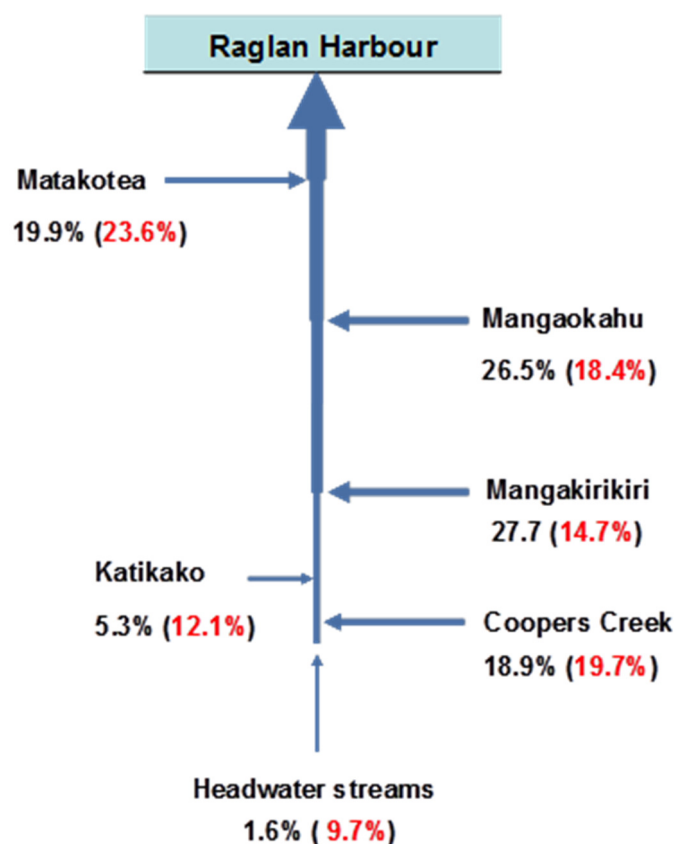


Figure 51: Schematic representation of the Waitetuna River showing the proportional contributions from the main tributaries for the summer storm event as estimated by the CSSI technique (black) compared with the proportional mean annual sediment yields estimated by the SPARROW regional regression model (red). The proportions have been normalised to 100% as the Waitetuna River enters the Raglan Harbour as described in section 4.4.

Using the procedures described in section 4.4, the proportional contribution of soil from each tributary was estimated (Figure 51). As a reality check the CSSI estimates were checked against mean annual sediment yields estimated by the GIS-based regional regression model, SPARROW (Elliott et al. 2008).

While some proportional contributions were in reasonable agreement, the CSSI technique indicated higher sediment contributions from the Mangaokahu and Mangakirikiri sub-catchments but lower sediment contributions from the headwater streams. The main reason for these differences was attributed to the uneven distribution of rainfall across the watershed during the storm. The heaviest rainfall occurred in the Mangaokahu and Mangakirikiri sub-catchments, which is consistent with the CSSI technique estimates. The GIS based model produced annual mean sediment yields and not the expected sediment yields from a single event.

These proportional contributions were converted to sediment yields using the total sediment discharged from the Waitetuna River as estimated by the physically-based model, SHETRAN, (Schmidt et al. 2008). The latter study monitored the flood event and used turbidity as a surrogate for suspended sediment concentration estimated that the total sediment yield for this storm event was 165t. In this case study scenario, 165t is the normalised 100% sediment yield from the river and the sediment yield from each tributary can be calculated from the proportional contributions to the river (Figure 51). (See later).

The FRN data from this study showed that, while ^7Be concentrations were readily measurable in the soil samples and in the sediment from the deposition zone at the mouth of the Waitetuna River, ^7Be concentrations were near or below detection level in the sediments in the river channel. This is interpreted as indicating either rapid movement of very fine material through the system or erosion of sub-soils associated with the large numbers of landslides that occurred during this storm event (Figure 9). Similar interpretations were made from the ^{137}Cs and ^{210}Pb data.

11.1.2 Whole watershed sediment contributions by land-use

GIS modelling produced a distribution map of the land-use in the Waitetuna watershed (Figure 52) and an estimate of the total areal proportion of each of the three main land-use types (combining dairy with pasture and scrub with native forest) in the watershed (Table 5). Multiplied by the mean annual sediment yield data, these data provide an estimate of the mean annual sediment yield from each land-use in the watershed. While these estimates may be suitable for annual reports, they may not be appropriate for the study of a single event.

In this case study, the CSSI technique was used to estimate the proportion of soil from each land-use in each sub-catchment contributing to the sediment at the mouth of each tributary. These sub-catchment proportions were then multiplied by the estimated sediment contribution from each tributary (Figure 51) to provide an estimate of the proportion of soil erosion from each land-use for the event being studied (Table 5).

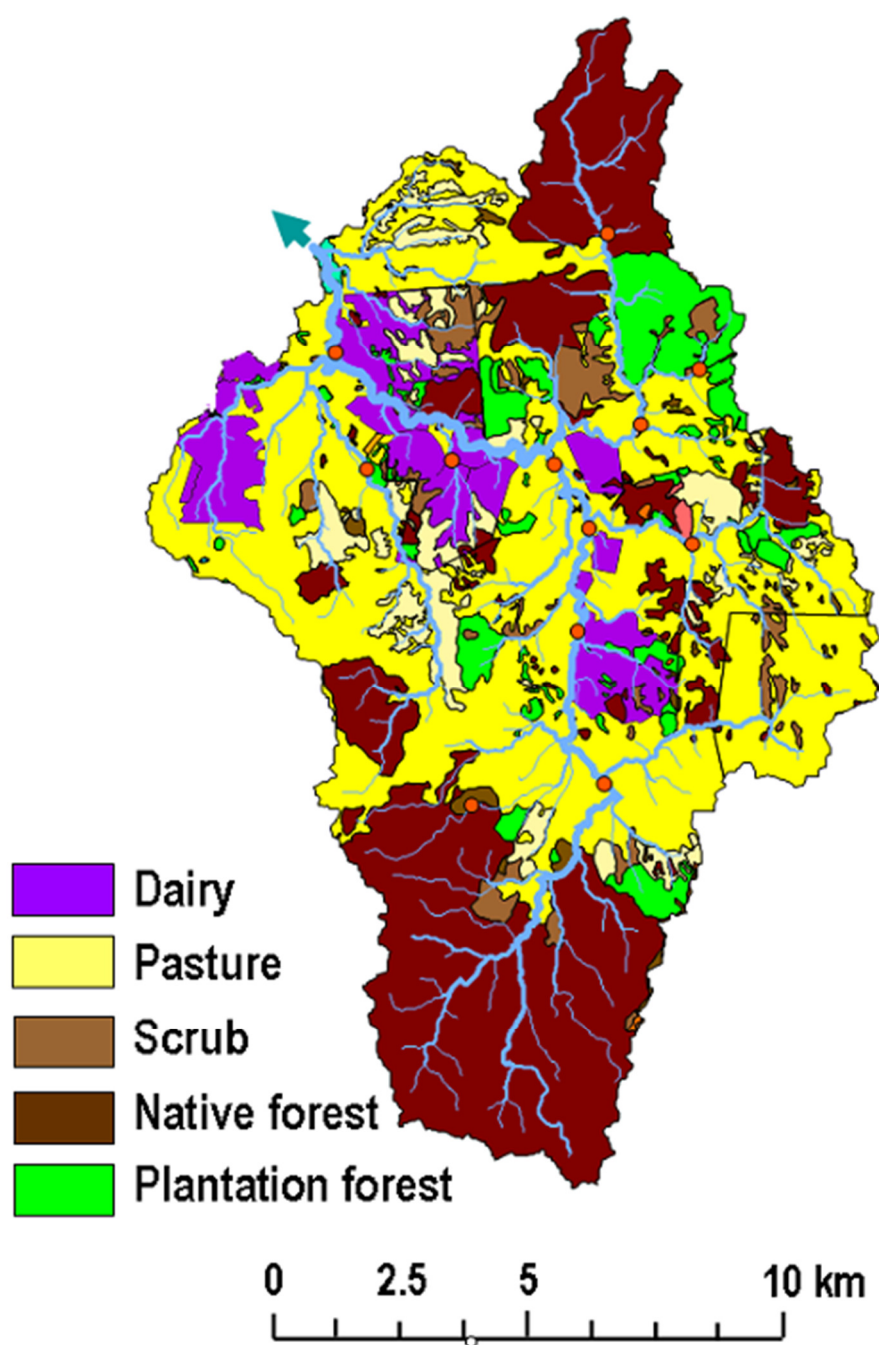


Figure 52: GIS-derived schematic land-use map of the Waitetuna watershed showing the five main land-uses and their relative distribution within the watershed.

The difference between the proportional contributions of main land-use types estimated by the GIS model and CSSI technique is that the CSSI technique values were for a single event and not a yearly average as in the GIS estimate. Notwithstanding this, the proportional contributions produced by the CSSI technique were comparable with those estimated by the GIS model even though the CSSI technique did not use any land-use area data – just the stable isotopic signatures of the bulk OC and the FA biomarkers.

Table 6: Three main land-use proportions as % derived from the CSSI technique for the summer storm event compared with mean annual sediment yield proportions from the GIS-based regional regression model, SPARROW. Level of uncertainty on the CSSI estimates <5%, values rounded to one decimal place. Proportional contributions of sediment from the sub-catchments by land-use were not estimated using the SPARROW model. (* not modelled individually in SPARROW - beef and sheep together contribute 42.4 % of the mean annual sediment yield). Land-use class

	Native	Pine/scrub	Pasture	Dairy	Sheep	Beef
Watershed (GIS)	35.8	13.4	50.8	8.5	(*)	(*)
Sub-catchments (CSSI)						
Matakotea	36.0	<2	62.0	20.0	41.0	<1
Mangaokahu	74.6	14.4	11.0	6.5	1.6	2.9
Mangakirikiri	3.5	1.7	94.7	16.7	1.6	76.4
Katikako	7.0	42.0	51.0	0.0	41.0	10.0
Coopers Ck	4.3	38.8	57.0	5.1	42.0	9.9
Headwaters	34.0	44.0	22.0	0.0	16.5	5.5
Watershed (CSSI)	30.0	15.0	55.0	11.0	17.0	27.0

Pasture sub-

The mass of soil erosion from each sub-catchment can be calculated by multiplying the proportional contributions of sediment (Figure 51) and the total mass of sediment discharged during the event. In this case study the total mass estimate was 165t, which was apportioned to each tributary (Figure 53).

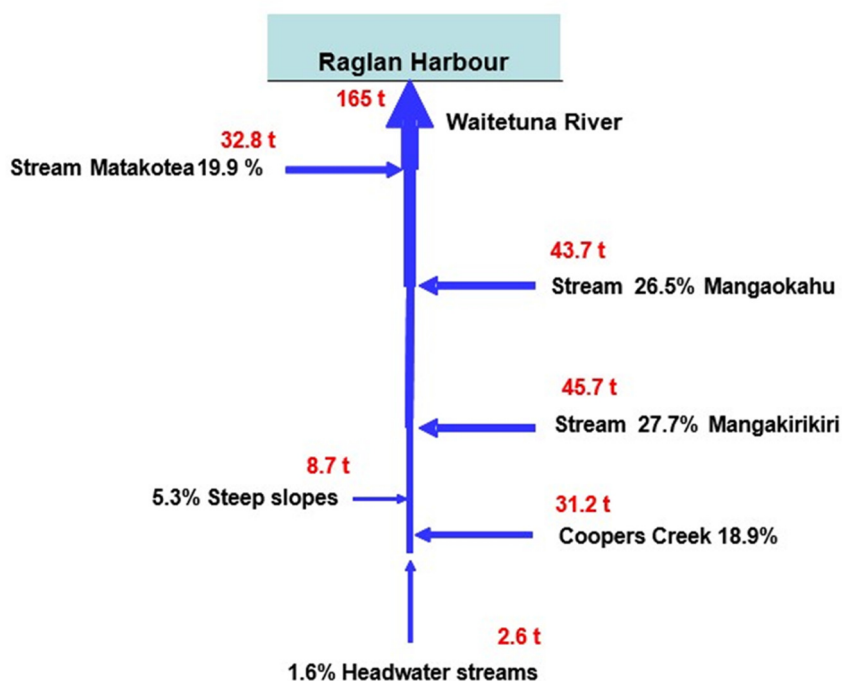


Figure 53: Schematic representation of the Waitetuna River showing the proportional soil contributions from the main tributaries and the mass in t.

The mass of soil eroded from each land-use source can be calculated by multiplying the landuse soil proportions in each subcatchment (Table 5) with the total mass eroded from that subcatchment (Figure 53) and summing these for the whole watershed (Table 7).

Table 7: Summary of erosion mass by land use in the Waitetuna watershed. For the storm event studied, the catchment average erosion factor was 9.88 kg/ha.

	Native Forest	Pine Forest	Pasture	Sheep	Beef	Dairy
Catchment area (ha)	5979	2238	8484	1697	1900	721
Erosion Mass (t)	49.5	24.75	90.75	15.3	24.5	10
Erosion factor (kg/ha)	8.28	11.06	10.70	9.02	12.89	13.87

These data show that dairy and beef (dry stock) land use produce the highest soil erosion in this catchment.

11.2 Bay of Islands historical land-use reconstruction

Combining FRN and CSSI techniques provides a powerful tool for assessing changes in land-use over time. Coupled with other non-isotopic techniques including colour changes in layers and X-ray assessment of grain size and density changes in a sediment core, it is possible to reconstruct historical land-use practices that produced those sediments.

In the following example from a study in the Bay of Islands, New Zealand (Figure 54-Figure 56), observations of coloured layers relative to SAR obtained from ^{210}Pb dating in the upper part of the core (Figure 54) define recent specific flood events, which can be confirmed from public records. X-ray imagery can confirm the depth of the surface mixed layer defined by the presence of ^7Be and identified changes in sediment grain size which indicate different magnitudes of events (e.g., Figure 17B). The CSSI values of selected FA biomarkers associated with specific depth layers (Figure 55). can identify the source of that sediment by land-use and changes in these CSSI values through the depth of the core indicate changes in land-use practices over the time period of the core (Figure 56).

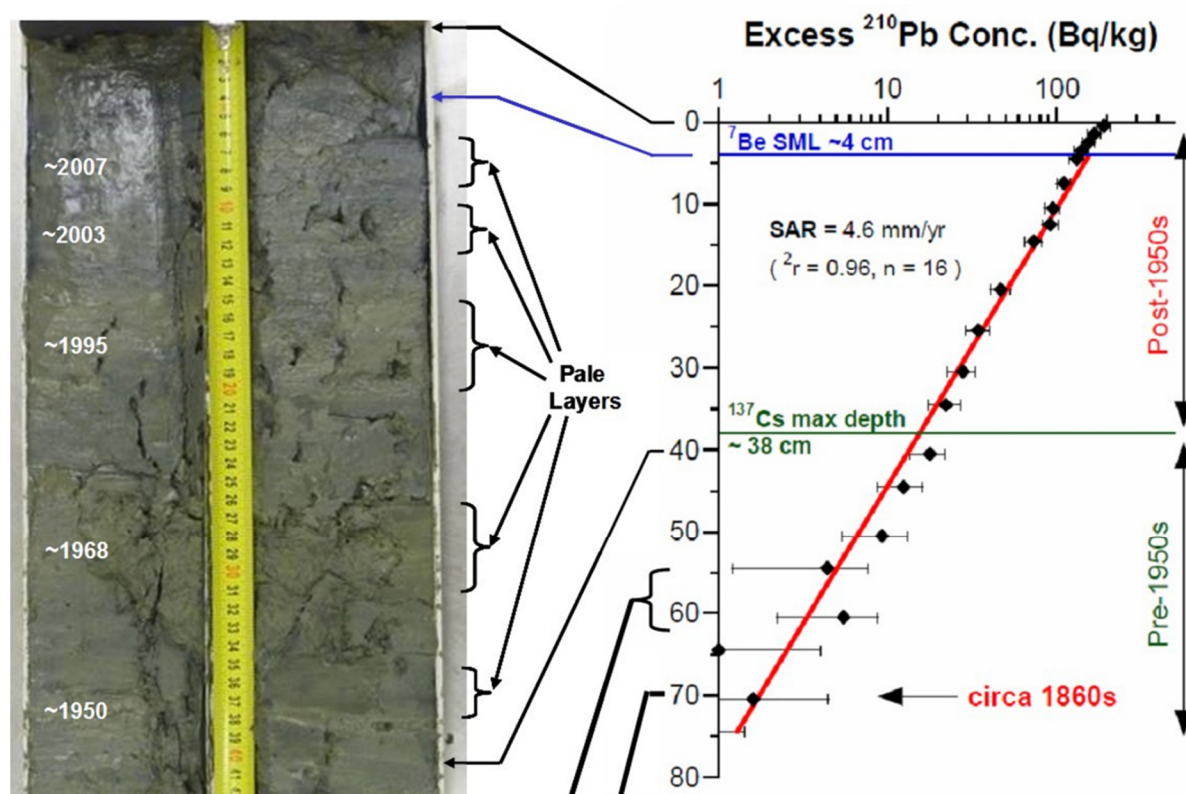


Figure 54: Changes in sediment colour can be dated using the sediment accumulation rate (SAR) estimates obtained from the ^{210}Pb data. In the upper core, these layers can be correlated with local records to improve the date precision. Deeper in the core, below 50 cm, the dating precision from ^{210}Pb decreases as the variability increases. In this example, the surface mixed layer (SML) is determined from the ^7Be data and the maximum ^{137}Cs depth is used to provide a date marker for the early 1950s.

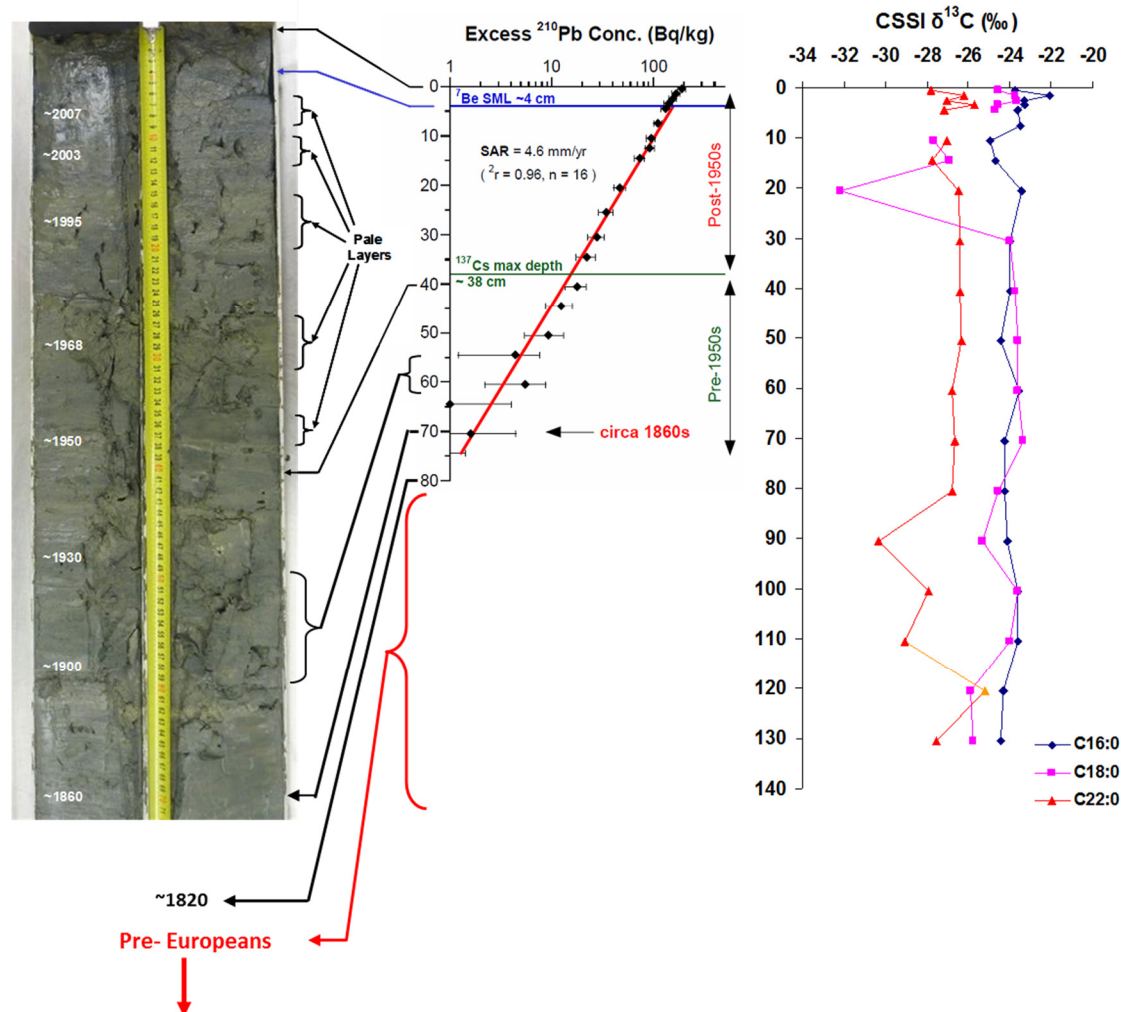


Figure 55: Accurate dating below 55 cm is not possible due to the high variability in the data. Best estimates are made by extrapolation for the rest of the core assuming the SAR data has not changed. The full core extends well beyond the range of the measurable FRNs but still has measurable CSSI values. The example CSSI profiles show large changes in one FA below 80 cm and changes at around 20 cm for another.

Full interpretation of the FA biomarkers using the CSSI technique assumes that the plants that produced those signatures 200-300 years ago are directly equivalent to the range of present day plants which label the soils in the soil land-use library for this area. The sources of the sediment at each analysis depth can be determined to give an estimate of the land-use practices producing sediment at those times (Figure 56). The proportional land-use contribution estimates have been shown as percentage shadow bars which have then been correlated with known dates and events from historical records (Figure 56) to reconstruct a chronology of historical land-use.

The interpretation of these estimates is based on the premise that for the soil to have been eroded, the soil in those land-uses must have been disturbed at those times. For example, the increase in proportional contribution of CSSI values from Kauri forest imply that soil from those forests would only be eroded if those trees were being harvested or the land under the Kauri forest was being disturbed. Coupled with the dating estimates from the SARs, the

presence of the first increase in Kauri signatures at around 1800 (Figure 56) would be related to early sailing ships harvesting these tall trees for replacement masts and building timber. Such activities were recorded in the log books of early sailing ships. The subsequent presence of the Kauri signatures a hundred years later (1900) corresponds with the arrival of the fortune seekers searching for Kauri gum in the wetlands and swamps in the area. Small amounts of swamp Kauri are still recovered to the present day.

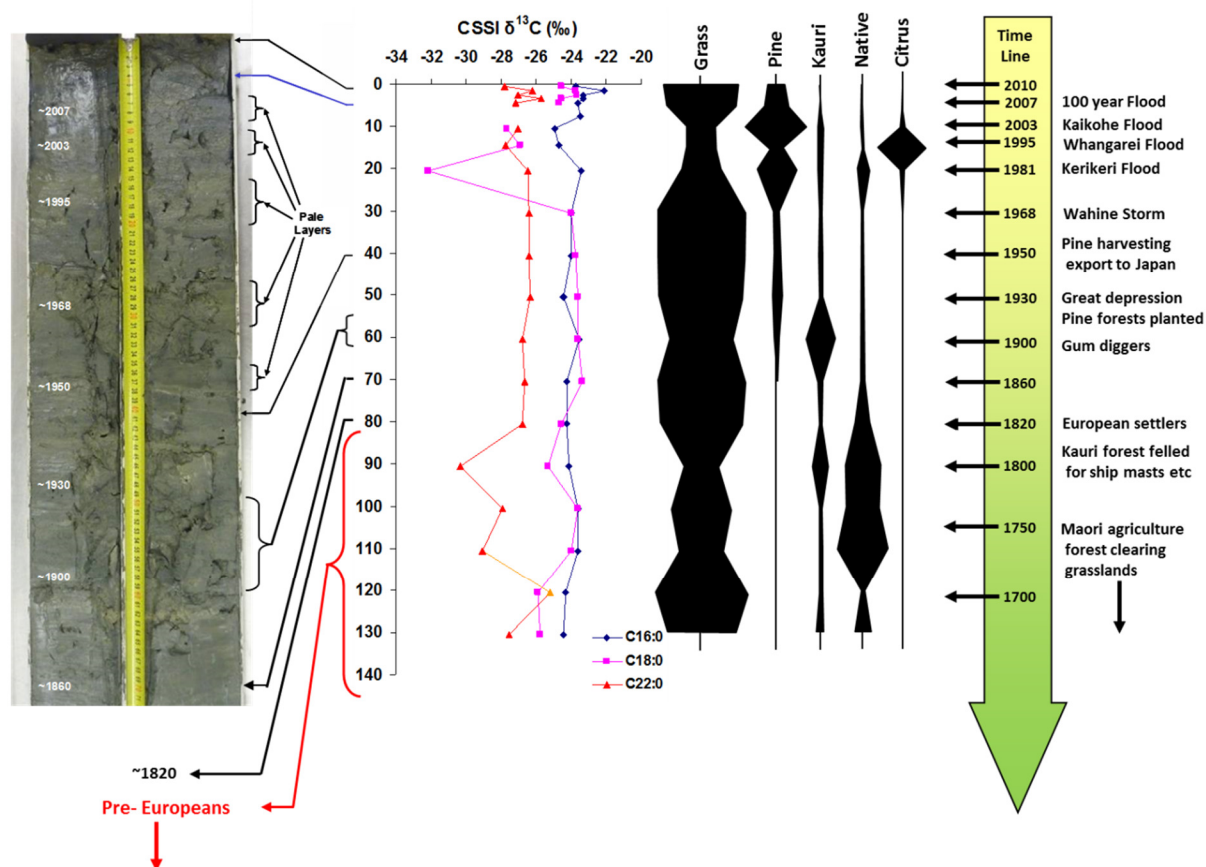


Figure 56: The CSSI data at each section depth was converted to a proportional land-use contribution and displayed as a percentage shadow bars (black graphs 0-100%). These proportional land-use contribution data were then correlated with known events and historical records to reconstruct the history of the area.

The presence of “grass” and native forest signatures before the arrival of Captain James Cook, who discovered New Zealand in 1769, is consistent with deforestation and agriculture by the large number of Maori in this watershed. Captain Cook wrote of the Bay of Islands: “The habitants of the Bay are far more numerous than at any other place we have yet been in”.

On his visit to New Zealand in 1835, Charles Darwin wrote in his diary:

“I think with much probability that all this extensive open country was once covered by forests & that it had been cleared in past ages by the aid of fire...digging in the barest spots, lumps of resin, which flows from the Kauri pine, are found”.

The increase in native forest signatures through the early 1800s and subsequent decline after 1860 is consistent with the clearance of the land by early European settlers. The planting of grass for farming is matched by an increase in grass signatures in the sediment. Pasture is still the dominant land-use today and the main source of sediment (Figure 47).

During the great depression after the First World War, large areas of New Zealand were hand planted in pine forest (*Pinus radiata*) to provide employment. Parts of the Bay of Islands were planted with pines and from 1920, there is a low level of pine signature in the sediments (Figure 56). Harvesting of these forests began about 25 years later but without a major increase in pine signature in the sediments. This may be due to the practice of burning the pine debris before replanting, a land-use practice which left the soil relatively undisturbed. The heat from the fires may have also volatilised the organic biomarkers. From the 1970s on, burning was discontinued and the debris was scraped into rows allowing mechanical planting on the bare ground. This change in land-use practice was accompanied by an increase in the proportion of pine signature in the sediment (Figure 56). This land-use practice continues to the present day.

The historical reconstruction (Figure 56) was of low temporal resolution due to the depth increments (10 cm) between the sediment layers analysed. With a SAR of 4.6 mm yr^{-1} , each sample point represents about 20 years. A smaller depth increment would have improved the temporal resolution, but may not have greatly increased the amount of information extracted from this core to warrant the increased cost.

12 Suess effect

The CSSI technique was developed to positively identify the source of soil by landuse to determine the provenance of sediment from contemporary watershed erosion. 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 $\delta^{13}\text{C}$ signature of atmospheric carbon dioxide (CO_2) due to the admixing of isotopically depleted CO_2 from the burning of fossil fuels.

Because the carbon in fossil fuels is isotopically depleted by around 18‰, the CO_2 released when that fuel is burnt is isotopically depleted (e.g., Andres et al. 2000; Verburg, 2007). This depleted CO_2 results in isotopic depletion of the CO_2 in the air. The effect began in the 1700s at the beginning of the industrial revolution and the rate of isotopic depletion has been gradually increasing since that time (Figure 57). Because it is caused by the burning of fossil fuels the rate of isotopic depletion appears to be a function of rate of increase in atmospheric CO_2 concentrations.

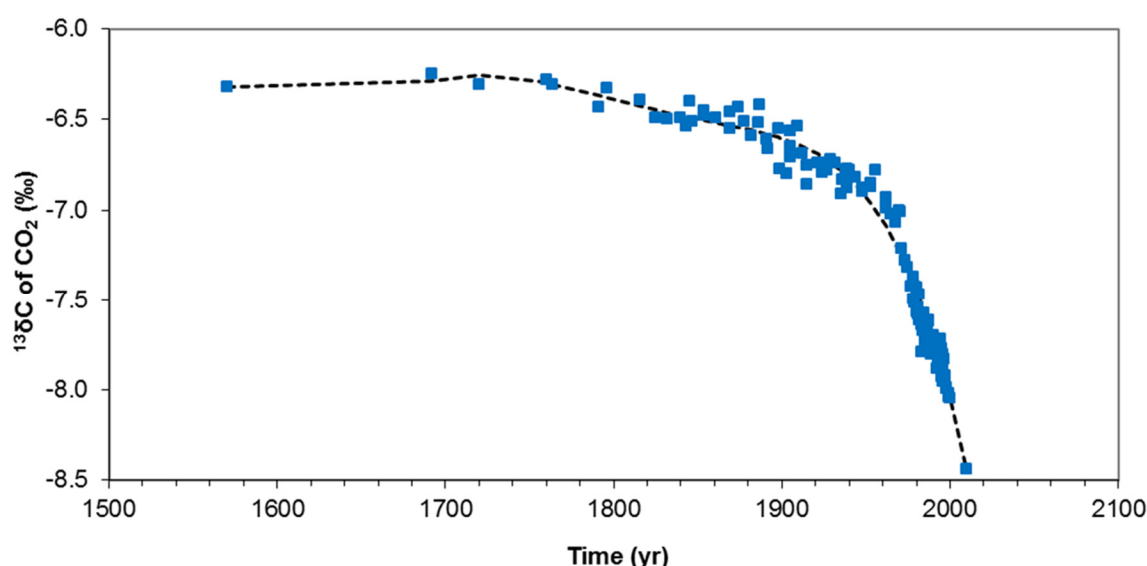


Figure 57: Time-series plot of the change in $\delta^{13}\text{C}$ abundance of atmospheric CO_2 that has occurred since pre-industrial times (AD 1700). The broken line is a 6th order polynomial curve fitted through the data. (Redrawn using data from Verburg, 2007 and papers therein).

The Suess effect only affects the CSSI technique when looking back in time as in the deconstruction of a sediment core to identify changes in land use over time. The earlier CSSI values can be as much as 2.3‰ more enriched than present day soil sources or sediments. Consequently, to use contemporary soil sources to deconstruct CSSI data from a sediment core the isotopic signatures of the present day sources need to be corrected to account for the Suess effect. This correction was applied to a sediment core from the Bay of Islands, New Zealand, to look back 2,500 years (Gibbs et al. 2012).

The CSSI values from the core sections between AD 1700 and present day were corrected by the isotopic depletion value calculated from the 6th order polynomial equation from

Verburg (2007), and adding the absolute $\delta^{13}\text{C}$ value (8.55‰) of present day CO_2 (year 2012) as an offset to obtain the change (Δ) in the $\delta^{13}\text{C}$ 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:

$$\text{Correction value} = 8.55 + 7.7738118 \times 10^{-16} \times Y^6 - 1.2222044 \times 10^{-11} \times Y^5 + 7.1612441 \times 10^{-8} \times Y^4 - 2.1017147 \times Y^3 + 3.3316112 \times 10^{-1} \times Y^2 - 273.715025 \times Y + 91703.261$$

In food-web studies where the lifespan of the organisms studied does not extend beyond a few tens of years, a simpler correction for the Suess effect has been applied as a time-dependent correction of -0.022‰ per year (Chamberlain et al. 2005; Hopkins and Ferguson 2012) to all sample isotope values, except the present day samples. This correction can also be used in the CSSI technique to correct isotopic data within the range of the ^{210}Pb dating, i.e. up to 100 years before present.

13 Glossary

^7Be	Berilium-7 (radioactive half-life 53.44 days)
^{137}Cs	Caesium-137 (radioactive half-life 30.23 years)
^{210}Pb	Lead-210 (radioactive half-life 22.3 years)
$^{210}\text{Pb}_{\text{ex}}$	Lead-210 excess
BF_3	Boron trifluoride [derivatisation catalyst]
Bq	Becquerel [unit of radioactivity: 1 Bq is 1 disintegration per second]
Biomarker	Naturally produced chemical specific to a source
CSSI	Compound-Specific Stable Isotope
DCM	Dichloromethane [solvent]
Deconstruction	Separation of the component soils in a soil mixture
FA	Fatty Acid
FAME	Fatty Acid Methyl Ester
FAO	Food and Agriculture Organisation of the United Nations
FRN	Fallout Radio Nuclide [^7Be , ^{137}Cs , ^{210}Pb]
GC	Gas Chromatograph
HCl	Hydrochloric acid
IAEA	International Atomic Energy Agency
IRMS	Isotope Ratio Mass Spectrometer
MeOH	Methanol [solvent]
OC	Organic Carbon
OM	Organic Matter
PDB	Pee Dee Belemnite [an international standard for ^{13}C]
PLFA	Phospho-Lipid Fatty Acid
QC	Quality Control
SAR	Sediment accumulation rate

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