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IAEA-TECDOC-1881

Guidelines for Sediment Tracing Using the Compound Specific Carbon Stable Isotope Technique

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

IAEA
International Atomic Energy Agency

GUIDELINES FOR SEDIMENT TRACING USING THE COMPOUND SPECIFIC CARBON STABLE ISOTOPE TECHNIQUE

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GUIDELINES FOR SEDIMENT TRACING USING THE COMPOUND SPECIFIC CARBON STABLE ISOTOPE TECHNIQUE

PREPARED BY THE
JOINT FAO/IAEA DIVISION OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

INTERNATIONAL ATOMIC ENERGY AGENCY
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FOREWORD

The IAEA and the Food and Agriculture Organization of the United Nations (FAO), through the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, support multidisciplinary scientists, policy makers, land managers and farmers around the world in developing suitable, cost effective isotopic techniques to enhance sustainable agricultural production.

With increasing attention being paid by both developing and developed countries to soil erosion and its associated sedimentation processes, the present publication addresses theoretical and practical aspects of the compound specific stable isotope technique, based on the determination of $\delta^{13}C$ signatures of fatty acids used as soil and sediment fingerprints. By establishing the origin of sediment (within agricultural systems and/or at the outlet of catchments), this innovative isotopic approach can support stakeholders in the effective application of climate smart agriculture.

The IAEA is grateful to M. Gibbs (New Zealand), for his instrumental role in developing the $\delta^{13}C$ fatty acid technique. The IAEA also wishes to thank all the other contributors and the participants in the IAEA coordinated research project on Nuclear Techniques for a Better Understanding of the Impact of Climate Change on Soil Erosion and Upland Agro-Ecosystems who were involved in the preparation of this publication. The IAEA officer responsible for this publication was L. Mabit of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

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SUMMARY

The global population is expected to double in the coming decades. In this context, soil is a crucial natural resource that allows for food production, but its degradation is increasing worldwide. As soil tracers, fallout radionuclides (FRNs) can provide distinct advantages over traditional soil erosion monitoring techniques by enabling retrospective estimates of soil redistribution. However, they cannot really provide reliable information regarding the origin of sediments, at the catchment scale.

To address this specific issue, compound-specific stable isotopes (CSSI) techniques with focus on fatty acids biomarkers (FAs) were proposed towards the end of the 2000's. Since then the technique has been tested in some countries (Australia, Austria, Chile, New Zealand, Switzerland, UK, etc…) and has been improved significantly.

This TECDOC details how to use the CSSI technique based on the determination of $\delta^{13}C$ signatures of FAs for investigating sediment tracing in agro-ecosystems. It provides guidance how to develop effective sampling strategy, to perform the various analytical steps, to generate data using stable isotope mixing models and to treat and interpret the information produced. While covering the fundamental concepts of the CSSI technique, this contribution distinguishes itself from others by providing step-by-step instructions for scientists, technicians and students on how to effectively use this isotopic technique.

It is important to mention that this method is still in its infancy. Readers are encouraged to test it under various agro-ecosystems and as well to update their knowledge about the latest development around this isotopic technique as new studies and methodological papers are regularly published in peer-reviewed soil and environmental science journals.

This publication is one of the first outputs associated with the on-going implementation of the Coordinated Research Project D1.50.17 *'Nuclear Techniques for a Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems'* led by the Soil and Water Management & Crop Nutrition Sub-programme of the Joint FAO/IAEA Division.

1. INTRODUCTION

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1.1. BACKGROUND

Global food security is challenged by the increase of the world population which is expected to double in the coming decades [1.1] [1.2]. As a vital natural resource, agricultural fertile soils should be protected and sustainably managed to support increased food production. Indeed, as highlighted by the FAO Director-General José Graziano da Silva during the 21st World Congress of Soil Science on 13 august 2018 in Rio de Janeiro, Brazil 'Sustainable soil management is an essential part of the zero-hunger equation'.

The yearly cost of the value of the world's ecosystem services that is lost due to land degradation processes was estimated at approximately 10 trillion USD [1.3] and recent value of soil loss at the global scale has been reported to be close to 36 Pg yr^{-1} [1.4].

To implement climate smart agriculture and to develop efficient soil conservation strategies in agroecosystems, there is a clear prerequisite to gain reliable scientific information regarding the origin of sediment and then provide key decision-support knowledge.

Off-site and on-site sediment sources tracing in agroecosystems is a scientific challenge that requires specific set of tracers. Such fingerprints should be 'conservative' and natural.

Geogenic, cosmogenic as well as anthropogenic fallout radionuclides (FRNs) tracers can provide distinct and additional advantages over traditional soil erosion monitoring techniques by enabling retrospective estimates of soil redistribution [1.5]. Radiotracers such as ^{137}Cs, $^{210}Pb_{ex}$, ^{7}Be [1.6] [1.7] and $^{239+240}Pu$ [1.8] generate trustworthy results on the extent and magnitude of soil erosion but do not provide detailed indication about the source of sediments (FIG. 1-1).

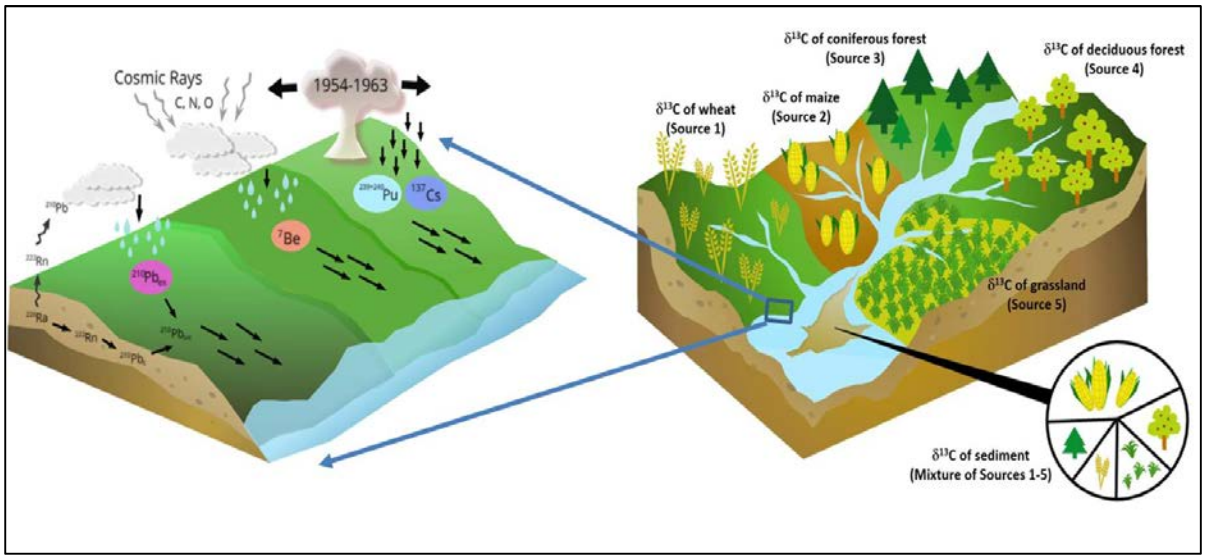

FIG. 1-1. Complementarity of FRN and CSSI techniques to broaden our understanding on sediment budget. (Adapted from [1.5])

Note that throughout this TECDOC, the term 'source' is used to describe the soil from a defined land use contributing to a sediment mixture, and the term 'mixture' will describe the material from one or more sources in suspension or in a deposition zone.

Discriminating one source from another requires a measurable difference in one or more parameters or physical characteristics between the sources, which is conservative over time. The discrimination capacity of these parameters or physical characteristics may change depending on external influences in the catchment being investigated. Consequently, different techniques may be required in different landscapes.

There are several existing methods that could be applied for identifying the sources of soil that are contributing to a sediment mixture with varying degrees of resolution over a range of spatial and time scales [1.9] [1.10].

A technique that is largely independent of the physical characteristics of the soil and therefore applicable across most landscapes is the CSSI technique (FIG. 1-1). The CSSI technique distinguishes itself from the traditional geochemical techniques because it is currently the only sediment source tracking approach that can positively identify and apportion the sources of soil, by land use, contributing to the suspended load or to the sediment in a deposition zone. It is a semi-quantitative technique, which uses mass flow data to obtain a quantitative assessment of the source contributions to the downstream environment.

Compound-specific stable isotopes (CSSI) technique based on the measurement of $\delta^{13}C$ signatures of natural fatty acids (FAs) which are specific organic biomarker compounds was proposed at the end of the 2000's to trace soil sediments to freshwaters [1.11].

As plants label the soil of the land where they are growing by exuding FAs biomarkers that have specific $\delta^{13}C$ isotopic values [1.12], those biomarkers can be used to determine the origin of sediment by land use. This soil and sediment fingerprint technique has been tested in different agroecosystems of some countries (e.g. Australia [1.13], Austria [1.12] [1.20], Chile [1.14], Switzerland [1.15], UK [1.16], Vietnam [1.17] [1.18]) and has been improved significantly since its inception.

The principles associated with CSSI analysis of FAs as soil fingerprints can be summarised through the following six statements [1.11] [1.19] [1.20]:

1) Land use is typically identified through the flora growing on it (FIG. 1-1);

2) While all plants produce the same suite of saturated FAs with straight carbon chain lengths from 14 to 26, each plant species produces those FAs with slightly different $\delta^{13}C$ isotopic signatures in the carbon atoms in the FA molecules due to isotopic fractionation along the different assimilation pathways of carbon dioxide (CO_2);

3) The $\delta^{13}C$ isotopic signatures of FAs is characteristic of the plant community defining the land use; they are transferred to the soil via the roots and dispersed throughout the upper soil layer by infiltrating rain water;

4) These values are stable when FAs get attached to fine soil particles and stay unchanged. i.e., they are highly conservative. Although the concentration may be reduced due to bacterial decomposition, the isotopic signatures do not change;

5) Based on the above statements, FAs and especially their $\delta^{13}C$ value can therefore be considered as robust agro-environmental biomarkers of land use;

6) A moderate change in the land use can be highlighted by a difference in the isotopic signature of FAs and sometime even in the bulk $\delta^{13}C$ value of the soil.

Based on existing literature, while the straight-chain saturated FAs with chain lengths between C14:0 and C26:0 have been used to identify sediment sources, it is the long carbon chain length FAs having an even number of carbon atoms (i.e. $\geq$ C20:0) that are recommended to be used [1.15] [1.20] [1.21] [1.22]. FAs with odd numbers of carbon atoms should be avoided as they are produced mostly by bacteria. Further, odd numbered FAs may have been produced locally in the deposited sediment due to decomposition processes and therefore will not represent the soil land use they originate from. FAs with carbon chains longer than C26:0 are essentially insoluble in water and may only label the soil directly in contact with the plant roots. Consequently, there may be increased variability in the amount of C28:0 and C30:0 FAs spread through the surface soils. C28:0 and C30:0 FA biomarkers can thus be used but with caution.

The first step in using the CSSI technique consists in establishing background information and identifying the source soils or 'library' of the area being investigated. This is one of the main prerequisite of the method.

These selected source soils should have contributed to the eroded soil mixture (i.e. the sediment transported and/or deposited), as the CSSI technique will only highlight the source soils that have been eroded and that have contributed to the mixture.

Then the relative contribution to the soil mixture of the different source soils identified can then be determined using a stable isotope mixing model (SIMM).

1.2. OBJECTIVES

The objectives of this TECDOC are to (i) provide guidance in the use of the CSSI technique for identifying areas at risk and the sources of sediment within various agro-ecosystems, and (ii) disseminate the technical knowledge gained within the on-going Coordinated Research Project D1.50.17 '*Nuclear Techniques for a Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems*', which started in mid-2016. One of the aims of the CRP is to refine isotopic techniques to assess the rates of soil erosion and its impacts in upland agro-ecosystems.

1.3. SCOPE

The scope of this TECDOC is to support scientists and experts in Member States in the effective use of the CSSI technique. This comprehensive illustrated guideline highlights new opportunities for improving area-wide soil conservation strategies in fragile agricultural landscapes.

1.4. STRUCTURE

This TECDOC consists of five sections. The first section introduces the concepts and assumptions behind the technique; the second section details the sampling strategy to optimise its field applications; the third section gives precise information on how to prepare and analyse soil and sediment samples collected; the fourth and fifth sections provide guidance on data treatment and interpretation of the results obtained.

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2. PLANNING AND PROTOCOLS FOR SAMPLING

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2.1. PLANNING

Before using the CSSI technique, there should be a clear understanding of the objectives of the proposed study and a written plan of what has to be done to achieve those objectives. The best approach is to write down the goals of the investigation and include a map or sketch a diagram of the study site being investigated.

Understanding the issues and potential problems before going into the field will avoid subsequent errors due to incorrect sampling.

CSSI studies can be undertaken for specific purposes and these may be:

1. Spatial investigations, e.g. what are the sources of soil, by land use, contributing to eroded sediments?

2. Temporal investigations, e.g. what are the effects of climate variability on soil erosion in upland agro-ecosystems?

3. Historical investigations, e.g. has there been any change in land use sources being eroded during major flood events?

Each type of investigation has its own set of protocols but they all begin with careful planning and are based on one clear research question.

In spatial investigations, the planning will be influenced by the size of the study site being investigated (from a couple of connected agricultural fields to a large catchment area) and the accessibility of potential sampling sites at key points. In small catchment scale, it may be possible to do a complete analysis of the whole area. In medium to large catchments, the catchment may need to be divided into sub-catchments that are easier to sample and understand. These would be recombined at the end of the analysis.
Note that geographical constraints apply where it is not possible for a specific land use source to influence a specific sediment mixture location. Conversely, library reference soils for specific land uses may be collected from adjacent catchments with the same land use to provide replicate samples.

In temporal investigations, the planning will be influenced by the time step between repeat samplings and the level of discrimination in the isotopic signatures that can be detected over natural variability in the isotopic signatures within a land use class.

In historical investigations, the planning will focus on finding a suitable site to collect sediment cores that can be dated and sectioned accordingly to answer the research question being asked. The $\delta^{13}C$ isotopic signatures of the FAs bound to different sediment layers will need to be corrected for the Suess effect [2.1] to allow contemporary land use soil sources to be used to deconstruct the different sediment layers from the past into equivalent land uses.

2.2. SAMPLING

Based on the study plan, the number of samples to be collected and the location of each sampling site will be established. The precise position for collecting the samples will be finalised on-site. Record all meta-data and for each site, include pictures, which show usable land features to enable a return to the exact location.

2.3. COLLECTING AND ESTABLISHING SOIL REFERENCE LIBRARY SAMPLES

- Sample each different major land use that has the potential to erode. Because of the limited spatial variability of the FAs signatures under a homogenous land use and in order to reduce the analytical cost of $\delta^{13}C$-FAs, composite sampling is encouraged when using CSSI techniques [2.2]. Each sample should be a composite of at least 10 equal sized divots from an area of at least 100 m^2 at each location, collecting just the top 20 mm of the soil layer;
- The sampling tool can be any mechanical device that will give a reproducible sized plug of the soil being sampled. For example, a simple hand operated corer was made from a 100mm diameter hole saw by attaching a handle and fitting a simple push plunger inside to help extrude the soil plug collected (FIG. 2-1). In this sampler, the soil plug taken is 40 mm thick. Consequently, the lower 20 mm layer of soil is removed and discarded before combining the upper soil layer from each plug in a 20 L plastic bucket;
- The soil is ‘rumbled’ or shaken from the plant roots;
- Sticks, stones, leaf material, insect and worms should be removed and discarded at the time of sampling;
- Mix the soil divots in the bucket and then take about 200-400 g of the mixture as the sample representing the sampled location;
- The soil should be sealed in a plastic bag, which is then sealed inside another plastic bag, with the label placed between the inner and outer bag, facing out so as to be readable.

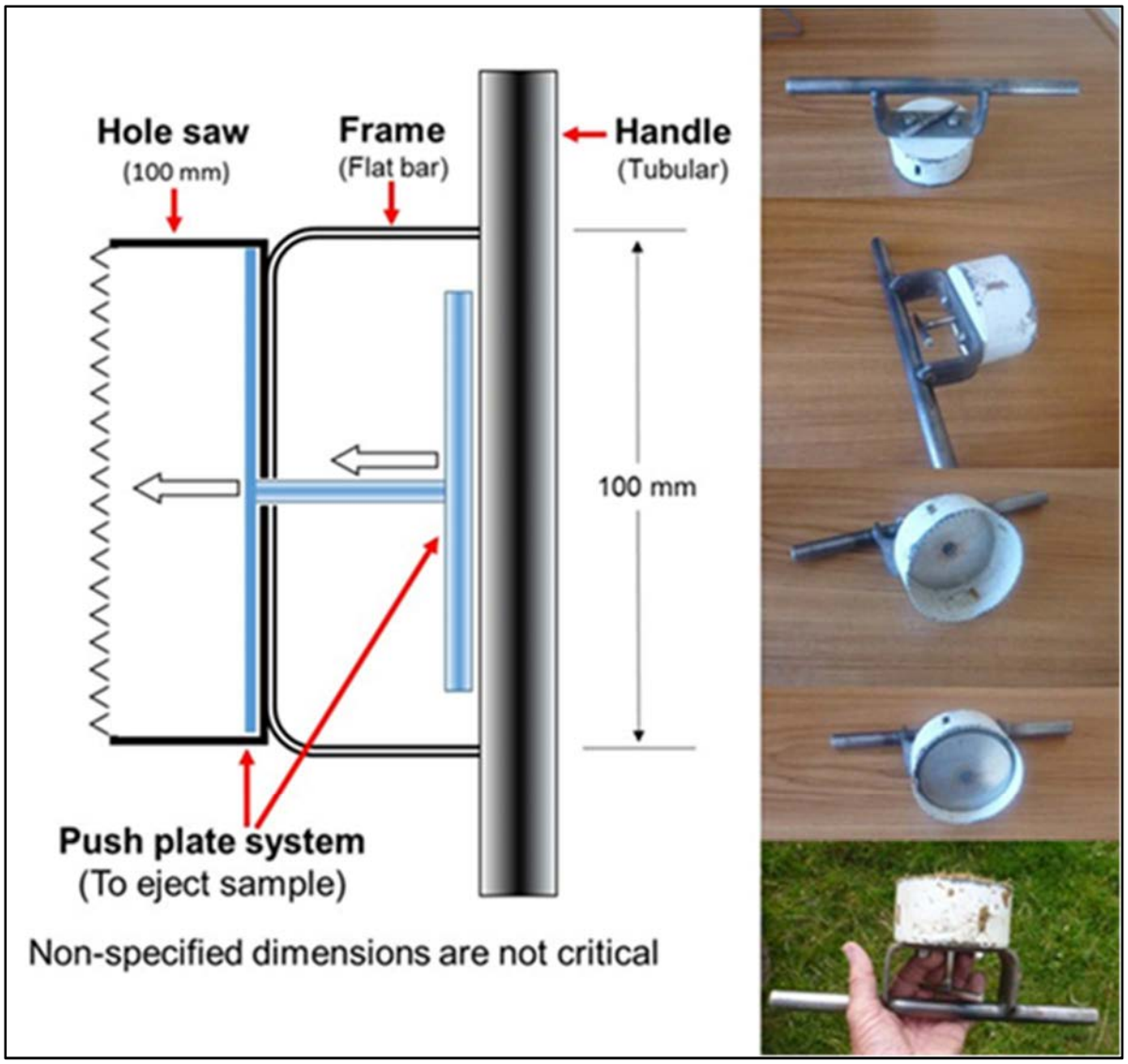

FIG. 2-1. Example of hand corer for collecting soil and some sediment samples. (Reproduced courtesy of M. Gibbs [2.3])

Precise incremental samples are typically required by some FRN techniques (e.g. ^{7}Be) to establish radionuclides depth profiles for erosion and sedimentation investigations [2.4]. The layer thicknesses are usually in the order of a few mm compared with the 20 mm thick layer recommended for the CSSI technique. While these samples may be used for CSSI analysis, the fine scale depth differences are likely to be compromised by plant root systems which can penetrate many cm down into the soil transferring FAs from the current land use throughout the upper soil layers. Consequently, scraper plate samples may not be useful in CSSI studies unless they are a specific part of the study plan.

2.4. STREAM BED AND ESTUARY SAMPLING

Because fine sediment is deposited as a thin layer, for contemporary sediments the surficial layer is scraped off as the representative sample. In a stream/river situation, the composite sample can be from a small area and may be just a single sample. This is an acceptable approach because the sediment will have been completely mixed by the water that carried it to that location.
In an estuary, multiple surface scrapes from a larger area using a spade (FIG. 2-2) should be combined, mixed and sub-sampled as for the soil reference library samples.

FIG. 2-2. Collection of a thin surface layer of sediment deposited on a mudflat in an estuary. (Reproduced courtesy of M. Gibbs)

Bank erosion sampling poses several problems, depending on the height of the bank and homogeneity of the bank material (FIG. 2-3). It could be sampled by scraping an even layer off a vertical section, after cleaning the surface of recent plant growth, and mixing the bank material in a bucket before subsampling. Conversely, if the river is cutting back into the original landscape, which has horizontal layering, the full height of the bank should be sampled as above, or the bank should be sampled from several layers, which can be combined to give a representative sample of the whole bank.

Old bank material will have the FA signatures of the contemporary plants growing on the surface being eroded. However, as the erosion bites deeper into the bank, the FA signatures of the original source material will become more dominant and there is the potential for the isotopic signatures associated with bank erosion to change. It may be necessary to use FRN techniques or archived land use records and historical photographs to estimate when each layer was deposited so that the FA signatures can be corrected for the Suess effect. Present day changes in the $\delta^{13}C$ of CO_2 are estimated to be -0.023‰ per year. For contemporary studies, it is the material being eroded that should be sampled.

FIG. 2-3. IAEA staff collecting sediment sources within the Petzenkirchen watershed located about 100 km west from Vienna (Austria). On the left sampling of agricultural fields; on the right streambank sampling.

2.5. SUSPENDED SOLIDS SAMPLING IN WATER

Sediment can be collected from flowing water using time-integration techniques such as the Phillips sampler [2.5]. This type of sampler typically collects the <63 μm particle size fraction, which is unlikely to settle in fast-flowing (water moving at >10 cm s^{-1}) streams, except in back waters. Ordinarily, this particle size range may never settle in the river system being studied. This effect must be considered when designing the study using the Phillips sampler and may be used to advantage where the study is specifically examining sediment runoff under light rain conditions. Fine sediment is always the first to be mobilised during a rainfall event. Under low turbidity conditions, collecting a sediment mass sufficient for CSSI analysis may be an issue (need about 20 g).

Another sediment sampler, which collects sediment faster, is a mat trap. Such traps are lined with artificial turf, which increases the roughness of the collecting surface and enhances settling. This type of trap will allow collection of a larger particle size range, more similar to the range found in natural sediment deposits. The sediment caught in the trap should be washed out of the fibres by placing the mat in a large plastic bag and shaking with a little water, which can be decanted off after settling.

2.6. SEDIMENT AND SOIL CORES FOR STUDING THE EVOLUTION OF PROCESSES IN THE PAST

Sediment cores can be used to look back in time to investigate changes in land uses that are eroding. Conversely, soil cores from open land, which is not a sediment sink, can be used to look back in time at changes in land use. This distinction is important to remember during planning. While a soil core will show all previous land uses, a downstream sediment core may have apparent missing land use components, if those land uses were not eroding at that time of sediment deposition. The sediment core may also have larger amounts of a particular land use source than indicated from the area of that land use in the catchment, indicating higher levels of erosion of that source at that time. Sediment and soil cores are processed the same way.

A set of three 100 mm diameter cores can be taken from each sampling site: one for X-ray slabbing and radionuclide dating, one for stable isotope analysis (both $\delta^{13}C$ of the whole soil as bulk $\delta^{13}C$ and of the FAs extracted from the soil) and one as a backup for re-analysis should that be necessary.

The cores must be immediately capped and sealed in the field then transported and stored horizontally. This is very important with soft sediment cores because, if stood vertical, the sediment will compact and that will alter the chronology of the layers for dating.

The technique used to obtain the core will vary depending the site being investigated.

For example, (1) soft sediment cores can be taken from a boat using a 100 mm diameter PVC core liner tube inside a heavy drop corer. This will allow the core to be collected from water depths of 0 m to 100 m or more; (2) soil cores can be collected using a motorized soil column cylinder auger or by hammering a stainless-steel tube into the ground. The core could then be retrieved using a tripod and hand winch.

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3. PROCESSING SAMPLES

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3.1. SPATIAL, CONFLUENCE AND SOURCE LIBRARY SAMPLES

The collected samples should be dried, either in an air fan oven at 60°C or by freeze drying. Each sample is placed in a labelled aluminium or stainless steel shallow tray (pie dish) and inserted into a new plastic bag with the end open. This is to reduce the risk of cross contamination while drying. Then one to two hundred grams of dry sieved material (<2 mm) of each sample should be sealed in a plastic bag and stored in the dark, at room temperature [3.1].

3.2. SEDIMENT CORES

Sediment cores need to be sectioned at different depths down the core from the surface into slices, which represent different times in the past. The FRN dating can use a three-stage process with a preliminary examination of the core by X-ray to look for sediment grain size changes and other structures to help in subsequent interpretation of the core. Of particular importance is to look for carbon material near the base of the core, such as shell, bone or wood fragments that can be used for ^{14}C dating of very long cores (e.g. 1 to 2 m). Ideally, this carbon material should be from as close to the bottom of the core as possible. Starting from the top of the core, a set of 4 or 5 preliminary sample slices could be taken from the core for lead-210 (^{210}Pb) analysis to investigate recent (<100 y) sediment accumulation rates. Based on these results, the core is sectioned into about 15 or more slices at selected depths.

3.2.1. Splitting the core

The core, in its tube, is laid horizontally on a flat work bench and marked with a water-proof marker pen along its length with two parallel lines 2 cm apart, on each side. These are the cut lines. Each line is cut almost through the tube wall with a small circular saw fitted with guides to keep it centred on the line (FIG. 3-1A). Once all saw cuts are made, the cuts are completed with a sharp knife blade to make slits just into the sediment. A set of approximately 1.5 mm thick, short stainless steel flat plates are driven through the slits and the sediment inside the core tube to isolate the centre section of sediment (FIG. 3-1B). Once all the plates are in position, thin stainless-steel shims are pushed through the core on top of the plates

(FIG. 3-1C). The core is rolled so the plates are vertical and wedges are used between one set of plates and shims to split that side of the core from the rest (FIG. 3-1D). The core is rolled back to horizontal and the plates are removed (FIG. 3-1E). The flat side of the core is covered with cling film and a 20 mm deep wooden tray, the length of the core, is laid on top of the cling film. The core and tray are rolled over, so the core is lying in the tray.

The other side of the core is split off and rolled off the wooden tray (FIG. 3-1F). After removing the plates and shims, both residual core sections are wrapped in cling film and stored horizontally ready for sectioning. The 20 mm thick slab in the wooden tray is also sealed in cling film and taken for X-ray examination. The cling film stops moisture loss as well as protecting the slab and core sections from contamination. Visual features are noted and photographed with an in-frame tape measure referenced from the surface end of the core.

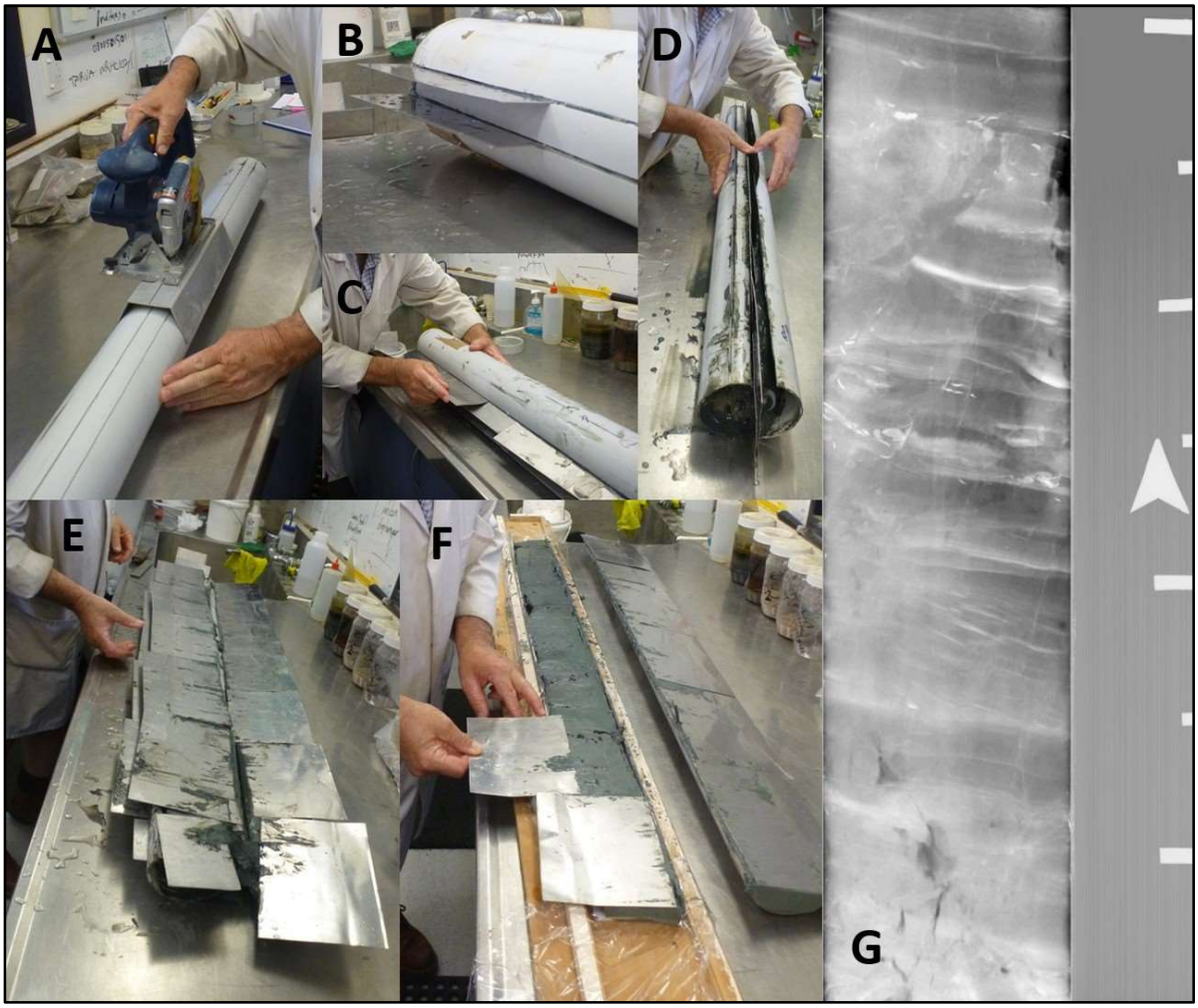

FIG. 3-1. Photo sequence of some steps in splitting a core. A) cutting the core tube with a circular saw; B) Inserting the 1.5 mm plates; C) Inserting the shims; D) Splitting the first part of the core; E) Separating the split part from the main core; F) Finishing the removal of the plates and shims from the 20 mm thick slab in the wooden X-ray tray; G) Example of reverse image X-ray from 1.0 to 1.35 m depth in an estuary (white = high density [sand, shell]; dark = low density [organic mud and silt], white bars are 5 cm intervals below 1 m depth.) (Reproduced courtesy of M. Gibbs)

If an X-ray facility is not available in the laboratory, the slab may be for example X-rayed in the radiology department of a local medical clinic.

The X-ray example (FIG. 3-1G) shows the level of detail possible from the X-ray image. This would allow targeted sectioning of the third core for investigating specific events. Each light-coloured layer is likely to represent a flood event or some other major soil disturbance that was capable of moving sand. The X-ray also allows carbon objects for radiocarbon dating to be more easily found.

3.2.2. Taking core sections

Once the X-ray has been taken and viewed, 1 cm thick slices are taken from the three parts of the core for FRN analysis and dating.

For CSSI sampling, the second core is split in half (after one longitudinal saw cut on each side) and 1 cm thick slices are taken down to a depth of 5 cm. Subsequent slices can be at 2 cm intervals down to 10 cm depth, then 5, 10 or 20 cm intervals for the remaining depth of the core, depending the precision required and the depth to which the study needs to answer the questions being asked. Alternatively, the slices can target specific layers identified in the X-ray examination to evaluate historical events. All CSSI samples can be processed as for the spatial, confluence and source library samples.

These core sections will be correlated with the ^{210}Pb dating data after the CSSI values have been corrected for the Suess effect (see Section 4).

3.3. BULK DELTA ^{13}C ANALYSIS

The analytical process for bulk $\delta^{13}C$ requires the sample to be acidified while for the CSSI analysis the FAs must be extracted from the non-acidified sample and converted to fatty acid methyl esters (FAMEs) for analysis.

Acidification, using HCL, is required to remove the inorganic carbonate from the soil and sediment samples. This allows the $\delta^{13}C$ isotopic value of the bulk organic carbon to be measured together with the total organic carbon content ($\%C_{org}$). Although the concentration of carbon is not used in the source modelling, $\%C_{org}$ is used in the conversion of isotopic proportions to soil proportions after modelling.

3.4. CSSI ANALYSIS

The analysis of CSSIs includes three steps:

1) extraction of FAs;

2) acidification for bulk organic carbon analysis or derivatisation for FA analysis;

3) isotopic $\delta^{13}C$ analysis of the organic carbon and the FAs.

3.4.1. Step 1: FA Extraction

Fatty acids bound to soil can be extracted using hot polar solvents such as dichloromethane (DCM) which has been double distilled from bulk or is analytical grade. The extraction will take a different amount of time depending on the technique used.

The easiest method is to use an accelerated solvent extractor (ASE). ASEs have a programmable extraction routine which can subject the sample, which has been loaded into a sample cell, to a soak in hot DCM at 100°C at a pressure of 2000 psi for 5 minutes, and then repeat the process, combining the extracts from each extraction cycle in a sealed bottle.

If the sample was not completely dry, moisture in the extract can be removed with 1 or 2 g of anhydrous sodium sulphate, which has been baked in a muffle furnace at 450°C and stored in a screw cap Pyrex glass bottle. To eliminate this problem, re-dry the sample in a 60° oven over night before extraction. The solvent extract is reduced to dryness in a 10ml screw cap Kimax digestion tube ready for derivatisation.

3.4.2. Step 2: Derivatization

Because FAs are polar molecules, they cannot be analysed directly using a gas chromatograph (GC). To solve this specific issue, the polar carboxylic acid radical (-COOH) is converted to a methyl ester by replacing the hydrogen atom with a methyl group ($-COOCH_3$). This results in a non-polar fatty acid methyl ester or FAME which can be analysed by GC.

There are several ways to produce the FAME from a FA, all using pure methanol as the source of the methyl group and a catalyst to facilitate the reaction. The methods used include the use of strong acid or alkali at high temperature for an extended period. These can have variable recovery results and should be used, with caution, exactly as described in the literature.

The alternative is the use of boron trifluoride (BF_3) as the catalyst. This method is more easily controlled, fast and produces highly reproducible results.

It is important to remember that a sample of the methanol utilized to create the FAMEs must also be sent to the analytical laboratory to obtain the $\delta^{13}C$ isotopic value of the methanol. This is used to correct the isotopic values of the FAMEs back to FAs.

3.4.3. Step 3: Analysis of bulk organic carbon and compound-specific stable isotopes

This step requires a competent analyst with sophisticated analytical equipment. If these conditions are not met, it may be best to select an analytical facility with the ability and capacity to provide that service and send the processed samples there for determination.

The $\delta^{13}C$ isotopic value of the acidified bulk organic carbon is measured after combustion in an elemental analyser using a continuous flow, isotope ratio mass spectrometer (IRMS). The IRMS $\delta^{13}C$ isotopic values are calibrated against known solid standards at the beginning and end of each analysis. IRMS analytical precision on repeat analyses is typically around 0.1‰.

The $\delta^{13}C$ isotopic values of the FAMEs are determined by GC-combustion-IRMS. There are several different instruments available to achieve this and all require a complex programme to enable the separation of the sequence of FAMEs in each sample.

The FAMEs are separated by the GC (FIG. 3-2) and the $\delta^{13}C$ isotopic value of each peak is measured. FAME identification is by reference standard retention times, which have been confirmed by GC-mass spectrometer. An internal C12:0 FAME standard may be added to each sample as a retention time reference because there is usually no interfering peak at the C12:0 position (FIG. 3.2). A mixed standard with 6 (or more) FAMEs is injected between each group of 10 samples being analysed. In each batch of samples, one should be the quality assurance (QA) sample and one should contain 6 (or more) FA standards that have been derivatized with the other samples in that batch.

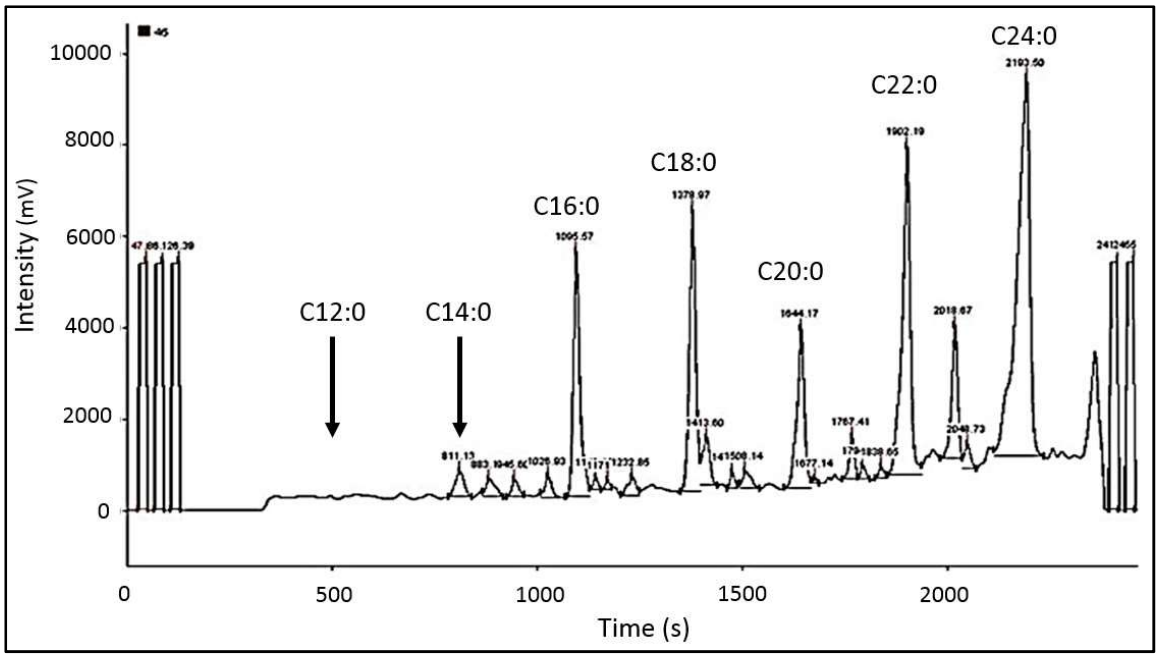

FIG. 3-2. Gas chromatogram of a pasture sample showing the main fatty acid components by chain length produced by the land use plant community. Small peaks are also fatty acids but are mostly produced by bacteria and are not used in the CSSI technique. (Reproduced courtesy of M. Gibbs)

In sediment and soil samples, saturated FAs from vascular plants have even numbers of carbon atoms at relatively high concentrations while those from bacteria mostly have odd numbers at low concentrations.

3.5. QUALITY ASSURANCE

A control sample should be included with a batch of samples to assess the efficiency of the FA extraction, the derivatisation, and the analysis. This QA sample can be any sample that has previously been analysed. Normally it would have been analysed at least 5 times and the statistics determined to account for analytical variability. When it is used in an analytical run, it is included at the extraction stage and will provide information on the efficiency of the extraction and subsequent derivatisation step. The analytical results will show whether there was any analytical drift that needs to be corrected. The QA sample is different from a standard in that it has a soil matrix with the fatty acids, whereas the standards do not. Both the QA sample and the standards are processed through the derivatisation step

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4. DATA TREATMENT AND INTERPRETATION

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4.1. CORRECTING THE DATA

For contemporary samples, the analytical results for bulk $\delta^{13}C$ and %C are used as supplied by the analyst. Conversely, the analytical results for $\delta^{13}C$ values of the FAMEs must be corrected for the extra carbon added from the methanol used during the derivatisation process to obtain CSSI values of the FAs.

For samples from sediment cores looking back in time, both the bulk $\delta^{13}C$ and the CSSI values (after methanol correction) must be corrected for the Suess effect.

4.1.1. Methanol Correction

During derivatisation, the methyl group from a methanol molecule replaces the H atom in the carboxylic acid radical to form the methyl ester group. The C atom in that methanol will have a different $\delta^{13}C$ value from the original FA and the resulting $\delta^{13}C$ value of each FAME will be different from the FA.

To determine the original isotopic signature of each FA, the effect of the additional C in that methyl group must be removed. This required correction can be directly performed using the below equation (4.1), [4.1] [4.2] [4.3]:

$$\delta^{13}C_{FA} = (\delta^{13}C_{FAME} - (1\text{-}X)\ \delta^{13}C_{Methanol}) / X \qquad (4.1)$$

Where:

- $\delta^{13}C_{methanol}$ is the bulk $\delta^{13}C$ value of the methanol used in the derivatisation process;
- FA is the fatty acid under investigation;
- X is the fractional contribution of carbon atoms in the FA to the FAME i.e. for C18:0, X = 18/19 = 0.9474).

4.1.2. Suess Correction

Since the industrial revolution in the 1700s, the burning of fossil fuels has released CO_2 into the atmosphere with $\delta^{13}C$ values reflecting the eon when coal and oil was created. This fossil CO_2 varies between fossil fuel sources [4.4] [4.5] and is thought to be more isotopically depleted than the $\delta^{13}C$ values of present day. The admixing of the fossil CO_2 causes the isotopic $\delta^{13}C$ values of the atmospheric CO_2 to become more depleted [4.6]. Because plants use atmospheric CO_2 for growth, the CSSI values of the FAs in the plants will reflect the contemporary isotopic $\delta^{13}C$ of the atmospheric CO_2. Consequently, the CSSI values of the FA biomarkers have been systematically changing since the mid-1700s and are presently about 2.2 ‰ more depleted than they would have been in 1700 AD. This is called the Suess effect.

As the Suess effect only began around 1700 AD, all CSSI values from core sections before that time are made more isotopically depleted by 2.2‰, i.e. they are corrected by -2.2‰. Between 1700 AD and present-day values must be modified using an isotopic depletion number calculated from a 6th order polynomial equation and adding the absolute $\delta^{13}C$ value (8.65‰) of present-day CO_2 (year 2016) as an offset to obtain the change in the $\delta^{13}C$ isotopic value for the year (Y) of the core section [4.2] [4.6].

$$\text{Correction value} = 8.65 + (7.7738118 \times 10^{-16} \times Y^6) - (1.2222044 \times 10^{-11} \times Y^5) + (7.1612441 \times 10^{-8} \times Y^4) - (2.1017147 \times 10^{-4} \times Y^3) + (3.3316112 \times 10^{-1} \times Y^2) - (273.715025 \times Y) + 91703.261 \quad (4.2)$$

Note that the polynomial coefficients in this equation (4.2) have been rounded to seven decimal places.

4.2. DISCRIMINATION BETWEEN SOURCE SAMPLES AND DATA INTERPRETATION

The bulk $\delta^{13}C$ and CSSI values in a source sample provide a set of isotopic values that are essentially unique as a ‘fingerprint’ for that specific source. The isotopic values that can be used in the modelling process are the $\delta^{13}C$ value of the bulk soil carbon and the $\delta^{13}C$ value of each FA i.e. C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C20:0, C22:0, C24:0 and C26:0. This represents 11 potential biomarkers for each source sample. These FAs are all slightly soluble in water and can be dispersed throughout the upper soil layer with infiltrating rainwater. Longer chain FAs including C28:0 and above, are not water soluble and are unlikely to disperse through the soil evenly, thereby not fulfilling all requirements of a biomarker (See Section 1).

Other similar, but different, sources may have a similar fingerprint but with minor differences. When the differences are small and the standard deviations of all the isotopic values of triplicate samples overlap between the two sources, it will not be possible to discriminate between these sources in the modelling process. This is an unusual situation because with 11 separate isotopic values, it is highly unlikely that they will all be that similar. Inspection of the data will identify those FAs which cannot be used because there may be missing values for that FA in some sources. A matrix of usable isotopic data should be prepared in a spreadsheet that can be manipulated electronically.

Source discrimination in the modelling process is only as good as the data available and inclusion of invalid source data in the modelling will produce erroneous results. High variability reduces the discrimination power between similar land use classes reducing the amount of detail that can be obtained from the study.

With careful study design and an adapted sampling strategy, pasture used for grazing sheep and pasture used for dairy farming can be discriminated. Even so, there will still be variability that must be tested against the null hypothesis that these two different land use sources are indistinguishable. Sources of variability of stable isotopes in food web studies have been discussed in the literature e.g. [4.7] and more recently the variability associated with the FAs used in the CSSI technique for soil source tracing [4.8]. That said, the level of variability will not be known until the samples collected have been analysed and the results are seen.

At this time, one needs to make the best use of the data. This may mean combining samples from similar land use classes or eliminating samples from land use classes which, because their isotopic values are essentially outliers, have a very low probability of contributing significantly as a source to the mixture being deconstructed.

Statistics
There is a move to use statistical analysis to test for discrimination between sources. Discrimination of similar and dissimilar land use classes based on their stable carbon isotopic composition can be pursued in graphical and statistical approaches, in two steps.

Step 1: Graphical analyses involves generation of Tukey boxplots for each of bulk $\delta^{13}C$ and the usable FAs, across all land use classes. Linear unconstrained ordinations can also be performed on centred and standardised variation in the bulk and usable FA $\delta^{13}C$ signatures of all samples following filtering for outliers. Principal components analysis (PCA) determines the underlying pattern of change (variance) in the usable FA and bulk $\delta^{13}C$ signatures, and then spreads all of the individual land cover samples as widely as possible along these gradients or 'principal components' of isotopic variation. A biplot of all of the samples distributed along the first and second principal components, using symmetric scaling, can be utilised to determine which land use classes were alike or dissimilar, as well as which FA or bulk isotopic signature varied most between land use classes.

Step 2: Using information provided by Tukey post-hoc testing and the PCA biplot, a subjective decision can be made to include, exclude or merge the land use classes to optimise variation between classes across all $\delta^{13}C$ indicators. Following this decision, the revised (fewer) land use classes can be subjected to 1-way ANOVA using revised land use classes as the factor examined, across all $\delta^{13}C$ indicators, inspecting the output for residual normality and equivalence of variance between land use classes.

Polygon test
Notwithstanding the power of statistics, it is mandatory that the sediment mixture data must lie within the bounds of a polygon drawn through the extreme values of the source samples for pairs of isotopes to be used in the modelling [4.7].

In practice, all combinations of isotope tracers should be tested against the bulk carbon $\delta^{13}C$ and each other using biplots of the isotope pairs to identify which isotopes could be used in the modelling.

Where the sediment mixture does not meet this criterion, that isotope tracer should not be used in the stable isotopic mixing model. The polygon test can also identify other data problems that may not be apparent from the statistical assessment (FIG. 4-1).

In FIG. 4-1A, the sediment mixture lies outside the polygon drawn through the extremes of the data points in the biplot graph (highlighted in yellow). If the biplot is redrawn using different FA tracers, and it produces biplots where the polygon encloses the mixture, one of the FA tracers in the first data pair cannot be utilized to identify the sources of soil in the sediment mixture. This type of result may also indicate that there is likely to be a missing source.

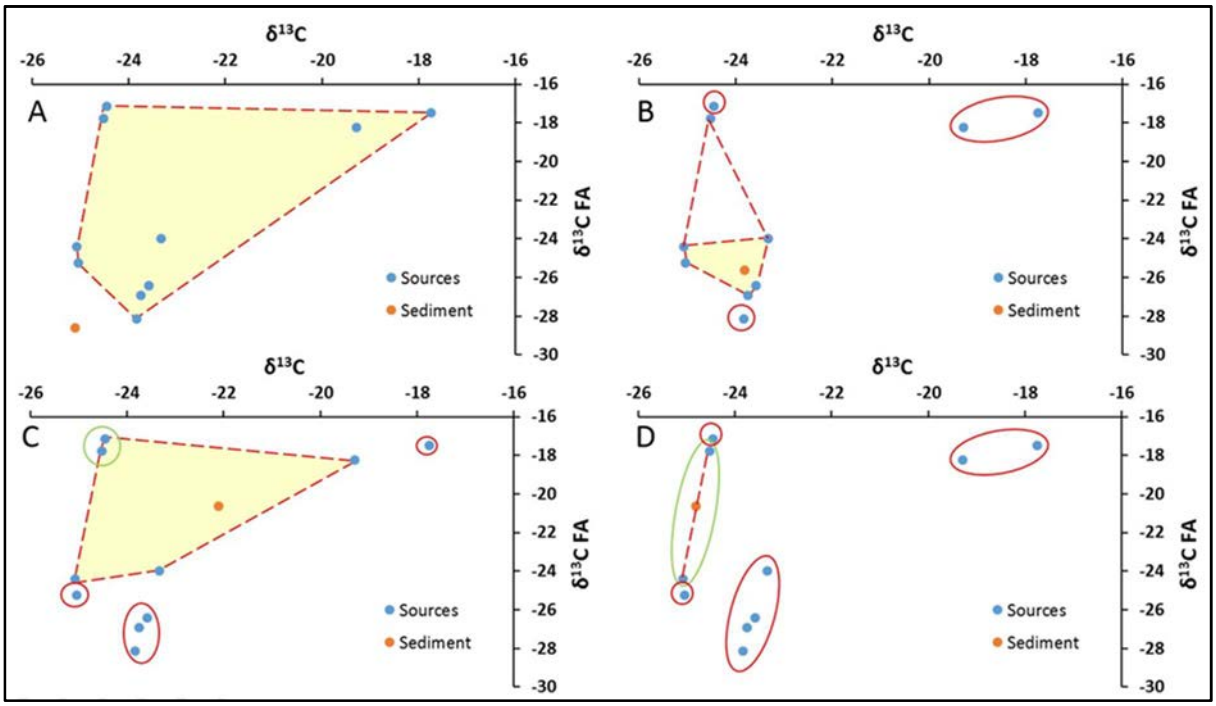

FIG. 4-1. Applying and interpreting the polygon test.
All example plots use the same source data (blue dots) with the sediment mixture (orange dot) in different positions to illustrate specific problems. See text for explanation. (Reproduced courtesy of M. Gibbs)

In (FIG. 4-1B), the sediment mixture lies inside the polygon drawn through the source points shown and highlighted in yellow. While it also lies within the bounds of the larger polygon drawn in (FIG. 4-1A) some of the source sample points (circled in red) are behind source points which are closer to the sediment mixture. In this situation, the closer sources 'shield' the more distant sources, which the model will define as having low or zero contribution to the sediment mixture. These shielded sources may be omitted from the modelling. However, removal of a source assumes that the same sources are shielded in the biplots with other tracer combinations. Redrawn using different FA tracers, may produce biplots where the source is not shielded, allowing that source to be retained in the modelling.

A similar ‘shielding’ effect occurs when sources are isotopically distant from the sediment mixture, even though there is clear path to the mixture (FIG. 4-1B), un-highlighted part of polygon). When modelled, the distant source will contribute very little sediment to the mixture and the sources forming the highlighted polygon will be the main contributing sources.

The shielding effect is often seen when the polygon encompasses a large isotopic area (FIG. 4-1C), and has multiple isotopes clustered near each polygon point , the shielded sources circled in red can be excluded from the modelling or, if the sources are close to the polygon point (lower left) and of similar land use class, they may be combined, and an average value used. The source points circled in green (upper left on polygon) should be averaged because they are too far away (isotopically) for the model to discriminate between them as separate sources.
In some cases, the mixture lies exactly between two sources (FIG. 4-1D) which indicates that the sources that plot to the side are having little or no contribution to the mixture. Only the two sources closest to the mixture and circled in green along the dotted line will have a major contribution to the mixture. If the sources at each end of the dotted line are of similar land use class, they can be combined and averaged. Otherwise, delete the shielded sources. This specific occurrence can be modelled with a two-endmember mixing model.

Several of these polygon types (FIG. 4-1) will possibly occur in the isotopic biplots as each tracer pair is tested. Source points which consistently plot outside the polygon should be excluded from the modelling.

For CSSI FAs fingerprinting and tracing investigation in agroecosystem, as highlighted by recent literature [4.9] [4.3], higher molecular weight tracers (C20:0, C22:0, C24:0) should be used in preference to the C14:0 and C16:0 tracers and, when possible, at least 4 and preferably 5 tracers should be used in the modelling.

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5. MODELLING

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5.1. STABLE ISOTOPE MIXING MODELS (SIMMs)

For the CSSI technique, stable isotope mixing models (SIMMs) are used to deconstruct a sediment mixture into the source soils contributing to that mixture. The SIMM uses two or more isotopic tracers to establish the proportional contribution of the possible soil sources to the sediment mixture. The isotopic tracers used are the $\delta^{13}C$ value of the bulk soil carbon and the $\delta^{13}C$ value of each FA i.e. C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C20:0, C22:0, C24:0 and C26:0. This represents 11 potential tracers for each sample provided the same tracers are present in both the sources and mixture.

The basic mixing model uses linear mass balance equations whereby the unique proportional contributions of n+1 sources can only be established by using n different isotopic tracers [5.1]. For example, in food web studies using bulk isotopes (e.g. $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$), it is only possible to model four different food sources in the predator's diet. If the number of potential sources exceeds n+1, a unique solution of source proportions cannot be found, and the model solution is undetermined.

There have been various approaches to solving this problem. In the CSSI technique, the number of isotope tracers have been increased to 11 potential tracers allowing 12 sources to be modelled. While this is theoretically possible, it is not practical in terms of computer processor capacity and run time. A more realistic number of tracers is 5 or 6 allowing the use of 6 or 7 sources. Even then, a unique solution may not be found.

Another approach has been the development of mixing models designed to 'unmix' the mixture. These models were especially developed for food web studies where the carbon from the prey is assimilated into the tissue of the predator with the expectation of one or two fractionation steps along the way.

Initially IsoSource was the only mixing model available to deconstruct the sediment into its source components [5.1]. This was a number-crunching model that multiplied all possible combination of the sources together to produce a matrix of isotopic values. Then the combination of sources that produced isotopic values that closely matched the isotopic values in the mixture were selected as feasible solutions. There was no estimate of uncertainty except that as the number of feasible solutions approach 1 (a unique solution), confidence increased in the results produced. In this approach, any of the feasible solutions could be correct, which means that the full range of results, and not just the mean, should be reported. The range of results between the 2.5% and 97.5% confidence levels was referred to as the credible interval.

Subsequently, new Bayesian mixing models such as SIAR [5.2], [5.3], [5.4] and MixSIAR [5.5], have been developed and are true models that can give reliable statistics.

It is important to keep in mind that most SIMMs have been adapted to sediment source tracing studies by omitting the calculations that are associated with isotopic fractionation, or by setting the fractionation value to zero. because there is no fractionation of the FAs attached to soil particles during transport or sedimentation.

There is no SIMM that is dedicated to sediment source tracing studies although there is one model being developed CSSIAR [5.6] that takes care of the data entry, the polygon test graphs, running the model SIAR and producing the results as soil proportions with appropriate statistics. This approach may be very useful for users of the CSSI technique who do not want to delve into the software script to manually run their data through a SIMM.

The potential danger of using SIMMs is that they will attempt to find a solution that includes all the sources entered into the model run. If a source is loaded that cannot be present because of geographical constraints (e.g. the source cannot contribute to the mixture site because it is downstream of the mixture), the SIMM output will be unrealistic, and no warning will be given to the user. Consequently, the discrimination process is extremely important, especially the polygon testing (see Section 4).

As IsoSource has already beneficiated of full detailed information in open-access literature [5.7], the models that will be described in the following section will be the two end-member models and MixSIAR.

5.2. TWO END-MEMBER MODEL ANALYSIS

A common question in the CSSI technique is ‘Where is the sediment coming from in a riverine catchment?’

The first step towards answering this question is to identify the source of the sediment by sub-catchment. This can be achieved using a two end-member mixing model. It requires a ‘confluence’ sampling approach where sediment samples are collected in each of the two upstream tributaries, A and B, and the mixture collected far enough downstream from the confluence to allow complete mixing of the sediment from the two sources (FIG. 5-1).

It requires just three samples per confluence to determine the proportional contribution of soil from each sub-catchment (FIG. 5-1). There are only two sources, so the n+1 criterion for linear models is met with just one tracer, and a unique result can be determined. Since the CSSI technique could produces up to 11 isotopic tracers (i.e., $\delta^{13}C$ value of the bulk soil carbon and the $\delta^{13}C$ value of each FA i.e. C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C20:0, C22:0, C24:0 and C26:0), each one of these can be used individually to model the confluence. Because each individual result is a unique solution, the mean and standard deviation about the mean of all of these results provides a statistical indication of the level of uncertainty in the result. This is useful because there may be small differences in the results from each FA tracer if the confluence mixture was not taken far enough downstream from the two tributary sources or a FA tracer doesn’t have sufficient discrimination power.

The $\delta^{13}C$ isotopic signatures of the bulk soil and the FAs extracted from the soil are collated with the %C values. This provides a set of at least 11 tracers.

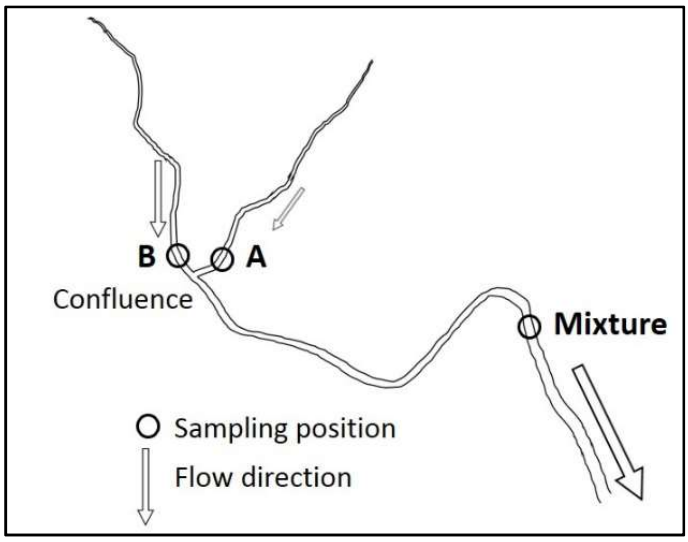

FIG. 5-1. Schematic diagram of sampling positions at a river confluence. Site A is the tributary; site B is the main stream source; site Mixture is the downstream sampling site. The mixture site must be far enough downstream to allow complete mixing. Sampling downstream of a bend improves the mixing. (Reproduced courtesy of M. Gibbs)

The river bed deposition samples are separated into confluence triplicates (A, B and mixture; (FIG. 5-1)) and the proportional contribution of the tributary at each confluence is determined using a two-end member linear mixing model. This model assumes that the %C or $\delta^{13}C$ isotopic value of each FA in the downstream site sediment mixture is the sum of the %C or the $\delta^{13}C$ isotopic values of the corresponding FA from the upstream sources, A and B, where A can be the tributary and B can be the main stem of the river (5.1).

$$\delta^{13}C_{mixture} = fA\delta^{13}C_A + fB\delta^{13}C_B \quad (5.1)$$

Where fA and fB are the fractions or proportions of each source. This equation (5.2) can also be rewritten

$$1 = fA + fB \quad (5.2)$$

To solve for fA, equation (5.3) is rewritten as:

$$fA = (\delta^{13}C_{mixture} - \delta^{13}C_B)/(\delta^{13}C_A - \delta^{13}C_B) \quad (5.3)$$

and for fB, the equation (5.4) is rewritten as:

$$fB = (\delta^{13}C_{mixture} - \delta^{13}C_A)/(\delta^{13}C_B - \delta^{13}C_A) \quad (5.4)$$

The caveat for the two-endmember mixing model is that the %C or the $\delta^{13}C$ value of each FA in the **mixture** must be between the corresponding %C or the $\delta^{13}C$ values of each FA in the **sources** A and B.

Theoretically, this should be the case where only tributaries upstream of the confluence contribute to the mixture downstream, and where both upstream sources are dissimilar. However, because current flow in a river system can rework deposited sediments, there may be variability in the isotopic signatures of mixtures in the deposition zone resulting in non-valid values. Non-valid results are either negative values or values >1 and are discarded (TABLE 5.1). Identification of valid feasible results from the full suite of two-endmember mixing model results can be confirmed with the polygon test (FIG. 5-2). In this example, the tracer pair C18:0 and C20:0 produce a valid polygon, i.e., a straight line through all points indicating that both tracers can be used in the two end-member mixing model.
In contrast, the tracer pair C18:1 and C20:0 (FIG. 5-2) do not fit to a straight line through all points, indicating that one of the pair of tracers should not be used in the two end-member mixing model. Because the tracer C20:0 has already be accepted as a valid tracer from the C18:0 vs C20:0 polygon, the tracer C18:1 is the cause of the polygon failure and should not be used.

The two end-member model can be run in a spreadsheet and the results can be averaged to give a mean and standard deviation (TABLE 5.1). Tracers %C, $\delta^{13}C$ and C18:1w9c were identified as invalid (see above) and were not included in the statistical evaluation of the results. Tracer C16:0 may also be invalid but has been include as an example to show that even borderline data will have only minor effects on the final result, although their inclusion will increase the variability of the output. In this example the results indicate that source A contributed 46% ± 9% and source B contributed 54% ± 9% of the sediment to the mixture in the deposition zone.

TABLE 5.1. EXAMPLE SET OF TWO END-MEMBER DATA WITH RESULTS INCLUDING MEANS AND STANDARD DEVIATION. SOURCE DATA ARE HIGHLIGHTED IN YELLOW. SEE TEXT FOR INVALID DATA.

	%C	D13C	C12:0	C14:0	C16:0	C18:0	C18:1w9c	C18:2w6c	C20:0	C22:0	C24:0	Mean	Stdev
Mixture	6.91	-25.51	-26.77	-28.29	-25.96	-28.32	-27.18	-26.17	-30.40	-30.15	-28.80		
Source A	4.26	-29.85	-30.45	-34.97	-34.77	-31.43	-30.94	-30.10	-32.37	-33.63	-33.53		
Source B	5.05	-26.13	-23.39	-24.36	-22.79	-24.46	-26.36	-22.60	-28.35	-26.89	-24.01		
FA	-2.36	-016	0.48	0.37	0.26	0.55	-0.18	-0.48	-0.51	-0.48	-0.50	0.46	0.09
FB	3.36	1.17	0.52	0.63	0.74	0.45	-0.82	-0.52	-0.49	-0.52	-0.50	0.54	0.09

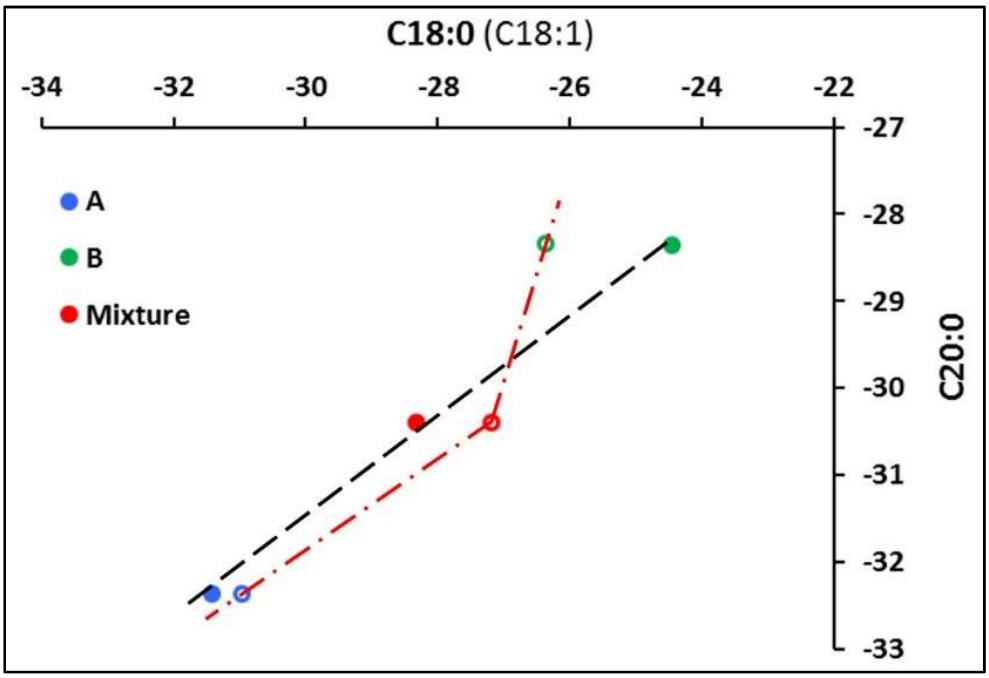

FIG. 5-2. Polygon test used to confirm valid data for the two end-member mixing model. Solid dots are valid data (C18:0 vs C20:0). Open circles are invalid data (C18:1 vs C20:0). (See text). (Reproduced courtesy of M. Gibbs)

5.2.1. Case Study

The two end-ended mixing model can be a powerful tool for examining a catchment to identify the main sources of sediment by sub-catchment. The case study demonstrates how this technique was used to focus limited resources into a sub-catchment that was producing more than 62% of the sediment reaching an estuary and causing degradation of the local fishery.

The combined sub-catchments shaded light grey produced <5% of the total sediment load on the estuary with the remainder coming from the yellow and green shaded sub-catchments (FIG. 5-3)

In the following description the sources are identified by their sample numbers, as provided in (FIG. 5-3) Starting at the top of the catchment, at the confluence of the Te Kowhai Stream (35) with the upper Wainui Stream (34) about 95% of the sediment in the deposition zone downstream (36) came from the upper Wainui Stream (34). At the Oruaiti river site (26) downstream of the confluence of the Mangawhero Stream (41) with the Wainui Stream (28), the sediment proportions were about 84% from the Mangawhero Stream and 16% from the Wainui Stream.

Further downstream, the Oruaiti River (13) below the confluence with Stony Stream (14) receives about 91% of its sediment from upstream sources and only about 9% from the two large sub-catchments draining into Stony stream (14). On a land area basis, the green shaded sub-catchments produced substantially less sediment than the yellow and orange shaded sub-

catchments. Subsequent calculations indicated that more than 62% of the sediment in the Oruaiti River at site 8 (FIG. 5-3) came from the Mangawhero Stream sub-catchment.

While the two end-member modelling technique can identify the sub-catchment producing the most sediment, it requires a SIMM, such as IsoSource or MixSIAR to deconstruct the sediment mixture into its source proportions by land use. In this case study, the dominant (<93%) of the sediment came from steep pasture unsuitable for dry stock farming and bank erosion where the lack of fencing allowed stock unrestricted access to the stream bed.

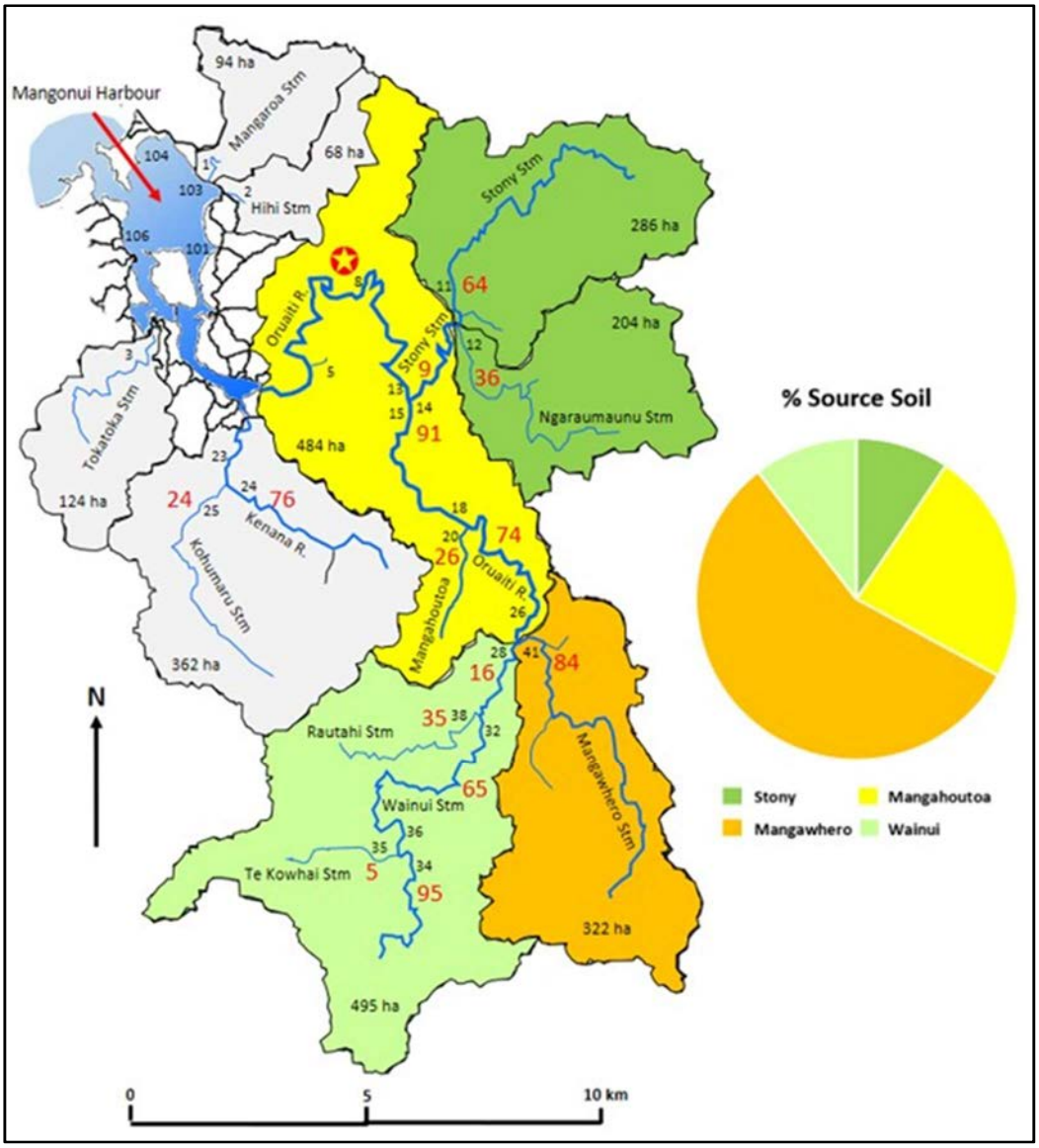

FIG. 5-3. Case study using the two end-member mixing model on the Oruaiti River flowing into Mangonui Harbour estuary, New Zealand. Sub-catchment areas are given in ha. The sample numbers are given at each confluence (small black) and the sediment contribution from each source at each confluence is given in % (large red). The pie chart represents the relative proportions of soil leaving each major sub-catchment. (Reproduced courtesy by M. Gibbs)

5.3. BAYESIAN MIXING MODELS

A detailed step-by-step protocol in using IsoSource has been already reported [5.1] [5.7]. The main failing of this SIMM is that it has no statistical evaluation of the credibility of the source proportions in its output. Instead it produces a statistical assessment based on the feasible solutions found. The model assigns the source proportions within the range of feasible solutions found and any feasible solution could be a correct solution. The statistics identify the most common feasible solution and provide a mean and standard deviation about that mean. However, in all applications the full range of feasible solutions should be reported rather than just the mean.

Therefore, following the development of IsoSource, the question of uncertainty in the results initiated a number of new models based on Bayesian Belief Networks to account for the variability in the transfer of $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures from prey to predator tissue in food web studies. In these studies, the model had to account for isotope specific trophic enrichment factors (TEF), assimilation efficiency and digestibility [5.8] [5.9], and concentration dependence: i.e., different food sources may contain different amounts of C/N – omnivores may have more $\delta^{15}N$ from animals than plants [5.10]. None of these factors are relevant in sediment source tracing studies because these is no isotopic fractionation and concentration is not used.

The first Bayesian mixing model was MixSIR [5.11]. It was written in MATLAB code and had a graphical user interface (GUI). Input data included source and consumer $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures and their isotope specific TEFs and can include priors from other studies. In Bayesian jargon, a 'prior' is a probability distribution representing prior knowledge. For example, the prior may indicate that the predator can consume any or all of the available food sources in the food spectrum or it may be constrained to a sub-set of the whole food spectrum. This model assumes that all consumers have the same mean diet and that variance of mixture is a function of proportions, source variances. Yeakel et al. have identified the assumption that the available biomass of all sources is equal as a potential flaw [5.12]. The next model developed was Stable Isotope Analysis in R [SIAR] [5.3]. It is a menu driven interface written in the software package R and it is freely available at http://cran.r-project.org/web/packages/siar/index.html. SIAR used the same basic assumptions as MixSIR and included estimation of extra isotope-specific additive residual error. SIAR needs multiple consumers to estimate residual error and the GUI also allows the model to be applied to different groups (factors). A user-friendly version of SIAR, CSSIAR has been specifically developed for use with sediment source tracing studies [5.6].

Fully Bayesian versions of MixSIR / SIAR were produced [5.13], which were flexible, and incorporated uncertainty in source means / variances. Further advances e.g. [5.14], [5.15] [5.16] have culminated in the development of the hierarchical model, MixSIAR [5.5], which is a fusion of the previous tools. It has a user-friendly GUI and the grouping of variables may be fixed or random. Data can be input in a number of formats including as source means with variances.

5.4. MIXSIAR

Section 5.4 is based on experiences using MixSIAR v3.0 [5.5]. The model is freely available from the internet and requires the software package R to be installed before the model is run. As the model is refined, newer versions will become available and there will be accompanying instructions to assist in the installation and operation of the software. For example, a new version of MixSIAR (D-MixSIAR) has been developed for deconvoluting sediment source apportionments for a river basin [5.17].

MixSIAR was designed for food web studies. When it is used for sediment source tracing studies, much of the complexity is left unused and the output is simplified. However, when it comes to convert isotopic proportions into soil proportions, additional information must be extracted from the software to allow the calculation of the mean isotopic proportions with true statistics (see below).

5.4.1. Model Description

Model-fitting to the observed data is based on a Markov Chain Monte Carlo (MCMC) method whereby the isotopic proportions of potential sources are estimated by repeated random sampling and discarding those which are not 'probabilistically consistent with the data' [5.18]. Subsequent estimates are required to be similar to previous ones, thereby creating a Markov Chain [5.18]. The model output consists of a sample of the posterior proportions derived from the MCMC simulation and represents a true probability distribution of source proportions that can be summarised by various descriptive statistics, including the 95% (i.e. 2.5–97.5%) credible interval. A key advantage of Bayesian mixing models, such as MixSIAR, over earlier linear mixing models is that uncertainty in source signature values is explicitly taken into account.

Bayesian estimates of source proportions can be informed by reliable priors based on data and thereby constrain the model and reduce uncertainty. For example, in food web studies, the gut contents of fish (e.g. relative abundance of prey species) can be used to construct 'informative priors' about prey preferences and digestibility. In sediment tracer studies the relative contributions of the various soil sources to the fatty acid pool preserved in deposition zone sediments is unknown so that an 'uninformative prior' is used. An uninformative prior is one were all combinations of isotopic proportions (sum = 1) are equally likely [5.5].

The following section uses an example from a catchment study looking at the apportionment of different land uses contributing soil to an estuary [5.19] as a case study to help explain how MixSIAR is used. MixSIAR is a complex model that allows a range of different run settings to constrain the output in food web studies. Not all of these are needed for sediment apportionment and can be turned off in the model settings.
The MCMC settings applied to MixSIAR in the example sediment tracer study were: chain lengths of 300,000 (i.e., **long run**), burn in of 200,000 and 'thin' value of 100, which generated 3000 estimates of posterior source proportions, with sum of mean values equal to one. A continuous effects model run, with a process only (i.e., **n = 1**) error structure, was employed to estimate the posterior distributions of sources for each individual sediment mixture sampled. (For more detailed information on these settings see [5.5].

The number of fatty acid tracers present in each sediment sample varied from a minimum of three (i.e., the most abundant fatty acids - C20:0, C22:0 and C24:0) and seven (i.e., with the

addition of C16:0, C18:0, C18:1 and C18:2). The bulk soil tracer $\delta^{13}C$ was also used where practicable but was often excluded from aquatic sediment mixtures during the polygon test. The influence of the number of tracers on model performance was important. Where possible, the number of sources included in each model simulation was $\leq n + 1$, where n is number of tracers. This avoided under-determined solutions [5.18]. Unlike food-web studies, estimates of isotopic diet-tissue discrimination factors were not required for sediment source applications, removing a key source of uncertainty.

5.4.2. Diagnostic Tests

A MCMC model run will always generate a result even if it is nonsensical. Consequently, it is important to check the validity of the result using the software generated diagnostic tests. Diagnostic tests of convergence of the Markov chain to a stationary distribution for all variables and a measure of model fit provided with MixSIAR output may be used to evaluate model performance.

1. The Gelman-Rubin test requires more than one MCMC to be calculated (default = 3), with a value of 1 at convergence. A value of less than 1.1 is generally acceptable [5.5]. In sediment source tracing studies, most model variables had Gelman-Rubin values of less than 1.05;

2. The Geweke test is a two-sided z-test comparing the means of MCMC segments. At convergence these means should be the same, with large z-scores indicating rejection [5.5].;

3. Trace plots of each model run also provided information on model convergence;

4. The deviance information criterion (DIC) provides another measure of model fit to the data and is commonly applied to Bayesian models where the posterior distributions have been estimated using MCMC methods [5.20]. In essence, model fit improves inversely with the DIC value. The DIC assumes that the posterior distribution is approximately multivariate normal.

5.4.3. Model Set up

For soil source sediment analysis, the data should be loaded into comma delimited tables and saved in .csv format (TABLE 5.2).

TABLE 5.2. EXAMPLES OF THE THREE TABLES REQUIRED. EACH DATA-SET IS SAVED AS A SEPARATE TABLE IN .CSV FORMAT.

Mixture.csv

Depth_cm	Year	C181	C182	C20	C22	C24
1.5	2015	-26.80	-25.67	-28.98	-25.72	-27.73

Sources.csv

Sources	MeanC181	SDC181	MeanC182	SDC182	MeanC20	SDC20	MeanC22	SDC22	MeanC24	SDC24	n
beech forest	-29.53	1.74	-29.85	0.99	-30.14	0.62	-29.12	1.26	-30.26	0.51	5
Brachen	-28.30	0.42	-28.43	0.16	-29.09	2.36	-29.50	1.46	-30.00	1.05	5
Inflow_stream	-25.13	0.20	-23.48	0.20	-28.54	0.20	-28.39	0.20	-29.47	0.20	5
Native Bush	-26.96	0.22	-29.15	2.07	-26.46	1.53	-29.65	0.79	-30.72	0.18	5
Pine harvested	-28.10	1.41	-29.02	1.00	-28.57	0.67	-27.56	0.10	-28.17	0.62	5
Ponga_fern	-28.59	0.75	-28.43	0.91	-32.97	3.19	-32.44	1.93	-32.55	1.21	5
sheep	-30.46	0.28	-29.99	0.84	-31.14	1.15	-30.77	0.77	-31.09	0.61	5
Subsoil	-30.44	0.76	-30.57	0.56	-31.99	0.15	-30.68	0.01	-31.00	0.15	5

Discrimination.csv

Sources	MeanC181	SDC181	MeanC182	SDC182	MeanC20	SDC20	MeanC22	SDC22	MeanC24	SDC24	n
beech forest	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	5
Brachen	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	5
Inflow_stream	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	5
Native Bush	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	5
Pine harvested	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	5
Ponga_fern	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	5
sheep	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	5
Subsoil	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	5

In all tables, the column headers have no spaces and do not start with non-alpha-numeric characters. The mixture.csv file contains the data for the mixture sample without standard deviations. There needs to be at least one column to the left of the data. In this example, the sample is the surface slice from a dated core and the information in the first two columns is not used in the modelling. The remaining 5 columns have the tracer name as the column header. These link to the column headers in the source.csv and discrimination.csv files.
The source file has the mean and standard deviation data for each tracer, for each land use source used. These names are written as Meantracername and SDtracername. The discrimination.csv file has exactly the same column header names and land use names as the source.csv file and in the same order but all data are set to ‘0’ for sediment source tracing because there is no fractionation.

It is important to sort the data entered into the source.csv and discrimination.csv files by name in the left-hand column. This is because MixSIAR outputs the results for each land use in alphabetical order, irrespective of the order the data is entered. This can cause unexpected errors unless the user is aware of this. Save these as individual .CSV files.
It is convenient to have these two files in a separate WORKING INPUT folder together with all the mixture.csv files to be run with these files. It is also useful to create a WORKING OUTPUT folder to receive the various data files from MixSIAR. It is a good idea to create a set of subfolders in the OUTPUT folder, one for each mixture, because there will be about 10 files created for each mixture and MixSIAR over-writes the output files and the output results could be lost.
Without these folders, MixSIAR writes all the output data to the 'R' programme folder.
In the following step-wise description, it is assumed that all the required input files have been put in the WORKING INPUT folder and that specific output sub-folders have been created for each mixture in the OUTPUT folder.

5.4.4. Model Run

For a first time use, establish the linkage with the nearest R mirror and load the library and other specified components as per the instructions in the Manual [5.5]. Install the R software in an easy-to-find directory on C: drive, but not in the 'C:/Program Files' folder as that folder (and the 'C:/Program Files (x86)' folders, as these folders are usually administrator access only and cannot be written to. Create an icon on the menu bar for R.
This installation is only done once. Subsequently, start R by clicking the icon. The introduction R Console screen (FIG. 5-3) will appear:

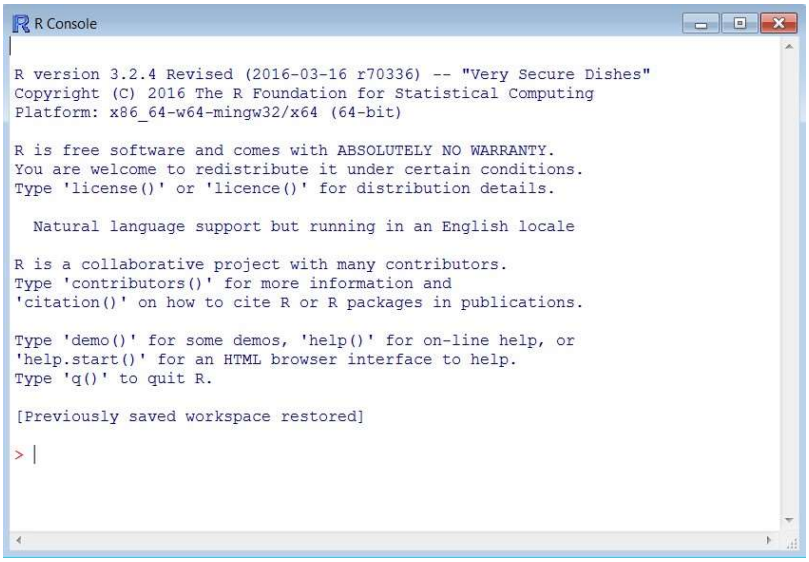

FIG. 5-3. Screen-shot of correctly loaded R software.

Enter the commands **source('mixsiar_gui.r')** and **mixsiar_gui ()** each line followed by enter, and the software for MixSIAR will load and run. The commands must be exactly as shown (FIG. 5-4).

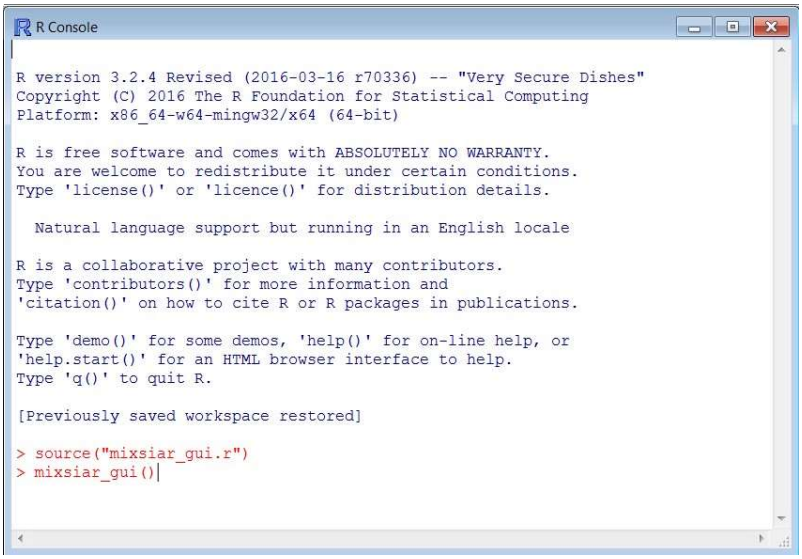

FIG. 5-4. Screen shot of the command lines entered to load MixSIAR.

If the response to this command is a non-fatal error message (e.g., FIG. 5-5), it is likely that the MixSIAR library is not where the icon link was pointing.

Non-fatal errors, such as this, do not affect the running of MixSIAR and the next valid text input will be executed correctly.

Clicking the [X] button in the top right-hand corner of the Windows Screen will close the programme and a question is asked 'Save workspace image?'. Answer 'No' as that feature is not needed for soil source tracing.

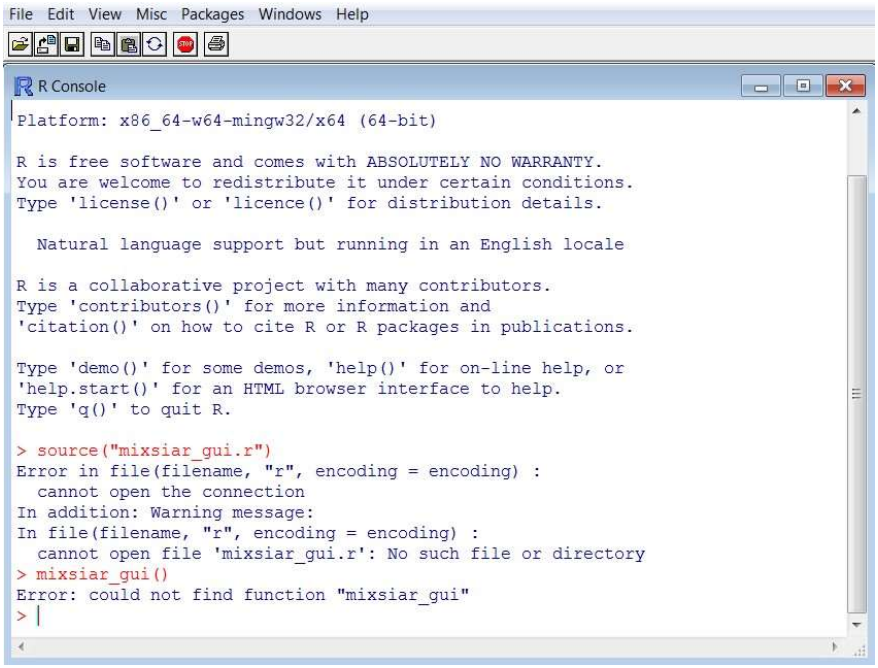

FIG. 5-5. Screen shot of the non-fatal error message.

To continue running MixSIAR, find the directory where the MixSIAR library is located then click on the [File] button at the top left of the screen and select ‘Change dir…’ from the pull-down menu (FIG. 5-6). Set this to the appropriate directory.

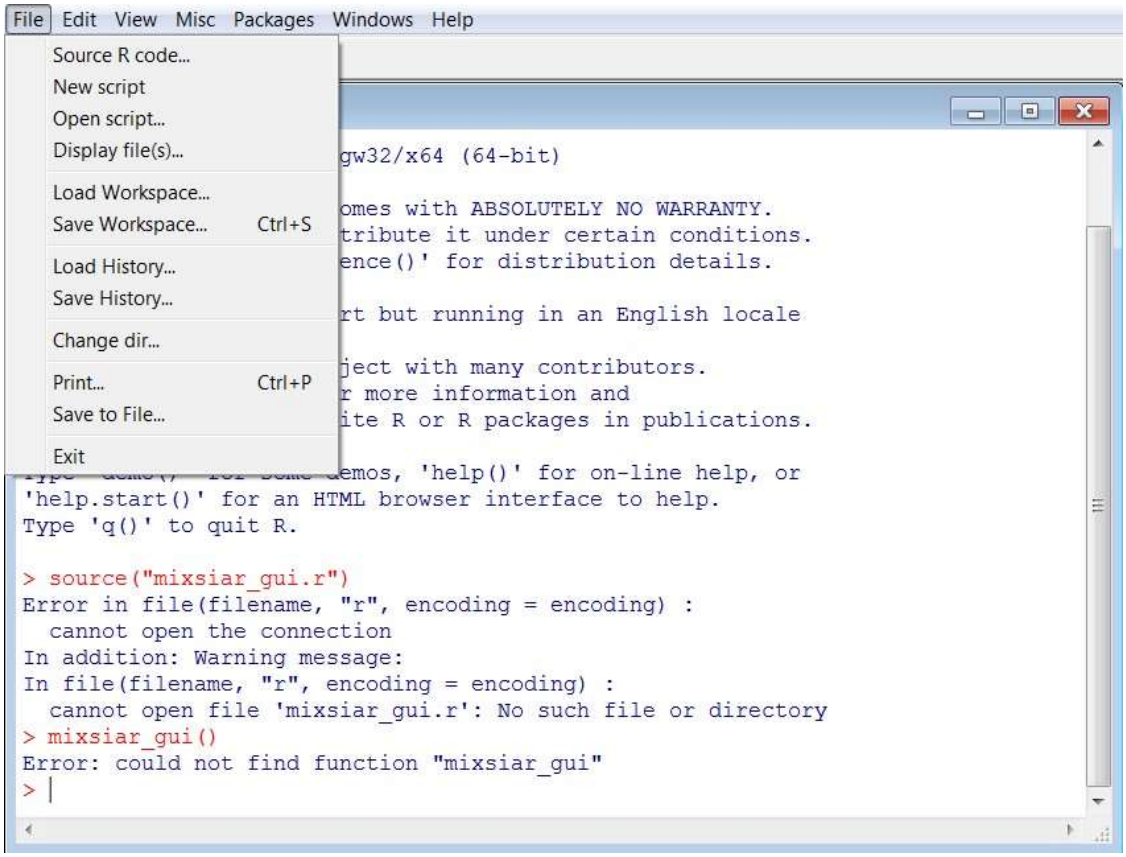

FIG. 5-6. Screen shot of pull-down file menu.

Re-enter the script source(‘mixsiar_gui.r’) and mixsiar_gui(). The script will be displayed in the R Console window along with other text and then the main MixSIAR GUI window will be displayed (FIG. 5-7). This is an interactive window. Any key strokes should be single only unless otherwise specified.

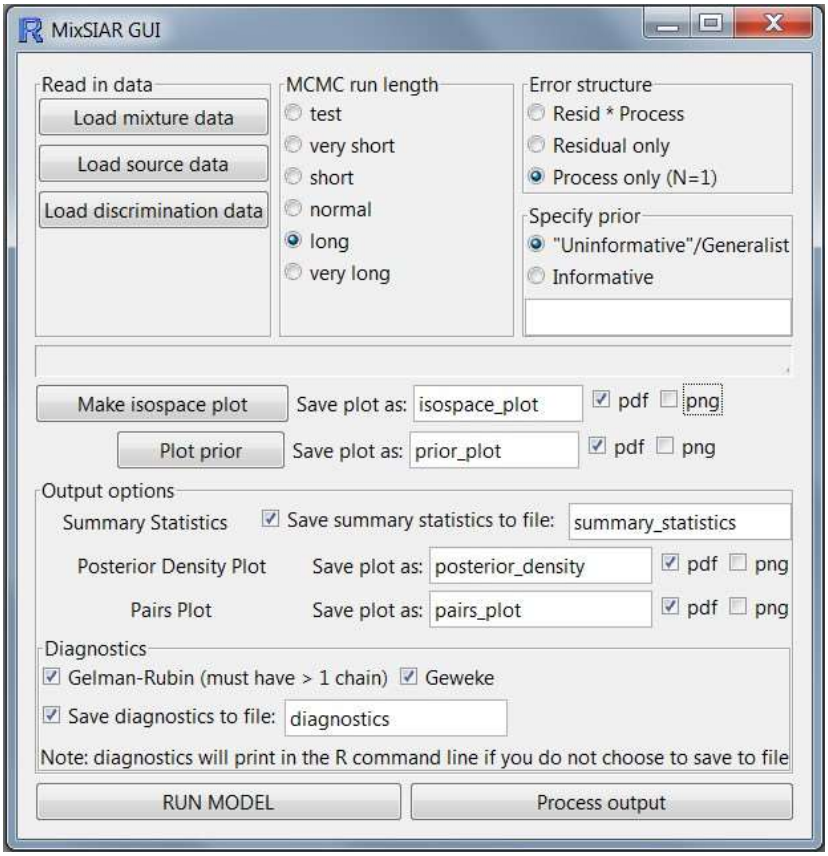

FIG. 5-7. Screen shot of main MixSIAR Gui command screen.Note that the various settings selected in this image are those used in the example sediment source tracing study and are appropriate whenever using MixSIAR for sediment source tracing.

To load the mixture data, click the 'Load mixture data' button on the main MixSIAR Gui command screen and the 'Read in your MIXTURE data' screen will appear (FIG. 5-8A).

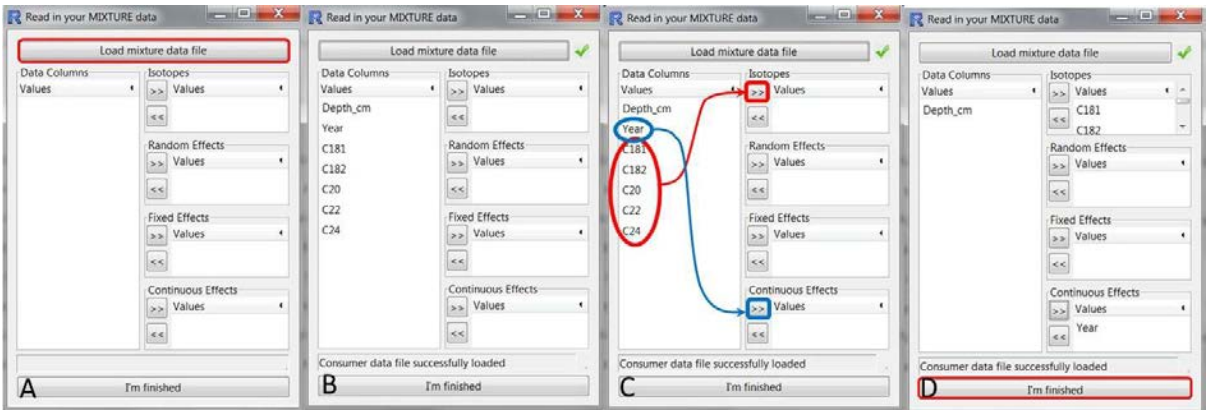

FIG. 5-8. Screen shots of 'Read in your MIXTURE data' with step-by-step explanations.

Click the ‘Load mixture data file’ button (FIG. 5-8A) and select the **mixture.csv** file from the WORKING INPUT folder. The column headings from the mixture .csv file appear in the ‘Data Columns’ panel (FIG. 5-8B). Sequentially highlight each tracer then click the [>>] button by ‘Values’ in the Isotopes panel (red lines, FIG. 5-8C) until all tracers have been transferred to the Values sub-panel. Repeat for the ‘Year’ header into the Continuous Effects panel (blue lines, FIG. 5-8C).
The completed data entry will show the values entered in the appropriate panels and there will be a green tick beside the ‘Load mixture data file’ button. Click the ‘I’m finished’ button to exit this screen and proceed (FIG. 5-8D).
If this process has been successful, a green tick will appear beside the ‘Load Mixture Data’ in the main MixSIAR Gui command screen (FIG. 5-7). If this step has not been successful, there will be no green tick and the mixture.csv file needs to be checked for errors such as incorrect column names or alpha characters in the numeric data fields. Correct the file and reload.

To load the source data, click the ‘Load source data’ button on the main MixSIAR Gui command screen (FIG. 5-7) and the ‘Read in your SOURCE data’ screen will appear (FIG. 5- 9).

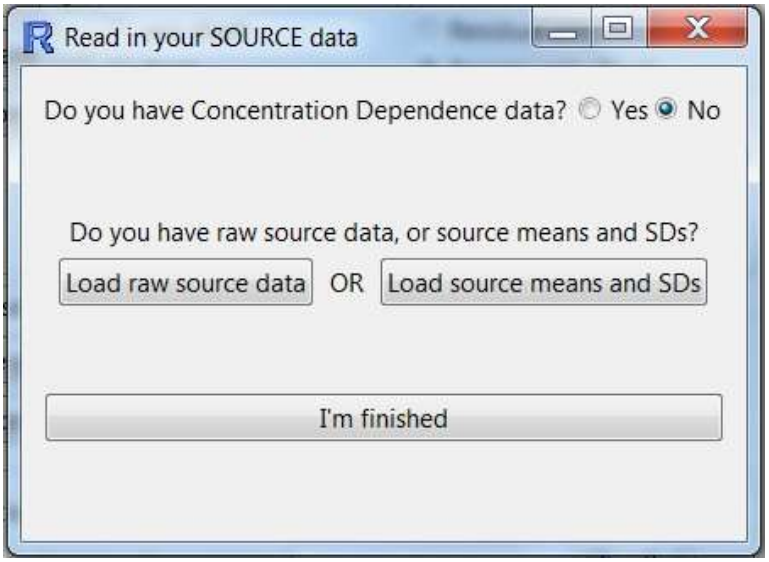

FIG. 5-9. Screen shot of ‘Read in your SOURCE data’ screen.

Concentration is not used in the CSSI data analysis, so the first question is set to **No**. Because the sources data includes both means and standard deviations about the mean data for each source, click the ‘Load source means and SDs’ button. Select the **sources.csv** file from the WORKING INPUT folder. When loaded a green tick will appear beside the ‘Load source means and SDs’ button. Click the ‘I’m finished’ button to exit this screen and proceed. If this process has been successful, a green tick will appear beside the ‘Load Source Data’ in the main MixSIAR Gui command screen (FIG. 5-7). If this step has not been successful, there will be no green tick and the sources.csv file needs to be checked for errors. Since this file includes the standard deviation data, a common error is leaving spreadsheet cell blank or including a space character before a column name or data point. Correct the file and reload.

To load the discrimination data, click the 'Load discrimination button' on the main MixSIAR Gui command screen and select the Discrimination.csv file from the WORKING INPUT folder. If successful, a green tick will appear beside the 'Load discrimination button' on the main MixSIAR Gui command screen (FIG. 5-7). If this step has not been successful, there will be no green tick and the discrimination.csv file needs to be checked for errors. The discrimination.csv file is identical the sources.csv except it has zeros (i.e. '0') in place of the numeric data values. The values of 'n' remain the same as for the sources.csv. Correct the file and reload. When all three data files have been loaded correctly, there will be three green ticks in the main MixSIAR Gui command screen (FIG. 5-10), and the model is ready to run.

Before proceeding, change the directory of where the output data is currently being written to the 'WORKING OUTPUT Sub-folder' where all subsequent data will be written.
Click 'File' on the top screen menu bar and select 'Change dir…' from the drop-down menu. This will bring up a 'Browse' window to allow the 'WORKING OUTPUT Sub-folder' to be selected. This step is needed for every time the model is run because the model continues writing the last WORKING OUTPUT Sub-folder and to the 'R' folder each time the program is started.

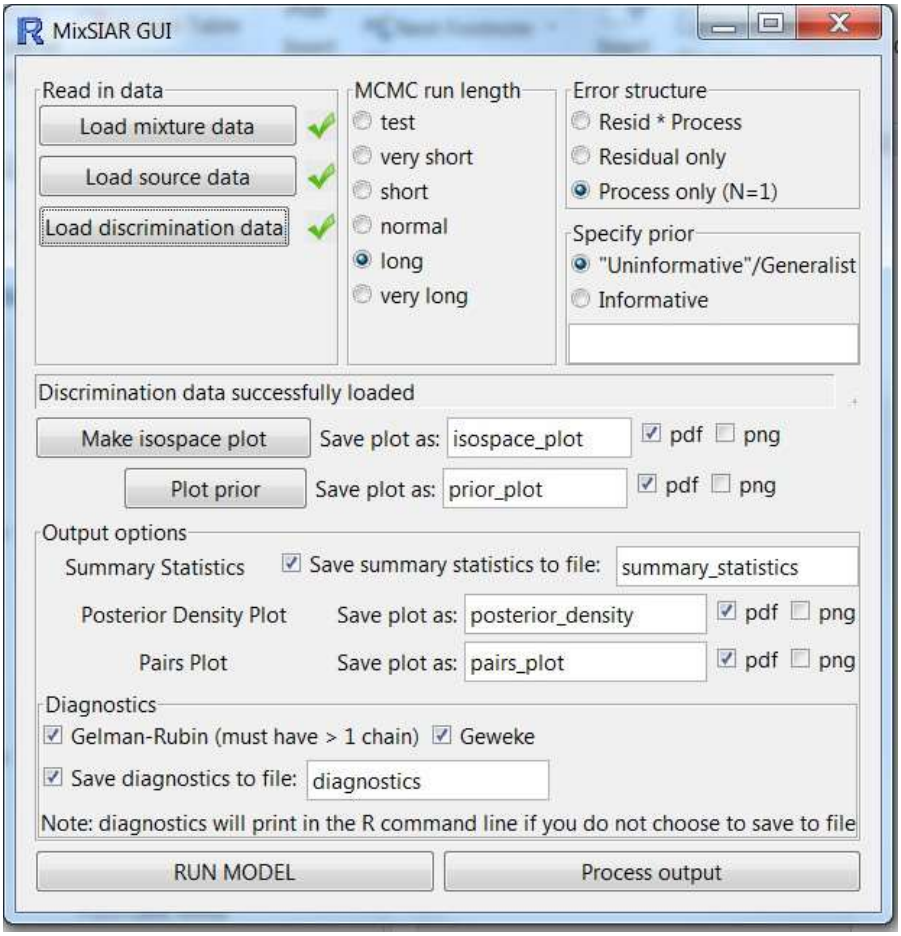

FIG. 5-10. Screen shot of main MixSIAR Gui command screen with the data loaded correctly.

The first output that can be created is an ‘isospace_plot’ (click ‘Make isospace plot’), which is equivalent to the polygon test. This will produce a plot of all the sources with error bars based on the standard deviation values entered in the source data file, as a bi-plot using the first two isotope tracers (FIG. 5-11). The mixture data is represented by a dot without error bars as no standard deviation values are include with the mixture data. This example plot from the case study indicates that the inflow stream is a major sediment source.

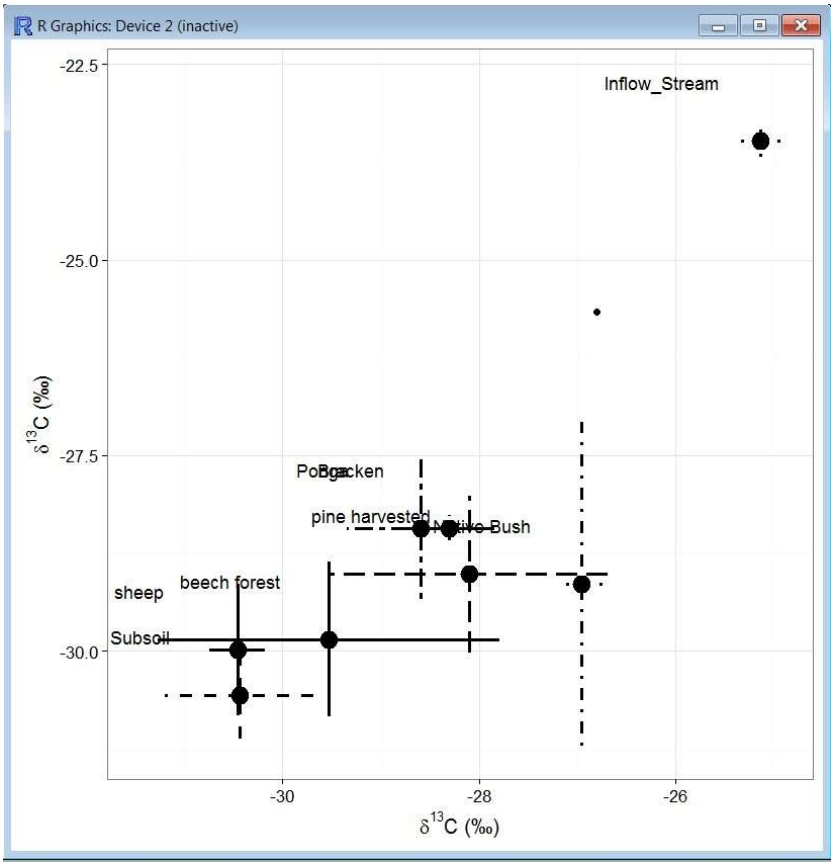

FIG. 5-11. Isospace_plot of data using two isotopes.Note that there is considerable overlap of source data, which may require revision of the source files to resolve after the diagnostic tests have been applied.

This plot can be saved in the WORKING OUTPUT Sub-folder by clicking on ‘File’ in the top menu bar, selecting ‘Save as’ then selecting the file type from the drop-down menu. Enter a filename and the file is saved. MixSIAR will have also saved a file in the folder but it will not have any data.

Close the Isospace_plot and check that all the model settings are correct as shown in (FIG. 5-7 and FIG. 5-10) before clicking the ‘RUN MODEL’ button. The R Console screen will display sequential progress bars as the initialising and modelling proceeds and will show 100% for each when the modelling is finished (FIG. 5-12).

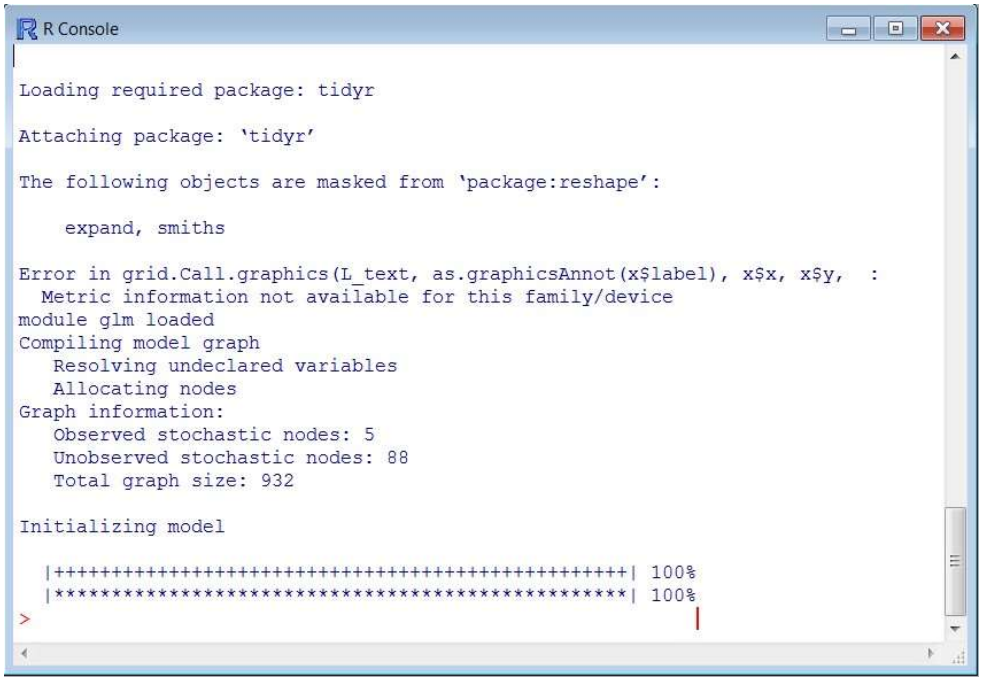

FIG. 5-12. Screen shot of the R Console screen showing the progress bars with 100% indicating the modelling is finished.

To complete the modelling and obtain an output, click the 'Process output' button. The summary statistics (FIG. 5-13) and diagnostics (e.g. Gelman-Rubin,) will be produced in the R Console screen and saved to the WORKING OUTPUT Sub-folder.

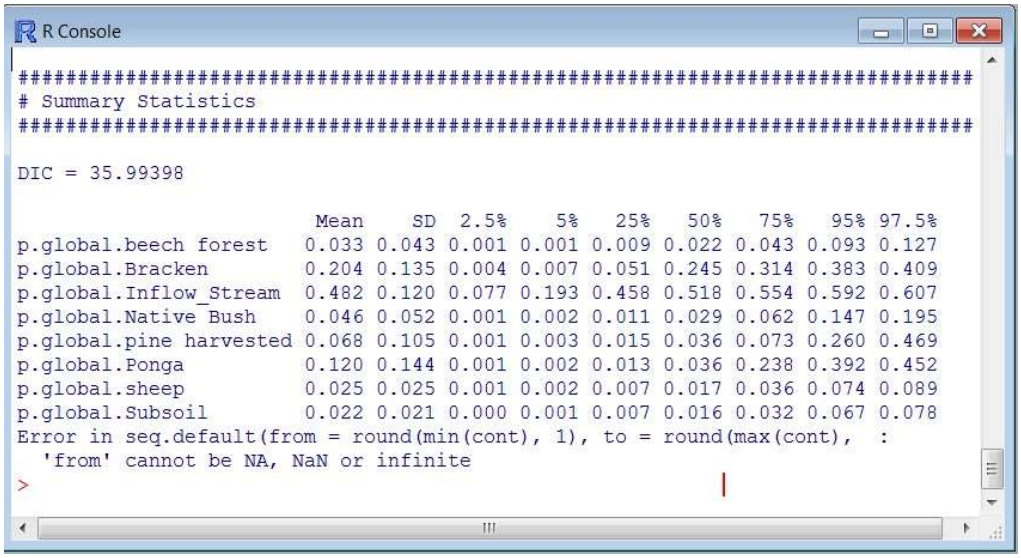

FIG. 5-13. Screen shot of the R Console screen showing the Summary Statistics.

The Summary Statistics (FIG. 5-13) show the means and standard deviations of the source isotopic proportions in the mixture being analysed and the range of the credible intervals. These results are the mean of 3000 estimates of posterior source proportions, with sum of mean values equal to one. Essentially the Summary Statistics output data appears reasonable, but is it?

Looking at the diagnostics data will define whether the output is usable or nonsensical. The deviance information criterion (DIC) value at 35.99398 (FIG. 5-13) is relatively high indicating a poor fit to the model. The model fit improves as the DIC value decreases.
The Gelman-Rubin diagnostics (FIG. 5-14) show that there were 2 values > 1.05 indicating that the modelling may have a problem.

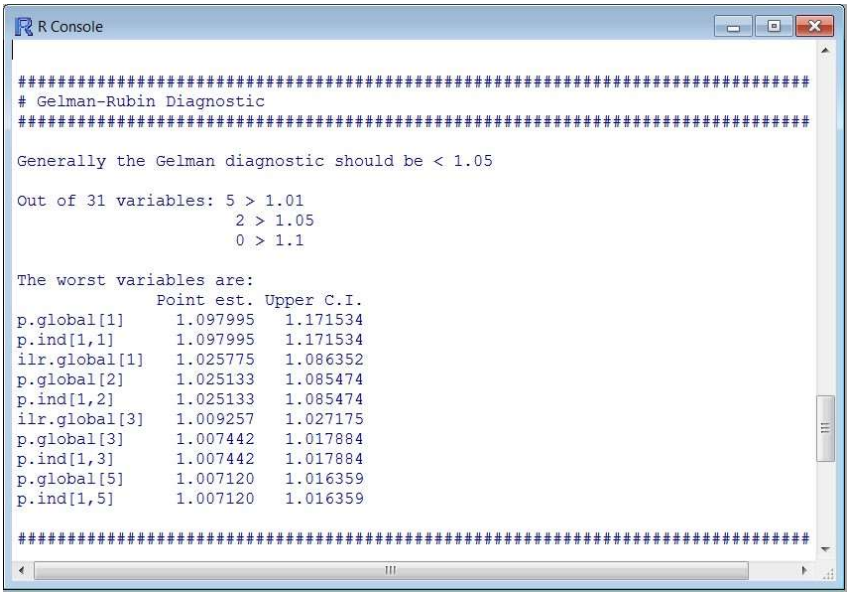

FIG. 5-14. Screen shot of the R Console screen showing the Gelman-Rubin diagnostics.

The Geweke Diagnostic (FIG. 5-15) is comparing means of MCMC segments. At convergence these means should be the same and the z-scores should be 0. Large z-scores indicate rejection.

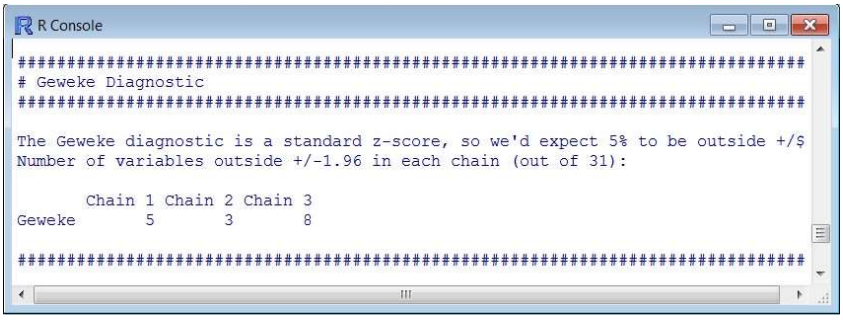

FIG. 5-15. Screen shot of the R Console screen showing the Geweke diagnostics.

MixSIAR also outputs the results in graphical form (FIG. 5-16) and it is here that bimodal population proportions (Proportion of diet) become apparent. This also indicated that there is something 'wrong' with the modelling. The most common problem is that there are too many sources and the separation between sources is insufficient for discrimination. This is seen as overlapping error bars in the Isospace plot (FIG. 5-11).

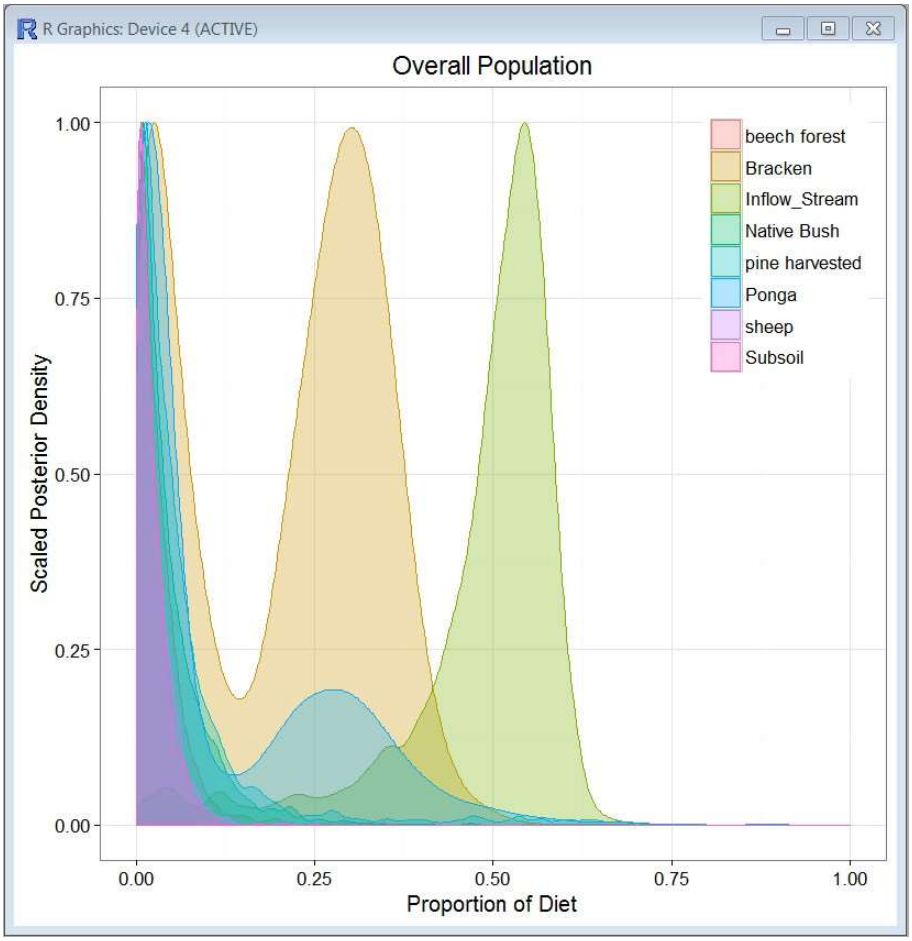

FIG. 5-16. Graphical output of the data produced by the MixSIAR model run (Axis labels were intended for food web studies).

To overcome this problem, some of the overlapping source data could be combined, if they are of similar land use, or some of the sources could be eliminated based on prior information.

5.4.5. Rerun

The data for Bracken and Ponga, as well as Sheep and Subsoil were combined (TABLE 5.3). Revised sources and discrimination files were produced, and the model was rerun.

TABLE 5.3. THE TWO REVISED TABLES.

Sources-1.csv

Sources	MeanC181	SDC181	MeanC182	SDC182	MeanC20	SDC20	MeanC22	SDC22	MeanC24	SDC24	n
Beech forest	-29.53	1.74	-29.85	0.99	-30.14	0.62	-29.12	1.26	-30.26	0.51	5
Brachen-Ponga	-28.45	0.59	-28.43	0.54	-31.03	2.78	-30.97	1.70	-31.28	1.13	5
Inflow_Stream	-25.13	0.20	-23.48	0.20	-28.54	0.20	-28.39	0.20	-29.47	0.20	5
Native Bush	-26.96	0.22	-29.15	2.07	-26.46	1.53	-29.65	0.79	-30.72	0.18	5
Pine harvested	-28.10	1.41	-29.02	1.00	-28.57	0.67	-27.56	0.10	-28.17	0.62	5
Sheep- subsoil	-30.45	0.52	-30.28	0.70	-31.57	0.65	-30.73	0.39	-31.05	0.38	5

Discrimination-1.csv

Sources	MeanC181	SDC181	MeanC182	SDC182	MeanC20	SDC20	MeanC22	SDC22	MeanC24	SDC24	n
Beech forest	0	0	0	0	0	0	0	0	0	0	5
Brachen-Ponga	0	0	0	0	0	0	0	0	0	0	5
Inflow_Stream	0	0	0	0	0	0	0	0	0	0	5
Native Bush	0	0	0	0	0	0	0	0	0	0	5
Pine harvested	0	0	0	0	0	0	0	0	0	0	5
Sheep- subsoil	0	0	0	0	0	0	0	0	0	0	5

Output from the rerun showed a small decrease in the DIC value from 35.99 to 32.33 and relatively small changes in the mean source isotopic proportions (FIG. 5-17).

The combined Bracken and Ponga sources proportions produced an isotopic proportion equivalent to the sum of the individual isotopic proportions. This implies that both sources were contributing to the mixture and that combining them was appropriate. Bracken and Ponga are species of fern that rapidly colonise disturbed soil and can be used to determine the proportion of earth flows or land-slides in a catchment. In contrast, the combined Sheep and Subsoil sources isotopic proportions (FIG. 5-17) were not the sum of the individual isotopic proportions, only increasing marginally relative to the individual isotopic proportions. That and their low isotopic proportional contribution to the isotopic mass balance for the mixture implies that these two sources are having minimal input to the mixture and could be removed.

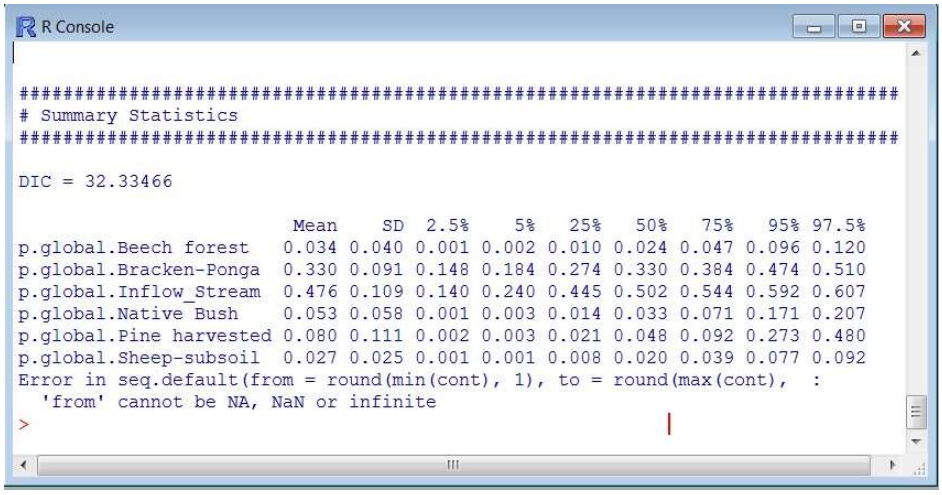

FIG. 5-17. Screen shot of the R Console screen showing the Summary Statistics of the revised data.

Justification for the removal of the combined Sheep and Subsoil source from the model can be seen in the Isospace_plot of the revised data (FIG. 5-18). The Sheep-Subsoil combined source is entirely within the variability range of the Beech Forest source and therefore cannot be discriminated from the Beech Forest source. The same argument could be used to eliminate the Bracken-Ponga mixture as it is within the variability range of the Pine-harvested source signature. This would be reasonable as the understory in the pine forest comprises mainly ferns, which would give a similar signature.

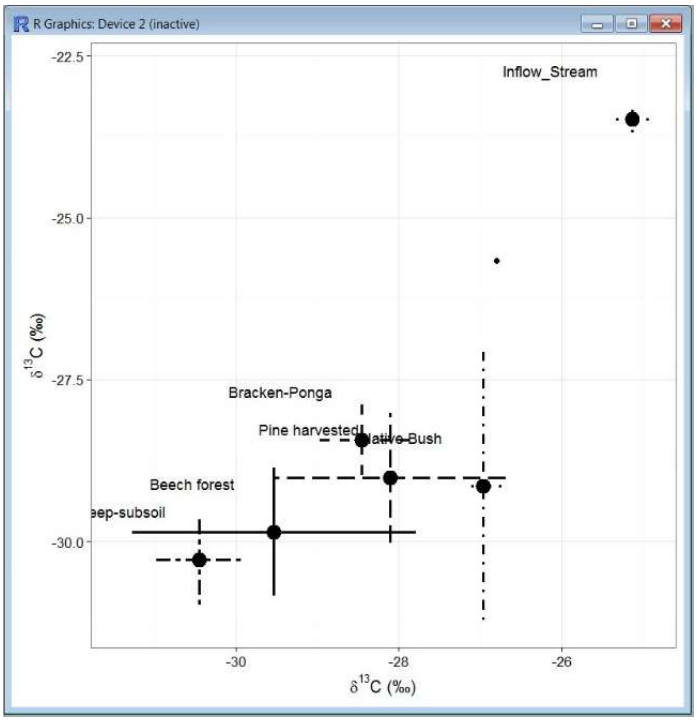

FIG. 5-18. Isospace_plot of the revised data using two isotopes.

Combining the two sets of sources improved the Gelman-Rubin diagnostic test by 1 > 1.01 (FIG. 5-19), but there were still 2 values > 1.05 indicating that the modelling may still have a problem. However, in the rerun data, the two values were 1.054 compared with the original model run where they were 1.098, and therefore only just outside the diagnostic criteria.

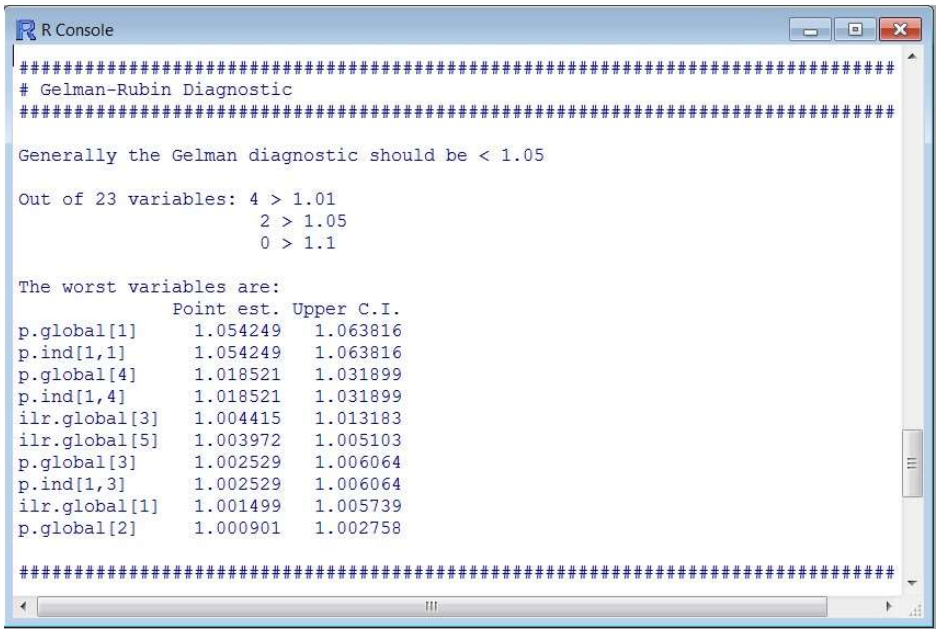

FIG. 5-19. Screen shot of the R Console screen showing the Gelman-Rubin diagnostics for the revised data.

There was a significant improvement in the Geweke diagnostic values with two of the three chain comparisons showing a good match with all z values less than the ± 1.96 threshold, and the third chain comparison with 2 values greater than 1.96 (FIG. 5-20). This is also an indication that the model rerun was almost acceptable.

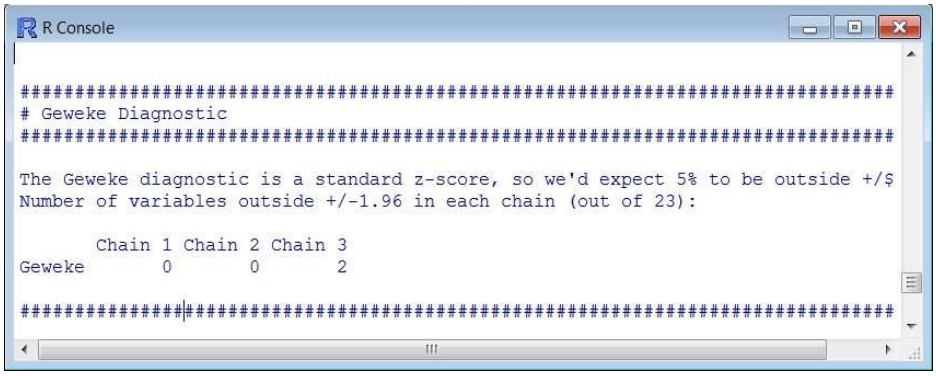

FIG. 5-20. Screen shot of the R Console screen showing the Geweke diagnostics for the revised data.

The graphical output (FIG. 5-21) shows that the double peaks produced in the initial run (FIG. 5-16) have mostly disappeared. There is still a small but 'ugly' tail to the Inflow-Stream curve between 0.00 and 0.30 (FIG. 5-21).

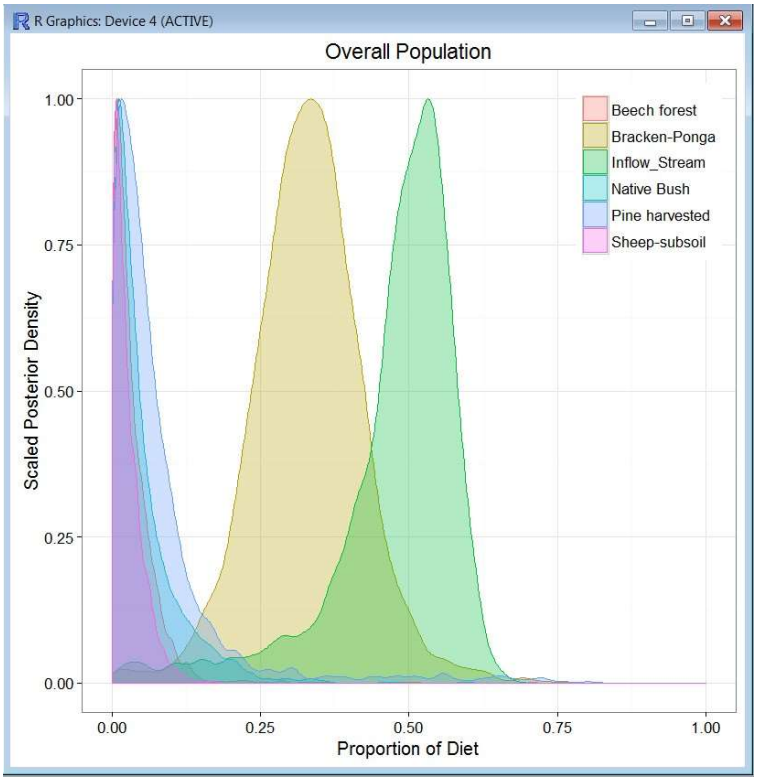

FIG. 5-21. Graphical output of the data for the revised data.

The improvements gained by combing the two pairs of sources might be further enhanced with further iterations of the model by, say, removing the Sheep-Subsoil source and/or the Bracken-Ponga source, until the diagnostics indicate an acceptable output.

5.4.6. Conversion of results from isotopic proportions to soil proportions

As it would be the case when using the SIMM IsoSource, the results obtained from the example data presented are all expressed in isotopic proportions. They must be converted into soil proportions with the following equation (5.5) which is using the total organic carbon ($\%C_{org}$) of the different sources [5.7] [5.21]:

$$S_n\% = \left(\frac{\left(\frac{I_n}{C_n\%}\right)}{\sum_n^1\left(\frac{I_n}{C_n\%}\right)}\right) * 100 \qquad (5.5)$$

Where I_n is the mean feasible isotopic proportion of source soil n in the mixture estimated using an isotopic mixing model and $C_n\%$ is the $\%C_{org}$ in the source soil.

It is important to point-out that in most of the existing literature the conversion of the carbon isotopic proportions to source soil proportions was performed using $\%C_{org}$, however some scientists have suggested - when available - to use instead the FAs concentration [5.22] [5.23].

While the summary statistics output provides mean and standard deviation values for the MixSIAR model run, these are for the isotopic proportions and may be little better than the mean data from IsoSource if applied to the scaling equation directly. To obtain the mean and standard deviation for the soil proportions, each of the 3000 isotopic proportion results must be converted to a soil proportion before the mean and standard deviation values can be determined. This requires an additional four lines of code to be entered at the end of each model run (FIG. 5-22). These are:

save.image('Filename-1.RData')

require(R2jags)

attach.jags(mixsiar$jags.1)

write.table(p.global,file=' Filename-1.csv',append=TRUE,sep=',',na='NA')

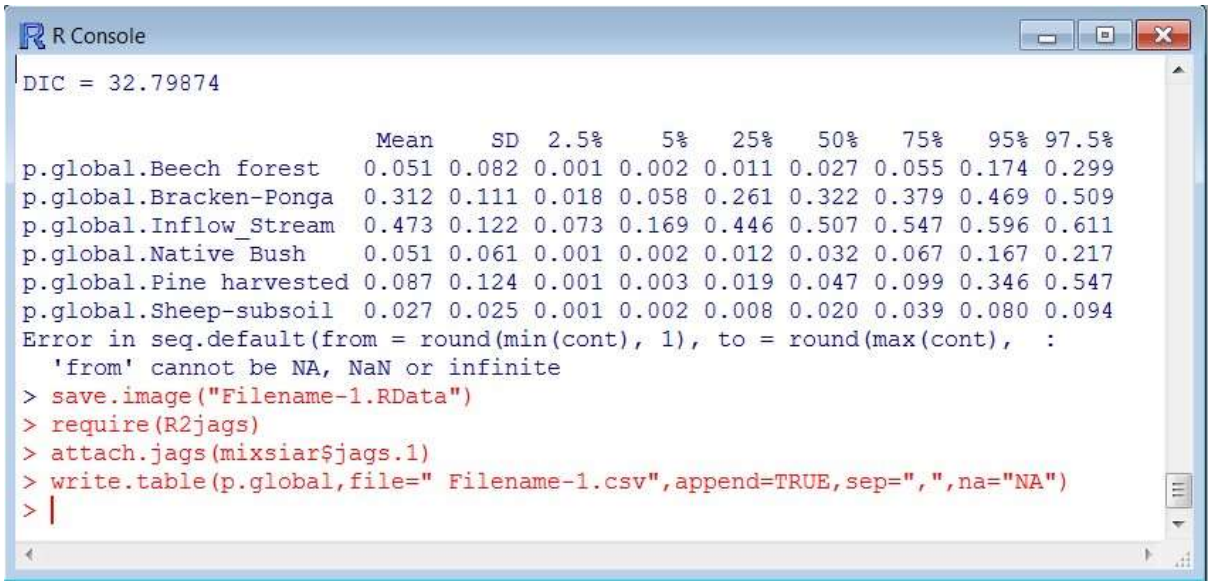

FIG. 5-22. Additional lines of code added to the MixSIAR run to output each line of isotopic proportions produced by the model. (The 'filename' can be changed to any name as required for the data analysed before using this script or the resultant 'Filename-1.csv' file can be renamed after the script is run).

The output file 'filename-1.csv' will have 3000 sets of isotopic proportions, which need to be converted individually to soil proportions. This data file has column headings as 'V1', 'V2', 'V3', … that should be changed to the source names obtained from the first column in the **source.csv** file, i.e., Beech forest, Bracken-Ponga, Inflow_stream, ... This is where the sorting of source names alphabetically, when preparing the data files, becomes important.

The linear scaling equation can be loaded into a spreadsheet and the isotopic proportion data in the filename.csv file can be copied and pasted into the spreadsheet for conversion to soil proportions. The outcome is a table with 3000 sets of soil proportions that can be statistically analysed for mean, median, SD, standard error, 2.5% percentile and 97.5% percentile.
Conversion of the mean soil proportions from the 3000 individual soil proportions produced values that were similar to the soil proportions produced from the mean isotopic proportions but the standard deviation values about the mean are generally smaller (TABLE 5.4).

TABLE 5.4. CONVERSION OF ISOTOPIC PROPORTIONS TO SOIL PROPORTIONS IN THE MIXTURE USING THE MEAN ISOTOPIC PROPORTIONS FROM THE RERUN SUMMARY STATISTICS TABLE (FIG. 5-17) COMPARED WITH TAKING THE MEAN OF THE 3000 INDIVIDUAL SOIL PROPORTIONS FROM THE RERUN MODEL.

Land use	**From isotopic proportions**		**From soil proportions**	
	Soil proportions		Soil proportions	
Source	**Mean** (%)	**SD**	**Mean** (%)	**SD**
Beech forest	1.36	7.88	1.6	3.8
Brachen-Ponga	11.05	10.56	11.4	5.0
Inflow_Stream	77.98	11.48	76.7	13.4
Native Bush	0.49	5.70	0.6	0.8
Pine Harvested	6.55	11.47	7.2	12.0
Sheep-subsoil	2.57	2.42	2.6	2.4

The reason for using a Bayesian mixing model with the CSSI technique is to provide information about the variability of the data and the uncertainty associated with the model predictions. For sediment tracking and tracing studies, the mean isotopic proportions given in the revised summary statistic table (FIG. 5-17) must be converted to soil proportions (TABLE 5.4, left hand columns). With the introduction of the technique for obtaining the 3000 individual soil proportion results, it is possible to determine the mean values for both the soil proportions and SD values, with statistical power (n = 3000) (TABLE 5.4, right hand columns). These data show that there were only minimal differences in the mean soil proportion values obtained using the mean isotopic proportion data and those obtained using the 3000 individual soil proportion results, but the SD values of the later were generally smaller, i.e., lower uncertainty.
This procedure is recommended over using the mean isotopic proportions from MixSIAR to generate the soil proportions and SD values.

To model another mixture using the same source and discrimination files without closing the MixSIAR programme, return to the main MixSIAR Gui command screen (FIG. 5-10), click on the ‘Load mixture data’ button and load the mixture data (FIG. 5-8). Change directory to the predefined output subfolder and repeat the model run from that point. New source and discrimination files could also be loaded for the new mixture, if required, before repeating the model run.

5.4.7. Cautionary note and future direction

Because Bayesian mixing model data fitting is based on the Markov Chain Monte Carlo method using repeated random sampling to obtain values which are probabilistically consistent with the mixture values, there is no realistic expectation for the model to produce a unique solution or, for that matter, to produce the same output values for different model runs using exactly the same input data. This implies that some of the variability seen in the summary statistics is an artefact of the model. Fortunately, the variability in the model isotopic proportion results from repeated runs of the same input data were small but sufficiently large to advise against the use of values to more than one decimal place or even question whether reporting to one decimal place is justified.

At present, the discrimination between sources contributing to a sediment mixture in sediment tracer studies relies on the use of Bayesian isotope mixing models designed for food web studies. Development of a Bayesian isotope mixing model specifically for sediment tracer studies, and which incorporates the routine for converting isotopic proportions to soil proportions along with statistically valid SD values, would be a big step forward.

REFERENCES TO SECTION 5

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