Stable Isotopes in Ecology and Environmental Science

SECOND EDITION

EDITED BY
ROBERT MICHENER
AND KATE LAJTHA

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Abbreviations

ANPP	above-ground net primary production
ANCA-MS	automated nitrogen and carbon analyzer mass spectrometry
BMP	best management practice
BSR	Bottom Simulating Reflector
BSTFA	*N*,*O*-bis(trimethylsiyl)trifluoroacetamide
BTEX	benzene, toluene, ethylbenzene, and xylenes
CAM	Crassulacean acid metabolism
CDT	Canyon Diablo Troilite
CF-GC/C/IRMS	continuous-flow gas chromatography/combustion/ isotope ratio mass spectrometry
CF-IRMS	continuous flow isotope ratio mass spectrometer
CP/MAS	cross polarization magic-angle-spinning
DHAP	dihydroxyacetone phosphate
DI-IRMS	dual-inlet isotope ratio mass spectrometer
DIC	dissolved inorganic carbon
DIN	dissolved inorganic nitrogen
DNRA	dissimilatory reduction of NO_3^- to ammonium
DOC	dissolved organic carbon
DON	dissolved organic nitrogen
EA-IRMS	elemental analyzer isotope ratio mass spectrometry
EBM	ecosystem-based management
EMMA	End Member Mixing Analysis
FAME	fatty-acid methyl esters
FEBS	Federation of European Biochemical Societies
GC-C-IRMS	gas chromatography combustion-IRMS
GIS	geographic information systems
GMWL	global meteoric water line
GNIP	global network of isotopes in precipitation
HPLC	high-performance liquid chromatograph
IAEA	International Atomic Energy Association
ICP-MS	inductively coupled plasma mass spectrometry
IHS	isotopic hydrograph separations
IRGA	infrared gas analyzer

IRMS	isotope ratio mass spectrometer
KE	kinetic energy
KIE	kinetic isotope effect
LAG	lines of arrested growth
LAVD	leaf area vapor deficit
LMWL	local meteoric water lines
LTER	Long Term Ecological Research
MDF	mass dependent fractionations
MIF	mass independent fractionation
MPA	Marine Protected Area
MTBE	methyl *tert*-butyl ether
NADP	National Atmospheric Deposition Program
*N*AIP	*N*-acetyl iso-propyl
NESIS	Non-Equilibrium Stable Isotope Simulator
NICCCE	Nitrogen Isotopes and Carbon Cycling in Coniferous Ecosystems
NMR	nuclear magnetic resonance
*N*TFA-IP	*N*-trifluoroacetyl iso-propyl
PAN	peroxyacetyl nitrate
PCR	polymerase chain reaction
PEP	phosphoenolpyruvate
PMIs	pentamethylicosanes
POC	particulate organic carbon
POM	particulate organic matter
PON	particulate organic nitrogen
PN	particulate nitrogen
PLFAs	phospholipids fatty acids
RCC	River Continuum Concept
SEM	standard errors of measurement
SIA	stable isotope analysis
SIMS	secondary ion mass spectrometry
SIP	stable isotope probing
SOB	sulfide-oxidizing bacteria
SOM	soil organic matter
SRB	sulfate-reducing bacteria
tBDMS	*tert*-butyldimethylsilyl
TCA	tricarboxylic acid
TDLAS	tunable diode laser absorption spectroscopy
TEF	trophic enrichment factor
TFA	trifluoroacetyl
TLE	total lipid extraction
TMS	trimethylsilylation
TOF-SIMS	time-of-flight secondary ion mass spectrometry
TRACE	Tracer Redistributions Among Compartments in Ecosystems

TTD	travel time distribution
V-PDB	Vienna Pee Dee Belemnite
V-SMOW	Vienna Standard Mean Ocean Water
WMO	World Meteorological Organization
WUE	water-use efficiency

Introduction

Since the first edition of our book, the field of stable isotopes has expanded tremendously. From its earliest uses, geochemists and paleooceanographers have developed a rigorous theoretical and empirical basis for the integration of isotopes into studies of global element cycles, past climatic conditions, hydrothermal vent systems, and tracing rock sources. Similarly, plant biologists, ecologists, and environmental chemists have developed the theoretical framework and the empirical database for the use of isotopes to study plants and animals. Natural abundance isotope signatures can be used to find patterns and mechanisms at the single organism level as well as to trace food webs, understand paleodiets, and follow whole ecosystem nutrient cycling in both terrestrial and marine systems. As a consequence, isotopic analysis has really become almost a standard tool for physiologists, ecologists, and all scientists studying element or material cycling in the environment.

As access to isotope ratio mass spectrometers has increased and prices for sample analysis have decreased, ecologists from a broad range of disciplines, not necessarily trained as isotope chemists, have increasingly added stable isotope analysis as another tool in their research. This second edition is intended as a review and assessment of the theory and practice of stable isotope analysis in a variety of ecological disciplines, with suggestions for both generalist ecologists who might be considering including such analyses to their studies, as well as for the more experienced isotope ecologist who is pioneering new uses and new directions in the field. We have taken a look at the field, and have chosen topics that are basic to ecologists, as well as new and emerging uses of stable isotope analysis in a variety of ecological subdisciplines. We have started with an excellent primer by Elizabeth Sulzman for those of you who are new to the field or who are teaching an introductory course in stable isotopes. From here, the book is divided into several broad areas beginning with terrestrial systems. John Marshall and his colleagues look at the variation of stable isotopes in plants. The next chapter by Charles Garten and colleagues looks at forest status and soil carbon dynamics, followed by Dave Evan's in-depth discussion of nitrogen isotopes in soil systems. Moving on to things both alive and fossilized, Paul Koch discusses the use of isotopes in the biology of vertebrates. Keith Hobson then discusses how migratory organisms can be traced using stable isotopes. The next three

chapters involve the marine environment, beginning with Joe Montoya's discussion of nitrogen in planktonic systems. Cindy Van Dover looks at the extreme environments of chemoautotrophic-based systems. Bob Michener and Les Kaufman discuss the use of stable isotopes in marine food webs, and how isotopes can be applied to marine conservation and management. Back on land, we take a look at freshwater systems and hydrology. Jacques Finlay and Carol Kendall apply isotopes to freshwater ecosystems, looking at both temporal and spatial variability in organic matter sources. Kevin McGuire and Jeff McDonnell take a look at where water goes when it rains, using isotopes as tracers in watershed hydrology. Following this, Carol Kendall and her colleagues discuss where the nitrogen goes, looking at the impact of anthropogenic N to ecosystems, especially as it applies to watersheds. Next, given the volume of data scientists can now generate, Bill Currie explores how to use that data to model ecosystem dynamics. We finish the book with a comprehensive chapter on compound-specific uses of stable isotopes put together by Richard Evershed and his colleagues.

Methods from a historical perspective

Many ecologists using stable isotopes will, and perhaps should, choose to send their samples to outside laboratories that specialize in the analysis of stable isotopes. Although the price of instrumentation has decreased, the costs for an individual to set up this type of laboratory are still quite high; typical startup budgets can be as high as $500,000. Maintenance of the mass spectrometer and the costs of having a full time, trained laboratory manager to run the laboratory (a necessity) are also steep. One can contrast this to the analysis costs of a typical study, which could be about $1000–$10,000 (with per sample charges averaging $7–$80, depending on the sample matrix and isotope in question). For many scientists it is much more cost-effective to use an outside laboratory.

Since the first edition, many of the methods to analyze samples have been automated, allowing for larger numbers of samples to be measured more quickly and inexpensively. More of this is covered in greater detail in chapters 1 and 14, and will be briefly discussed here. However, it is also useful to take a look at how scientists used to measure stable isotopes just 20 years ago, to give one an appreciation for how far the field has come, as well as seeing how it was done in the "dark ages" of stable isotope analysis. Fortunately, we have come a long way from the chart recorder and ruler! For those of you who are brand-new to the field and are not quite sure what mass spectrometers or stable isotopes are, we encourage you to first read chapter 1, an introduction to the terminology and chemistry of stable isotopes. After that, feel free to come back and get a historical perspective on stable isotope analysis.

Methods of sample preparation vary for each isotope. The goal in stable isotope analysis is to convert a sample quantitatively to a suitable purified gas (typically CO_2, N_2, or H_2) that the mass spectrometer can analyze. Sulfur can be analyzed as SO_2 or SF_6. Usually, organic samples are first dried (either in a 60°C oven or freeze-dried) and then ground to a fine powder. The samples can then be stored indefinitely in closed containers (such as scintillation vials or plastic bags), provided they are kept dry. If the investigator is interested in carbon isotopes for samples that may contain inorganic carbonates, the samples must first be acidified (usually with 1 N HCl, although some investigators are using dilute H_3PO_4; Showers & Angle 1986), since carbonate isotopic values are quite distinct from organic values and will skew the results (Haines & Montague 1979; Fry 1988).

Carbon and nitrogen in organic matter

In the early days of stable isotope measurement, most researchers used an oxidation reaction either "off-line" (sealed tubes in a muffle furnace, referred to as a Dumas combustion) or "on-line" (sample preparation line connected directly to the mass spectrometer) to combust the organic sample to a gas. The off-line combustion involves mixing the sample (typically 5–20 mg, depending on the sample's organic content) with an oxidant, usually cupric oxide, in a vycor (quartz) tube. In this procedure the sample must be in intimate contact with the CuO, which can be done in several ways: shaking the sample vigorously with the CuO within the tube (Fogel "shake method", M. Fogel, pers. comm.), grinding both in a mortar and pestle, or using a Wig-L-bug (Crescent Dental Manufacturing, Lyons, Illinois, USA). Shaking or using a Wig-L-bug is preferred, since there is less chance of sample cross-contamination. Approximately 1 g of CuO is used, then about 0.5 g of Cu is placed on top of the sample mix within the vycor tube in order to absorb the excess oxygen and convert N_2O to N_2. Once all sample tubes are prepared, they are then sealed under vacuum and combusted at 900°C for 1–2 h in a muffle furnace, and allowed to cool overnight to room temperature. It is then possible to cryogenically separate and purify the combined gases of CO_2, N_2, and H_2O (Stump & Frazer 1983; Boutton et al. 1983; Minagawa et al. 1984; Nevins et al. 1985). With manual samples, this can be done on a vacuum line using liquid nitrogen and an ethanol/dry ice slush. An important point to note for these and all following procedures is that the combustions and collections must be quantitative and close to 100% efficiency in order to prevent any fractionation. At this point the purified gas samples can be introduced into the isotope ratio mass spectrometer.

From this time-consuming, laborious process (generally 10–15 samples per day), the isotope ratio mass spectrometer (IRMS) manufacturers developed semi-automated combustion systems using elemental (CN) analyzers coupled to cryogenic purification systems that reduced sample preparation times and

cost per analysis (Fry et al. 1992) and allowed simultaneous analysis of carbon and nitrogen isotopic compositions. This type of system was appropriate for most organic tissue samples, sediment and soil samples containing sufficient organic matter, as well as materials such as collagen and some plankton samples. Note that encapsulators were also available which allowed liquid samples to be analyzed. Some of the next generation of cryogenic systems were able to analyze samples containing as little as 1 μmol N and 1 μmol C. Depending on the type of system used and the type of sample being analyzed, 1–20 mg of material is loaded into a tin boat, folded, then placed in the sample carousel. In automated systems, combustion and separation of the gases is, in principle, similar to the manual method. The sample is flash-combusted at 1600–1800°C in an oxygen stream, then the combustion gases are carried in the helium stream through a series of cryogenic traps, which are maintained at specific temperatures to collect H_2O, CO_2, and N_2. The gas of interest is then introduced into the mass spectrometer for analysis by appropriate timing of a valve that shunts the gases either to waste or to the mass spectrometer.

The next evolution of automated combustion system involved introducing the helium stream containing the combusted gases directly into the mass spectrometer, otherwise known as continuous flow analysis, which is very rapid and can analyze around 100 samples per day.

Carbonates and dissolved inorganic carbon

Inorganic carbonate samples (e.g., foraminifera for paleotemperature studies) are reacted under vacuum with 100% phosphoric acid, which results in a complete conversion of carbonate to purified CO_2 (Craig 1953). This allows for the analysis of both $\delta^{13}C$ and $\delta^{18}O$ from the same sample, provided the phosphoric acid is pure and contains no water (Coplen et al. 1983).

Dissolved inorganic carbon (DIC) in water samples is prepared by acidifying a water sample and stripping the water with CO_2-free gas under a partial vacuum (Kroopnick 1985; McCorkle & Emerson 1988), then isolating and purifying the gas. The same principle can be applied to samples of bicarbonate in blood for tracer studies (Moulton-Barrett et al. 1993).

The latest methods for both types of samples have evolved with the development of automated continuous flow systems (Revesz & Landwehr 2004). Instead of evacuating the vials completely for carbonates, a helium stream displaces any atmosphere in the vial before the acid is added. After a set reaction time, the CO_2 is transferred into a sampling loop before being introduced into the mass spectrometer through either a helium stream or a dual inlet system. A similar technique is used for DIC in water.

Ammonia and nitrate $\delta^{15}N$ in water samples

In the dark days of dissolved inorganic nitrogen (DIN) analysis, ammonium in water samples was isolated using various steam distillation techniques (Velinsky et al. 1989) or later using passive diffusion within a closed container (Brooks et al. 1989). Both procedures involve making the pH of the water sample basic, then trapping or collecting the ammonium in an acid trap. Steam distillation techniques are good for large water samples containing low levels of NH_3, can be used on salt water solutions, and take about 30 min per sample (Velinsky et al. 1989). Once the ammonia is collected in an acid trap, zeolite is used to remove NH_3 from solution. The zeolite is then dried and can be analyzed using the sealed tube Dumas combustion method (see above). As with all methods, care is needed to trap all of the NH_3 in all steps in order to avoid fractionation. Nitrate-N can be distilled using the same techniques after first reducing the nitrate in the water to ammonia with Devarda's Alloy, a chemical reagent.

These passive diffusion techniques work well for samples such as soil solutions or Kjeldahl digests, and can be done in a batch fashion. Two different procedures are used, one involving suspending an acidified filter paper (usually with H_2SO_4) above the solution, the other wrapping the filter paper in Teflon tape and floating the packet in the solution (Downs et al. 1999). The solution is made basic and, using the same principle as the distillation technique, the ammonia diffuses onto the acidic filter paper. After the diffusion is complete (anywhere from 3–5 days), the filter paper is dried and can be combusted using the automated CN-mass spectrometer system.

These are by no means the only techniques to measure NH_3 and NO_3 in water samples, and we refer the reader to the volume by Knowles & Blackburn (1993) for further details on these and other methods. The latest techniques involve using denitrifying bacteria and a gas concentration system interfaced to the mass spectrometer; interested readers should see chapter Chapter 12 and papers by Chang et al. (1999), Sigman et al. (2001) and Casciotti et al. (2002).

Oxygen in water

The measurement of ^{18}O in water samples can be accomplished using several different procedures. One of the earliest procedures used by oceanographers, applicable to larger volume water samples (such as groundwater), uses 200 µL to 1 mL of water (Socki et al. 1992; Wong et al. 1987; Taylor 1973). The water sample is first placed in a suitable vessel such as a vacutainer or serum bottle. After removing the headspace atmosphere, a measured aliquot of CO_2 of known isotopic composition is introduced into the headspace. The water is incubated at a controlled temperature for a

period of time that allows the oxygen in the water to completely exchange with the oxygen in the CO_2, after which the headspace CO_2 is removed using cryogenic techniques, then analyzed on the mass spectrometer. Modifications of this technique, using automated continuous flow analysis, routinely use 100–200 μL of water with excellent reproducibility (Horita & Kendall 2004).

Small-volume samples in the range of 3–10 μL, which may be generated from samples such as small animal metabolic studies, plant water, or combusted organic matter, are more problematic, given the difficulty of balancing the amount of headspace CO_2 to water volume. One technique that was used to get around this was a chemical procedure utilizing guanidine hydrochloride to release the oxygen. For the details of the technique, see Wong et al. (1987). This was an incredibly time-consuming and labor-intensive procedure, but produced good results.

The latest technique utilizes pyrolysis, where the water sample is combusted in an oxygen-free environment and the oxygen converted to CO, which is then analyzed by the mass spectrometer (Farquhar et al. 1997). The debate rages concerning carryover between water samples, but many laboratories have developed protocols to eliminate this problem (P. Brooks pers. comm.; Ghosh & Brand 2003; Gehre et al. 2004).

Deuterium

In the past, in order to measure ^2H, or deuterium, from organic tissue, the sample was combusted using an off-line, sealed tube procedure and the resulting water collected quantitatively (Schiegl & Vogel 1970). The water was then reduced to H_2 using either a vacuum line and uranium furnace, or using zinc in a sealed vessel (Krishnamurthy & DeNiro 1982, Coleman et al. 1982). The procedure could be used for other types of water samples, such as plant water, ground water, and water obtained for metabolic studies. Many investigators used the zinc method, as it could be done in a batch fashion and avoided any problems associated with obtaining uranium for the furnace.

Modern procedures now use one of two automated techniques. The first is similar to the CO_2 equilibration procedure for water samples. Instead of flushing the water vials with a CO_2/helium mix gas, the technique uses a hydrogen/helium mix gas; the vials also contain Hokko beads (platinum on a polymer base) that are suspended out of the water (Coplen et al. 1991). The beads act to enhance the exchange of hydrogen in the water with the gaseous hydrogen in the headspace. After a fixed time, the hydrogen is extracted and introduced via continuous flow into the mass spectrometer. The second technique is a pyrolysis procedure involving chromium. Water is injected onto hot chromium, which is contained in a combustion column in an elemental analyzer. The water reacts with the chromium

and is converted to hydrogen gas, which goes directly into the mass spectrometer. This procedure is very rapid and very precise (Kelly et al. 2001; Nelson & Dettman 2001). Certainly a long way from the off-line uranium furnace days!

Sulfur

The analysis of sulfur isotopes depends on the starting matrix, but in essence involves converting sulfur in the sample to SO_2 or SF_6. Sulfur hexafluoride has the advantage that fluorine has only one isotope, but the techniques involved are somewhat hazardous, therefore most laboratories use SO_2 gas. Most of the early procedures to isolate sulfur from its matrix (water, plant and animal tissue, soils, sulfides) generally involved oxidizing sulfur to sulfate in solution. The sulfate could then be precipitated as $BaSO_4$ using a 10% barium chloride solution. From here the sample was oxidized to SO_2 gas and introduced into the mass spectrometer via a dual inlet. These procedures were generally not done in the laboratory of an ecologist, due to the labor, materials, and time involved. For a more detailed description of early sulfur preparation, see Krouse & Tabatabai (1986).

Once again, continuous flow has really revolutionized sulfur analysis. It is still not easy, but it certainly has evolved from those early days. Samples are combusted in an elemental analyzer and then passed through a gas chromatography (GC) column to separate the various combustion gases. Sulfur is much stickier and will take longer to elute. One has to make quite a few modifications to the elemental analyzer, due to the amount of water produced and the possibility that the water will make the sulfur "stick" in the system (C. Cook, pers. comm.; Giesemann et al. 1994).

The reproducibility of isotope measurements will depend on the procedure and laboratory techniques of the investigator, but is typically ±0.2‰ or better for carbon, oxygen, nitrogen, and sulfur, and 0.3–2‰ for hydrogen. This methods section is but a brief introduction to the procedures involved in preparing stable isotope samples. Our aim was to give you an idea of how modern day procedures have evolved out of the early days of stable isotope analysis. For more elaboration and further details on other methods, we refer the reader to volumes by Coleman & Fry (1991), Knowles & Blackburn (1993), and de Groot (2004).

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Finally, it is with great sadness that we report the death of Elizabeth Sulzman on June 10, 2007. She was 40 years old, and was a scientist, colleague, teacher, mentor, mother, and wife. We will remember her kindness, her laughter, and great conversations about science and life. We will miss her greatly.

ROBERT MICHENER AND KATE LAJTHA

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