1 Introduction

1.1 Discovery

I remember the first time I discovered the power of stable isotopes. It was by accident. It was thirty years ago, when I was a beginning graduate student along the south Texas coast. That summer I helped a visiting professor collect rodents (mice, rats, and ground squirrels) in a coastal sand dune community. Yes, I worked with the rodent traps, but I also got bored and wandered off during the hot afternoon hours, collecting plants and grasshoppers from the dunes. One evening later in that summer of 1976 we were at the mass spectrometer, watching the chart recorder display the isotope results for our collections. It was fascinating. One sample was very enriched in the heavy carbon stable isotope, ^{13}C, and the next sample was depleted in ^{13}C. A great divide was evident in the isotopes of the sand dune community. We watched the chart recorder for hours as sample after sample showed the basic ^{13}C distinction, or variations on this ^{13}C isotope theme.

We had discovered something new, something unexpected. Plants fell into two categories, recognizable as the C_3 and C_4 plants that had been described recently as distinct plant types (Bender 1968; Smith and Epstein 1971). But grasshoppers also fell into these same categories, so that the different grasshopper species were specialists in their diets. Rodents were intermediate in their ^{13}C values, and therefore dietary generalists. That evening at the mass spectrometer a food web took shape. And I realized that as I had wandered the dunes, making my collections, I had missed a fundamental order in the natural world. The isotope measurements showed carbon connections and flows in the sand dune community, from plants to specialist grasshoppers and generalist rodents (Fry et al. 1978). I could not perceive the isotopes with my senses, nor could many months of observation have shown so powerfully the distinctive C_3–C_4 structure of that coastal food web. The isotopes illuminated an unknown ecology. My experience was and is not unique, for scientists worldwide recognize that there is an "isotopically ordered world" (Wada et al. 1995) within ecological systems. As you embark on your own ecological adventures, I wish you good discovery with

the isotopes. They will help you see and test ecological interactions, powerfully tracing otherwise invisible connections.

1.2 General Introduction

Ecologists make many types of measurements to understand ecological systems, measurements such as length, timing, or pH. Isotope measurements are chemical measurements that allow detailed nuanced views of element cycling in all systems that interest ecologists. It takes some time and practice to learn how to use isotopes, just as it takes time and practice to learn how to use a taxonomic key or how to use statistics. Learning to work with isotopes is usually time well spent, for it provides a different way to view ecological connections, a distinct tracer-based perspective that often leads to new discoveries.

The goal of the book is to help you use isotope tracers in correct and creative ways to solve environmental problems. This book works on accomplishing this goal in several ways. First, it focuses on fundamental principles of mixing and fractionation that govern isotope circulation in the biosphere, and aims to help you understand and use these principles. Second, it presents a new modeling approach that is fairly simple to use with computer spreadsheets. This approach enables you to circulate isotopes for yourself as you sit at your desk, mimicking in virtual reality the ways that isotopes circulate in nature. Lastly, this book presents many stories and illustrations to help you learn, hear, and speak the rich isotope language that permeates the natural world. Although the book is factual in essence, it seeks to stimulate your creativity.

A complete course of study for a graduate or post-graduate student interested in learning stable isotope ecology might include three elements: reading this book, finding and reading current research articles as supplements, and planning and carrying out her or his own pilot project using stable isotopes (Box 1.1). You should also realize that no matter what you encounter here, it is only a small part of what there is to learn about isotopes, so many sections point you towards further readings.

This book also emphasizes "learning by doing" in the living examples portrayed in many spreadsheet models. You can read about these models in the book, then go to the accompanying CD and open up the computer models to interactively enter parameter values and watch the isotope action unfold before your eyes. This book also contains a more traditional type of learning by doing, problems posed for each chapter to increase your isotope expertise.

For better and worse, this book reflects the author's experience and bias garnered over thirty years, and so focuses primarily on isotopes of the three elements, carbon, nitrogen, and sulfur. The majority of examples concern aquatic ecology. Nonetheless the book expounds and emphasizes

Box 1.1. How to Start Your Own Isotope Project

The most meaningful way to get started in stable isotope ecology is usually to design and carry out your own pilot project. This is much easier than you might think. Analyzing 10 to 25 samples is often enough to see if your idea is worth pursuing, and costs less than $300. Here are some of the steps you might take for your project.

1. Think about what interests you. Remembering that isotopes are everywhere and in everything, think about how isotopes might be circulating in something that interests you.
2. Literature review. Go to the library or check the Internet and the Web of Science to see if anyone is doing related work. Type in search words such as "isotope," ^{15}N, ^{13}C and the like and see what you find.
3. Think about a field site. What kinds of samples are available at your site that would be interesting to sample?
4. Find an isotope lab. Look up isotope laboratories on the Internet for prices and how to prepare samples.
5. Contact the isotope lab and discuss your idea. People working at the isotope lab may run a few trial samples at no cost, especially if they know you are just trying to get started and you seem to have a good idea. They will have some good advice in any case. If the isotope laboratory is nearby, go visit and see what is really involved with sample preparation.
6. Collect and analyze samples. Plant, animal, and soil samples can be collected by hand without fear of contamination, and small samples <100 mg typically suffice for isotope work. Laboratory preparation is also simple, typically drying at 60°C in an oven, then grinding to a fine power. Your contacts at the isotope lab will help you with specialized sampling, and how much sample you need to weigh out for the actual analysis.
7. Data interpretation. When results come back from the isotope lab, examine the data to see if your ideas worked out. Make this project an activity for one of your classes, so that you share the results and get some feedback discussion from your fellow students about your interpretations.

Following steps 1 to 7 is the fast track to learning about isotopes, and will probably teach you more than you will learn from reading several books (including this one). Louis Agassiz, one of the great scientists of the 19th century put it this way: "Study Nature, Not Books." (You can see this framed handwritten motto in Woods Hole, Massachusetts when you walk into the main library at the Marine Biological Laboratory. The motto provides a somewhat ironical introduction to a great collection of books and journals.) Overall, an isotope project will put you in touch with the world's greatest teacher, the natural world. And if you work through these seven project steps, you deserve promotion to the ranks of the working isotope scientists.

a centralized view of isotope circulation in the biosphere that underlies all branches of isotope research, so that the book is intended to be useful to all scientists working with stable isotope tracers.

This book generally has a very informal, conversational style, and represents my attempt to make a technical subject accessible in an uncomplicated and often fun way. You can read about my own scientific evolution in Section 1.3, and how I earned the nickname "Mr. Polychaete." But Mr. Polychaete has his own persona in this book where he offers wise isotope advice with a bit of humor and a wiggle. He is an isotope enthusiast who likes to laugh, and might have called this book *Isotope Sorcery for Ecologists* or *A Traveler's Guide to the Mysterious and Wondrous Realm of Isotopia*. Mr. Polychaete appears in this book to help explain isotopes to ecologists. Mr. Polychaete will also let you know when detailed technical sections are present that can be skipped during the first reading of this book.

A Little Encouragement for the Novice

Starting out as an amateur in the science of isotopes may seem daunting, but in reality, being an amateur is often a good thing in science. Here is something for you to think about, quoted from C.H. Hapgood's book, *Maps of the Ancient Sea Kings*.

> Every scientist is an amateur to start with. Copernicus, Newton, [and] Darwin were all amateurs when they made their principal discoveries. Through the course of long years of work they became specialists in the fields which they created. However, the specialist who starts out by learning what everybody else has done before him is not likely to initiate anything very new. An expert is a man who knows everything, or nearly everything, and usually thinks he knows everything important, in his field. If he doesn't think he knows everything, at least he knows that other people know less, and thinks that amateurs know nothing. And so he has an unwise contempt for amateurs, despite the fact that it is to amateurs that innumerable important discoveries in all fields of science have been due . . . when a difficult problem was being discussed, Thomas A. Edison said it was too difficult for any specialist. It would be necessary, he said, to wait for some amateur to solve it.

In other words, amateurs are great for their new thinking and initiatives. Your new start in this technical world of isotopes is welcome.

Isotopes and Their Elements

Isotopes are forms of the same element that differ in the number of neutrons in the nucleus. Extra neutrons in the nucleus of an element generally impart only subtle chemical differences, small differences that keep almost identical isotopes from being truly identical. In the world of chemicals, the real differences among elements lie in the numbers of protons and electrons (Figure 1.1). The negatively charged electrons react to form the bonds between atoms. The electrons also balance the number of positively charged

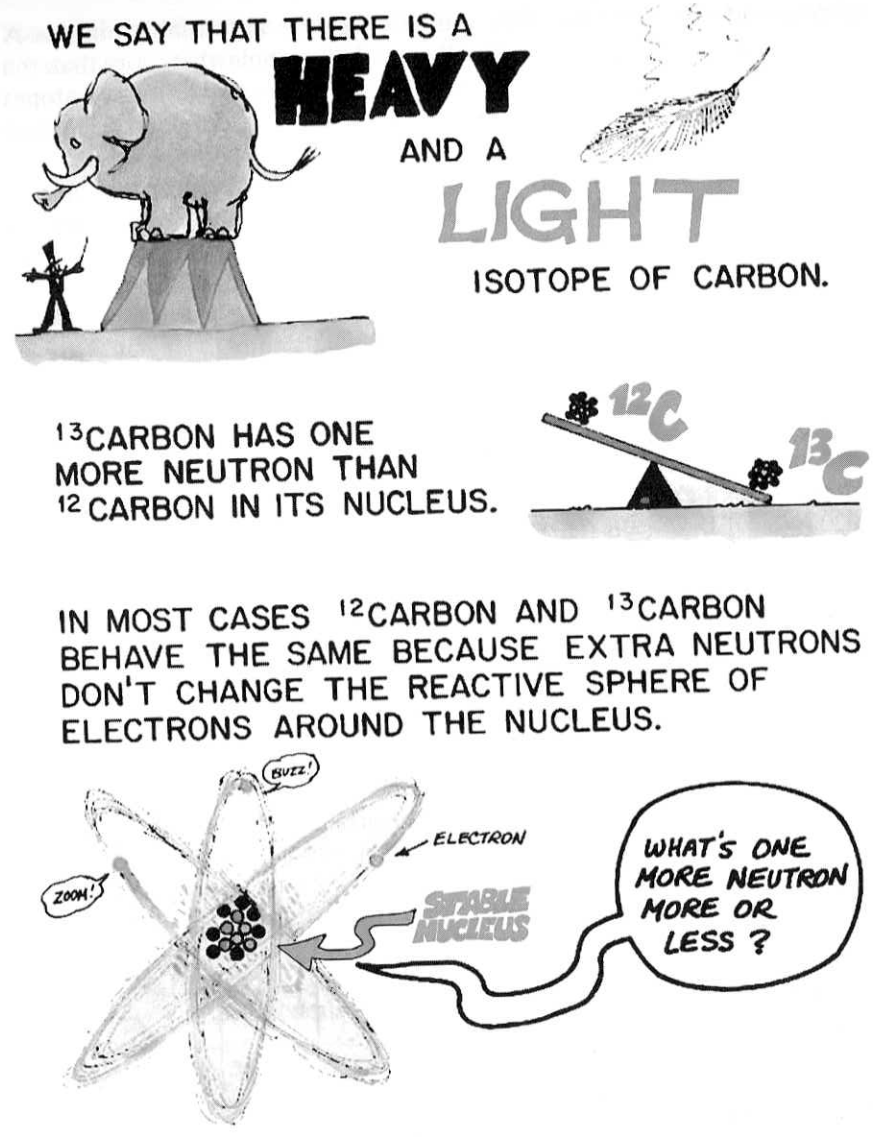


FIGURE 1.1. An extra neutron in the ^{13}C isotope makes the nucleus more massive or "heavier" than the ^{12}C isotope, but does not affect most chemistry that is related to reactions in the electron shell.

protons in the nucleus. So with this positive–negative balance in good working order, you might wonder who needs neutrons? The nontechnical answer is that having one or more neutrons is important because neutrons are the peacekeepers of the nucleus, keeping the highly charged, mutually repulsive protons from getting too close together. But overloading the

nucleus with too many neutrons can also result in an unstable nucleus. A moderate number of neutrons, usually equal to or somewhat more than the number of protons, is the key ingredient for long-term stability of isotopes and their elements. Only the elements hydrogen (H) and helium (He) have stable isotopes with fewer neutrons than protons.

We humans cannot directly detect these neutron differences that exist at the level of atoms. And in fact, it was less than 100 years ago that the first machines were built to detect isotopes. In 1919, Francis W. Aston built an early double-focusing mass spectrometer in Cambridge, England while working in the laboratory of J.J. Thompson. With this machine, Aston proved that neon was in fact an isotope triplet, not just a singlet element. All three members of the neon triplet had the same number of electrons and protons, but extra neutrons accounted for the neon triplet at masses 20, 21, and 22. Each neon isotope had 8 electrons and 8 protons, but had different numbers of neutrons. There were 12, 13, or 14 neutrons in the neon isotopes, making the atomic number (protons + neutrons) totals come up to 20 (= 8 + 12), 21 (= 8 + 13), and 22 (= 8 + 14). Aston went on to discover that many elements come in more than one flavor or isotope form, and quickly received the 1922 Nobel Prize in Chemistry for his efforts (see: http://nobelprize.org/chemistry/laureates/1922). In the same year, he also published the seminal book in this area, *Isotopes*.

The word "isotope" comes from consideration of the periodic table of the elements (Figure 1.2), and means that isotopes of an element all occupy the same (*iso*) place (*topos*) in this table. In a sense, isotopes are whole hidden families of nearly identical forms of the same element packed into the boxes of the periodic table. Dr. Margaret Todd of Edinburgh coined the term

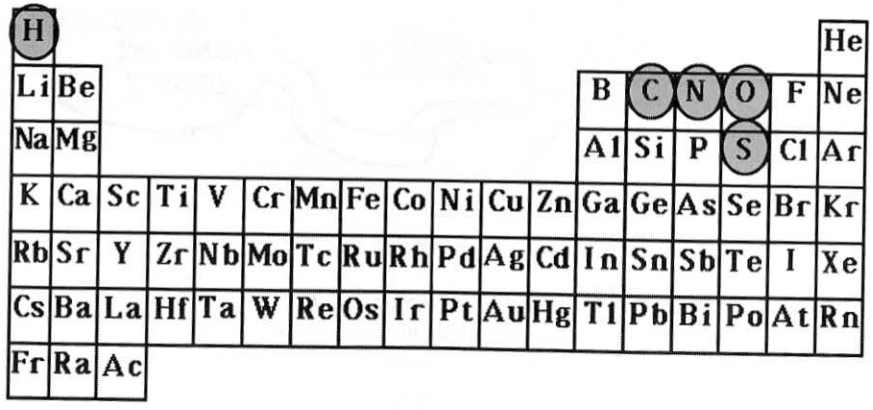


FIGURE 1.2. An abbreviated periodic table of the elements. Many elements have more than one isotope variety and differ in the number of neutrons. Stable isotopes of the circled elements HCNOS (hydrogen, carbon, nitrogen, oxygen, and sulfur) are emphasized in this book. Details about isotopes for many of these elements are available at the Web site http://wwwrcamnl.wr.usgs.gov/isoig/period/.

"isotope" (see footnote 15–90 in Dahl's *Flash of the Cathode Rays*), and it came to be preferred over a rival word, "pleiad," used for a group of stars recognized as sisters by the ancient Greeks. Frederick Soddy first introduced the term "isotope" in a formal way during a speech to the British Royal Society on February 27, 1913. Soddy was one of the first to suspect the presence of isotopes, and went on to win the 1921 Nobel Prize in Chemisty for "his investigations into the origin and nature of isotopes" (see http://nobelprize.org/chemistry/laureates/1921).

Today we know that all elements have multiple forms or isotopes. The ultimate source of elements and isotopes lies in the Big Bang that started our universe and in stars where nuclear reactions of fission and fusion produce new atoms (Penzias 1979, 1980; Broecker 1985; Clayton 2003). Given these sources, it is perhaps not surprising to learn that the fundamental proportions of heavy and light isotopes here on earth derive from ancient syntheses billions of years ago. Further reactions in the cold emptiness of interstellar space somewhat modify these proportions, especially for hydrogen isotopes (Clayton 2003). And radioactive decay on earth is also active, slowly but surely producing a mildly distinctive local mixture of elements and isotopes on this planet. At the time of this writing, scientists on our planet Earth recognize or suspect the existence of approximately 120 elements and 3100 accompanying isotopic forms or nuclides. However, most of the 3100 nuclides are very short-lived radionuclides. Among the nuclides, there are only 283 stable isotopes that do not undergo radioactive decay, so that stable isotopes comprise <10% of all known isotopes.

The term nuclide comes from a physicist's appreciation of fission and fusion processes in stars and Earthside reactors. For these nuclear reactions, the total number of neutrons plus protons (the nuclide number) is the important aspect of an element, not the chemistry deriving from electron interactions. Physicists keep track of the nuclides in a "chart of the nuclides." This chart is a fundamental starting point for understanding the physics of elements and isotopes, just as the periodic table is a fundamental starting point for chemistry. In the chart of the nuclides, all the isotopes are listed in plain view in separate boxes. There is quite a bit of chemical and physical "biographical" detail listed in each box, summarizing basic facts for each isotope. So in the chart of the nuclides, the isotopes are liberated from their crowded, hidden existence in the same place or *isotopos* element compartments of the periodic table. The chart of the nuclides is the reference to consult when you want to find out details about an isotope. Clayton (2003) provides excellent isotope-by-isotope information in a reader-friendly commentary on the early part of the chart of the nuclides that includes the elements of most interest in this book.

A current listing of the periodic table of the elements is available on the Web at http://pearl1.lanl.gov/periodic/default.htm. Current versions of chart of the nuclides are given at http://wwwndc.tokai.jaeri.go.jp/CN03/index.html and http://t2.lanl.gov/data/nuclides/map8.html. The number of stable iso-

topes given above as 283 comes from counting the stable isotope nuclides listed in those charts. Ecological applications for isotopes of many of these elements are described on numerous Web sites, for example, at http://wwwrcamnl.wr.usgs.gov/isoig/period/ and http://ecophys.biology.utah.edu/sirfer.html.

The most famous isotopes are undoubtedly the uranium isotopes used in nuclear reactors and in atomic bombs. The common form of uranium (^{238}U) has three more neutrons than the rarer, more reactive form (^{235}U), and much of the secret of dealing with uranium is figuring out how to separate these two isotopes which generally have very similar chemical qualities. You may hear in the news that nuclear proliferation worries often focus on centrifuge technology, because centrifugation is one of the ways that you can separate a more massive isotope twin (^{238}U) from its lightweight uranium counterpart (^{235}U). Radioisotopes such as these two uranium isotopes emit various kinds of particles and decay or change into other elements, liberating energy that can be used for both destructive and beneficial purposes.

But this book focuses on a different set of isotopes, the stable isotopes that persist in the same form for eons after they are formed. The stable isotopes have survived over billions of years of geological time here on Earth. They provide some of the few surviving records about early life on Earth and the early ecology of our planet. Stable isotopes are safe isotopes that do not decay and unlike the radioactive isotopes, are not at all hazardous to human health. In fact, stable isotopes are quite abundant and natural parts of each one of us (Figure 1.3; Wada et al. 1995).

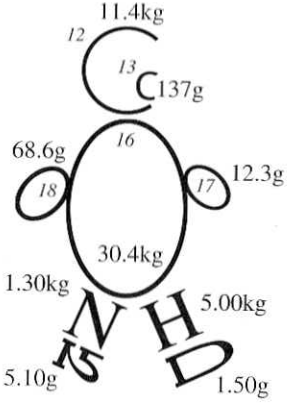


FIGURE 1.3. You are what you eat: stable isotopes in a 50 kg human who is composed mostly of light isotopes with a small amount of heavy isotopes. People are mostly water, so hydrogen and oxygen isotopes dominate at >35 kg. Next come C isotopes at >11 kg, then N isotopes. S isotopes are missing; they should be here at about 220 g for the light isotope ^{32}S and 10 g for the heavy isotope ^{34}S. Have you had your isotopes today? (From Wada and Hattori, 1990; reproduced with permission of CRC Press LLC.)

TABLE 1.1. Isotopes for the Light Elements HCNOS (Hydrogen, Carbon, Nitrogen, Oxygen and Sulfur).[a]

Element	Isotope Abundance: Low Mass		Isotope Abundance: High Mass		Mass Difference[b] (Relative)	Range in δ[c] (‰)
Hydrogen[d]	^{1}H	99.984	^{2}H	0.016	2.00	700
Carbon	^{12}C	98.89	^{13}C	1.11	1.08	110
Nitrogen	^{14}N	99.64	^{15}N	0.36	1.07	90
Oxygen	^{16}O	99.76	^{18}O	0.20	1.13	100
Sulfur	^{32}S	95.02	^{34}S	4.21	1.06	150

[a] For each of these elements, the low-mass or "light" isotope is by far the most abundant of the isotopes, >95%. These fundamental isotope abundances prevailing on our planet Earth were determined long ago during element synthesis at the start of our universe, in interstellar space and in stars (Penzias 1979, 1980; Clayton 2003).

[b] Mass difference = high mass/low mass, e.g., 2/1 = 2 for the hydrogen isotopes.

[c] The listed range in δ values is representative for most natural samples that have not been artificially enriched with heavy isotopes (data from Anderson and Arthur, 1983). δ values are the common way to express isotope abundances (see Section 2.1).

[d] Hydrogen isotopes especially are in a different class in the isotope world, with large fractionations associated with the large 2× mass difference between protium (^{1}H) and deuterium (^{2}H, or also "D").

Stable isotopes often have skewed distributions on Earth, mostly reflecting details of their synthesis long ago in stars. For example, the lightest stable isotope accounts for more than 95% of all the isotopes for elements such as hydrogen (H), carbon (C), nitrogen (N), oxygen (O), and sulfur (S) (Table 1.1). But the reverse is true for some elements such as boron (B) and lithium (Li) where the heavy stable isotopes are the abundant isotopes, >80% of the total. Only a few elements such as bromine (Br), silver (Ag), and europium (Eu) show a roughly equal, 50–50, distribution between light and heavy stable isotopes. The element tin (Sn) has the most stable isotopes (10 isotopes), and there are elements such as fluorine (F) and phosphorus (P) that are endowed with only a single stable isotope form. Ecologists can only regret that stars did not make a second stable P isotope so that we could use differences between the isotope pair to track natural P dynamics in the biosphere.

But what about radioactive phosphorus, ^{32}P? In fact, ecologists added ^{32}P to terrestrial and aquatic ecosystems in the 1950s and 1960s to study P dynamics (reviewed by Odum, 1971). But today ecologists generally refrain from introducing radioactive isotopes into outdoor field settings. Instead, they study natural radiotracer distributions, or increasingly add stable isotopes instead to field experiments. There are also many medical uses for the nontoxic, safe stable isotope tracers (Boutton 1991; Fischer and Wetzel 2002).

For ecologists, stable isotopes provide a natural way to directly follow and trace details of element cycling. The isotopes function as natural dyes,

colors, or tracers and their use can resolve many environmental problems. We use special machinery, especially an improved version of Aston's early mass spectrometer, to follow these isotope colors, like using special 3-D glasses at the movies to follow the action. Chapter 2 considers details of isotope measurements and the δ notation used to express measured isotope values. The special measurements let us follow the origins and fates of the many elements as they circulate in the biosphere. Elements of particular interest in today's environment are those that cycle tightly with organic matter (Table 1.1), especially H, C, N, O, and S. All these elements are blessed with two or more stable isotopes, that is, isotope twins, triplets, and quadruplets (Figure 1.4). The HCNOS elements are generally lightweights among the elements, but comprise most of the mass present in organic materials (see, e.g., Figure 1.3). Stable isotope studies of the sources and cycling of the HCNOS elements are sometimes supplemented by natural

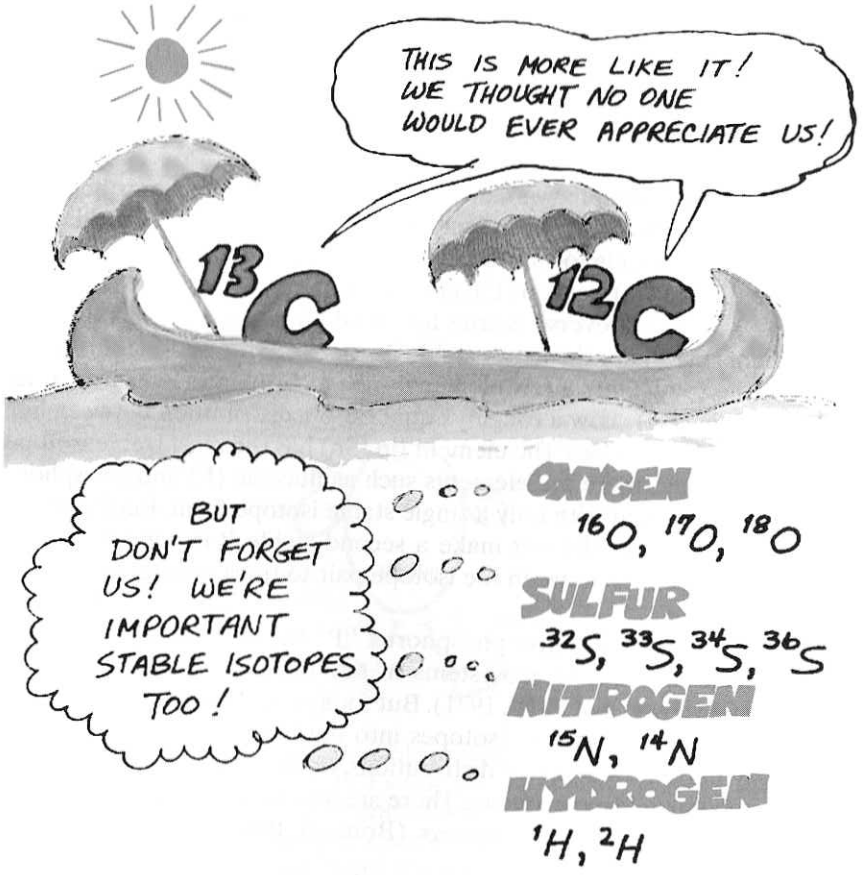


FIGURE 1.4. This book focuses on the five elements (hydrogen, carbon, nitrogen, oxygen, and sulfur) and their 13 stable isotopes.

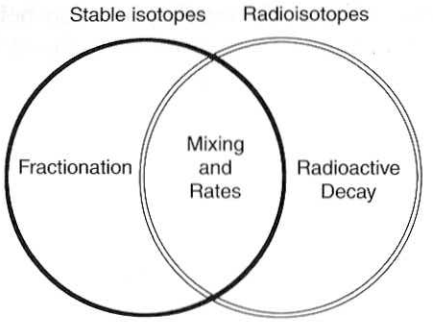


FIGURE 1.5. Stable isotopes are especially valuable for studying the origins and cycling of organic matter in the biosphere. Ecologists also use radioisotopes (especially ^{3}H, ^{14}C, and ^{32}P) to study cycling rates and to determine ages. Stable isotopes that pose no health risk are increasingly substituted for the radioisotopes in ecological and medical research.

radioisotope (especially ^{14}C) studies about rates of element cycling (Figure 1.5). Studies of natural radioisotope distributions are extremely valuable (e.g., Broecker and Peng 1982; Schell 1983) but the analytical work involved is currently too costly for most routine ecological applications. Fortunately, advances in measurement technologies have made HCNOS stable isotope analyses affordable for current ecological work, with costs in the $5–15 range for most samples.

This book deals with the HCNOS stable isotopes in the context of organic matter dynamics, and Chapter 3 gives five introductory reviews of how ecologists use isotopes in ecological research. The ecological focus of this book supplements other books that deal with isotope applications in geology (Hoefs 2004; Faure and Mensing 2004), H and O isotope applications in hydrology (Clark and Fritz 1997; Kendall and McDonnell 1998; Criss 1999), and six books of collected papers that deal with aspects of stable isotope ecology (Rundel et al. 1988; Ehleringer et al. 1993; Lajtha and Michener 1994; Wada et al. 1995; Griffiths et al. 1997; Ehleringer et al. 2005).

Isotopes are something like a mysterious hidden language written everywhere in the common chemicals and compounds circulating in the biospehere. We live surrounded by isotopes, in a sea of isotope information, with isotopes appearing even in the alphabetical letters of this text you are reading. The fundamental isotope information actually exists at an extremely fine and detailed level, on an atom-by-atom or "position-specific" basis within molecules (Rossman et al. 1991; Brenna 2001). But this book aims to help you work with a more aggregated isotope language used for ecological work with plants, animals, soils, and gases, so that you can start to write your own isotope ecology stories. Besides the HCNOS elements that are the focus of this book, isotope languages for other elements such

as silicon (Si), calcium (Ca), and iron (Fe) are also being deciphered and read, yielding important results for ecological studies (Clementz et al. 2003; Varele et al. 2004; Basile-Doelsch et al. 2005; Rouxel et al. 2005).

Mixing and Fractionation

The two overarching themes of this book are mixing and fractionation. Chapter 4 outlines a modeling approach that deals with both mixing and fractionation, and Chapters 5 and 6 deal more carefully with mixing dynamics. Mixing is generally very easy to understand: we do this in cooking recipes every day, so it is easy to think about mixing isotope flavors. Mixing combines substances into a homogeneous whole.

But we also turn to the more difficult subject of fractionation, where the effects of an extra neutron are considered more closely. As the chemists and physicists studied isotopes, they found whole ranges of subtle behavior where an extra neutron or two made a detectable difference. For example, it turned out that right at the heart of the chemical universe, in the making and breaking of bonds, isotopes were not behaving exactly alike. Here the emphasis is on *exactly*, because it was very, very close, but still not *exactly*. Where bonds are forged or broken in forward-moving reactions, these slight rate or kinetic differences are important, leading to the important rule that

In kinetic reactions, the light isotopes usually react faster

(Figure 1.6). This differential isotope behavior known as fractionation means isotopes do not react exactly alike, as considered in detail in Chapter 7. In addition to this kinetic rule for reactions that move forward at slower and faster rates, there is also an important equilibrium rule for exchange reactions in which reactions proceed both forwards and backwards, eventually coming to a balanced equilibrium. This second rule is that

In exchange reactions, heavy isotopes concentrate where bonds are strongest.

This differential concentration during exchange reactions is also a type of fractionation. Fractionation is the hidden power controlling isotope distributions on this planet, and the fundamentals of fractionation are in the chemical details.

Today, chemists can calculate maximum potential fractionations for many reactions, but unfortunately these detailed calculations are often of little help in understanding isotope distributions in most biological systems. The detailed chemical understanding is difficult to scale up for complex biological systems, so ecologists have been using isotopes in more empirical ways. Chapter 7 deals with these chemical and ecological approaches to understanding fractionation, why isotopes don't react exactly the same even though they are nearly identical in chemical structure. Most modern isotope studies focus on whole organisms or molecules, but with future technolog-

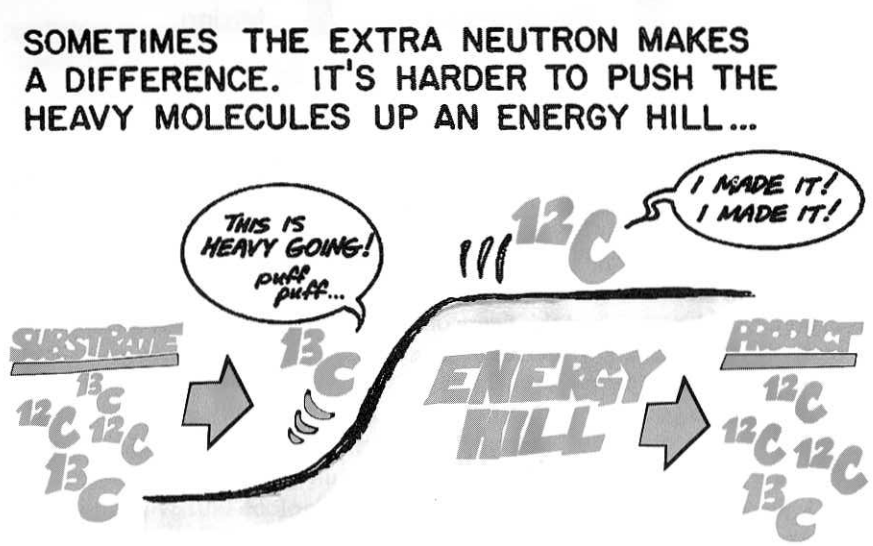


FIGURE 1.6. The extra neutron does make a very slight difference in some reactions; having an extra neutron usually results in slower reactions. This reaction difference is fractionation.

ical advance, the focus may shift downwards to the fundamental atomic level, where fractionation routinely alters isotope compositions of the atoms within molecules.

In reality, fractionation and mixing are just two sides of a coin, with fractionation acting to separate isotopes whereas mixing reunites them. These two processes of fractionation and mixing oppose and complement each other. You can think that these two processes operate separately and independently (Figure 1.7), but in reality they are linked in most contexts (Figure 1.8). They combine to continually recycle isotopes in the natural world.

Many ecologists do not like the complexities of fractionation. But fractionation is perhaps a necessary evil that sets the stage for mixing and tracing. Without fractionation, there would be only a uniform boring distribution of isotopes. Fractionation creates the artist's palette, the isotope colors that are later mixed and arrayed to form the grand isotope masterpieces of nature. Although many scientists think of two source materials uniting to create mixtures, Figure 1.9 also shows that fractionation of the mixture is necessary in the longer term to regenerate the source materials.

Chapter 6 considers how the individual scientist can create her or his own isotope tapestry and experiment. One important aim of these isotope

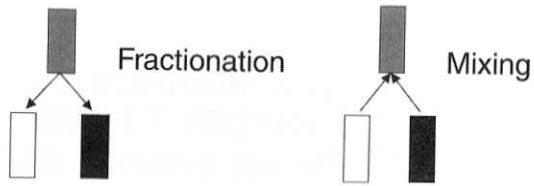


FIGURE 1.7. The two main themes of the book are fractionation and mixing. Fractionation splits apart mixtures to form source materials. These sources recombine via mixing. There is a strong general analogy between isotopes and colors, so that isotopes can be thought of as dyes or tracers. In this example, fractionation separates grey into black and white components, and conversely, black and white mix to form grey. (A color version of this figure on the accompanying CD gives this mixing in terms of blue and yellow sources mixing to form the intermediate green color, and conversely, fractionation regenerates blue and yellow from green.)

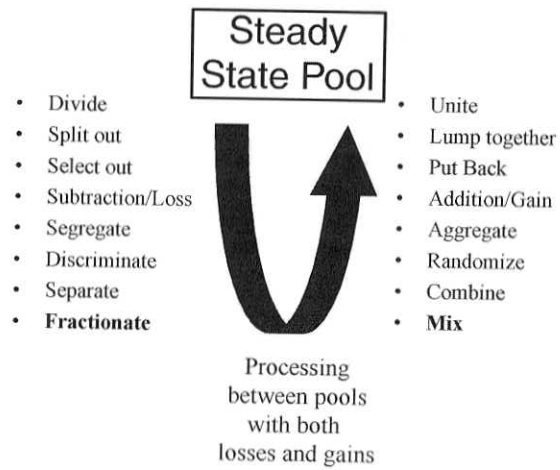


FIGURE 1.8. Fractionation and mixing together control isotope cycling and circulation. There are many words to use when thinking about isotope "fractionation" or "mixing," and as long as you remember that these words do not imply human intervention, control, or intent, most of these words can help you understand isotope cycling.

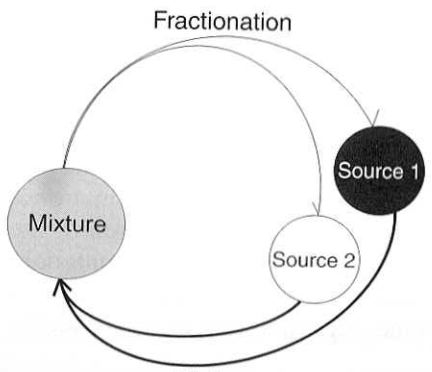


FIGURE 1.9. Isotopes cycle via fractionation and mixing, with fractionation splitting apart mixtures to form source materials. These sources recombine via

addition experiments is to create very strong mixing signals so that fractionation becomes unimportant. To do this, scientists purchase isotope-labeled materials from a normal chemical catalogue, then add the label to a field or laboratory experiment to follow the downstream flow and fates of the tracer and element. In effect, these experiments try to outdo Mother Nature, putting the isotope signals where we humans think they will do most good so that we can see the mixing dynamics. In Chapter 6, we show that these isotope addition experiments are elegant, but not always simple.

Structure of This Book

Chapter 1 introduces stable isotopes and Chapter 2 presents the δ notation used to express isotope values. Chapter 3 reviews how ecologists use stable isotope tracers in modern research. Chapter 4 presents a new modeling approach for combining mixing and fractionation, circulating stable isotopes in any virtual ecological system you care to simulate or create. Chapters 5 and 6 give examples where mixing is the dominant force controlling isotope distributions, with Chapter 5 showing natural examples and Chapter 6 focusing on examples where experimenters add their own tracers. Chapter 7 shows how fractionation works in model systems and in real-world examples. Chapter 8 concludes the main part of the book by illustrating that isotopes are just part of the toolkit ecologists should use when investigating the natural world. The appendix gives the equations used throughout this book. The CD that accompanies this book contains a reading list, cartoons, and figures (many in color), interactive spreadsheet models, problems for the interested student, and answers to these problems. The CD also contains several detailed technical supplements for reference; the interested reader can print these out for use with this book.

Because every aspect of human endeavor has its own drama, you will find quite a few isotope stories throughout the book. These stories are definitely fiction, but build on the principles of mixing and fractionation to illustrate how isotopes move and act in the real world. These story examples are also extended problems, showing how scientific thinking can progress using isotope-based calculations.

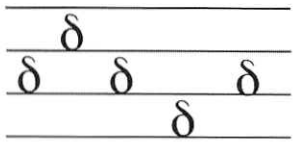
Many of these story examples come equipped with practice spreadsheets on the attached CD. As you read these stories, open the accompanying spreadsheet models on the CD and start to manipulate isotopes for yourself to see what isotopes do in these living illustrations. The combination of stories, spreadsheet illustrations, and problems at the end of each chapter provides an underlying mathematical structure to this book to help readers develop a sound working understanding of stable isotope ecology.

Overall, about half the value of the book lies in reading the text for comprehension, and half in working through problems. If you neglect the simple algebra of the spreadsheets and problems, you will miss much of the point of this book. The point is that the spreadsheet math enables you to easily

use and manipulate isotopes to explore the future, what isotopes might be doing in a system that interests you.

In many ways, this book has a how-to-do-it philosophy, teaching concepts and practice with stable isotopes. You can acquire the important concepts by reading, but also you have to work through problems to gain real mastery of isotope ecology. So I wish you both good reading and good problem-solving as you proceed through this book.

For those scientists who have little time and want the condensed version, reading the following sections will give a good overview: all chapter overviews and summaries, then Sections 1.1, 1.2 (this section), 2.1, 3.1–3.5, 4.1–4.4, 4.7, 5.1–5.5, 5.7, 5.9, 6.1, 7.1, 7.2, 7.5–7.7, and 8.2. These sections comprise about half the book in total. The appendix recapitulates the book in condensed mathematical form, summarizing equations used throughout the book. All mathematics in this book are simple algebra, so that advanced mathematical abilities are not needed for a good understanding of stable isotope ecology.



1.3 Just for Fun—An Isotope Biography of Mr. Polychaete

Sometimes it is good to know more about the author of a book you are reading. Here is a short sketch of my time in Isotopia, looking back through thirty years of isotope grime and fun.

Once upon a time those many years ago, I was an unsuspecting fellow sunnily and blissfully ignorant of all things isotope, just like you are now or like you were once upon a different time. But things began to change one summer on the Texas coast when I started graduate studies. It was a time spent out on the mudflats sifting through the sands looking for wiggly marine worms (yes, now you know what kind of person is writing this). I wasn't looking for worms for fishing, but searching for those polychaete worms because they were so unexpected, so full of color, and so cool to find. That summer, they called me Mr. Polychaete at the student awards festival, a distinction I wore with honor (and looking back, perhaps a true pinnacle of achievement in my life—something else that should warn you about this writing). Anyway, there I was and somehow, through family connections it was, I entered the school chemist's (Pat "The Chief" Parker's) lab, never to truly emerge again. There were these things, isotopes, that no one had ever heard of but were everywhere, all around, like secret writing. Definitely cool. Pick up something and put it in the measurement gizmo and get the secret decoded message, a new way to see the invisible connections out there on the mudflats. Sunburn and isotopes—who could ask for more!

It was a good summer, 1975. I decided to work towards a degree with isotopes in mind. I was driving down the island road that Fall and there was a dead coyote on the road. Road kill you might think and drive right on by. Not me, no sir. I jammed on the brakes and stalked back and darted between the cars to get a sample of coyote hair for isotopes. Yep, nothing could escape the young and budding Isotopeteer: isotopes everywhere, excitement at every turn, every twist in the road, every hair follicle. Glorious! Those singing youngsters on the Mickey Mouse program of my youth were proud "Mouseketeers," and now I had entered a similar exalted realm, that of the flushed "Isotopeteer."

But with every peak there comes a valley. And so it was. Not everything worked out all the time, and there were days of depression. One of my friends took pity on me, and asked what was wrong. I replied that as scientists we were supposed to test things, but sometimes the tests weren't working out. The isotopes were not the eternal key to life. In fact, sometimes they were worthless for finding out important stuff! I had morphed from an "Isotopeteer" into an "Isotopist," pissed off and angry at the isotope betrayal. My friend looked at me thoughtfully, then observed that for many months I had been so enthusiastic there must have been something to all this isotope business, even if it wasn't perfect. He told me to take the long-term view; remember the big picture. You will know that kind of free advice, I'm sure. So I thought and thought and thought some more. And I decided that my friend was right, that I was going to go where the isotopes would lead me, where the isotopes were useful in their own right, and quit trying to stuff them into my preconceived ways of thinking. That way I could sit back and observe the way isotopes were distributed over time and space in a topological manner, and could make grand pronouncements about the space–time continuum as the isotope Einstein. The grand view spread out before me as an "Isotopologist" and I entered another happy time.

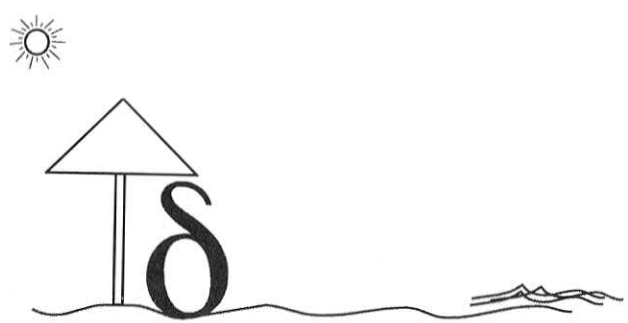
This was a long and fun chapter in my career that took me to topics such as the origins of life, acid rain, and the cycling of organic matter in the sea. I also ran a big isotope lab, with samples sent in from all over the world. I helped scientists interpret their isotope results, and spent many hours imparting isotopological wisdom.

I found that the isotopes gave a curious combination of source and fractionation information, with most scientists wanting to use isotopes as source markers, dyes, or tracers. The idea is that Nature or the experimenter adds a few coded colors somewhere in the system, then tracks the colors, like releasing yellow and blue dyes in two upstream branches of a river, then

seeing which stream contributes more color and water (the source information) and how fast the colors combine as the streams come together (the rate, process, or fractionation information). The demand was to help figure out the source information from the isotopes. With practice, I learned to part the curtains of isotope fractionation and extract the much-sought source information, becoming an "Isotope Sourcerer" magician. (Note: the true English word for a magician is sorcerer, not sourcerer.)

As I grew older, I decided to work on telling the many great isotope stories, imparting sage advice and wisdom about Isotopology and Isotope Sourcery from an ecological perspective. Mr. Polychaete still retains a sense of humor and fun about isotopes. He participates in this book as a wise, wiggly counsel, to help develop your skills with Isotopology and Isotope Sourcery.

Now that you have read this far, take some wise and wiggly advice from Mr. Polychaete and open up the CD that accompanies this book. There you should check out the several color cartoons and figures developed for this introductory Chapter 1. Also try out your new-found isotope knowledge with a set of ten true/false problems. Test yourself and see if you are indeed learning something with all this reading. And while you linger there on the CD, browse around in the various folders, to get an idea of what Mr. Polychaete has planned for you in the rest of this voyage into Isotopia. After your electronic explorations, which should leave you wiser and wigglier, return to the printed text and Chapter 2.



Further Reading

Section 1.1

Bender, M. 1968. Mass spectrometric studies of carbon 13 variations in corn and other grasses. *Radiocarbon* 10:468–472.

Fry, B., W.L. Jeng, R.S. Scalan, P.L. Parker, and J. Baccus. 1978. $\delta^{13}C$ food web analysis of a Texas sand dune community. *Geochimica et Cosmochimica Acta* 42:1299–1302.

Smith, B.N. and S. Epstein. 1971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. *Plant Physiology* 47:380–384.

Wada, E., T. Ando, and K. Kumazawa. 1995. Biodiversity of stable isotope ratios. In E. Wada, T. Yoneyama, M. Minagawa, T. Ando, and B.D. Fry (eds.), *Stable Isotopes in the Biosphere*. Kyoto University Press, Japan, pp. 7–14.

Section 1.2

Anderson, T.F. and M.A. Arthur. 1983. Stable isotopes of oxygen and carbon and their application to sedimentologic and paleaoenvironmental problems. In M.A. Arthur, T.F. Anderson, I.R. Kaplan, J. Veizer, and L.S. Land (eds.), *Stable Isotopes in Sedimentary Geology*. SEPM Short Course #10, Society of Economic Paleontologists and Mineralogists, Dallas, TX, pp. 1–1 to 1–151.

Aston, F.W. 1922. *Isotopes.* E. Arnold & Co., London.

Basile-Doelsch, I., J.D. Meunier, and C. Parron. 2005. Another contintental pool in the terrestrial silicon cycle. *Nature* 433:399–402.

Bigeleisen, J. 1965. Chemistry of isotopes. *Science* 147:463–471.

Boutton, T.W. 1991. Tracer studies with ^{13}C-enriched substrates: Humans and large animals. In D.C. Coleman and B. Fry (eds.), *Carbon Isotope Techniques*. Academic Press, New York, pp. 219–242.

Brenna, J.T. 2001. Natural intramolecular isotope measurements in physiology: Elements of the case for an effort toward high-precision position-specific isotope analysis. *Rapid Communications in Mass Spectrometry* 15:1252–1262.

Broecker, W.S. 1985. *How to Build a Habitable Planet.* Eldigio. Palisades NY.

Broecker, W.S. and T.-H. Peng. 1982. *Tracers in the Sea.* Lamont-Doherty Geological Observatory. Palisades, NY.

Clark, I.D. and P. Fritz. 1997. *Environmental Isotopes in Hydrogeology.* Lewis. Boca Raton, FL.

Clayton, D. 2003. *Handbook of Isotopes in the Cosmos.* Cambridge University Press, New York.

Clementz, M.T., P. Holden, and P.L. Koch. 2003. Are calcium isotopes a reliable monitor of trophic level in marine settings? *International Journal of Osteoarchaeology* 13:29–36.

Criss, R.E. 1999. *Principles of Stable Isotope Distribution.* Oxford University Press, Oxford, UK.

Dahl, P.F. 1997. Flash of the Cathode Rays. Institute of Physics Publishing, Bristol.

Ehleringer, J.R., T.E. Cerling and M.D. Dearing 2005. *A History of Atmospheric CO_2 and Its Effects on Plants, Animals and Ecosystems.* Springer, New York.

Ehleringer, J.R., A.E. Hall, and G.D. Farquhar. 1993. *Stable Isotopes and Plant Carbon–Water Relations* (Physiological Ecology Series of Monographs, Texts, and Treatises). Academic Press, New York.

Faure, G. and T.M. Mensing. 2004. *Isotopes: Principles and Applications.* John Wiley and Sons, New York.

Fischer, H. and K. Wetzel. 2002. The future of ^{13}C-breath tests. *Food and Nutrition Bulletin* 23:53–56.

Griffiths, H. 1997. *Stable Isotopes: Integration of Biological, Ecological and Geochemical Processes (Environmental Plant Biology Series).* BIOS Scientific. Oxford, UK.

Griffiths, I.W. 1997. J.J. Thompson—The centenary of his discovery of the electron and of his invention of mass spectrometry. *Rapid Communications in Mass Spectrometry* 11:1–16.

Hapgood, C.H. 1996. *Maps of the Ancient Sea Kings.* Adventures Unlimited, Kempton IL.

Hoefs, J. 2004. *Stable Isotope Geochemistry*, 5th ed Springer-Verlag, New York.

Kendall, C. and J.J. McDonnell. 1998. *Isotope Tracers in Catchment Hydrology.* Elsevier Health Sciences, New York.

Lajtha, K. and R.H. Michener. 1994. *Stable Isotopes in Ecology and Environmental Science.* Blackwell Scientific Publications, Oxford, UK.

Odum, E.P. 1971. *Fundamentals of Ecology*, 3rd ed, especially pp. 459–464 and references cited there. W.B. Saunders, New York.

Penzias, A.A. 1979. The origin of the elements. *Science* 205:549–554.

Penzias, A.A. 1980. Nuclear processing and isotopes in the galaxy. *Science* 208:663–669.
Rossman, A., M. Butzenlechner, and H.-L. Schmidt. 1991. Evidence for a nonstatistical carbon isotope distribution in natural glucose. *Plant Physiology* 96:609–614.
Rouxel, O.J., A. Bekker, and K.J. Edwards. 2005. Iron isotope constraints on the Archean and Paleoproterozoic ocean redox state. *Science* 307:1088–1091.
Rundel, P.W., J.R. Ehleringer, and K.A. Nagy. 1988. *Stable Isotopes in Ecological Research.* Springer Verlag, New York.
Schell, D.M. 1983. Carbon-13 and carbon-14 abundances in Alaskan aquatic organisms: delayed production from peat in Arctic food webs. *Science* 219:1068–1071.
Varele, D.E., C.J. Pride, and M.A. Brzezinski. 2004. Biological fractionation of silicon isotopes in Southern Ocean surface waters. *Global Biogeochemical Cycles* 18, GB1047, doi:10.1029/2003GB002140.
Wada et al. 1995. Listed above; see Section 1.1 readings.
Wada. E. and A. Hattori. 1990. *Nitrogen in the Sea: Forms, Abundance and Rate Processes.* CRC, Boca, Raton, FL.

2
Isotope Notation and Measurement

Overview

This chapter gives an introduction to isotope notation, calculations, and measurement. The beginner should probably read only the first section, 2.1, then skip on to Chapter 3 which reviews ecological applications of these isotope tracers. Reading Section 2.1 should allow you to understand the rest of the book, and reading the remaining sections 2.2 to 2.4 of this chapter should deepen your understanding as you read the wider isotope literature. There are also three detailed technical supplements for this chapter on the accompanying CD.

2.1. *The Necessary Minimum for Ecologists.* This section introduces the basics of isotope notation and how isotope measurements are made. If you are a beginner, read only this section, then skip the rest of Chapter 2 and go on to Chapter 3. After you finish the book, you can come back and read the rest of Chapter 2, as you have time and interest.

2.2. *Why Use the* δ *Notation?* Isotope values are expressed in the δ notation which turns out to be slightly inexact but convenient notation. This book uses the convenient δ notation, but this section also shows how to convert from the δ notation to exact alternative notations, the ratio (R) notation and the atom percent (AP) notation.

2.3. *Why Is* δ *a Good Substitute for % Heavy Isotope?* For natural samples, δ values are linearly related to % heavy isotope, and this section gives the algebra that explains why this is so.

2.4. δ *and the Ratio-of-Ratios.* If you examine the δ definition carefully, you find a ratio-of-ratios which is often hard to understand when first encountered. This section explains the advantages of a ratio-of-ratios definition.

Technical Supplement 2A. The Atom Percent Notation and Measuring Spiked Samples (see accompanying CD, Chapter 2 folder). Stable isotopes can be separated commercially and added to ecological systems, enriching natural abundances. This section shows that measuring enriched samples

usually requires calculations made with the atom percent notation rather than the δ notation, and can also require some modifications of normal measurement systems.

Technical Supplement 2B. Ion Corrections (see accompanying CD, Chapter 2 folder). Most isotope measurements are made with mass spectrometers that produce a variety of ions from gases such as H_2, N_2, O_2, CO_2, and SO_2. The main ion beams provide the desired isotope information, but are also contaminated by a variety of minor ion products. This section shows some details of how calculations routinely assess and correct for these ion problems.

Technical Supplement 2C. The Ratio Notation and the Power of 1 (see accompanying CD, Chapter 2 folder). Because the light and heavy isotopes of elements react nearly identically, reaction ratios are very close to 1. As it turns out, many special mathematical properties apply to calculations made near the value of 1, making algebraic shortcuts possible and convenient in isotope ratio calculations. The technical supplement also shows the easy algebra for writing exact fractionation equations in ratio notation.

Main Points to Learn. Isotope notation is confusing in many ways, yet simple at its core. The δ values are difference measurements made with respect to recognized standards, and are related in a straightforward, essentially linear way to % heavy isotope. This leads to the rule that the higher the δ value, the greater the amount of heavy isotope, and the lower the δ value, the lower the amount of heavy isotope, or "higher heavier, lower lighter." Most isotope ecology applications use simple addition and subtraction with δ values to understand isotope circulation, but in special cases, using the alternative ratio and atom percent notations becomes important. Currently, mass spectrometer machines developed over the last 85 years routinely measure most isotope values with great precision and accuracy. But fast real-time lasers may eventually replace these expensive machines for isotope measurements as laser technology improves.

2.1 The Necessary Minimum About Isotope Notation and Measurement

Isotope values have their own special notation, the δ notation that signifies difference. The δ values denote a difference measurement made relative to standards during the actual analysis. The isotope compositions of standards are given in Table 2.1, and are used routinely in the calculation of δ values where they appear as the R_{STANDARD} term:

$$\delta^{\text{H}}X = [(R_{\text{SAMPLE}}/R_{\text{STANDARD}} - 1)] * 1000.$$

TABLE 2.1. Isotope Compositions of International Reference Standards.

	Ratio, H/L[a]	Value, H/L[a]	% H	% L
Standard Mean Ocean Water (SMOW)	$^{2}H/^{1}H$	0.00015576	0.015574	99.984426
	$^{17}O/^{16}O$	0.0003799	0.03790	99.76206
	$^{18}O/^{16}O$	0.0020052	0.20004	99.76206
PeeDee Belemnite (PDB) and Vienna-PDB (VPDB)	$^{13}C/^{12}C$	0.011180	1.1056	98.8944
	$^{17}O/^{16}O$	0.0003859	0.0385	99.7553
	$^{18}O/^{16}O$	0.0020672	0.2062	99.7553
Air (AIR)	$^{15}N/^{14}N$	0.0036765	0.36630	99.63370
Canyon Diablo Troilite (CDT) and Vienna-Canyon Diablo Troilite (VCDT)	$^{33}S/^{32}S$	0.0078772	0.74865	95.03957
	$^{34}S/^{32}S$	0.0441626	4.19719	95.03957
	$^{36}S/^{32}S$	0.0001533	0.01459	95.03957

[a] H and L indicate heavy and light isotope components, respectively.

Source: Ratio values are taken from Hayes (2002) for H, C, N, and O isotopes and from Ding et al. (2001) for S isotopes. Historical values for PDB and newer values for VPDB are considered equivalent (based on data in Coplen 1983, 1996), and similarly, historical values for CDT (Coplen and Krouse 1998) and newer values for VCDT also are considered equivalent (Ding et al. 2001). See Ding et al. (2001) and Hayes (2002) for errors associated with the ratio measurements.

In this definition, the δ notation is specified for a particular element (X = H, C, N, O or S), the superscript H gives the heavy isotope mass of that element (^{2}H, ^{13}C, ^{15}N, ^{18}O, or ^{34}S), and R is the ratio of the heavy isotope to the light isotope for the element, $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, or $^{34}S/^{32}S$. The $\delta^{2}H$ measurements are also known as δD, where D stands for deuterium, the heavy stable isotope of hydrogen.

The δ definition involves a final multiplication by 1000, and this multiplication amplifies very small differences measured between samples and standards. Small differences of 1 percent become 10 permil δ units, because of the final multiplication by 1000. Thus, the δ definition makes the small neutron-related isotope differences seem large. The units of δ are "‰" or "permil" (also per mill), from Latin roots for parts per thousand, just as "percent" or "%" is derived from Latin roots for parts per hundred. A sample that measures 10‰ (ten permil) is only 1% (one percent) different than the standard, and even a seemingly large 100‰ difference is still only a 10% difference. Most of us should say "permil" out loud a few times to get familiar with this term, until it begins to sound different than "percent."

Most δ values range between –100 and +50‰ for natural samples, the so-called "natural abundance" range, with the exception that δ measurements for hydrogen span a broader range. Many δ values are negative values, and these negative δ values are usually quite confusing when we first encounter them. It often takes some time before these negative numbers start to seem

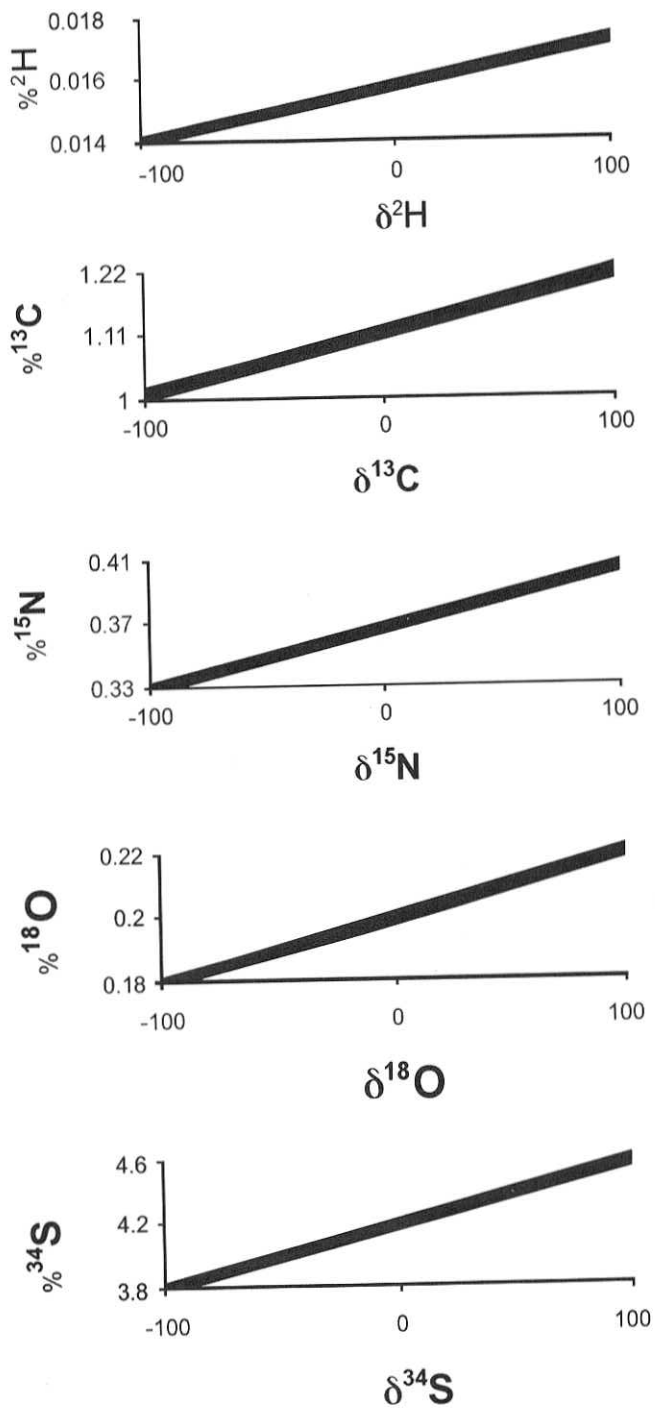
natural and familiar. But negative δ values just indicate relatively less heavy isotope than is present in the standard.

Standards have a δ value of 0‰, which makes sense from the δ definition because when a standard is measured versus itself, the difference will be zero. Standards contain appreciable, nonzero amounts of heavy and light isotopes (Table 2.1), so that 0‰ means no difference from the standard, not "0% isotope," not "no isotope," and not "no heavy isotope."

Samples with higher δ values are relatively enriched in the heavy isotope and are "heavier." Samples with lower δ values are relatively enriched in the light isotope and are "lighter." This leads to the convenient mnemonic for δ values, "higher heavier, lower lighter." Remembering this mnemonic will help when we think about fractionation and how light isotopes react slightly differently than heavy isotopes.

Viewed in a more detailed, technical way, the δ definition actually contains two separate ratios (R_{SAMPLE} and $R_{STANDARD}$) and a ratio-of-ratios, $R_{SAMPLE}/R_{STANDARD}$. This leads many scientists to write about isotope variations in terms of ratios. Although use of the ratios has its advantages, practical use of the δ values does not involve a focus on "ratios." Instead, δ values are straightforward indicators of "% heavy isotope" because there is a simple, essentially linear relationship between δ values and isotope content (Figure 2.1). Thus in this book, the terms "heavier" and "enriched" refer to

FIGURE 2.1. Linear relationships of H, C, N, O, and S heavy isotope contents to δ values. Large natural abundance δ variations from −100 to +100‰ correspond to only slight variations in percent heavy isotope, so that the effect of using δ values is to greatly magnify the small natural differences found in nature. Also, the strong relationships shown between δ values and "% heavy isotope" means that δ values can be used to track heavy isotope dynamics in accounting and budget equations used later in this book for I Chi modeling. Lastly, the range shown here is for natural samples. Isotope can be purchased and added to natural systems, raising values to 1000‰ and above. Outside the natural abundance range, the depicted linear relationships do not hold, and become increasingly curvilinear. Data used for the lines in these graphs were calculated from the definition of δ and using standards listed in Table 2.1, with SMOW used as the standard for oxygen isotopes. The basic equation used for the calculations derives from the δ definition as ${}^{H}AP = 100*(\delta + 1000)/[(\delta + 1000 + (1000/R_{STANDARD})]$ where % heavy isotope is atom % of the heavy isotope, or ${}^{H}AP$. Calculations with this equation were modified for oxygen and sulfur that have more than two stable isotopes, assuming that the minor O and S isotopes were fractionated according to mass-dependent rules (Hulston and Thode 1965; Hoefs 2004; $\delta^{17}O = 0.515*\delta^{18}O$, $\delta^{33}S = 0.515*\delta^{34}S$, and $\delta^{36}S = 1.9*\delta^{34}S$). Depicted best-fit lines reflect natural conditions and have r^2 values of 1.0000. Equations for the lines are as follows: hydrogen ($\%\ ^{2}H = 0.0000156*\delta^{2}H + 0.0155726$), carbon ($\%\ ^{13}C = 0.00109*\delta^{13}C + 1.10559$), nitrogen ($\%\ ^{15}N = 0.000365*\delta^{15}N + 0.366295$), oxygen ($\%\ ^{18}O = 0.000200*\delta^{18}O + 0.200041$), and sulfur ($\%\ ^{34}S = 0.00400*\delta^{34}S + 4.19652$).



samples that have a higher % heavy isotope and higher δ values, whereas "lighter" and "depleted" refer to samples that have lower % heavy isotope and lower δ values.

The % heavy isotope is also termed atom percent, atom %, or abbreviated as ${}^{H}AP$ where the superscript H indicates the heavy isotope. When isotopes are expressed on a percentage basis, it becomes clearer that isotopes are components of total amounts of the HCNOS elements. The isotope percentages partition the larger circulation of an element, and allow a separate subtotal budget within the larger total budget for that element. This subtotal budgeting provides an independent way to view element circulation, an internal isotope audit within the larger element circulation.

Also please note that $\%^{13}C$ and $\%^{15}N$ are not the same as %C and %N. The %C and %N values do not allow separate budgeting of the heavy and light isotopes, and so should not be confused with % heavy isotope, atom %, or ${}^{H}AP$.

One of the most unfortunate aspects of isotope work is that at least three notations are used for isotope accounting. Exact equations for mixing are written in one notation (the atom percent or AP notation, or also the fractional F notation when atom percent values are divided by 100), but exact equations for fractionation are written in terms of ratios (R values, the ratio notation). Combining the exact results from the mixing and fractionation notations requires mathematical dexterity. The third notation, the δ notation, functions as a good compromise. It allows mixing and fractionation calculations with simple algebra, with results that are still accurate at the level of most experimental data. However, this compromise fails for some calculations involving hydrogen isotopes and for samples that have been spiked with heavy isotope tracer. Calculations in this book are done generally with the δ notation, with exact solutions given in the AP and R notations as needed. The next section shows how to convert between these notations, and how to make the exact isotope calculations.

An important notation-related observation for ecologists is that an error term often used with averages, the coefficient of variation or CV, needs to be calculated using the atom % or F notation (see Appendix A.2 in Fry 2003). Use of δ notation for CV calculations (e.g., Lancaster and Waldron 2001) leads to incorrect CV results.

Fractionation occurs during reactions and is commonly denoted by the Greek symbol Δ (Box 2.1). Perhaps the simplest equation of fractionation applies to a reaction where a product is formed from a source material,

$$\delta_{\text{PRODUCT}} = \delta_{\text{SOURCE}} - \Delta,$$

or

$$\Delta = \delta_{\text{SOURCE}} - \delta_{\text{PRODUCT}}.$$

For example, when a plant fixes carbon dioxide during photosynthesis, a fractionation of 20‰ occurs between the source atmospheric CO_2 at –8‰ $\delta\ {}^{13}C$ and the –28‰ plant sugar product that is formed from atmospheric CO_2. The Δ fractionation values are expressed in positive permil units (e.g., Δ = 20‰), and are usually quite similar to the simple difference between two δ values. Box 2.1 considers fractionation definitions and terminology in more detail, as do other authors (Farquhar et al. 1989; Mook 2000; Hayes 2002, 2004).

Box 2.1. What Is a Fractionation Factor?

There are several terms scientists use to denote fractionation factors, and several derivations of these fractionation terms. The following gives a derivation of fractionation factors for common biological reactions that are one-way kinetic reactions, following the derivations presented by Farquhar et al. (1989). For these kinetic reactions, one can think of two rates: one for molecules with the heavy isotope substitution, and one with the more usual light isotopes. These reaction rates or kinetic "k" constants can be designated for the light (L) and heavy (H) isotope molecules as ${}^{L}k$ and ${}^{H}k$. The ratio of rate constants gives the fractionation "alpha" or "$\alpha_{L/H}$" or most simply "α":

$$\alpha = \frac{{}^{L}k}{{}^{H}k}.$$

If there is no effect of the isotope substitution of light for heavy isotopes, then the reaction rates would be equal and α would have a value of 1. But because molecules with a light isotope substitution usually react slightly faster, this ratio is normally slightly greater than 1. A 1% faster reaction of ${}^{L}k$ versus ${}^{H}k$ translates to an α value of 1.01. You might note that if you look at the decimal places following the 1, you can see that a value shows this 1% difference in reaction rates as the fraction .01. To make this fractionation difference easier to see and work with, Δ values are derived from α values:

$$\Delta = (\alpha - 1) * 1000,$$

where Δ gives the fractionation in positive permil (‰) units. Thus, if there is a 1% faster reaction rate for the light-isotope molecules, this is also a 10‰ faster reaction. The α and Δ terms express these differences: α = 1.01, and Δ = 10‰. When scientists talk about isotope fractionation, they commonly use the positive permil units of the Δ values, such as 10‰. Because common parlance favors expressing fractionation in this way, positive Δ values are used in this book.

You should also know that many scientists use alternative definitions of α and Δ. Hydrologists and geochemists especially favor a definition of $\alpha = {}^{H}k/{}^{L}k$ for the common case where the lighter isotope reacts faster so that α is typically less than 1 by this definition, and $(\alpha - 1) * 1000$ is redefined as ε, a negative permil number (Mook 2000). This usage is based on inversions of the terms given above, *H/L* instead of *L/H*. Choosing between *H/L* and *L/H* resembles the disputes of the Lilliputians who fought over which end of the egg is better, the top or bottom. This book adopts the *L/H* formulations that perhaps are less conventional but favored by chemists and some biologists. In case you need them, here are the formulas to convert the hydrological and geochemical H/L fractionations to the L/H fractionations used in this book:

$$\alpha_{L/H} = (1/\alpha_{H/L}) \quad \textit{and} \quad \Delta_{L/H} = (-1000 * \varepsilon_{H/L})/(1000 + \varepsilon_{H/L})$$

or approximately $\Delta_{L/H} = -\varepsilon_{H/L}$ for values in the 0 to 20‰ range. For completeness, and not forgetting hydrologist and geochemist readers of this book, it is also possible to calculate the *H/L* fractionation values from the *L/H* values of this book, using the formulas:

$$\alpha_{H/L} = (1/\alpha_{L/H}) \quad \textit{and} \quad \varepsilon_{H/L} = (-1000 * \Delta_{L/H})/(1000 + \Delta_{L/H}).$$

Lastly, no matter how you define α, Δ, and ε there are occasional "inverse" isotope effects that have the opposite sense of your chosen definition, when the heavy isotope molecules react faster than their light isotope counterparts. We meet one of these inverse cases in Section 7.8.

Here we also consider how the isotope measurements are currently made, usually with an isotope ratio mass spectrometer. But first you collect a plant, animal, soil, or gas sample from nature or the laboratory, then grind, pulverize, and combust your precious sample until it emerges as a simple gas that the isotope machine can conveniently analyze. For this preparation work, you can use an elemental analyzer, a gas chromatograph, or a laser. These different devices plus various combustion interfaces are the front-end engines that convert samples to the common denominator gases. For the HCNOS isotope measurements, these common denominator gases are generally hydrogen (H_2), carbon dioxide (CO_2), nitrogen (N_2), oxygen (O_2), and sulfur dioxide (SO_2). Most ecologists currently use dual CN isotope measurements made with elemental analyzers coupled to mass spectrometers. Technical advances now are allowing triple CNS isotope measurements from the same sample, and future advances will likely allow quadruple HCNS isotope measurements all from the same sample at affordable costs of <$10 U.S. per sample.

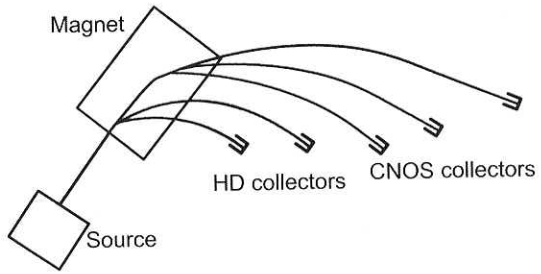


FIGURE 2.2. Schematic of an isotope ratio mass spectrometer used to make isotope determinations. In the source region, gas molecules are ionized as they encounter electrons boiling off a hot filament. The charged ions are accelerated via electric fields through a stainless steel flight tube (not shown) maintained under vacuum. In the central magnetic field, charged ions are separated according to inertia, and dispersed towards collectors for automated counting by computers. Due to their small masses and consequent low inertia, the hydrogen ion beams are sharply bent by magnet focusing. Magnet focusing results in much more gradual bends in flight paths of the ion beam for gases with higher masses, especially N_2, O_2, CO_2, and SO_2.

The mass spectrometer (Figure 2.2) uses carefully calculated physics to make the actual measurement, with the following main steps. Gases first enter a source region where a white-hot filament is boiling off electrons. Close encounters with these electrons are violent, and the sample gas molecules are ionized, losing electrons of their own, and often even fragmenting to simpler molecules. Ionized molecules lacking an electron have a positive charge, and via electric fields, they are accelerated out to the flight tube. The positively charged ions pass through a magnetic field that separates them according to their atomic mass and isotopes, with the resulting ion beams focused into collectors for counting (Figure 2.2).

The main principle of the mass spectrometer separation is inertia, simple inertia. The gas molecules with the extra neutrons require more force to displace them from their flight paths. Thus their flight paths are straighter than those of their lighter-isotope counterparts (Figure 2.2).

In the end, computers tally up the counts from the multiple collectors and calculate the final isotope values. Simple algebra outlined in the appendix gives common ways to recalculate these laboratory results relative to international standards for final use in talks and publication. Technical Supplements 2A and 2B on the accompanying CD give details about how computers actually count the ion beam currents and calculate "raw" or laboratory δ values. These calculations are done routinely by computer software in most modern laboratories, so that most users don't have to bother with these calculations. But these calculations are included in the Technical Supplements for the interested reader.

Mass spectrometers are not the only means of detecting and measuring isotope values. There are recent advances in using lasers to detect isotope values in rapid and precise ways. Lasers detect isotope differences in gas molecules such as CO_2 by tuning to different infrared absorption bands, with heavy-isotope $^{13}CO_2$ absorbing at different wave numbers than light-isotope $^{12}CO_2$. Commercial companies are now marketing laser devices that permit field measurements of isotope values at better than 0.5‰ precision for gases such as CO_2, CH_4, and H_2O (Los Gatos Research, www.LGRinc.com). Many thousands of isotope numbers can be generated each day by lasers to track real-time field experiments, and this new technology contrasts with the slower mass spectrometers that typically produce 50 to 500 values per day in laboratory conditions.

Here are some final notes on terminology. (1) Many ecologists currently write about isotopes in terms of "isotope signatures" or "isotope fingerprints." Although isotopes are often good "descriptors" of processes, and multiple isotope measurements can indicate a fairly distinctive isotope "profile," isotope values rarely provide a truly unique fingerprint. Also, there is usually substantial natural time and space variation for isotope compositions of plants, animals, and soils. A more neutral terminology that recognizes this variability is "isotope values" rather than "isotope signatures." And common parlance favors use of "isotope values" rather than "isotopic values." For these reasons of common parlance and respect for natural variation in isotope compositions, this book consistently uses "isotope values" or "δ values" when discussing stable isotopes. (2) Future isotope scientists may adopt the recent recommendations that ϕ values be substituted for δ values (Brenna et al. 1997; Corso and Brenna 1997). ϕ values are defined in a parallel manner to δ, but with fractional abundance F values substituted for ratio R values:

$$\phi^{H}X = [(F_{\text{SAMPLE}}/F_{\text{STANDARD}} - 1)] * 1000.$$

For most natural samples, the ϕ notation gives nearly identical values to the commonly used δ values, and algebraic calculations are generally both precise and easier with ϕ. However, the ϕ notation is not widely used at present by isotope scientists, and for this reason and for continuity with the published literature, this book uses the traditional δ values.

2.2 Why Use the δ Notation?

There are actually four important notations used in isotope work. These notations are: δ, R, F, and AP or atom %. This section helps you navigate among these notations, with the δ notation providing the starting point. The definition of δ already involves 3 of these notations, δ, R, and F:

$$\delta = [(R_{\text{SAMPLE}}/R_{\text{STANDARD}} - 1)] * 1000,$$

where

$$R = {}^{H}F/{}^{L}F \quad \text{and}$$

F = fractional abundance of the heavy $({}^{H}F)$ or light $({}^{L}F)$ isotope.

The fourth notation is AP = atom percent = $F * 100$. Although it seems a little complex at first, it is actually easy to calculate R, F, and AP from δ using the formulas above and a computer spreadsheet.

Usually, δ suffices because of the close correlations between δ and the other notations (Figures 2.1, 2.3). Because of these correlations, we usually use δ instead of R or F values. But using δ does generate very small errors in calculations of fractionation and mixing and when considering enriched samples. Most of the time we ignore all these small problems, but occasionally we have to fall back from δ to the R, F, and AP values. For mixing, it is exact to use the F values. For fractionation, it is exact to use the R values (which are actually ratios of F values, so using F values is also exactly correct for fractionation calculations). And for some algebra, you have to learn to navigate among all three notations. Here are several examples where the F notation is needed: (1) for some statistics, especially the coefficient of variation, (2) when truly exact values are needed for mixing, (3) when working with hydrogen isotopes that have δ values outside the −100 to +100‰ range,

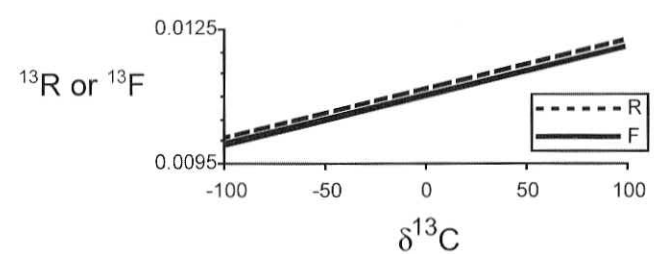


FIGURE 2.3. There is a linear relationship between the three types of isotope notation (δ, R, and F) for natural samples in the −100 to +100‰ δ range. This example shows how R and F are related to δ for carbon isotopes, $\delta^{13}C = [(R_{\text{SAMPLE}}/R_{\text{STANDARD}}) - 1] * 1000$ where $R = {}^{H}F/{}^{L}F = {}^{13}C/{}^{12}C$, ${}^{H}F = {}^{13}C$, ${}^{L}F = {}^{12}C$, the standard is VPDB (see Table 2.1), and $R_{\text{STANDARD}} = {}^{H}F/{}^{L}F_{\text{STANDARD}} = 0.011056/0.988944 = 0.011180$.

and (4) when working with artificially enriched samples that have very high enrichments, >10% heavy isotope. For all these cases, one converts between the notations using the δ definition above. An important final rule is that the most fundamental quantity in these calculations is F or AP.

Calculations based on F or AP are always the correct ones, whereas calculations based on δ and R are sometimes misleading.

Table 2.2 illustrates some of these problems. When the δ notation is used, small errors arise for both mixing and fractionation, especially when δ values differ greatly from the natural abundance range near 0‰. For example, in an added tracer experiment, you might consider a 50/50 mixture from two sources whose δ values were 0 and 601‰. Using the δ notation, the answer for this mixing problem is 300.5‰, but the correct value is actually 299.5‰, obtainable only by basing the calculations on the F notation. This is still a small error of 1‰, but an error that grows larger at higher enrichments.

Errors can also arise during fractionation calculations with δ. For example, in an enrichment experiment where the starting substrate pool is labeled at 1000‰, the actual correct δ value for a product made with a 10‰

TABLE 2.2. Examples of Errors Encountered in Working with the Three Different Isotope Notations, δ, R, and F.[a]

	Results of Mixing or Fractionation			Difference (‰) vs. Correct, F-Based Solution		
	δ	R	F	δ	R	F
A. Mixing, average of two samples, one has δ = 0‰, δ of second sample as follows.						
2nd Sample:						
−100	−50	−50	−50.028	0.028	0.028	0.000
20	10	10	9.999	0.001	0.001	0.000
60	30	30	29.990	0.010	0.010	0.000
190	95	95	94.900	0.100	0.100	0.000
601	300.5	300.5	299.5	1.000	1.000	0.000
B. Fractionation, Δ = 10‰ or α = 1.01 versus the following starting δ value.						
Starting δ value:						
−100	−110	−109	−109	−1.000	0.000	0.000
−50	−60	−59.5	−59.5	−0.500	0.000	0.000
0	−10	−10	−10	0.000	0.000	0.000
50	40	39.5	39.5	0.500	0.000	0.000
100	90	89	89	1.000	0.000	0.000
1000	990	980	980	10.000	0.000	0.000
10000	9990	9890	9890	100.000	0.000	0.000

[a] Results are given as δ values (left four columns), or Δδ values (difference by simple subtraction) versus correct, F-based calculations (right three columns).

Source: Used with permission from Fry, B. 2003. Steady-state models of stable isotope distributions. *Isotopes in Environmental and Health Studies* 39:219–232, Published by Taylor & Francis Ltd, http://www.tandf.co.uk.

fractionation is 980‰, not 990‰. Oddly, the overall effect of working with the δ notation is that fractionation is underestimated at high enrichments, but at enrichments with light isotope that drive values towards no heavy isotope (δ = −1000‰), the opposite effect occurs, and fractionation effects are overestimated with the δ notation. The consequence of all of this is that when working outside the natural abundance range, it is good to check calculations based on δ against calculations based on F. The right answers are the F-based calculations.

Lastly, it is instructive to consider the relative importance of errors in mixing and fractionation, using the −100‰ examples listed at the top of the mixing and fractionation sections in Table 2.2. In these examples, the error for mixing is about 0.0278‰, and the error for fractionation is 1‰, a 36-fold larger error. This large difference in errors is representative for many situations, so that with the δ notation, fractionation calculations have much larger errors than do mixing calculations. For this reason, first efforts to reduce errors should be focused on fractionation terms, and using R notation is often adequate for eliminating major errors. Technical Supplement 2C in the Chapter 2 folder on the accompanying CD shows ways to routinely use the R notation instead of δ notation in detailed isotope fractionation calculations.

You may well ask, given all these uncertainties, why would anyone actually use δ? The answer is this: because it is more convenient, and because all these uncertainties are normally very, very small and safely ignored compared to other normal sources of sampling and measurement error for most HCNOS natural abundance samples. Here is an example that actually converts among all these notations, and looking at such examples has convinced most scientists that it is simpler to use δ.

The problem is this. If the $\delta^{13}C$ value of the atmospheric CO_2 is currently −8‰ versus the VPDB standard, that is, $\delta^{13}C = -8.0‰$, what are the corresponding values for R_{SAMPLE}, ${}^{H}F$, and AP (atom %)? Here is the solution.

To calculate R_{SAMPLE} for carbon isotopes, remember first that the $R_{STANDARD}$ value for VPDB is 0.01118 (from Table 2.1). Rearranging the definition of δ,

$$R_{SAMPLE} = [(\delta/1000)+1]*R_{STANDARD} = [(\delta/1000)+1]*0.01118 = 0.0110906.$$

The next step is to calculate ${}^{13}F$ from R_{SAMPLE} from the δ definition

$${}^{H}F = (\delta+1000)/[(\delta+1000+(1000/R_{STANDARD}))]$$

so that

$${}^{13}F = (\delta+1000)/[(\delta+1000+(1000/0.01118))] = 0.0109689.$$

Calculating atom $\%{}^{13}C$ from ${}^{13}F$ is easy, ${}^{13}AP = \text{Atom } \% = 100*F = 1.09689$.

When you look at these numbers, $\delta = -8‰$, $R_{SAMPLE} = 0.0110906$, $^{13}F = 0.0109689$, and $^{13}AP = 1.09689$, you might agree that to prevent headaches from looking at too many decimal places, it is better to use the δ notation that gives –8‰. But when exactness is truly needed, you should use the R, F, and AP notations.

2.3 Why Is δ a Good Substitute for % Heavy Isotope?

Here we start with the definition of δ and show how it contains a very nearly linear equation for the relationship between δ and the fractional abundance of heavy isotopes. The definition for δ is:

$$\delta = [(R_{SA}/R_{ST}) - 1] * 1000.$$

$R = H/L$ where H is the fraction of heavy isotope and L is the fraction of light isotope. H and L range from 0 to 1. The subscripts SA and ST denote sample and standard, respectively.

The next step is to realize that R_{ST} is a constant. For carbon isotopes, for example, the PDB standard has an R_{ST} value of 0.01118 (Table 2.1), and dividing R_{SA} by this amount yields:

$$\begin{aligned}\delta &= [(R_{SA}/0.01118) - 1] * 1000 = (89.44544 * R_{SA} - 1) * 1000 \\ &= 89{,}445.44 * R_{SA} - 1000.\end{aligned}$$

The third step is to realize that R_{SA} can be rewritten as $H/(1 - H)$, so that $R_{SA} = H/(1 - H)$, and

$$\delta = (89{,}445.44/(1 - H)) * H - 1000.$$

This is still not the equation for a line, and now comes the most nonintuitive part of this derivation. When H varies over a narrow range corresponding to 100‰ or less (for example, H varies from about 0.010 to 0.011 for most natural carbon isotope measurements, a range of 0.001), the denominator term is nearly constant and can be approximated as a constant c. This approximation yields the equation for a linear relationship between δ and H:

$$\delta = (89{,}445.44/c) * H - 1000.$$

Overall, it is the small variation in the $(1 - H)$ term, the term used in the denominator of the ratio $H/(1 - H)$ in the above equations, that accounts for the linear relationships between H and δ. This small variation is relative, and is small only across the relatively "narrow" isotope ranges of 100‰.

Also, although the denominator minimizes impacts of H variations, the numerator vastly multiplies any slight variations in H. The combination of these two factors determines the almost perfect straight-line relationships between δ and H at natural abundance levels.

However, as isotope ranges increase from 100‰ to 1000‰ and beyond, when both natural and isotope-enriched samples are being studied together, the variation in H in the denominator of the term $H/(1 - H)$ cannot be ignored, and the relationship between H and δ is increasingly nonlinear. Technical Supplement 2A on the accompanying CD illustrates this problem that occurs when combining work with two types of samples, natural abundance samples and highly enriched samples. In these cases, it is necessary to convert the δ values to fractional abundances (H values) or $100 * H$ = atom percent so that natural and enriched samples can be compared directly. But for almost any "narrow" range of 100‰ variation, be it at natural abundance or 99% enrichment, the linear relationship between δ and H applies.

2.4 δ and the Ratio-of-Ratios

The δ definition used throughout the isotope world is an odd parameter, because it is calculated from a ratio-of-ratios:

$$\delta = (R_{SAMPLE}/R_{STANDARD} - 1) * 1000,$$

where the ratio-of-ratios is

$$R_{SAMPLE}/R_{STANDARD} = (H_{SAMPLE}/L_{SAMPLE})/(H_{STANDARD}/L_{STANDARD}),$$

where H and L represent the fractions of heavy and light isotope, respectively. Although odd in several ways, this ratio-of-ratios definition has four useful functions.

Foremost, the δ definition expands very small absolute differences in isotope compositions into much larger numbers that typically fall in the –100 to +100‰ range, numbers that are easier to use in everyday communication. And, as it turns out, for narrow ranges such as –100 to +100‰, the relationship between δ and % heavy isotope is almost exactly a straight line (see Figure 2.1). So, in spite of the confusing ratio-of-ratios calculation, δ values give very good approximations of the % heavy isotope and, by difference, also % light isotope in a sample. The conclusion is that δ values can be understood rather simply as percentages of heavy isotope, or atom %.

A second use has to do with the ratio-of-ratios itself, for this "double ratio" is a way to normalize for initial conditions when the standard is the starting point. To see this more clearly, we rearrange

$(H_{SAMPLE}/L_{SAMPLE})/(H_{STANDARD}/L_{STANDARD})$ by multiplying by

$(L_{STANDARD} * L_{SAMPLE})/(L_{STANDARD} * L_{SAMPLE})$ to obtain

$(H_{SAMPLE} * L_{STANDARD})/(H_{SAMPLE} * L_{SAMPLE})$ then restate

$L_{STANDARD}/L_{SAMPLE}$ as $1/(L_{SAMPLE}/L_{STANDARD})$ to obtain

$(H_{SAMPLE}/H_{STANDARD})/(L_{SAMPLE}/L_{STANDARD})$.

In this formulation, one sees that the heavy isotopes and the light isotopes in the sample are both normalized to contents of the standard material (H_{SAMPLE} /$H_{STANDARD}$) and (L_{SAMPLE}/$L_{STANDARD}$), respectively. Using this normalizing ratio-of-ratios, it becomes apparent that deviations from the standard are what count, regardless of the starting composition of the standard. In practice, this gives a common scale to isotope variations for elements such as carbon and hydrogen that differ a great deal in their normal H/L compositions. For example, a 1% deviation from the standard yields the same 10‰ δ value for carbon or hydrogen, even though the heavy isotope contents of standard materials differ more than 70-fold, about 1.1% heavy isotope for carbon and 0.015% heavy isotope for hydrogen. Although it seems odd, the double normalization of heavies and lights via the ratio-of-ratios calculation makes the δ notation flexible and comparable across elements. That is, a 10‰ difference means the same 1% difference for H isotopes as for C isotopes.

A third advantage to using a ratio-based definition of δ can be found in laboratory measurements. Mass spectrometer measurements are typically more precise when a ratio is monitored, rather than just monitoring the light isotope ion beam by itself or the heavy isotope ion beam by itself. Machine noise and fluctuations in the source and magnet focusing affect all ion beams and largely cancel out when multiple ion beams are monitored and used to calculate a ratio.

A last advantage of the ratio-of-ratios lies in consideration of equilibrium reactions, where one is comparing the isotope fluxes in an exchange between two substances. The forward fluxes derive from the reaction rates for the light and heavy isotopes, $L_{FORWARD}$ and $H_{FORWARD}$, and from the reverse fluxes, $L_{REVERSE}$ and $H_{REVERSE}$. The fractionation in the forward direction is $L_{FORWARD}/H_{FORWARD}$ and the fractionation in the reverse reaction is $L_{REVERSE}/H_{REVERSE}$, with the overall fractionation α between two substances in equilibrium given as

$$\alpha = (L_{FORWARD}/H_{FORWARD})/(L_{REVERSE}/H_{REVERSE})$$

or

$$\alpha = (H_{REVERSE}/L_{REVERSE})/(H_{FORWARD}/L_{FORWARD}).$$

This last quantity is again a ratio-of-ratios that can be calculated from δ values of substances participating in the exchange reaction. In effect, the δ values are convenient expressions of the overall isotope fractionation occurring between the two equilibrated substances (see also Section 7.6 in Chapter 7).

In conclusion, using ratios allows tracking of two quantities at once, in this case the differential behavior of the light versus heavy isotopes. Although ratios can be confusing, they are invaluable at many levels for tracking the many isotope twins, triplets, and "multiplets" of the HCNOS elements.

2.5 Chapter Summary

Most ecologists currently do not make isotope measurements themselves, and instead send off samples to specialized laboratories for the analytical work. But it is helpful to have some idea of how the measurements are made in those analytical laboratories, and to understand the notation used in the reports that come back from the isotope labs. This chapter aims to help the novice understand the isotope notations and how isotope measurements are made.

Mass spectrometers currently make most isotope measurements, comparing samples to standards for a difference measurement, or δ value. Most standard materials currently used in isotope work are distributed from the International Atomic Energy Agency in Vienna, Austria. But some standards are free, especially nitrogen and oxygen gas in the atmosphere that provide reference points for $\delta^{15}N$ and $\delta^{18}O$ measurements.

Measurement shows that natural materials can be enriched or depleted in heavy isotopes relative to the standard materials, with positive δ values reflecting enrichment and negative δ values reflecting depletion. It is often confusing for beginners that the isotope values can be positive or negative, but this confusion starts to clear once one remembers that δ values are a difference measurement, not an absolute concentration measurement. With this in mind, it is also good to reiterate that a δ value of zero means no difference from the standard, or the same as the standard, not zero amounts or zero concentrations. All natural samples and standards have appreciable, nonzero isotope concentrations. Negative δ values mean a smaller percentage of heavy isotope than is present in the standard, not negative amounts of isotopes.

The δ difference measurements also have unfamiliar units, units given as ‰, parts per thousand or permil. Note that permil is similar to percent, the difference being that permil denotes parts per thousand whereas percent denotes parts per hundred. Samples that are ten permil different than a standard are one percent different, so that 10‰ = 1%. Most ecologists should say "permil" out loud a few times to get used to these ‰ units used for the δ measurements.

These are basic points for beginners to read about in Section 2.1 of this chapter. More advanced readers can consult the remaining sections of this chapter for details of notation and measurement. Unfortunately, many isotope terms are used differently throughout the literature, but consulting these more advanced sections can help clarify alternative usages and terminologies. Sections 2.2 to 2.4 present some of the philosophy and algebra that underlies the δ scale, and show how to convert to the main alternate notations, the ratio (R) notation and the atom percent (AP) notation. The atom percent notation is generally recommended when dealing with enriched samples, and Technical Supplement 2A on the accompanying CD shows that using the δ notation for enriched samples often will result in errors that can be easily avoided by converting the δ values to atom percent values. Technical Supplement 2B on the accompanying CD shows how mass spectrometer data is used to calculate the δ isotope values. Technical Supplement 2C on the accompanying CD shows some elegant mathematics of the ratio notation, and how to write exact fractionation equations with this notation.

Most of the important information about notation and measurement is contained in Section 2.1, and beginning readers should probably focus on that section. The advice is thus to read Section 2.1 then skip on to Chapter 3, until more advanced information about notation and measurement is needed. The more advanced material is in Sections 2.2 to 2.4 and in Technical Supplements 2A, 2B, and 2C in the Chapter 2 folder on the accompanying CD.

Further Reading

Section 2.1

Avak, H., A. Hilkert A, and R. Pesch. 1996. Forensic studies by EA-IRMS. *Isotopes in Environmental and Health Studies* 32:285–288.

Barrie, A. and S.J. Prosser. 1996. Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry. In T.W. Boutton and S. Yamasaki (eds.), *Mass Spectrometry of Soils.* Marcel Dekker, New York, pp. 1–46.

Brand, W.A. 1996. High precision isotope ratio monitoring techniques in mass spectrometry. *Journal of Mass Spectrometry* 31:225–235.

Boutton, S.W. 1991. Stable carbon isotope ratios of natural materials: I. Sample preparation and mass spectrometric analysis. In D.C. Coleman and B. Fry (eds.), *Carbon Isotope Techniques.* Academic Press, San Diego, CA, pp. 155–172.

Bowling, D.R., S.D. Sargent, B.D. Tanner, and J.R. Ehleringer. 2003. Tunable diode laser absorption spectroscopy for stable isotope studies of ecosystem-atmosphere CO_2 exchange. *Agricultural and Forest Meteorology* 188:1–19.

Brenna, J.T., T.N. Corso, H.J. Tobias, and R.J. Caimi. 1997. High-precision continuous-flow isotope ratio mass spectrometry. *Mass Spectrometry Reviews* 16:227–258.

Coplen, T.B. 1983. Comparison of stable isotope reference standards. *Nature* 302:236–238.

Coplen, T.B. 1996. Ratios for light-element isotopes standardized for better interlaboratory comparion. *EOS* 77:255–256.

Coplen, T.B. and H.R. Krouse. 1998. Sulphur isotope data consistency improved. *Nature* 392:32.

Corso, T.N. and J.T. Brenna. 1997. High-precision position-specific isotope analysis. In *Proceedings of the National Academy of Sciences* 94:1049–1053.

Ding, T., S. Valkiers, H. Kipphardt, P. De Bievre, P.D.P. Taylor, R. Gonfiantini, and R. Krouse. 2001. Calibrated sulfur isotope abundance ratios of three IAEA sulfur isotope reference materials and V-CDT with a reassessment of the atomic weight of sulfur. *Geochimica et Cosmochimica Acta* 65:2433–2437.

Farquhar, G.D., J.R. Ehleringer, and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40:503–537.

Fry, B. 2003. Steady state models of stable isotope distributions. *Isotopes in Environmental and Health Studies* 39:219–232.

Ghosh P. and W.A. Brand. 2003. Stable isotope ratio mass spectrometry in global climate change research. *International Journal of Mass Spectrometry* 228:1–33.

Griffiths, I.W. 1977. J.J. Thompson—the centenary of his discovery of the electron and his invention of mass spectrometry. *Rapid Communications in Mass Spectrometry* 11:1–16.

Hayes, J.M. 2002. Practice and principles of isotopic measurements in organic geochemistry. http://www.nosams.whoi.edu/docs/IsoNotesAug02.pdf

Hayes, J.M. 2004. An introduction to isotopic calculations. http://www.nosams.whoi.edu/docs/IsoCalcs.pdf

Hoefs, J. 2004. *Stable Isotope Geochemistry*, 5th Edition. Springer-Verlag, New York.

Hulston, J.R. and H.G. Thode. 1965. Variations in the S^{33}, S^{34} and S^{36} contents of meteorites and their relation to chemical and nuclear effects. *Journal of Geophysical Research* 70:3475–3484.

Lancaster, J. and S. Waldron. 2001. Stable isotope values of lotic invertebrates: Sources of variation, experimental design, and statistical interpretation. *Limnology and Oceanography* 46:723–730.

Los Gatos Research. www.LGRinc.com.

Mook, W.G. 2000. *Environmental Isotopes in the Hydrological Cycle, Principles and Applications.* Available on-line from http://www.iaea.org/programmes/ripc/ih/volumes/volumes.htm

Mulvaney, R.L. 1993. Mass spectrometry. In R. Knowles and T.H. Blackburn (eds.), *Nitrogen Isotope Techniques.* Academic Press, San Diego, CA, pp. 11–58.

Section 2.2

Fry, 2003. Listed above; see Section 2.1 readings.