

# STABLE ISOTOPES IN ECOSYSTEM STUDIES

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## INTRODUCTION

The use of stable isotopes to solve biogeochemical problems in ecosystem analysis is increasing rapidly because stable isotope data can contribute both source-sink (tracer) and process information. The elements C, N, S, H, and O all have more than one isotope, and isotopic compositions of natural materials can be measured with great precision with a mass spectrometer. Isotopic compositions change in predictable ways as elements cycle through the biosphere. These changes have been exploited by geochemists to understand the global elemental cycles. Ecologists have not until quite recently employed these techniques. The reasons for this are, first, that most ecologists do not have the background in chemistry and geochemistry to be fully aware of the possibilities for exploiting the natural variations in stable isotopic compositions, and second, that stable isotope ratio measurements require equipment not normally available to ecologists. This is unfortunate because some of the more intractable problems in ecology can be profitably addressed using stable isotope measurements. Stable isotopes are ideally suited to increase our understanding of element cycles in ecosystems.

This review is written for ecologists who would like to learn more about how stable isotope analyses have been and can be used in ecosystem studies. We begin with an explanation of isotope terminology and fractionation, then summarize isotopic distributions in the C, N, and S biogeochemical cycles, and conclude with five case studies that show how stable isotope measurements can provide crucial information for ecosystem analysis. We restrict this review to studies of natural variations in C, N, and S isotopic abundances, excluding from consideration  $^{15}\text{N}$  enrichment studies and hydrogen and oxygen isotope studies. Our focus on C, N, and S derives in part from our

experience that combinations of these measurements are often complementary and in part from the need to limit the scope of the review.

## TERMINOLOGY AND MEASUREMENT

Most ecological studies express isotopic compositions in terms of  $\delta$  values, which are parts per thousand differences from a standard:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3, \quad 1.$$

where  $X$  is  $^{13}\text{C}$ ,  $^{15}\text{N}$ , or  $^{34}\text{S}$ , and  $R$  is the corresponding ratio  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$  or  $^{34}\text{S}/^{32}\text{S}$ . The  $\delta$  values are measures of the amounts of heavy and light isotopes in a sample. Increases in these values denote increases in the amount of the heavy isotope components (Figure 1). Conversely, decreases in  $\delta$  values denote decreases in the heavy isotope content, and a reciprocal increase in the light isotope component. Standard reference materials are carbon in the PeeDee limestone, nitrogen gas in the atmosphere, and sulfur from the Cañon Diablo meteorite. The precision of the measurements is typically  $\pm 0.2\text{‰}$  or better, and current analysis costs typically range from \$30–100 per sample through commercial firms.

Many reactions alter the ratio of heavy to light isotopes, or “fractionate” stable isotopes, but the degree of fractionation is typically quite small. Even very large changes of  $100\text{‰}$  (10%) between reactants and products involve only minute absolute changes of 0.04%, 0.11%, and 0.44% for the heavy isotopes of nitrogen, carbon, and sulfur, respectively (Figure 1). A mass spectrometer is required for accurate detection of these small differences and gaseous samples are required for the isotopic determinations.

Sample preparation differs from many other ecological measurements in that quantitative yields (a complete conversion of sample to gas) are required. When yields are not quantitative, isotopic fractionation between product and residual materials may result in a false apparent isotopic composition of the samples. Many combustion schemes have been developed to quantitatively break down diverse molecules into the simple gases most suitable for mass spectrometry. Most laboratories currently employ a high temperature sealed tube combustion to convert carbon and nitrogen compounds to  $\text{CO}_2$  and  $\text{N}_2$  (70).  $\text{N}_2$  can also be prepared from Kjeldahl digestions or ammonia (70). Sulfur-containing materials are typically converted to sulfates and sulfides, which are in turn converted quantitatively to  $\text{SO}_2$  (30, 41, 122). Pure  $\text{CO}_2$ ,  $\text{N}_2$ , and  $\text{SO}_2$  are separated from one another and from water using various cold traps that allow differential volatilization and trapping under high vacuum conditions. A pure gas is then introduced into a dual or triple collector isotope ratio mass spectrometer, and its isotopic composition measured relative to a known standard.

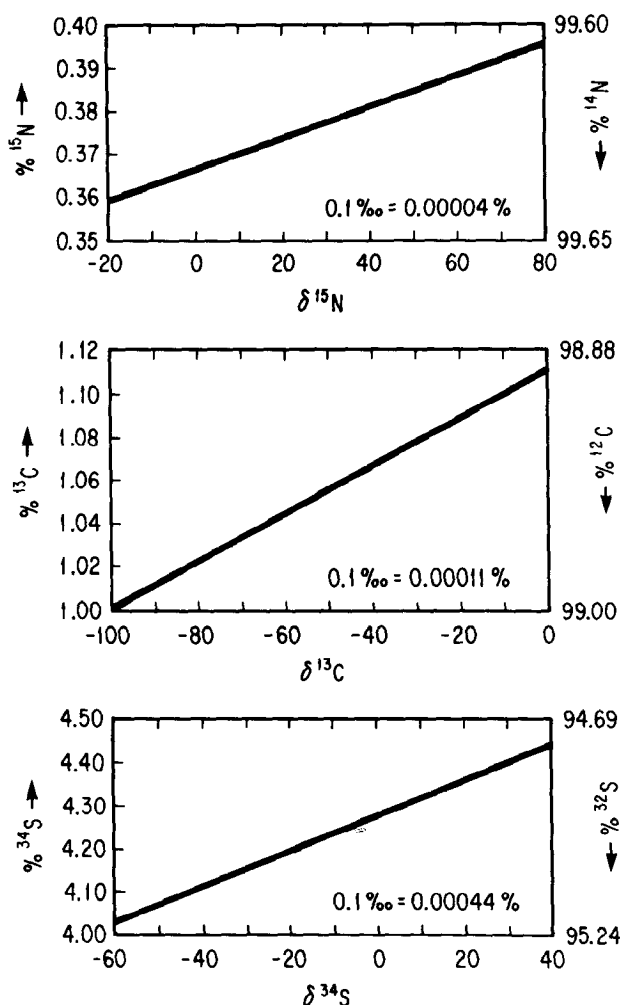


Figure 1 Relationship of N, C, and S stable isotope content to  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values. Large variations of  $100\text{‰}$  correspond to only slight variations in percent  $^{13}\text{C}$ ,  $^{15}\text{N}$  or  $^{34}\text{S}$ . The linear relations shown were derived from simultaneous solution of Equation 1 (text), and the following isotope ratios for standards:  $^{13}\text{C}/^{12}\text{C}_{\text{PDB}} = 0.0112372$ ,  $^{15}\text{N}/^{14}\text{N}_{\text{AIR}} = 0.0036765$  and  $^{34}\text{S}/^{32}\text{S}_{\text{CDT}} = 0.045004$  (15, 51, 77). By definition (Equation 1) standards have  $0\text{‰}$   $\delta$  values.

### Isotopic Fractionation

Stable isotopes record two kinds of information. Where physical and chemical reactions fractionate stable isotopes, the resulting isotopic distributions reflect reaction conditions (process information). Stable isotope distributions also record information about the origins of samples (source

information). The source sets an isotopic baseline that can subsequently be shifted by isotopic fractionation. A well-studied example is carbon isotope fractionation in photosynthesis. A 1974 study showed that terrestrial  $C_3$  plants average  $-27.8\text{‰}$  (115). This was about  $20\text{‰}$  more negative than the source of carbon for plants,  $CO_2$  in air ( $\delta^{13}C_{CO_2} = -7.4\text{‰}$  in 1974; 55). The overall plant isotopic composition thus reflected both source ( $-7.4\text{‰}$ ) and fractionation ( $-20.4\text{‰}$ ) information:  $-27.8_{\text{PLANT}} = -7.4_{\text{SOURCE}} - 20.4_{\text{FRACTIONATION}}$

Isotopic fractionation is a mass-dependent phenomenon. The addition of neutral mass (neutrons) does not alter most aspects of chemical reactivity so that different isotopes of the same element are functionally equivalent in most chemical and biochemical reactions. The slight differences that do occur are measured as a partial isotopic separation or fractionation. In this introduction, we focus on two kinds of isotope effects, equilibrium and kinetic, and on how isotopic fractionation is expressed in ecosystems.

Where chemical exchange occurs between two molecules, small equilibrium isotope effects are common. One example is the exchange of carbon between  $CO_2$  in air and bicarbonate in the ocean (74). Bicarbonate equilibrated with  $CO_2$  in air is enriched in  $^{13}C$  by 10.8 to  $7.4\text{‰}$  relative to the  $CO_2$  at 0–30°C. Under conditions of free exchange, the general rule is that heavy isotopes concentrate in the molecule where bond strengths are greatest, in this case, in bicarbonate ( $HCO_3^-$ ) rather than  $CO_2$ . Equilibrium isotopic fractionations vary in magnitude with temperature and can be predicted from bond strength measurements for reactions involving simple molecules (23, 116).

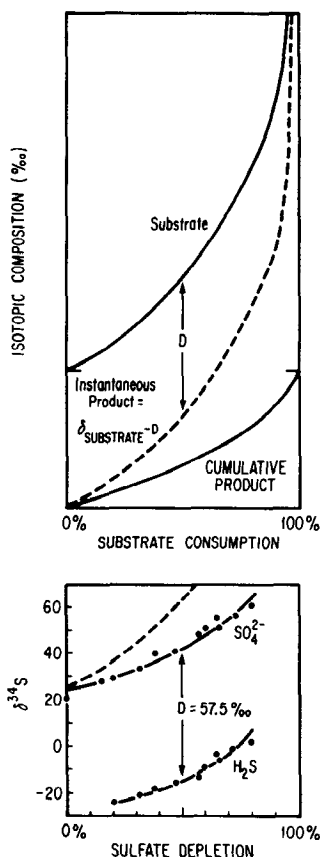
Most biological reactions are more complex than simple equilibrations, and such reactions involve kinetic isotope effects. Kinetic isotope effects are most simply illustrated in physical processes such as diffusion. For example, diffusion of  $^{34}SO_2$  (mass 66) is about 1.6% or  $16\text{‰}$  slower in a vacuum than diffusion of  $^{32}SO_2$  (mass 64). This is because application of the same force displaces the mass 66 sulfur dioxide more slowly than its light-isotope mass 64 counterpart. The  $\text{‰}$  isotope effect or discrimination,  $D$ , ranges from 0 to  $100\text{‰}$  for most kinetic reactions involving C, N, and S stable isotopes. The discrimination ( $D$ ) values are positive in sign when light isotope species react faster than their heavy isotope counterparts and can be closely approximated as the  $\text{‰}$  difference between a substrate and product formed at an instant in time from that substrate (83):

$$D \equiv \delta_{\text{SUBSTRATE}} - \delta_{\text{PRODUCT}}$$

The maximum isotopic discrimination represented by a  $D$  value is an important unit used in modeling isotopic distributions. This maximum discrimination is often not realized, and the degree of observed fractionation depends strongly upon reaction conditions. The simplest case in which full

discrimination occurs in a one-step reaction starting with an essentially unlimited supply of substrate. Under these open system conditions, the substrate and product isotopic compositions are related simply by  $\delta_{\text{PRODUCT}} = \delta_{\text{SUBSTRATE}} - D$ .

A somewhat more complex case occurs in closed systems where a limited amount of substrate is initially available, and this substrate is completely converted to product over time. Closed and partially closed systems include laboratory bottle incubations and some sediments and soils. In closed systems, the substrate gradually increases in heavy isotope content (Figure 2,



**Figure 2** Top: Model of closed system isotopic changes where substrate is converted to two kinds of products—a cumulative product and an instantaneously forming product. Bottom: Isotopic changes in marine sediment core 2092, a partially closed system (43). Sulfides produced from sulfate are an average of 57.5‰ depleted in  $^{34}\text{S}$ . Isotopic values of sulfate increase with depth (sulfate depletion) but not as fast as expected for a 57.5‰/‰ fractionation (dashed line), possibly because of increased diffusion  $^{32}\text{SO}_4^{2-}$  vs.  $^{34}\text{SO}_4^{2-}$  from overlying seawater (50) or sulfide oxidation (2).

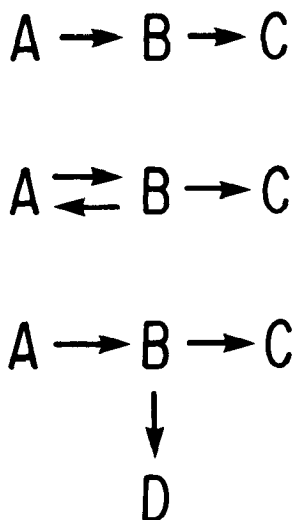
top) due to isotopic fractionation during the formation of heavy-isotope depleted (isotopically light) products. This change in substrate isotopic composition contrasts with open systems where substrate is continually renewed and hence does not change in isotopic composition. The cumulative product in closed systems follows an isotopic trajectory that preserves mass balance as substrate is consumed (Figure 2). As in open systems, however, the product forming at any instant in time differs from the substrate isotopic composition by  $D$  (Figure 2).

The decomposition process of sulfate reduction, for example, occurs in a semi-closed system in marine sediments and results in marked sulfur isotopic changes. As sulfate is consumed in sediments, the  $^{34}\text{S}$  content of the residual sulfate increases from a surface  $\delta^{34}\text{S}$  value near  $+21\text{‰}$  to values  $> +60\text{‰}$  at depth (Figure 2, bottom). A large isotope effect during sulfate reduction (typical  $D = 30$  to  $70\text{‰}$ ; 38) is responsible for this increase, as production of  $^{34}\text{S}$ -depleted sulfides leaves residual sulfate increasingly enriched in  $^{34}\text{S}$ . Marine sediments are not truly closed systems because of continual, albeit slow substrate inputs (50), but models of closed system isotopic behavior are nonetheless useful for obtaining a qualitative understanding of isotopic changes accompanying many diagenetic reactions (67).

This discussion of fractionation has thus far been limited to consideration of single reactions. Multiple coupled reactions, perhaps the common reality in ecosystems, have also been extensively modeled. As examples, we consider three cases: a tightly linked reaction sequence with several enzymatic steps, a two-step reaction in which the first step is reversible, and a branched reaction chain with two products (Figure 3).

In many biological reactions, chemical intermediates are nearly quantitatively converted to a final product in a series of linked, essentially unidirectional enzymatic steps. If fractionation is observed in the final product, this fractionation must have occurred in the first step of the reaction. It is the first step that determines the isotopic input into the reaction chain; by mass balance, the final product, or output, must match in isotopic composition with this input. This finding is a more general formulation of the rule that when reactions go to completion (all substrate is converted to product) there is no opportunity for isotopic fractionation to occur.

A second example concerns a reaction sequence with an early reversible step (Figure 3, middle). This occurs in photosynthetic carbon fixation by  $\text{C}_3$  terrestrial plants (83). The diffusion of  $\text{CO}_2$  from the atmosphere into internal leaf air spaces is reversible, since  $\text{CO}_2$  can diffuse back out to the atmosphere. The observed isotopic fractionation depends in part on this back diffusion, and in part on the carboxylation of  $\text{CO}_2$  into simple plant sugars. Where back diffusion is negligible, the overall fixation reaction is unidirectional into the leaf and all  $\text{CO}_2$  entering the leaf is carboxylated. The only isotope effect expressed in this circumstance is that associated with diffusion ( $D \approx 4.4\text{‰}$ ;



*Figure 3* The outlined reaction sequences contain starting substrate (A), intermediate (B), and product(s) (C & D). Each reaction step could result in isotopic fractionation if measured in isolation, but linking reactions modifies expression of the individual fractionations. In a sequence of tightly linked reactions (top), overall isotopic fractionation is associated with the first step ( $A \rightarrow B$ ). Where a reversible equilibration occurs before a unidirectional reaction (middle), isotopic fractionation depends on the fraction of B converted to C versus the back reaction of B to A. In branched reactions (bottom), the proportion of mass flow diverted from product C to product D strongly influences isotopic compositions of both of these products.

25), the first step in the overall fixation process. The other extreme occurs when carboxylation is much slower than diffusion, and the internal leaf  $\text{CO}_2$  concentration approaches that of the atmosphere. Diffusion no longer controls reaction rates, and a larger carboxylation discrimination of about  $29\text{‰}$  (97) will be expressed. Actual fixation occurs in an intermediate situation where neither diffusion nor carboxylation completely limits the overall fixation rate, and the observed fractionation is intermediate between  $4.4$  and  $29\text{‰}$ , at about  $21\text{‰}$  (25). The reversibility of the diffusional step accounts for this intermediate value; if the flow of  $\text{CO}_2$  were unidirectional into the leaf, only the  $4.4\text{‰}$  diffusional fractionation would be expressed. Reversibility of diffusional fluxes may be important for isotopic fractionation in other uptake reactions involving  $\text{HCO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  etc. To the extent that nutrient uptake steps are not simply unidirectional, the magnitude of isotopic fractionation will reflect the balance in the rates of nutrient fixation and nutrient efflux from the sites of uptake. Very low substrate concentrations or slow diffusion steps often result in small overall fractionations since isotope effects for diffusion of inorganic nutrients in air and water are typically small,  $<5\text{‰}$  (83, 84, 95, 120).

Our photosynthesis example is actually a special case of branched reactions, with back-branching occurring during  $\text{CO}_2$  efflux from the leaf. For branched reactions with one or more side products (Figure 3, bottom), the product isotopic compositions depend not only upon the isotopic discrimination factors for individual steps, but also upon how the mass flow is partitioned between these steps (44). The isotopic compositions of many metabolites such as amino acids, proteins, and lipids are controlled by branched reactions (1, 42, 62). Investigations of branched reaction networks show that isotopic compositions of especially small pools with rapid turnover can vary widely depending on the reactions leading away from these pools. Isotopic compositions record source information most faithfully when there is no fractionation in reactions leading away from or to the pool in question.

In summary, isotopic fractionation in most biochemical reactions arises when similar molecules of slightly different mass react at different rates. Chemical and biochemical studies typically divide reactions into a series of individual steps that, when combined in kinetic models, yield the appropriate fractionation for the overall reaction. These models can be expanded and applied at an ecosystems level to understand fluxes of materials but have not yet been intensively used by ecologists to do so.

## OVERVIEW OF C, N, AND S DISTRIBUTIONS IN NATURE

The natural abundance isotopic compositions of C, N, and S in ecosystems are illustrated in Figures 4–6. Our objective with these figures is to give a comparative overview of C, N, and S stable isotopic distributions at the ecosystem level, rather than to give detailed values for all parts of each cycle. Our values are representative but do not encompass the full spectrum of observed values. Because space prevents us from a detailed treatment, we suggest that interested readers consult the references cited in the figure legends for further information.

### *The Carbon Cycle*

The carbon cycle involves active exchanges of  $\text{CO}_2$  between the atmosphere and both terrestrial ecosystems and the surface ocean (Figure 4). The  $\delta^{13}\text{C}$  value of atmospheric  $\text{CO}_2$  is decreasing in response to inputs of  $^{13}\text{C}$  depleted  $\text{CO}_2$  from fossil fuel plus biomass burning and decomposition (88). Over the past 30 years the decrease may have been almost  $1^\circ/\infty$ . Carbon uptake by the dominant  $\text{C}_3$  plants on land involves a net fractionation of about  $21^\circ/\infty$  between the atmosphere ( $-7^\circ/\infty$ ) and plant biomass ( $-28^\circ/\infty$ ) (17). Carbon uptake by  $\text{C}_4$  plants, mainly tropical and salt grasses, involves a small fractionation of about  $6^\circ/\infty$ . Soil organic matter globally contains several-



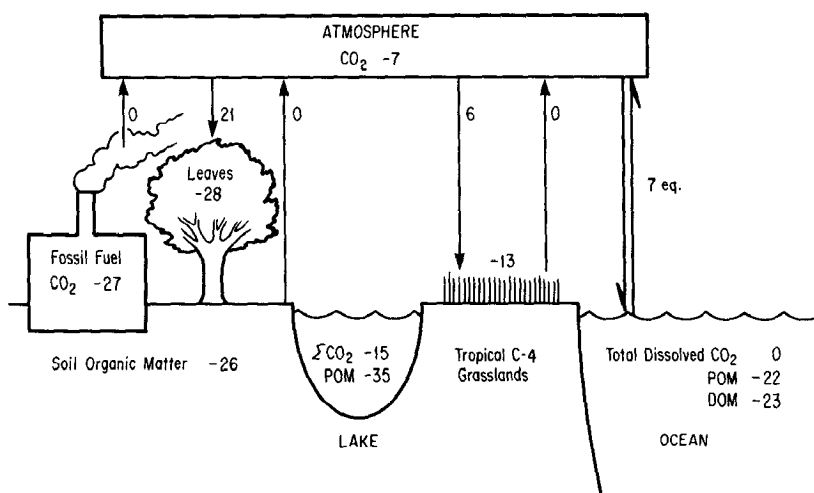


Figure 4 The  $\delta^{13}\text{C}$  distribution in ecosystems. Single arrows indicate  $\text{CO}_2$  fluxes. The double arrow signifies an equilibrium isotope fractionation. Numbers for pools indicate  $\delta^{13}\text{C}$  values (‰) and numbers for arrows indicate the fractionation (D) occurring during transfers. POM = Particulate Organic Matter, DOM = Dissolved Organic Matter. Sources: 5, 16, 17, 35, 83, 104, 121.

fold more carbon than either the atmosphere or living plant biomass and in general is similar or slightly enriched in  $^{13}\text{C}$  in comparison with the dominant vegetation. While either differential preservation or mineralization of soil components with different  $\delta^{13}\text{C}$  values does lead to gradual shifts in soil  $^{13}\text{C}$  content (6, 22), on average there is little fractionation of respired  $\text{CO}_2$  (83).

The exchange of  $\text{CO}_2$  between the atmosphere and the surface of the ocean involves an equilibrium chemical fractionation between atmospheric  $\text{CO}_2$  ( $-7\text{‰}$ ) and the total  $\text{CO}_2$  ( $\Sigma \text{CO}_2$ , mostly bicarbonate) in surface ocean water ( $0\text{‰}$ ) (74). The withdrawal of carbon to form carbonates involves little isotopic fractionation whereas uptake of dissolved inorganic carbon in planktonic photosynthesis involves larger kinetic fractionation that results in algal values of about  $-19$  to  $-24\text{‰}$ . Both the dissolved and the particulate organic matter in the oceans reflect predominantly a marine planktonic origin (121).

The  $^{13}\text{C}$  contents of components of the carbon cycle of fresh waters vary widely depending on the source of dissolved  $\text{CO}_2$  in the waters—from carbonate rock weathering, from mineral springs, from the atmosphere or from respired organic matter. Where respiration inputs are strong,  $\delta^{13}\text{C}$  values for dissolved inorganic carbon may approach  $-20\text{‰}$ , and algae that further fractionate during carbon uptake can measure  $-45\text{‰}$  (93, 94, 99).

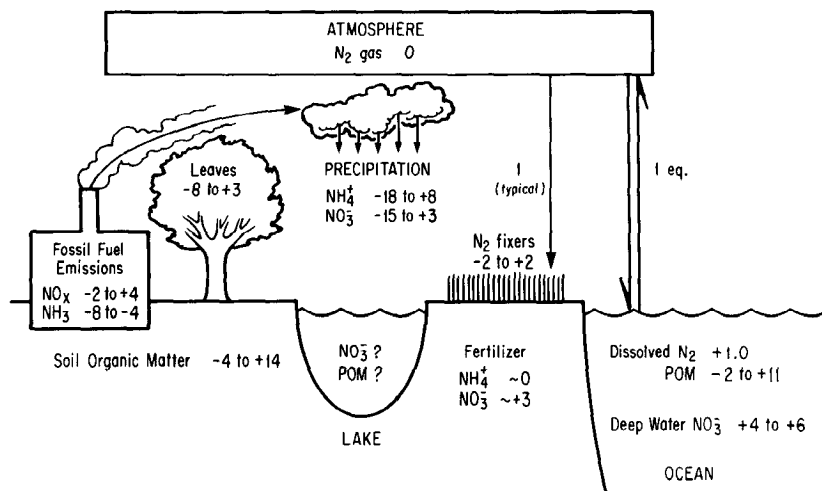


Figure 5 The  $\delta^{15}\text{N}$  distributions in ecosystems. See Figure 4 legend for explanation of symbols. Sources: 26, 27, 14, 48, 52, 60, 63, 71, 75, 76, 106, 108, 109.

## The Nitrogen Cycle

Most nitrogen in the biosphere is present as  $\text{N}_2$  gas in the atmosphere. This massive reservoir is well-mixed with an isotopic composition that is essentially constant at  $0\text{‰}$  (63). Nitrogen in most other parts of the biosphere also has an isotopic composition near this  $0\text{‰}$  value, from  $-10$  to  $+10\text{‰}$  (Figure 5), primarily because the rate of nitrogen supply often limits reactions such as plant growth and bacterial mineralization. Under these conditions all available nitrogen can be consumed without isotopic fractionation. The rates of supply and amounts of substrate N are thus important in understanding nitrogen isotopic distributions.

Some cumulative and large fractionations do occur in the nitrogen cycle. A cumulative faster loss of  $^{14}\text{N}$  than  $^{15}\text{N}$  during particulate N decomposition in soils (66) and in the sea (100) results in  $^{15}\text{N}$  increases of  $5\text{--}10\text{‰}$  with increasing depth. Nitrification and denitrification in the sea both proceed with substantial isotope effects ( $D = 10\text{--}40\text{‰}$ ; 14, 65, 85), and where nitrate is abundant, assimilation by phytoplankton proceeds with a smaller effect ( $D = 4$  to  $6\text{‰}$ ; 3, 119). With two exceptions (24, 86), nitrogen isotopic values in lakes have received little study, although large contrasts might be expected between lakes in which primary production is limited by N (no fractionation by phytoplankton) vs P (abundant N  $\rightarrow$  large fractionations during N uptake by phytoplankton). Where phytoplankton have different  $\delta^{15}\text{N}$  values than terrestrial vegetation, the nitrogen isotopes may function as source markers

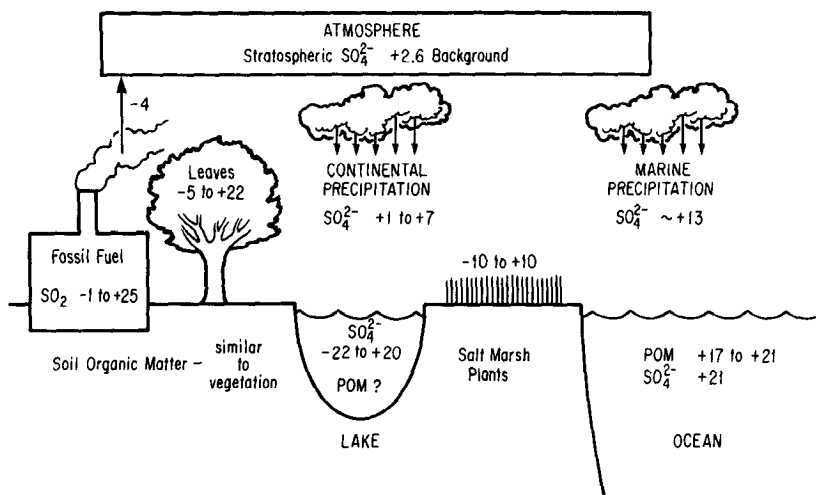


Figure 6 The  $\delta^{34}\text{S}$  distributions in ecosystems. See Figure 4 legend for explanation of symbols. Sources: 8, 9, 13, 46, 52, 53, 54, 58, 77, 79, 91, 101.

for autochthonous and allochthonous organic matter. This approach has been successfully applied in marine environments (90).

There is a wide range reported for nitrogen isotopic values for ammonium and nitrate in precipitation from about  $-20$  to  $+10$ ‰ (Figure 5) (48, 75, 76). Some of the more negative values are related to soil and anthropogenic emissions in highly industrialized areas (26, 27). Further study may show that stable isotope studies are helpful in identifying the sources and fates of N that human activities are currently adding to many forests and lakes.

### The Sulfur Cycle

Sulfate in the ocean is a large well-mixed sulfur reservoir whose isotopic composition is  $21$ ‰ heavier than primordial sulfur in the meteorite standard, Cañon Diablo Troilite (96). Fixation of sulfate by phytoplankton occurs with a small isotope effect ( $D = 1$ – $2$ ‰; 53), but dissimilatory sulfate reduction in marine sediment occurs with a large effect of  $30$ – $70$ ‰ (10, 38). Over geological time, and partially in response to global-scale fluctuations in sulfate reduction activities, the  $\delta^{34}\text{S}$  values of oceanic sulfate have varied from about  $+10$  to  $+33$ ‰ (77). Uplift and preservation of marine sulfides and sulfate-containing evaporites on land have produced a patchwork of terrestrial environments, each with different  $\delta^{34}\text{S}$  values for bedrock sulfur (e.g. 46). Thus, large  $\delta^{34}\text{S}$  ranges must be assigned in general sulfur cycle diagrams (Figure 6). In spite of this, continental vegetation seems to average

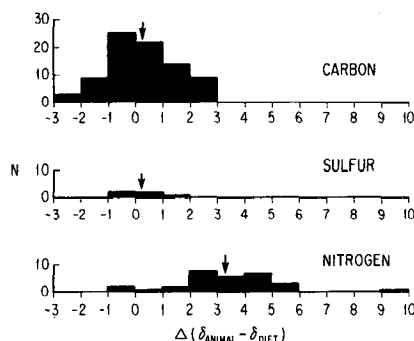
near  $+2$  to  $+6\text{‰}$  over large areas (13) and is quite distinct from the  $\sim +17$  to  $+21\text{‰}$  values of marine plankton and seaweeds.

The stable isotopic composition of sulfur entering the atmosphere can also be quite variable. For instance,  $\text{SO}_2$  emissions from a sour gas plant in Alberta, Canada vary between  $+8$  and  $+25\text{‰}$  (58), but in eastern Canada and the northeastern United States initial studies show average ambient  $\delta^{34}\text{S}$  values of  $0$  to  $2\text{‰}$  for  $\text{SO}_2$  (79, 101). The oxidation of  $\text{SO}_2$  to sulfate occurs with an overall inverse effect ( $D = -4\text{‰}$ ) that favors concentration of the heavy  $^{34}\text{S}$  isotope in the product sulfate; this inverse effect arises from an equilibrium step between  $\text{SO}_2$  and  $\text{HSO}_3^-$  prior to final oxidation to sulfate (101). Oxidation of other sulfur-containing molecules also occurs with small isotope effects,  $D < 5\text{‰}$  (37).

Rainfall sulfate over the open oceans has a significantly lower  $\delta^{34}\text{S}$  value than sea-spray sulfate ( $\sim +13$  vs  $+21\text{‰}$ ; 13), possibly because of slow oxidation of reduced sulfur gases (45). The isotopic compositions of these gases— $\text{H}_2\text{S}$ , carbonyl sulfide and dimethyl sulfide—are poorly known, but further study may clarify the relative contributions of human vs natural sources of these atmospheric sulfur compounds.

### *Stable Isotopes in Animals*

Animals are not represented in our C, N, and S diagrams (Figures 4–6), but they are important components of ecosystems. Animals are similar in isotopic compositions to their diets for carbon and sulfur, but average  $3$  to  $5\text{‰}$  heavier than dietary nitrogen (Figure 7). Diet-switching and turnover experiments for carbon clearly show that the diet is the primary determinant of



**Figure 7** Relationships between animal and diet isotopic compositions for carbon, sulfur and nitrogen stable isotopes. Animals are on average only slightly enriched in  $^{13}\text{C}$  and  $^{34}\text{S}$  by  $0.2\text{‰}$  (have positive  $\Delta$  values) vs their diets (arrows denote averages). A larger  $3.2\text{‰}$  average enrichment occurs for nitrogen isotopes due to excretion of  $^{15}\text{N}$  depleted nitrogen. Sources: 31, 35, 61, 68, 69, 92.

animal isotopic compositions (34, 59, 114). For example, gerbils that were switched from a  $-12.2\text{‰}$  corn diet to a  $-21.8\text{‰}$  wheat-based diet approached the wheat carbon isotopic composition with time (114). This should be true for sulfur as well, and also for nitrogen, with the caveat that animals should be somewhat enriched in  $^{15}\text{N}$  vs the new diet.

The  $^{15}\text{N}$  enrichments vs diet are mainly due to excretion of isotopically light nitrogen in urine. Initial studies with cows, fish, and zooplankton show that animals and feces are enriched in  $^{15}\text{N}$  vs the diet, but urinary nitrogen (both  $\text{NH}_3$  and urea) is depleted in  $^{15}\text{N}$  (11, 12, 69, 73, 109). For example, cow urine is  $-1$  to  $-4\text{‰}$  depleted in  $^{15}\text{N}$  vs diet; cow feces are  $\sim +2\text{‰}$  enriched in  $^{15}\text{N}$ , and milk and blood are  $+4\text{‰}$  enriched in  $^{15}\text{N}$  (109). As they must be to preserve mass balance, the urinary losses of  $^{14}\text{N}$  are thus offset by  $^{15}\text{N}$  enrichments in other nitrogen pools.

When whole food webs are examined, differences between animal processing of C, N, and S isotopes stand out even more clearly. Nitrogen isotopic values increase by 10 to  $15\text{‰}$  in many food webs; these increases are due to the presence of 3 to 5 successive trophic transfers, each of which effectively boosts the  $^{15}\text{N}$  content by 3 to  $5\text{‰}$  (69). The opposite effect—no change with increasing trophic level—is observed for sulfur (68, 92), making the sulfur measurements particularly good indicators of which plant or bacterial food sources are most important for consumers. Carbon appears to be intermediate between N and S, showing modest increases between 0.0 and  $1.0\text{‰}$  per trophic level (19, 35, 36). This small enrichment may be due to carbon isotopic fractionation during assimilation or respiration. The distinct behavior of each isotope allows a cross-referenced trophic structure analysis through combined C, N, and S measurements (32, 91).

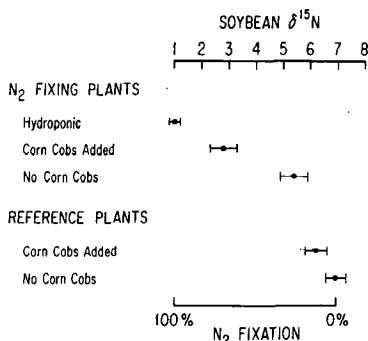
Although diets control the overall isotopic compositions of animals, considerable isotopic variation still exists among different tissues and metabolites within individual animals. The bone protein collagen, for instance, is 2 to  $6\text{‰}$  enriched in  $^{13}\text{C}$  vs the diet, while lipids in fat reserves are 2 to  $8\text{‰}$  depleted in  $^{13}\text{C}$  (87, 117). These enrichments and depletions arise during the many internal enzymatic steps that fractionate stable isotopes after dietary uptake in animals (1, 102, 123). Use of stable isotopes to study diets is based on use of animal tissues that bear a fixed isotopic enrichment or depletion vs the diet. In some cases, whole animals are used, while in others, analyses of muscle or protein fractions have proven adequate indicators of diet. These analyses complement other methods of studying diets in that the stable isotopic compositions of tissues are a measure of the assimilated (not just ingested) diet, reflect both long-term and short-term diets in slow and fast turnover tissues, and provide a unique way to study food webs of the past, detrital food webs, and diets of animals that are hard to observe. Two of our case studies illustrate how stable isotopes can be used to reconstruct diets when other direct dietary information is lacking.

## CASE STUDIES

Rather than attempting an exhaustive review of a rapidly growing field, we have chosen five case studies to illustrate how ecosystems scientists are currently using stable isotopes as natural tracers. These studies show that stable isotopes are being used to indicate sources ( $N_2$  fixation, archaeology, detrital food webs), to help estimate large-scale fluxes (global carbon), and to discern how sulfate loading affects an important decomposition process in lake sediments (acid deposition in lakes). This wide variety of applications is remarkable but represents only a small part of the spectrum of current uses of stable isotopes.

### $\delta^{15}N$ Measures of $N_2$ Fixation

Nitrogen stable isotope measurements can be used to estimate plant fixation of atmospheric  $N_2$ . Plants fixing  $N_2$  from the atmosphere have  $\delta^{15}N$  values of about  $-2$  to  $+2$ ‰, close to the 0‰ value of atmospheric  $N_2$  (107). Plants that cannot fix  $N_2$  from the atmosphere have  $\delta^{15}N$  values that are usually closer to those of total nitrogen in soils and vary widely from  $-8$  to  $+10$ ‰, depending on location (66, 107; K. Nadelhoffer & B. Fry, unpublished). For plants whose nitrogen source is unknown, values close to 0‰ may indicate strong reliance on  $N_2$  derived from the atmosphere (Ndfa), especially if soil organic nitrogen has a  $\delta^{15}N$  value very different from 0‰. An agricultural experiment with soybeans illustrates this approach (Figure 8). Nodulating soybeans grown hydroponically in greenhouse trials had values of  $+1$ ‰ for total plant N, but in field planting experiments, nodulating soybeans had values close to those of reference plants that did not fix  $N_2$  (in this case, the reference plants were soybeans that lacked  $N_2$ -fixing



**Figure 8** The nitrogen stable isotopic composition of nitrogen in soybeans depends on cultivation conditions. Soybean  $\delta^{15}N$  varies according to the fraction derived from the atmosphere vs soils (reference plants). Sources: 56, 57.

nodules). Presumably, the nodulating soybeans derived most of the nitrogen from the soil, as did reference plants. Addition of corn cobs changed these results. Corn cobs provide a rich carbon source for soil microorganisms that deplete available nitrogen in the soil. Enhancement of microbial growth in soils sets up a competition for soil nitrogen stocks so that plants capable of fixing atmospheric  $N_2$  may increasingly do so. The lower  $\delta^{15}N$  values of soybeans grown in corn-cob amended soils are consistent with a greater dependence on atmospheric nitrogen (Figure 8).

This natural abundance method of measuring the fixation of atmospheric nitrogen has been extensively tested against other methods and appears accurate at the 5 to 15% level in many situations (107). Simply collecting leaves of presumed  $N_2$ -fixers and reference plants to estimate in situ patterns of N fixation is a nonmanipulative procedure that has great simplicity and intrinsic appeal for ecosystems studies. Interesting results have been found in comparative field studies. For instance, recent findings show that presumed  $N_2$  fixers can range widely in their reliance on Ndfa in different habitats (108). The extent to which Ndfa enters and contributes to nutrition of neighboring reference plants, and also acts as a source of new nitrogen in ecosystems, has not been fully explored in spite of some initial attempts (7, 64).

### *The Global Carbon Cycle and the CO<sub>2</sub> Problem*

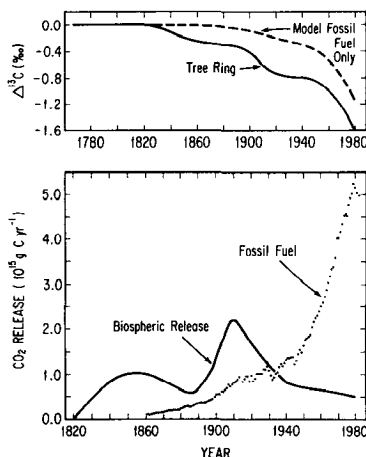
Carbon isotope research is making substantial contributions to understanding the factors controlling the CO<sub>2</sub> balance in the atmosphere. While it is well known that the CO<sub>2</sub> content of the atmosphere has been increasing steadily in recent decades, the relative importance of CO<sub>2</sub> from combustion of fossil fuels and from oxidation of forest and soil organic matter in causing this rise has been debated (47). While the fossil fuel CO<sub>2</sub> emission history is known fairly well, the timing and amount of carbon dioxide releases from burning and decay of terrestrial organic matter is not. However, the addition of terrestrial CO<sub>2</sub> to the atmosphere can be calculated through use of carbon cycle models that incorporate measurements of the time-course and magnitude of the changes in  $^{13}C$  content of atmospheric CO<sub>2</sub> from 1800 to the present.

The approach uses the fact that the addition of large quantities of CO<sub>2</sub> from both fossil fuel combustion ( $\delta^{13}C \sim -27\text{‰}$ ) (110) and oxidation of terrestrial organic matter ( $\delta^{13}C \sim -28\text{‰}$ ) has lowered the  $\delta^{13}C$  values of atmospheric CO<sub>2</sub> ( $\delta^{13}C \sim -7\text{‰}$ ). Since the time course and amount of fossil fuel use are well known (98), it is possible to calculate by difference both the time course and the magnitude of release of terrestrial biomass carbon to the atmosphere. The calculations require both an accurate time course of atmospheric  $\delta^{13}C$  values and the use of models of the carbon cycle

since atmospheric  $\text{CO}_2$  exchanges continuously with oceanic  $\text{CO}_2$  and biospheric carbon.

Carbon isotopes in tree rings have been analyzed as a surrogate to measure trends in  $\delta^{13}\text{C}$  values of atmospheric  $\text{CO}_2$ . While different trees show a wide variability in their isotopic records due to climatic and physiological factors, the grand average of many tree-ring records indicates a  $1.5\text{‰}$  decrease in tree ring  $\delta^{13}\text{C}$  values from 1750 to 1980 (Figure 9) (88). The inference is that atmospheric  $\delta^{13}\text{C}$  values have also gradually declined from 1800 to 1950 and declined more rapidly since 1950. Recent analyses of atmospheric  $\text{CO}_2$  trapped in Antarctic ice cores have supported the approximate timing and magnitude of the atmospheric  $\delta^{13}\text{C}$  changes deduced from tree ring analysis (29). The interesting point is that the initial decline in atmospheric  $\delta^{13}\text{C}$  values was occurring during the 1800s well before the major upswing in fossil fuel use. This has been attributed to a long-term and large-scale release of  $^{13}\text{C}$ -depleted  $\text{CO}_2$  caused by the clearing and burning of forests and oxidation of soil organic matter ( $\delta^{13}\text{C} - 28\text{‰}$ ). The more rapid recent  $\delta^{13}\text{C}$  decrease correlates with the increase in fossil fuel use.

Through use of carbon cycle models, the time course and amount of biospheric carbon release required to account for the observed tree ring  $\delta^{13}\text{C}$  decrease have been computed (88, 89, 110). The calculations are sensitive to small changes in the  $^{13}\text{C}$  trends, but the general conclusions are (a) that



**Figure 9** Top. The changes in  $\delta^{13}\text{C}$  values ( $\Delta$  from historical tree ring baseline) observed in tree rings from 1870 to 1980 (solid line) and the calculated change expected due solely to increased fossil fuel use (dashed line). Note the decline in  $^{13}\text{C}$  (declining  $\Delta$  value) in tree rings prior to the decline expected from fossil fuel use. The difference between the two lines may be due to carbon release from terrestrial biomass and soils accompanying clearing of forests and prairie (88). Bottom. The time course of fossil fuel  $\text{CO}_2$  emission (98) and, using  $\delta^{13}\text{C}$  data, the calculated biospheric  $\text{CO}_2$  emissions from terrestrial soils and forests (88).



carbon input from the terrestrial carbon stocks to the atmosphere was greatest between 1840 and 1940 but (b) that after 1958 fossil fuel became the predominant cause of the atmospheric  $\text{CO}_2$  increase (Figure 9; 88). The total integrated  $\text{CO}_2$  input from each of the two sources until 1980 was about equal, roughly  $140 \times 10^9$  tons from the biosphere and  $170 \times 10^9$  tons from fossil fuels, according to recent model calculations (88). This model allows back calculation of the preindustrial atmospheric  $\text{CO}_2$  level that provides the best fit to both the isotopic trends and the recently measured atmospheric  $\text{CO}_2$  values. The best estimate of 266 ppm is consistent with ice core values of 258 to 289 ppm (28).

This example illustrates the usefulness of the isotopic tracer approach for solving mass balance problems, especially where a well-mixed reservoir such as the global atmosphere can be sampled over time. Alternative methods of calculating the loss of biospheric carbon through analysis of land-use patterns and deforestation are uncertain due to the lack of accurate historical land-use records (49). The existence of two independent methods of computing changes in biospheric carbon stocks ( $\delta^{13}\text{C}$  vs historical land use records) is important considering the urgency of gaining accurate understanding of the controls of atmospheric  $\text{CO}_2$  levels.

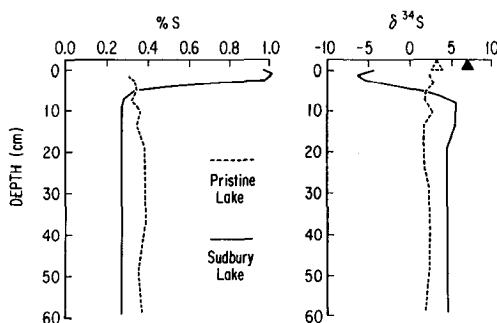
### *Sulfur and Acid Deposition*

Human perturbation of the natural sulfur cycle is pronounced, as the majority of sulfur currently emitted into the atmosphere is probably anthropogenic in origin (4; R. Howarth, personal communication). Anthropogenic sulfur emissions ( $\text{SO}_2$ ,  $\text{SO}_3$ ,  $\text{SO}_4^{2-}$ ) have increased dramatically with industrialization, and these changes have been recorded by changes in the sulfur content of lake sediments. The studies of Canadian lakes by Jerome Nriagu and colleagues illustrate these changes (78, 79, 80, 81).

A very large smelter of copper-nickel sulfide ores located in Sudbury, Ontario, has accounted for about 20% of total Canadian S emissions. Sudbury smelters began operation in about 1889, and sharp increases in the percentage of sulfur in the sediments of nearby lakes have been dated to the 1880–1890 horizons (80). The isotopic composition of the sulfur in lake sediments has also changed since the 1880s (Figure 10). The weighted average  $\delta^{34}\text{S}$  value of sulfur in ores from seven mines in the Sudbury area is  $+2.1\text{‰}$  (range =  $0.5$  to  $7.2\text{‰}$ ; 105), and this is slightly depleted in  $^{34}\text{S}$  relative to sulfur collected from chimney stacks, precipitation, lake water, and older pre-industrial lake sediments that all average about  $4\text{--}5\text{‰}$  in and near Sudbury (78, 79, 80). Simple addition of ore-derived sulfur should only slightly depress  $\delta^{34}\text{S}$  values in the Sudbury area towards the  $+2.1\text{‰}$  average value of ore sulfides, but in fact much larger  $^{34}\text{S}$  depletions of  $-30\text{‰}$  have been found in sulfur from recent lake sediments.

Analysis of the chemical forms of the sulfur in recent sediments provided a key to understanding how additions of sulfur at  $+2$  to  $+5^\circ$  could lead to  $-10$  to  $-30^\circ$  values in surface sediments of lakes. Reduced sulfur species ( $\text{H}_2\text{S}$ ,  $\text{S}^0$ , and pyrite) characteristically formed during dissimilatory sulfate reduction have been found in large quantities in two of the Sudbury lakes (81). Bacterial sulfate reduction in these lake sediments appears to be stimulated by atmospheric sulfate additions; low sulfate levels in lake water normally limit sulfate reduction. Increased sulfate loading stimulates sulfate reduction, increases the production of  $^{34}\text{S}$  depleted sulfides, and since sulfides react with iron and organic matter in sediments, results in decreases in the  $\delta^{34}\text{S}$  value of total sulfur in lake sediments. The decrease in lake sediment  $\delta^{34}\text{S}$  is thus not primarily due to a change in the isotopic composition of source sulfur but rather to changes at the process level as sulfate reduction is stimulated.

As in the preceding example of  $\delta^{13}\text{C}$  and global  $\text{CO}_2$  changes, this sulfur case study shows that isotopic measurements can be used to identify fluxes of anthropogenic pollutants. However, because sulfate concentrations limit an important ecosystem process (sulfate reduction), the sulfur isotopic response is not governed by simple mixing as was the case for anthropogenic  $\text{CO}_2$  additions. Models that relate sulfur isotopic distributions in lake sediments to increased sulfate loading are rudimentary at this time (33), but further development and testing should improve our understanding of long-term changes in both sulfate reduction activities and historical rates of anthropogenic sulfate loading in lakes.



**Figure 10** Sedimentary sulfur profiles differ between a pristine lake in western Ontario and a lake with high sulfate loading near Sudbury, Canada. Triangles denote lake water sulfate  $\delta^{34}\text{S}$  values. The increases in  $\%S$  and decreases in  $\delta^{34}\text{S}$  in the Sudbury lake contrast strongly with the uniform vertical profiles from the pristine lake. Increased sulfate loading from smelter activities at Sudbury has led to production and storage of  $^{34}\text{S}$ -depleted sulfides in lake sediments, causing the recent increases in  $\%S$  and decreases in  $\delta^{34}\text{S}$  of sediments. Sources: 80, 81.

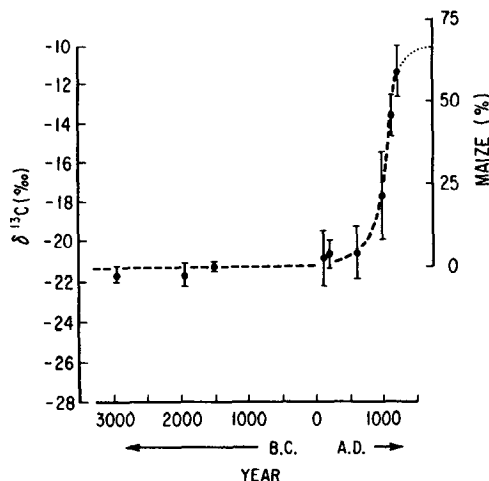


Figure 11 Change in diets of North American Indians are recorded in  $\delta^{13}\text{C}$  contents of the collagen bone protein. Older collagen averages  $-21.5\text{‰}$ , about  $5\text{‰}$  enriched in  $^{13}\text{C}$  relative to common  $\text{C}_3$  plants that average  $-26.5\text{‰}$  (117). With the introduction of maize,  $^{13}\text{C}$  contents rise to  $\sim -11\text{‰}$ , indicating a 60% reliance on this  $\text{C}_4$  species (a 100% dependence would produce a  $-4\text{‰}$   $\delta^{13}\text{C}$  value for collagen). Sources: 116, 117, 107.

## Stable Isotopes in Archaeology

The study of human diets in ancient settings is a key to understanding both cultural evolution and human modification of ancient ecosystems. For example, a civilization that relied heavily on corn was probably organized around farming practices and differed in social structure and land use from a hunter-gatherer civilization. Archaeologists glean evidence about ancient diets from charred remnants of foods and from skeletons. Stable isotopic studies have been used to quantify prehistoric human diets and have proven valuable supplements of the more qualitative visual estimates.

A case in point is the use of bone collagen  $\delta^{13}\text{C}$  to document the transition to maize cultivation by North American Indians (Figure 11). Collagen extracted from Indian bones averaged  $-21$  to  $-22\text{‰}$  from 3000 BC to 500 AD, but underwent a rapid increase in  $^{13}\text{C}$  to  $\sim -11\text{‰}$  by 1300 AD. This increase stems from the advent of widespread maize cultivation by the Upper Mississippian Indians, and the  $^{13}\text{C}$  increase is such that  $\sim 60\%$  of the dietary carbon was derived from maize. This finding is interesting because traces of maize plants were found in earlier sites and might have been judged important. However, the isotopic analyses of collagen from those earlier peoples do not show values expected for a dietary dependence on maize or maize-fed animals. This supports the idea that for these earlier peoples maize was a

minor dietary item whose presence was probably due to an extensive trade network (118).

Subsequent archaeological investigations have been extended to coastal areas where marine and terrestrial foods differ in both C and N stable isotopic compositions, with marine foods being relatively enriched in both  $^{13}\text{C}$  and  $^{15}\text{N}$ . Counterintuitive results have sometimes emerged from those investigations. For example, prehistoric humans living within 1 km or so of coastal waters might be expected to depend heavily on marine foods, but this has not always been the case. Analyses of collagen from skeletons of prehistoric Danish coastal dwellers showed that mesolithic Danes (before 4000 BC) had relatively heavy C and N isotopic compositions ( $\sim -12\text{‰}$   $\delta^{13}\text{C}$ ,  $\sim +14\text{‰}$   $\delta^{15}\text{N}$ ), reflecting a consumption of predominantly marine foods. Postmesolithic Danes had substantially lighter C and N values ( $\sim -20\text{‰}$   $\delta^{13}\text{C}$ ,  $\sim +9\text{‰}$   $\delta^{15}\text{N}$ ; 103, 112). The isotopic shifts near 4000 BC are consistent with shifts from an early subsistence-level marine-based ecology to that involving increased agriculture and trade. Farming and trade items apparently came to constitute the bulk of the diet of even the coastal Danes after about 4000 BC (112).

The use of stable isotopes in anthropology has thus led to a more quantitative understanding of food webs and civilizations from the last 6000 years. Stable isotope studies may be extended to decipher the dietary basis of human subsistence for other areas and times where archaeological evidence is much less extensive. Caution must be exercised in selection of samples and interpretation of results, however, as structural analyses show that collagen in bones slowly degrades and undergoes replacement, sometimes resulting in large C and N isotopic changes (20, 21, 42). Uses of stable isotopes in archaeology have been the subject of two excellent reviews (18, 117).

### *Detrital Organic Matter in Salt Marshes*

The need for chemical tracers of organic matter flow and trophic relationships is greatest in ecosystems dominated by detritus because the origins of detritus can not be determined visually. Tidal salt-marsh estuaries contain vast areas of highly productive salt-marsh grasses, often predominantly *Spartina alterniflora*. Large amounts of detrital *Spartina* become available to the decomposers and detrital food webs, and it has long been thought that export of this detritus is largely responsible for the high secondary productivity of salt-marsh estuaries (82, 113).

One way to test the hypothesis that *Spartina* detritus is the dominant source of organic matter in estuarine waters and for consumers is through stable isotope analysis. *Spartina* is enriched in  $^{13}\text{C}$  ( $\delta^{13}\text{C} -13\text{‰}$ ) relative to either plankton ( $-22\text{‰}$ ) or upland C-3 plants ( $-28\text{‰}$ ). Haines (39) was

the first to point out that seston in tidal creeks at Sapelo Island, Georgia was not isotopically similar to *Spartina* but was in the range expected for organic matter derived from phytoplankton. Subsequent studies of consumers indicated that filter feeders such as the oyster (*Crassostrea virginica*) also had  $\delta^{13}\text{C}$  values more similar to plankton than to *Spartina*. Other consumers such as ribbed mussels had  $\delta^{13}\text{C}$  values intermediate between plankton and *Spartina* (40, 72).

The carbon isotope data provided strong evidence that *Spartina* was probably not the primary source of detritus or of food for filter feeders in Sapelo Island salt-marsh creeks. However, since other sources of organic matter, such as benthic algae ( $-17\text{‰}$ ) or upland organic matter ( $-28\text{‰}$ ) carried by rivers, might be present, the  $\delta^{13}\text{C}$  values could not identify the origin of the detritus unambiguously. This is a common problem of isotopic tracer studies when more than two sources are present and the sample has an intermediate isotopic value.

One way of increasing the ability to identify organic matter sources is to employ simultaneously additional tracers such as sulfur and nitrogen isotopes. In marshes and estuaries, sulfur is especially appropriate because upland plants, marine phytoplankton, and *Spartina* have different  $\delta^{34}\text{S}$  values reflecting their different sources of sulfur. Phytoplankton use seawater sulfate ( $+21\text{‰}$ ), whereas *Spartina* also uses  $^{34}\text{S}$ -depleted sulfides which are produced via sulfate reduction in anoxic sediments (8). Upland plants obtain sulfates from precipitation or weathering and often have  $\delta^{34}\text{S}$  values intermediate between *Spartina* and phytoplankton. A plot of  $\delta^{34}\text{S}$  versus  $\delta^{13}\text{C}$  for organic matter producers in and near Sippewissett salt marsh on Cape Cod illustrates the clear two-dimensional separation (analogous to a two-dimensional chromatograph) that can be achieved in favorable circumstances with this dual isotope approach (Figure 12). Analyses of pooled samples of ribbed mussels from nine locations along an ocean-to-inner marsh transect demonstrated that the isotopic composition of these filter-feeding mussels was a function of location where the organisms grew. Mussels collected near the ocean were isotopically similar to plankton, whereas mussels from the smallest creeks and the marsh surface were isotopically similar to *Spartina*. The food resources available to mussels at various sites in the marsh appeared to be determined in large part by the geometry and physical characteristics of the ecosystem. Transect sampling of stable isotopes in sediments, plants, and animals can be a powerful way to define gradients and boundaries of biogeochemical processes and cycles that are not otherwise apparent.

The multiple isotope approach might be carried further through addition of more isotopes or other types of tracers. For example, if carbon and sulfur

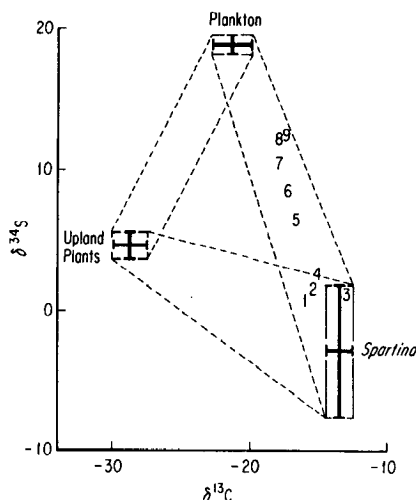


Figure 12 The  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values for upland plants, plankton and *Spartina alterniflora* at Sippewissett salt marsh. The numbers 1–9 indicate the isotopic composition of filter-feeding ribbed mussels, *Geukensia demissa*, collected along a transect from the inner marsh (1–4) to the ocean (9). Source: 91.

isotopes can identify the ultimate sources of the organic matter, the  $\delta^{15}\text{N}$  value could give an estimate of trophic level. At our present level of understanding, there are problems in doing this. For example, there are potential foods in estuaries such as benthic microalgae that have not been well characterized isotopically, and there are sometimes changes in  $\delta^{15}\text{N}$  values of detritus as it ages (124). Nonetheless, the use of multiple tracers has significantly increased our ability to trace organic matter flows in the detrital food webs of marshes and estuaries. Determining the importance of marsh grass production for secondary production in estuaries is critical because of the intense development pressures on coastal ecosystems, and stable isotopic tracer studies can help evaluate these relationships.

## CONCLUSIONS

Stable isotope tracers are already present and circulating in natural systems, and their natural distribution reflects an integrated history of physical and metabolic processes within ecosystems. As our case studies show, the major advantage of the stable isotope approach lies in field studies where measurements of existing isotopic distributions show how components of ecosystems

are connected. Where clear-cutting, fertilization, burning, or other kinds of ecosystem-level perturbations alter flows of elements, stable isotopes can be used to follow these changes and show which processes or components are most sensitive to perturbation.

It is often impossible to judge on theoretical grounds alone whether or not stable isotopes will be useful in solving a particular field research problem. However, by analyzing a few carefully selected samples, one can often determine whether further analyses will contribute significantly to a solution. One initial objective is to determine the signal-to-noise ratio. If isotopic differences between pools are very large and the variation within pools is small, isotopes may provide a very powerful tool, and a few samples may be very effective. For example, it is easy to distinguish between  $C_4$  ( $\delta^{13}C - 13\text{‰}$ ) and  $C_3$  ( $\delta^{13}C - 28\text{‰}$ ) plants using stable C isotopes. On the other hand, it would be very difficult to estimate nitrogen fixation using the  $\delta^{15}N$  technique where soil organic matter is about  $+2\text{‰}$  since atmospheric  $N_2$  is  $0\text{‰}$ . It would be far better to choose an ecosystem where the soil organic matter had  $\delta^{15}N$  values of  $+6$  to  $+10\text{‰}$  which contrast strongly with the atmospheric value.

Measurements of isotopic compositions of standing stocks need to be balanced by process studies that define fractionations under natural conditions in ecosystems; the isotopic compositions of pools are determined not only by inputs but also by any isotopic fractionation occurring during efflux of materials. Precise understanding of isotopic fractionation allows judgment as to when the stable isotopes record source information and when they record process information. It is this combination of source and process information encoded in the natural distributions of stable isotopes that make these measurements valuable independent indicators of C, N, and S cycling in ecosystems.

The specialized chemical knowledge and the sophisticated analytical equipment required for isotope determinations has resulted until now in a partial but real segregation of isotope studies from other ecological investigations. A productive development for the future would be an increasing integration of stable isotope information into a variety of ecosystem studies.

#### ACKNOWLEDGMENTS

The authors thank Luis Cifuentes, Jon Cole, David DeMarais, Marilyn Fogel, Anne Giblin, Robert Howarth, Daniel Kohl, and Georgia Shearer for comments on initial drafts of this manuscript. We also thank the many scientists too numerous to list individually who provided reprints, theses, comments, and ideas for our consideration. This effort was supported by the Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA.



## Literature Cited

1. Abelson, P. H., Hoering, T. C. 1961. Carbon isotope fractionation in formation of amino acids by photosynthetic organisms. *Proc. Natl. Acad. Sci. USA* 47:623-32
2. Aller, R. C. 1986. Anoxic oxidation of sulfides in marine sediments. *Eos* 67:996 (Abstr.)
3. Altabet, M. A., McCarthy, J. J. 1985. Temporal and spatial variations in the natural abundance of  $^{15}\text{N}$  in PON from a warm-core ring. *Deep-Sea Res.* 32:755-72
4. Anderson, J. W. 1978. Sulphur in biology. *Inst. Biol. Stud. Biol. No. 101*. 60 pp.
5. Anderson, T. F., Arthur, M. A. 1983. Stable isotopes of oxygen and carbon and their application to sedimentologic and paleoenvironmental problems. In *Stable Isotopes in Sedimentary Geology*, ed. M. A. Arthur, T. F. Anderson, I. R. Kaplan, J. Veizer, L. S. Land, 1:1-151. Dallas: Soc. Econ. Paleontol. Mineral.
6. Benner, R., Fogel, M., Hodson, R., Sprague, K. 1986. Depletion of  $^{13}\text{C}$  in the lignin fraction of plants: Implications for source identification of detritus. *Eos* 67:990 (Abstr.)
7. Binkley, D., Sollins, P., McGill, W. B. 1985. Natural abundance of nitrogen-15 as a tool for tracing alder-fixed nitrogen. *Soil Soc. Am. J.* 49:444-47
8. Carlson, P. R. Jr., Forrest, J. 1982. Uptake of dissolved sulfide by *Spartina alterniflora*: Evidence from natural sulfur isotope abundance ratios. *Science* 216:633-35
9. Castleman, A. W. Jr., Munkelwitz, H. R., Manowitz, B. 1974. Isotopic studies of the sulfur component of the stratospheric aerosol layer. *Tellus* 26: 222-34
10. Chambers, L. A., Trudinger, P. A. 1979. Microbiological fractionation of stable sulfur isotopes: A review and critique. *Geomicrobiol. J.* 1:249-93
11. Checkley, D. M. Jr., Entzeroth, L. C. 1985. Elemental and isotopic fractionation of carbon and nitrogen by marine, planktonic copepods and implications to the marine nitrogen cycle. *J. Plankton Res.* 7:553-68
12. Checkley, D. M. Jr., Miller, C. A. 1986. Nitrogen isotope fractionation by oceanic zooplankton. *Eos* 67:988 (Abstr.)
13. Chukhrov, F. V., Ermilova, L. P., Churikov, V. S., Nosik, L. P. 1980. The isotopic composition of plant sulfur. *Org. Geochem.* 2:69-75
14. Cline, J. D., Kaplan, I. R. 1975. Isotopic fractionation of dissolved nitrate during denitrification in the eastern tropical north Pacific Ocean. *Mar. Chem.* 3: 271-99
15. Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. *Geochim. Cosmochim. Acta* 12:133-49
16. Degens, E. T. 1969. Biogeochemistry of stable carbon isotopes. In *Organic Geochemistry*, ed. G. Eglington, M. T. J. Murphy, pp. 304-29. Berlin: Springer-Verlag
17. Deines, P. 1980. The isotopic composition of reduced organic carbon. In *Handbook of Environmental Isotope Geochemistry*, Vol. I., ed. P. Fritz, J. Ch. Fontes, pp. 326-406. Netherlands: Elsevier Sci. Publ.
18. DeNiro, M. J. 1987. Stable isotope and archaeology. *Am. Sci.* 75:182-91
19. DeNiro, M. J., Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* 42:495-506
20. DeNiro, M. J., Epstein, S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim. Cosmochim. Acta* 45:341-51
21. DeNiro, M. J., Schoeninger, M. J., Hastorf, C. A. 1985. Effect of heating on the stable carbon and nitrogen isotope ratios of bone collagen. *J. Archaeol. Sci.* 12:1-7
22. Dzurec, R. S., Boutton, T. W., Caldwell, M. M., Smith, B. N. 1985. Carbon isotope ratios of soil organic matter and their use in assessing community composition changes in Curlew Valley, Utah. *Oecologia* 66:17-24
23. Epstein, S., Buchsbaum, R., Lowenstam, H. A., Urey, H. C. 1953. Carbonate-water isotopic temperature scale. *Bull. Geol. Soc. Amer.* 64:1315-26
24. Estep, M. L. F., Vigg, S. 1985. Stable carbon and nitrogen isotope tracers of trophic dynamics in natural populations and fisheries of the Lahontan Lake System, Nevada. *Can. J. Fish. Aquat. Sci.* 42:1712-19
25. Farquhar, G. D., O'Leary, M. H., Berry, J. A. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust. J. Plant Physiol.* 9:121-37
26. Freyer, H. D. 1978. Preliminary  $^{15}\text{N}$



- studies on atmospheric nitrogenous trace gases. *Pageoph* 116:393-404
27. Freyer, H. D. 1978. Seasonal trends of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  nitrogen isotope composition in rain collected at Jülich, Germany. *Tellus* 30:83-92
28. Friedli, H., Moor, E., Oeschger, H., Siegenthaler, U., Stauffer, B. 1984.  $^{13}\text{C}/^{12}\text{C}$  ratios in  $\text{CO}_2$  extracted from Antarctic ice. *Geophys. Res. Lett.* 11: 1145-48
29. Friedli, H., Lotscher, H., Oeschger, H., Siegenthaler, U., Stauffer, B. 1986. Ice core record of the  $^{13}\text{C}/^{12}\text{C}$  ratio of atmospheric  $\text{CO}_2$  in the past two centuries. *Nature* 324:237-38
30. Fritz, P., Drimmie, R. J., Nowicki, V. K. 1974. Preparation of sulfur dioxide for mass spectrometer analyses by combustion of sulfides with copper oxide. *Anal. Chem.* 46:164-66
31. Fry, B. 1981. *Tracing shrimp migrations and diets using natural variations in stable isotopes*. PhD thesis. Univ. Tex., Austin. 225 pp.
32. Fry, B. 1983. Fish and shrimp migrations in the northern Gulf of Mexico analyzed using stable C, N and S isotope ratios. *Fish. Bull. U.S.* 81:789-801
33. Fry, B. 1986. Stable sulfur isotopic distributions and sulfate reduction in lake sediments of the Adirondack Mountains, New York. *Biogeochemistry* 2:329-43
34. Fry, B., Arnold, C. 1982. Rapid  $^{13}\text{C}/^{12}\text{C}$  turnover during growth of brown shrimp (*Penaeus aztecus*). *Oecologia* 54:200-04
35. Fry, B., Sherr, E. B. 1984.  $\delta^{13}\text{C}$  measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contrib. Mar. Sci.* 27:13-47
36. Fry, B., Macko, S. A., Ziemann, J. C. 1987. Review of stable isotopic investigations of food webs in seagrass meadows. In *Proc. Subtropical-Tropical Seagrasses of the Southeastern U.S.*, ed. M. Durako, R. Lewis, R. C. Phillips. *Fla. Mar. Res. Publ.* 42. In press
37. Fry, B., Cox, J., Gest, H., Hayes, J. M. 1986. Discrimination between  $^{34}\text{S}$  and  $^{32}\text{S}$  during bacterial metabolism of inorganic sulfur compounds. *J. Bacteriol.* 165:328-30
38. Goldhaber, M. B., Kaplan, I. R. 1975. Controls and consequences of sulfate reduction rates in recent marine sediments. *Soil Sci.* 119:42-55
39. Haines, E. B. 1977. The origins of detritus in Georgia salt marsh estuaries. *Oikos* 29:254-60
40. Haines, E. B., Montague, C. L. 1979. Food sources of estuarine invertebrates analyzed using  $^{13}\text{C}/^{12}\text{C}$  ratios. *Ecology* 60:48-56
41. Halas, S., Shakur, A., Krouse, H. R. 1982. A modified method of  $\text{SO}_2$  extraction from sulphates for isotopic analysis using  $\text{NaPO}_3$ . *Isotopenpraxis* 18:433-35
42. Hare, P. E., Estep, M. L. F. 1983. Carbon and nitrogen isotopic composition of amino acids in modern and fossil collagens. *Carnegie Inst. Wash. Yrbk.* 1982-1983 410-14
43. Hartmann, V. M., Nielsen, H. 1969.  $\delta^{34}\text{S}$ -werte in rezenten meeresedimenten und ihre deutung am beispiel einiger sedimentprofile aus der westlichen ostsee. *Geol. Rund.* 58:621-55
44. Hayes, J. M. 1983. Practice and principles of isotopic measurements in organic geochemistry. In *Organic Geochemistry of Contemporaneous and Ancient Sediments*, ed. W. G. Meinschein, 5:1-31. Bloomington, Ind: Great Lakes Sect., Soc. Econ. Paleontol. Mineral.
45. Hitchcock, D. R., Black, M. S. 1984.  $^{34}\text{S}/^{32}\text{S}$  evidence of biogenic sulfur oxides in a salt marsh atmosphere. *Atmos. Environ.* 18:1-17
46. Hitchon, B., Krouse, H. R. 1972. Hydrogeochemistry of the surface waters of the Mackenzie River drainage basin, Canada-III. Stable isotopes of oxygen, carbon and sulphur. *Geochim. Cosmochim. Acta* 36:1337-57
47. Hobbie, J. E., Cole, J., Dungan, J., Houghton, R. A., Peterson, B. J. 1984. The controversy on the role of the biota in the global  $\text{CO}_2$  balance. *BioScience* 34:492-498
48. Hoering, T. 1957. The isotopic composition of the ammonia and the nitrate ion in rain. *Geochim. Cosmochim. Acta* 12:97-102
49. Houghton, R. A., Hobbie, J. E., Melillo, J. M., Moore, B., Peterson, B. J., et al. 1983. Changes in the carbon content of terrestrial biota and soils between 1860 and 1980: A net release of  $\text{CO}_2$  to the atmosphere. *Ecol. Monogr.* 53:235-62
50. Jorgensen, B. B. 1979. A theoretical model of the stable sulfur isotope distribution in marine sediments. *Geochim. Cosmochim. Acta* 43:363-74
51. Junk, G., Svec, H. 1958. The absolute abundance of the nitrogen isotopes in the atmosphere and compressed gas from various sources. *Geochim. Cosmochim. Acta* 14:234-43
52. Kaplan, I. R. 1983. Stable isotopes of sulfur, nitrogen and deuterium in recent marine environments. In *Stable Isotopes in Sedimentary Geology*, ed. M. A. Arthur, T. F. Anderson, I. R. Kaplan, J.

- Veizer, L. S. Land, 2:1-108. Dallas: Soc. Econ. Paleontol. Mineral.
53. Kaplan, I. R., Rittenberg, S. C. 1964. Microbiological fractionation of sulphur isotopes. *J. Gen. Microbiol.* 34:195-212
  54. Kaplan, I. R., Emery, K. O., Rittenberg, S. C. 1963. The distribution and isotopic abundance of sulphur in recent marine sediments off southern California. *Geochim. Cosmochim. Acta* 27: 297-331
  55. Keeling, C. D., Mook, W. G., Tans, P. P. 1979. Recent trends in the  $^{13}\text{C}/^{12}\text{C}$  ratio of atmospheric carbon dioxide. *Nature* 277:121-23
  56. Kohl, D. H., Shearer, G. 1980. Isotopic fractionation associated with symbiotic  $\text{N}_2$  fixation and uptake of  $\text{NO}_3^-$  by plants. *Plant Physiol.* 66:51-56
  57. Kohl, D. H., Shearer, G., Harper, J. E. 1980. Estimates of  $\text{N}_2$  fixation based on differences in the natural abundance of  $^{15}\text{N}$  in nodulating and nonnodulating isolines of soybeans. *Plant Physiol.* 66:61-65
  58. Krouse, H. R. 1980. Sulphur isotopes in our environment. In *Handbook of Environmental Isotope Geochemistry*. Volume 1, ed. P. Fritz, J. Ch. Fontes, pp. 435-72. Netherlands: Elsevier Sci. Publ.
  59. LaCroix, M., Mosora, F. 1975. Variations du rapport isotopique  $^{13}\text{C}/^{12}\text{C}$  dans le métabolisme animal. In (Isotope Ratios as Pollutant Source and Behaviour Indicators.) Atomic Energy Agency, pp. 343-58. Vienna
  60. Libes, S. M. 1983. *Stable isotope geochemistry of nitrogen in marine particulates*. PhD thesis. Woods Hole Oceanogr. Inst./MIT. WHOI-83-9, 288 pp.
  61. Macko, S. A., Lee, W. Y., Parker, P. L. 1982. Nitrogen and carbon isotope fractionation by two species of marine amphipods: Laboratory and field studies. *J. Exp. Mar. Biol. Ecol.* 63:145-49
  62. Macko, S. A., Estep, M. L. F., Hare, P. E., Hoering, T. C. 1983. Stable nitrogen and carbon isotopic composition of individual amino acids isolated from cultured microorganisms. *Carnegie Inst. Wash. Yrbk* 1982-1983, 404-10
  63. Mariotti, A. 1983. Atmospheric nitrogen is a reliable standard for natural  $^{15}\text{N}$  abundance measurements. *Nature* 303:685-87
  64. Mariotti, A. J., Mariotti, F., Amarger, N. 1983. Use of natural  $^{15}\text{N}$  abundance in the measurement of symbiotic fixation. In *Nuclear Techniques in Improving Pasture Management*, 61-77. Vienna: Int. Atomic Energy Agency
  65. Mariotti, A., Lancelot, C., Billen, G. 1984. Natural isotopic composition of nitrogen as a tracer of origin for suspended organic matter in the Scheldt estuary. *Geochim. Cosmochim. Acta* 48:549-55
  66. Mariotti, A., Pierre, D., Vedy, J. C., Bruckert, S., Guillemot, J. 1980. The abundance of natural nitrogen 15 in the organic matter of soils along an altitudinal gradient. *Catena* 7:293-300
  67. Mariotti, A., Germon, J. C., Hubert, P., Kaiser, P., Letolle, R., et al. 1981. Experimental determination of nitrogen kinetic isotopic fractionation: Some principles; illustration for the denitrification and nitrification processes. *Plant Soil* 62:413-30
  68. Mekhtiyeva, V. L., Pankina, R. G., Gavrilov, Y. Y. 1976. Distributions and isotopic compositions of forms of sulfur in water animals and plants. *Geochem. Int.* 13:82-87
  69. Minagawa, M., Wada, E. 1984. Step-wise enrichment of  $^{15}\text{N}$  along food chains: Further evidence and the relation between  $\delta^{15}\text{N}$  and animal age. *Geochim. Cosmochim. Acta* 48:1135-40
  70. Minagawa, M., Winter, D. A., Kaplan, I. R. 1984. Comparison of Kjeldahl and combustion methods for measurement of nitrogen isotope ratios in organic matter. *Anal. Chem.* 56:1859-61
  71. Miyake, Y., Wada, E. 1967. The abundance ratio of  $^{15}\text{N}/^{14}\text{N}$  in marine environments. *Rec. Oceanogr. Works, Jpn* 9:37-53
  72. Montague, C. L., Bunker, S. M., Haines, E. B., Pace, M. L., Wetzell, R. L. 1981. Aquatic macroconsumers. In *The Ecology of a Salt Marsh*, ed. L. R. Pomeroy, R. G. Wiegert, pp. 69-85. New York, Springer-Verlag, 271 pp.
  73. Montoya, J. P., Horrigan, S. G. 1986. Rapid changes in the natural abundance of  $^{15}\text{N}$  in plankton and dissolved nitrogen in the Chesapeake Bay. *Eos* 67:988 (Abstr.)
  74. Mook, W. G., Bommerson, J. C., Staverman, W. H. 1974. Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. *Earth Planet. Sci. Lett.* 22:169-76
  75. Moore, H. 1974. Isotopic measurement of atmospheric nitrogen compounds. *Tellus* 26:169-74
  76. Moore, H. 1977. The isotopic composition of ammonia, nitrogen dioxide and nitrate in the atmosphere. *Atmos. Environ.* 11:1239-43
  77. Nielsen, H. 1978. Sulfur Isotopes in

- nature. In *Handbook of Geochemistry* II-2, ed. K. H. Wedepohl, 16B:1-40. Springer-Verlag, Berlin
78. Nriagu, J. O., Harvey, H. H. 1978. Isotopic variation as an index of sulphur pollution in lakes around Sudbury, Ontario. *Nature* 273:223-24
79. Nriagu, J. O., Coker, R. D. 1978. Isotopic composition of sulphur in atmospheric precipitation around Sudbury, Ontario. *Nature* 274:883-85
80. Nriagu, J. O., Coker, R. D. 1983. Sulphur in sediments chronicles past changes in lake acidification. *Nature* 303:692-94
81. Nriagu, J. O., Soon, Y. K. 1985. Distribution and isotopic composition of sulfur in lake sediments of northern Ontario. *Geochim. Cosmochim. Acta* 49:823-34
82. Odum, E. P., de la Cruz, A. A. 1967. Particulate organic detritus in a Georgia salt marsh-estuarine ecosystem. In *Estuaries*, ed. G. H. Lauff, 83:383-88. AAAS Publ. New York
83. O'Leary, M. H. 1981. Carbon isotope fractionation in plants. *Phytochemistry* 20:553-67
84. O'Leary, M. H. 1984. Measurement of the isotope fractionation associated with diffusion of carbon dioxide in aqueous solution. *J. Phys. Chem.* 88:823
85. Owens, N. J. P. 1985. Variations in the natural abundance of  $^{15}\text{N}$  in estuarine suspended particulate matter: A specific indicator of biological processing. *Estuarine, Coastal Shelf Sci.* 20:505-10
86. Pang, P. C., Nriagu, J. O. 1977. Isotopic variations of the nitrogen in Lake Superior. *Geochim. Cosmochim. Acta* 41:811-14
87. Parker, P. L. 1964. The biogeochemistry of the stable isotopes of carbon in a marine bay. *Geochim. Cosmochim. Acta* 28:1155-64
88. Peng, T.-H., Freyer, H. D. 1986. Revised estimates of atmospheric  $\text{CO}_2$  variations based on the tree-ring  $^{13}\text{C}$  record. In *The Changing Carbon Cycle: A Global Analysis*, ed. J. R. Trabalka, D. E. Reichle, pp. 151-59. New York: Springer-Verlag
89. Peng, T.-H., Broecker, W. S., Freyer, H. D., Trumbore, S. 1983. A deconvolution of the tree ring based  $\delta^{13}\text{C}$  record. *J. Geophys. Res.* 88:3609-20
90. Peters, K. E., Sweeney, R. E., Kaplan, I. R. 1978. Correlation of carbon and nitrogen stable isotope ratios in sedimentary organic matter. *Limnol. Oceanogr.* 23:598-604
91. Peterson, B. J., Howarth, R. W., Garritt, R. H. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science* 227:1361-63
92. Peterson, B. J., Howarth, R. W., Garritt, R. H. 1986. Sulfur and carbon isotopes as tracers of salt-marsh organic matter flow. *Ecology* 67:865-74
93. Rau, G. H. 1978. Carbon-13 depletion in a subalpine lake: Carbon flow implications. *Science* 201:901-02
94. Rau, G. H. 1980. Carbon-13/carbon-12 variations in subalpine lake aquatic insects: Food source implications. *Can. J. Fish. Aquat. Sci.* 37:742-46
95. Rees, C. E. 1973. A steady-state model for sulphur isotope fractionation in bacterial reduction processes. *Geochim. Cosmochim. Acta* 37:1141-62
96. Rees, C. E., Jenkins, W. J., Monster, J. 1978. The sulphur isotopic composition of ocean water sulphate. *Geochim. Cosmochim. Acta* 42:377-81
97. Roeske, C. A., O'Leary, M. H. 1984. Carbon isotope effects on the enzyme-catalyzed carboxylation of ribulose-bisphosphate. *Biochemistry* 23:6275-84
98. Rotty, R. M., Marland, G. 1986. Fossil fuel combustion: Recent amounts, patterns, and trends of  $\text{CO}_2$ . In *The Changing Carbon Cycle: A Global Analysis*, ed. J. R. Trabalka, D. E. Reichle, pp. 474-90. New York: Springer-Verlag
99. Rounick, J. S., Winterbourn, M. J. 1986. Stable carbon isotopes and carbon flow in ecosystems. *BioScience* 36:171-77
100. Saino, T., Hattori, A. 1980.  $^{15}\text{N}$  natural abundance in oceanic suspended particulate matter. *Nature* 283:752-54
101. Saltzman, E. S., Brass, G. W., Price, D. A. 1983. The mechanism of sulfate aerosol formation: Chemical and sulfur isotopic evidence. *Geophys. Res. Lett.* 10:513-16
102. Schoeller, D. A., Minagawa, M., Slater, R., Kaplan, I. R. 1986. Stable isotopes of carbon, nitrogen and hydrogen in the contemporary North American human Food web. *Ecol. food Nutr.* 18:159-70
103. Schoeninger, M. J., DeNiro, M. J., Tauber, H. 1983. Stable nitrogen isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric human diet. *Science* 220:1381-83
104. Schwarcz, H. P. 1969. Isotopes in nature. I. The stable isotopes of carbon. In *Handbook of Geochemistry* II-1, ed. K. H. Wedepohl, 6B:1-16. Berlin: Springer-Verlag
105. Schwarcz, H. P. 1973. Sulfur

- isotope analyses of some Sudbury, Ontario, ores. *Can. J. Earth Sci.* 10:1444-59
106. Schwarcz, H. P., Melbye, J., Katzenberg, M. A., Knyfe, M. 1985. Stable isotopes in human skeletons of southern Ontario: Reconstructing Palaeodiet. *J. Archaeol. Sci.* 12:187-206
  107. Shearer, G., Kohl, D. H. 1986.  $N_2$ -fixation in field settings: Estimations based on natural  $^{15}N$  abundance. *Austr. J. Plant Physiol.* 13:699-757
  108. Shearer, G., Kohl, D. H., Virginia, R. A., Bryan, B. A., Skeeters, J. L., et al. 1983. Estimates of  $N_2$ -fixation from variation in the natural abundance of  $^{15}N$  in Sonoran Desert ecosystems. *Oecologia* 56:365-73
  109. Steele, K. W., Daniel, R. M. 1978. Fractionation of nitrogen isotopes by animals: A further complication to the use of variations in the natural abundance of  $^{15}N$  for tracer studies. *J. Agric. Sci.* 90:7-9
  110. Stuiver, M. 1978. Atmospheric carbon dioxide and carbon reservoir changes. *Science* 199:253-58
  111. Tans, P. 1981.  $^{13}C/^{12}C$  of industrial  $CO_2$ . In *Carbon Cycle Modelling*, SCOPE 16, ed. B. Bolin, pp. 127-129. New York: Wiley
  112. Tauber, H. 1981.  $^{13}C$  evidence for dietary habits of prehistoric man in Denmark. *Nature* 292:332-33
  113. Teal, J. M. 1962. Energy flow in the salt marsh ecosystem of Georgia. *Ecology* 43:614-24
  114. Tieszen, L. L., Boutton, T. W., Tesdahl, K. G., Slade, N. A. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for  $\delta^{13}C$  analysis of diet. *Oecologia* 57:32-37
  115. Troughton, J. H., Card, K. A., Hendy, C. H. 1974. Photosynthetic pathways and carbon isotope discrimination by plants. *Carnegie Inst. Wash. Yrbk* 73:768-80
  116. Urey, H. C. 1947. The thermodynamic properties of isotopic substances. *J. Chem. Soc.* 1947:562-81
  117. van der Merwe, N. J. 1982. Carbon isotopes, photosynthesis, and archaeology. *Am. Sci.* 70:596-606
  118. van der Merwe, N. J., Vogel, J. C. 1978.  $^{13}C$  content of human collagen as a measure of prehistoric diet in woodland North America. *Nature* 276:815-16
  119. Wada, E. 1980. Nitrogen isotope fractionation and its significance in biogeochemical processes occurring in marine environments. In *Isotope Marine Chemistry*, ed. E. D. Goldberg, Y. Horibe, K. Saruhashi, pp. 375-98. Tokyo: Uchida Rotakuho
  120. Wada, E., Hattori, A. 1978. Nitrogen isotope effects in the assimilation of inorganic nitrogenous compounds by marine diatoms. *Geomicrobiol. J.* 1:85-101
  121. Williams, P. M., Gordon, L. I. 1970. Carbon-13: carbon-12 ratios in dissolved and particulate organic matter in the sea. *Deep-Sea Res.* 17:19-27
  122. Yanagisawa, F., Sakai, H. 1983. Thermal decomposition of barium sulfate-vanadium pentoxide-silica glass mixtures for preparation of sulfur dioxide in sulfur isotope ratio measurements. *Anal. Chem.* 55:985-87
  123. Yoneyama, T., Ohta, Y., Ohtani, T. 1983. Variations of natural  $^{13}C$  and  $^{15}N$  abundances in the rat tissues and their correlation. *Radioisotopes* 32:330-32
  124. Zieman, J. C., Macko, S. A., Mills, A. L. 1984. Role of seagrasses and mangroves in estuarine food webs: Temporal and spatial changes in stable isotope composition and amino acid content during decomposition. *Bull. Mar. Sci.* 35:380-92



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