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P.W. Rundel, J.R. Ehleringer, and K.A. Nagy
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Stable Isotopes in Ecological Research

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1. Stable Isotopes: History, Units, and Instrumentation

J.R. Ehleringer and P.W. Rundel

Isotopic Abundance

Elements exist in both stable and nonstable (radioactive) forms. Most elements of biological interest have two or more stable isotopes, although one isotope is usually present in far greater abundance. Table 1.1 lists the average natural abundances of the stable isotopes of the major elements used in environmental studies. In addition to the five light elements of importance for biological studies, strontium isotopes are assuming greater importance in understanding ecological transport processes and have therefore been included (see Chapters 14 and 28 for applications of strontium isotopes). Calcium, chlorine, magnesium, potassium, and silicon are additional elements of biological interest having more than one different stable isotope, but unfortunately very little information is available on these elements. While there is no evidence for biological fractionation of these elements, they may serve as potentially useful markers of ecosystem process studies.

Units of Isotopic Expression and Standards

Natural isotope variation or fractionation depends on thermodynamic equilibria and kinetic processes affecting the individual isotope. In both cases, fractionation is a function of slight variation in the physical and chemical properties

Table 1.1. Average Terrestrial Abundances of the Stable Isotopes of Major Elements of Interest in Ecological Studies

Element	Isotope	Abundance (%)
Hydrogen	^{1}H	99.985
	^{2}H	0.015
Carbon	^{12}C	98.89
	^{13}C	1.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{16}O	99.759
	^{17}O	0.037
	^{18}O	0.204
Magnesium[a]	^{24}Mg	78.70
	^{25}Mg	10.13
	^{26}Mg	11.17
Silicon[a]	^{28}Si	92.21
	^{29}Si	4.70
	^{30}Si	3.09
Sulfur	^{32}S	95.00
	^{33}S	0.76
	^{34}S	4.22
	^{36}S	0.014
Chlorine[a]	^{35}Cl	75.53
	^{37}Cl	24.47
Potassium[a]	^{39}K	93.10
	^{40}K	0.0118
	^{41}K	6.88
Calcium[a]	^{40}Ca	96.97
	^{42}Ca	0.64
	^{43}Ca	0.145
	^{44}Ca	2.06
	^{46}Ca	0.0033
	^{48}Ca	0.18
Iron[a]	^{54}Fe	5.82
	^{56}Fe	91.66
	^{57}Fe	2.19
	^{58}Fe	0.33
Copper[a]	^{63}Cu	69.09
	^{65}Cu	30.91
Zinc[a]	^{64}Zn	48.89
	^{66}Zn	27.81
	^{67}Zn	4.11
	^{68}Zn	18.57
	^{70}Zn	0.62
Strontium	^{84}Sr	0.56
	^{86}Sr	9.86
	^{87}Sr	7.02
	^{88}Sr	82.56

[a] Isotopes not discussed further in the chapters that follow.

of the isotopes and is proportional to differences in their masses (Broecker and Oversley 1976). The differences in the equilibrium and kinetic characteristics of isotopic species are usually small (on the order of a few percent), and thus absolute variations in isotopic abundances based on physical factors may be small. Enzymatic discrimination for or against an isotopic species will affect the absolute abundances, but again these variations are on the order of one or two percent. Therefore, in any isotopic analysis, very precise and analytical techniques are required. Isotopic composition is measured by determining the ratios of the two stable isotopes present in the sample. Most often, it has been found that measuring the absolute isotopic composition is not as reliable and/or convenient as measuring isotopic differences between a sample and a given standard. This is because while obtaining high precision in absolute isotopic composition of a sample is not difficult over the short term, it is very difficult over the long term (Hayes 1983). In contrast, analyses based on the measurement of the differences between a defined standard and sample provide high precision and repeatability over both short-term and long-term periods. Furthermore, the differential analysis approach allows very small differences in the isotopic composition of two samples to be accurately and reliably determined. The technique of differential comparison of sample and standard has been the approach used in stable isotopic analyses since it was first introduced almost forty years ago (McKinney et al. 1950).

Isotopic composition of a sample is usually expressed with the differential notation. That is:

$$\delta X_{\text{std}} = (R_{\text{sample}}/R_{\text{std}} - 1) \cdot 1000$$

where δX_{std} is the isotope ratio in delta units relative to a standard, and R_{sample} and R_{std} are the absolute isotope ratios of the sample and standard, respectively. Multiplying by 1000 allows the values to be expressed in parts per thousand (‰), more commonly as referred to on a "per mil" basis. Since the isotopic composition of two samples will not differ extensively in their absolute values, the differential notation allows one to focus in on the differences between samples. For instance, one sample may have a ^{13}C composition of 1.1230% while another has a value of 1.1210%. The useful information in this composition is in the third decimal place and is somewhat obscured by the preceding unchanging numbers. However, when expressed on a delta unit basis, these numbers become −0.6‰ and −2.4‰, respectively, relative to the PDB carbon standard (which has a value of 1.1237%), and the significance of the third- and fourth-place decimal place changes becomes more obvious.

There are presently four accepted isotopic standards for the five principal light elements of biological interest. These are standard mean ocean water (SMOW) for hydrogen and oxygen, PeeDee belemnite (PDB) for carbon and occasionally oxygen, atmospheric air for nitrogen, and the Canyon Diablo meteorite (CD) for sulfur. Estimated absolute ratios of these standards are listed in Table 1.2. While there is some variance in the estimates of the absolute ratios in these standards, the use of the differential or deviation from standard mea-

Table 1.2. Isotopic Compositions of Primary Standards[a]

Primary Standard	Isotope Ratio	Accepted Value ($\times 10^6$) (with 95% Confidence Interval)
Standard mean ocean water (SMOW)	$^2H/^1H$	155.76 ± 0.10
	$^{18}O/^{16}O$	2005.20 ± 0.43
	$^{17}O/^{16}O$	373 ± 15
PeeDee belemnite (PDB)	$^{13}C/^{12}C$	11237.2 ± 9.0
	$^{18}O/^{16}O$	2067.1 ± 2.1
	$^{17}O/^{16}O$	379 ± 15
Air	$^{15}N/^{14}N$	3676.5 ± 8.1

[a] From Hayes (1983).

surement approach overcomes these concerns and provides far greater precision and long-term reliability. The original supplies of both SMOW and PDB have been exhausted and replaced by other materials which had been carefully compared to the original standards. These standards are available to investigators for use in calibrating the working standards within individual mass spectrometer laboratories. The International Atomic Energy Agency (IAEA) in Vienna has mixed various waters together to produce V-SMOW (Vienna SMOW), which has an isotopic composition nearly identical to that of the original SMOW, and SLAP (standard light antarctic precipitation), obtained from Plateau Station, Antarctica. SLAP has a D/H ratio of 89.02 ± 0.05 (Gonfiantini 1978). The National Bureau of Standards provides a number of carbon isotope reference samples, including a graphite, NBS-21, with a carbon isotope ratio of −28.10‰ on the PDB scale. Coplen et al. (1983) have shown how oxygen on the PDB scale may be related to the V-SMOW scale (see below) through SMOW. Problems of calibration with atmospheric nitrogen have been discussed by Mariotti (1984).

Isotopic Abundance in Nature

Carbon

The first limited data on $^{13}C/^{12}C$ isotope ratios in natural materials were published by Nier and Gulbransen in 1939, using what would now be considered primitive technical approaches. However, these workers were able to establish that limestones, atmospheric CO_2, marine plants and terrestrial plants each possessed characteristic carbon isotope ratios. With the development of the isotope-ratio mass spectrometer (Nier 1947), and improved techniques (McKinney et al. 1950), much more intensive studies were made possible. Naturally occurring variations in carbon isotopic composition exceed 100‰, ranging from heavy carbonates with values of +20‰ to light methanes of about −90‰ (Hoefs 1980). Typically though, the range of values for carbon in geological and biological materials is much less (Figure 1.1).

The earliest observations of the carbon isotopic composition of plant materials

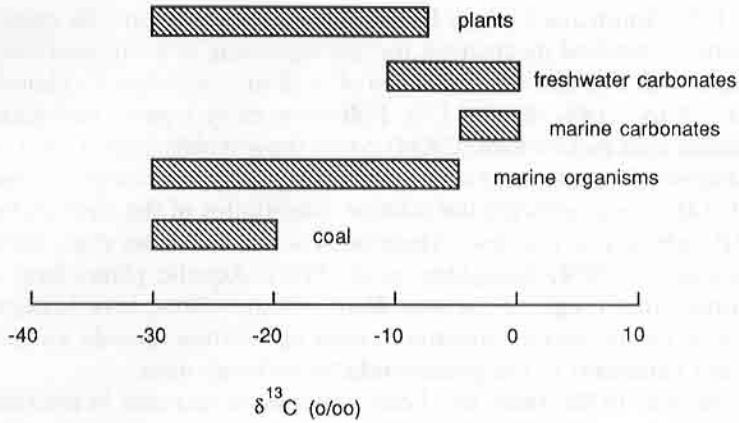

Figure 1.1. Observed ranges of carbon isotope ratios from various substances.

were those by Craig (1953, 1954) and Wickman (1952) in the United States and Baertschi (1953) in Switzerland. Craig (1953) observed that most plant material had a $\delta^{13}C$ value close to $-27‰$, and unknowingly made the first report of a $\delta^{13}C$ value for a C_4 species, on an unidentified grass from the midwestern United States with a $\delta^{13}C$ value of $-12‰$. Unaware of C_4 metabolism, he mistakenly attributed this high value to a possible limestone substrate. In his 1954 paper, Craig discussed the survey results in considerable detail and speculated on the relative importance of CO_2 diffusion, chemical absorption of CO_2, and respiration as possible mechanisms of isotopic fractionation.

This early discussion of carbon isotope fractionation was confirmed and extended by the pioneering experimental studies of Park and Epstein. Working with tomato plants, Park and Epstein (1960) were able to demonstrate that ribulose bisphosphate carboxylase discriminated against $^{13}CO_2$ and suggested that the differences in isotopic composition between plant and atmosphere were a function of this enzyme discrimination. In an attempt to verify their model, they measured the isotopic composition of "internal CO_2" by treating leaves with acid. There were problems, however, in identifying the origin of CO_2 evolved in these experiments, and thus in determining the relevance of its isotopic composition to the true internal CO_2 pool. Park and Epstein (1961) also studied the metabolic fractionation of carbon isotopes within plant tissues and established that lipids were depleted in ^{13}C by as much as 8‰. These data were used to explain the relatively low or lighter $\delta^{13}C$ values of petroleum in comparison to either coal or land plants.

It is interesting to note that all of these early studies on mechanisms of carbon isotope fractionation in terrestrial plants were conducted by geochemists rather than biologists. The development of biological interest in carbon fractionation was heightened in the mid-1960s by the discovery of C_4 metabolism (Kortschak et al. 1965; Hatch and Slack 1970). Isotope studies were able to demonstrate that C_4 plants had less negative $\delta^{13}C$ values than those found in C_3 plants (Bend-

er, 1968, 1971; Smith and Epstein 1971). This difference in isotopic composition has become a standard mechanism for distinguishing plant tissues from these two groups, with C_3 plants having ratios of −20 to −35‰ and C_4 plants having values of −9 to −14‰ (Figure 1.2). Following early reports that plants with Crassulacean acid metabolism (CAM) could show widely ranging $\delta^{13}C$ values, it was established by a number of research groups that the isotopic composition of such CAM plants reflected the relative magnitudes of the PEP carboxylase and RuBP carboxylase reactions (Osmond et al. 1973; Bender et al. 1973; Lehrman and Queiroz 1974; Troughton et al. 1977). Aquatic plants have carbon isotope ratios that range all the way from −8 to −30‰; here isotope ratios depend both on the carbon substrate taken up (carbon dioxide versus bicarbonate) and variations in the photosynthetic pathway used.

Since the mid 1970s, there has been a geometric increase in interest in the

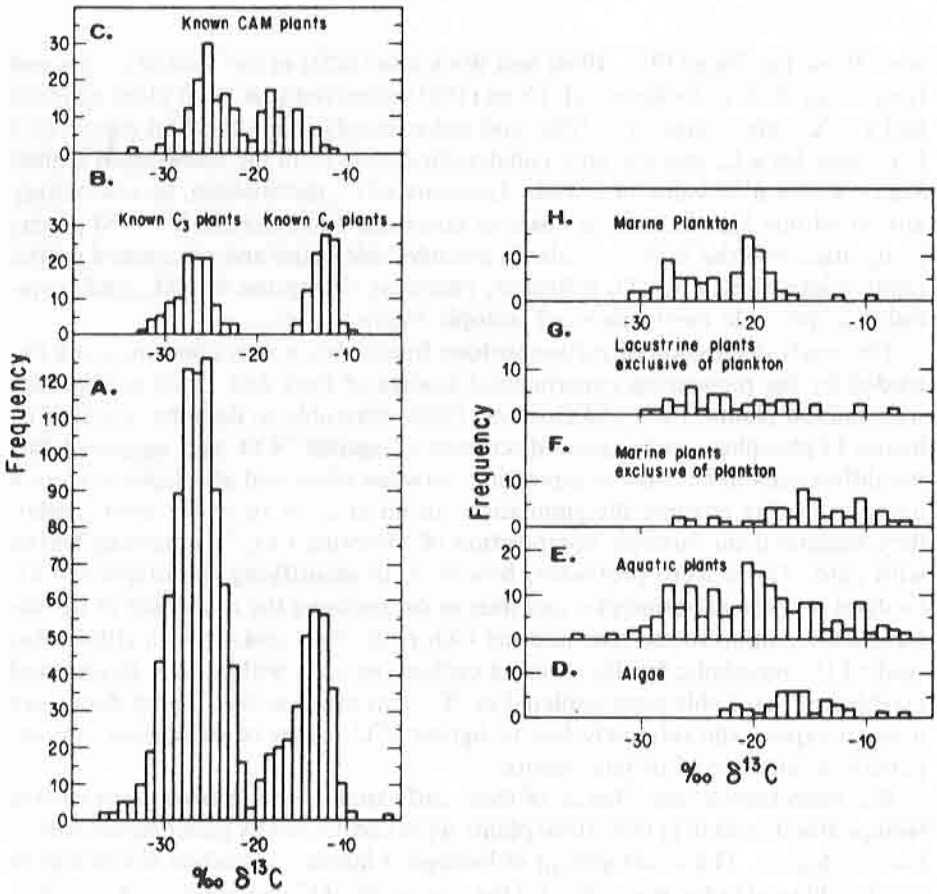

Figure 1.2. Leaf carbon isotope ratios for C_3, C_4, and CAM photosynthetic pathway plants. From Deines (1980).

physiological significance of carbon isotope ratios in plant tissues. These studies have ranged from environmental effects on $\delta^{13}C$ to problems of interpretation of intra- and interspecific variation in $\delta^{13}C$, to fractionation in aquatic plants, and even to metabolic processes of fractionation. Many of these exciting areas of research are discussed in this volume.

Hydrogen and Oxygen

The existence of natural isotopes of oxygen and hydrogen has been known for more than half a century. The oxygen isotopes ^{17}O and ^{18}O were discovered by Giaque and Johnston in 1929, and deuterium was later identified by Urey and his associates in 1932. Since these initial discoveries, the description of the relative ratios of these isotopes in various geochemical and biological systems has been a major focus of research. Thus, there is a rather large body of literature documenting variations in the natural abundance of oxygen and hydrogen isotopes in different chemicals and in water derived from various sources. These variations result from fractionations caused by phase transitions, chemical or biological reactions, and transport processes (Gat 1982). The fields of isotope hydrology and paleoclimatology are based on the fact that different molecular species of H_2O have different vapor pressures. Predictably, the vapor pressures of the nine different stable isotopic forms of H_2O are invariably proportional to their masses (Dansgaard 1964).

The two stable isotopes of hydrogen, ^{1}H and ^{2}H (more commonly referred to as deuterium, D), are present in relative proportions of 99.9844 and 0.0156% of the total hydrogen atoms. Hydrogen isotopes are particularly interesting because of their large relative mass differences. Perhaps not too surprisingly, the largest variations in isotope ratios are found for hydrogen (Figure 1.3). Isotope ratios of hydrogen from geological and biological materials vary up to 700‰ (Hoefs 1980), with a range of over 400‰ in precipitation values alone (Figure 1.4). The depleted oxygen and hydrogen isotope ratios in precipitation are a

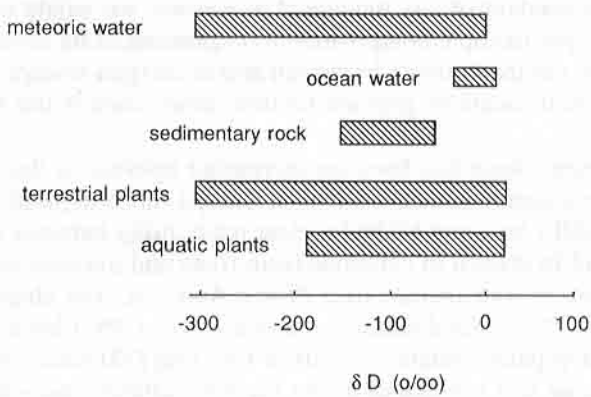

Figure 1.3. Observed ranges of hydrogen isotope ratios from various substances.

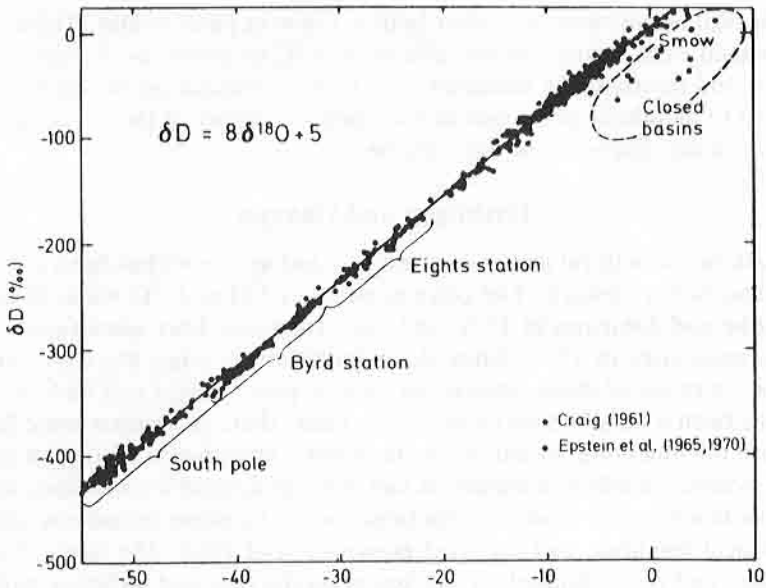

Figure 1.4. Hydrogen versus oxygen isotope ratios for various meteoric surface waters. From Taylor (1974).

function of isotopic fractionation from the evaporation of seawater and the subsequent condensation of cloud moisture. Mean annual isotope ratios for hydrogen and oxygen for different regions show a tight linear relationship between δD and $\delta^{18}O$ with a ratio of 8:1 (Craig 1961). This pattern is referred to as the meteoric water line (MWL).

The hydrogen isotope ratios of both plants and animals are very much dependent on the isotopic ratio of the water in that environment which they use for growth (Taylor 1974; Yapp and Epstein 1982a). Since the isotopic composition of precipitation depends on latitude, altitude, and temperature, this means that independent of any biological processes, we would expect the hydrogen and oxygen isotopic composition in organisms to be different between locations. Thus, comparisons of hydrogen and/or oxygen isotope ratios for tissues from different locations provide limited information if the source values are unknown.

In recent years, there has been an increasing interest in the paleoclimatic and physiological significance of hydrogen isotope ratios in plant tissues. Yapp and Epstein (1982b) have established a clear relationship between the δD values of carbon-bound hydrogen in cellulose from trees and average annual temperature for a range of tree species over North America. The slope of this relationship is 5.8‰ $°C^{-1}$. Krishnamurthy and Epstein (1985) have used this approach to develop paleoclimatic records of tree ring D/H ratios in East Africa. While this volume will not elaborate on the paleoclimatic area of research, it does provide discussions of applications of hydrogen isotope ratios to studies of plant source water, leaf water, and dry matter.

Observations of isotopic fractionation in plant tissues were first made half a century ago by Washburn and Smith (1934), who determined through density measurements that sap water from leaves of *Salix nigra* was isotopically heavier with respect to hydrogen than was river water in the same area. Since they found no evidence of fractionation in the passage of water through root membranes, Washburn and Smith concluded that hydrogen isotope fractionation resulted from transpiration and/or photosynthesis. With the advent of mass spectrometers, enriched levels of deuterium in leaf tissues were substantiated (Warshaw et al. 1970). That work has led to the modern studies included in this volume.

There are three stable isotopes of oxygen: ^{16}O (99.759%), ^{17}O (0.037%), and ^{18}O (0.204%). Primarily because of the greater relative abundance of ^{18}O and because $^{18}O/^{17}O$ ratios are constant, only the $^{18}O/^{16}O$ ratios are normally determined. Naturally occurring variation oxygen isotope ratios exceeds 90‰, with heavy sedimentary carbonates having values of +40‰ at one end of the scale and light meteoric waters of −50‰ at the other extreme. Geologically, ^{18}O analyses have assumed great importance in understanding thermodynamic processes and as indicators of paleotemperatures (Urey et al. 1951; Epstein 1959; Taylor 1974). Atmospherically, oxygen isotope ratios vary by over 50‰ globally, and have been most useful in tracing and describing water movement in soils and as indicators of humidity regimes (Figure 1.5). Meteoric waters have been shown to range from −40 to +6‰ in their $\delta^{18}O$ values (Craig 1961).

The phenomenon of heavy isotope accumulation of oxygen in plant leaves was established by Gonfiantini and his associates in 1965 in studies with twelve species of vascular plants. Subsequent physiological research has focused on the observed diurnal cycle of $H_2{}^{18}O$ of leaves. Problems is the analysis of oxygen isotopes in organic tissues have limited their use in physiological studies. However, DeNiro and Epstein (1979) have established that oxygen derived from CO_2 undergoes a complete exchange with the oxygen of the water in the plant during the synthesis of cellulose, and thus the $\delta^{18}O$ of tissue water is the primary influence on the $\delta^{18}O$ of fixed oxygen in cellulose. The significance of this finding is discussed further in this volume.

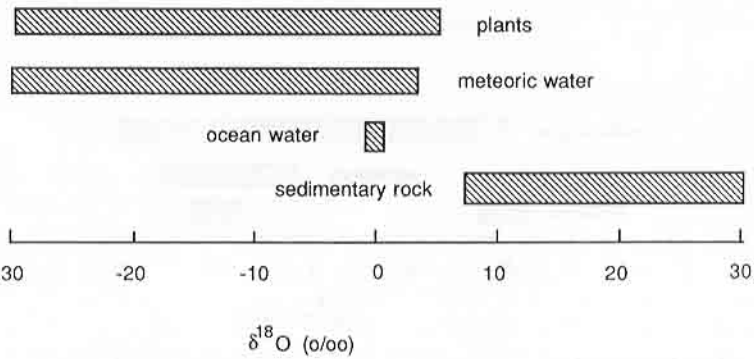

Figure 1.5. Observed ranges of oxygen isotope ratios from various substances.

Nitrogen

There are two stable isotopes of nitrogen: ^{14}N and ^{15}N. The average natural abundance of ^{15}N in air is a constant 0.366% (Nier 1950; Sweeney et al. 1978) and is therefore used as the standard for nitrogen analyses. Nitrogen has received relatively little attention from geochemists, since its composition in rock materials is low and, for the most part, the isotopic composition is largely determined by biological reactions rather than inorganic thermodynamic processes. Early studies of natural variation of nitrogen isotopes were made by Hoering (1955, 1957). While enriched ^{15}N tracer studies have been used in agriculture for many years (Bremmer 1965), biological interest in nitrogen at natural abundance levels began in the late 1960s, essentially regarding the question of whether or not ^{15}N levels of nitrates in soil water were an indication of its source or origin. Kohl et al. (1971) were among the first to suggest that it was possible to distinguish between nitrogen derived from fertilizers and that derived from compounds of natural origin.

Natural abundance levels of ^{15}N values range from −20 to +20‰ (Figure 1.6). Animal tissues are almost always enriched in ^{15}N relative to values measured for plants, and this progressional enrichment increases along advancing trophic levels (Miyake and Wada 1967; Minegawa and Wada 1984; Schoeninger and DeNiro 1984). This enrichment is due to catabolic pathways which favor the elimination of the lighter isotope (Gaebler et al. 1966; Macko et al. 1986, 1987). Nitrogen within the organic materials of soils also tends to be enriched in ^{15}N relative to that of the above-ground plant tissues, implying that there is microbial discrimination during the decomposition process. The conversion of diatomic nitrogen to organic forms by nitrogen fixation processes appears to discriminate little against ^{15}N. Consequently, the $\delta^{15}N$ values of leguminous plants are often close to 0‰.

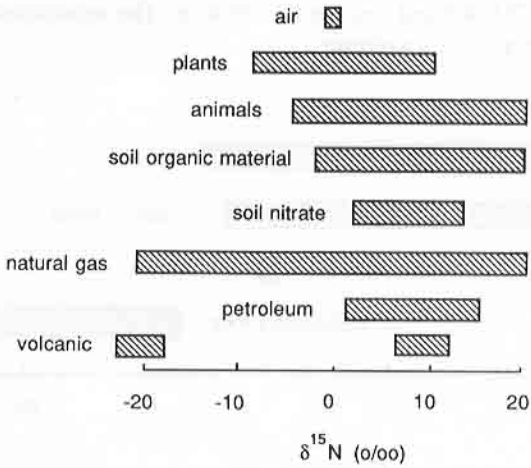

Figure 1.6. Observed ranges of nitrogen isotope ratios from various substances.

The isotopic component of fixation of molecular nitrogen by living organisms was first investigated in detail by Hoering and Ford (1960), who were unable to identify and fractionation between atmospheric nitrogen and synthesized organic matter. Later experiments by Delwiche and Steyn (1970) demonstrated an average depletion of $\delta^{15}N$ of about 5‰ in plant tissues utilizing soil nitrogen. They proposed that N_2-fixing plants would differ from nonfixing plants in their values of $\delta^{15}N$, thereby laying the groundwork for the ^{15}N natural abundance technique for estimating symbiotic nitrogen. This modern approach is described in detail in two chapters of this volume.

Sulfur

There are four stable isotopes of sulfur: ^{32}S (95.00%), ^{33}S (0.76%), ^{34}S (4.22%), and ^{36}S (0.014%). Geologically, sulfur is present as a minor component of most igneous and metamorphic rocks and as a component of organic substances such as coals and crude oils. Sulfur is a major component in some ores as sulfide (for instances, ferric sulfide) and in evaporates as sulfate. Early studies by Thode et al. (1949) established the large variability of sulfur isotope ratios in natural substances. Isotope ratios of sulfur are usually in terms of $^{34}S/^{32}S$ and range about 150‰ (Figure 1.7), with the heaviest sulfates being greater than +90‰ and the lightest sulfides having values of about −60‰ (Krouse 1980).

Plants and soil $\delta^{34}S$ values are typically from −30 to +30‰, although the range of natural values in unpolluted areas is considerably less (Krouse 1980). It is the large variation in $\delta^{34}S$ values betwee pollutant sulfur and natural plant and soil sulfur that has led to the important use of sulfur isotope ratios as tracers of pollutant transfer. Natural differences in $\delta^{34}S$ values between marine and terrestrial biota also provide an important tracer of food chains.

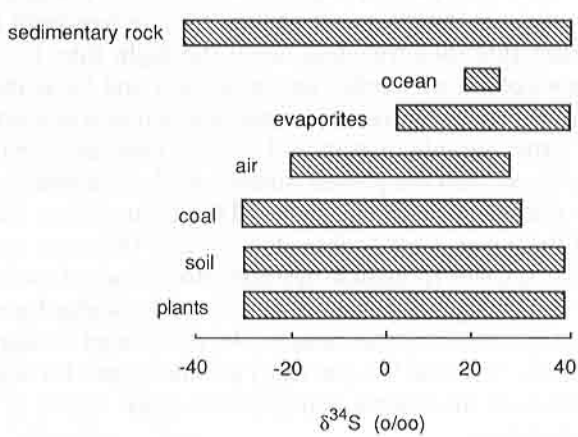

Figure 1.7. Observed ranges of sulfur isotope ratios from various substances.

Mass Spectrometry

Isotope ratios are measured on a mass spectrometer, which is an instrument that basically separates charged atoms and molecules on the basis of their mass differences. Stable isotope mass spectromers currently in use are based on the original design of Nier (1947) and consist of three essential components: a source to ionize the molecules, a magnetic field to deflect and thus separate the charged particles as they move down a flight tube, and a series of ion collectors to trap the ions at the opposite end of the flight tube.

Most isotope-ratio mass spectrometers are capable of measuring only low-molecular-weight compounds (usually less than mass 64). The compounds are introduced into the instrument as gases, most often as H_2, CO_2, N_2, and SO_2. Thus, with such an instrument, the isotope ratios of H, C, N, O, and S can be individually determined. Heavier elements and elements that do not readily form gases are measured with a thermal emission mass spectrometer.

In an isotope-ratio mass spectrometer, the pure gas is introduced into one end of the flight tube. At this point, ionization of the gas is achieved by an electron beam source which bombards the gas. The positively charged ions are accelerated and collimated into a fine beam. The ionized beam enters a magnetic field, which deflects the ions into circular paths whose radii are proportional to the masses of the isotopes. The beam is thus divided into its component masses, and these charged particles travel on to strike a series of collectors (Faraday cups) at the opposite end of the flight tube. Amplifiers attached to the collectors convert the ionic impacts into a voltage, which is then coverted into a frequency. The absolute intensity of the signals is not the critical measurement, because this will depend to a large extent on the amount of gas which is introduced into the mass spectrometer. Rather, the critical parameter is the ratio of the signals into the different collector cups.

One of the first requirements of an isotope-ratio mass spectrometer is a good vacuum system. The flight tube operates at a vacuum of approximately 10^{-8} torr. Since the mean free path of a gas molecule is inversely proportional to the pressure, a vacuum that low will ensure a mean free path length of over 500 m. This means that ions traveling down the flight tube (usually only 1 m in length) will not collide with other gas molecules and be scattered.

The gas inlet into the mass spectrometer is symmetrically arranged for the introduction of either sample or standard gases. Gases are temporarily stored in a metal bellows and then are passed through a set of capillaries (one for each side) to ensure viscous flow of the gases. This ensures that there will be no fractionation of the gases prior to introduction into the mass spectrometer. A changeover valve is used to switch between the standard and sample gases. The difference in the signals between sample and standard gases is used to calculate the isotope ratio for the sample. As mentioned earlier, even though absolute ratios (i.e., $^{45}/_{44}$ and $^{46}/_{44}$ for CO_2) are measured for a given gas, it is the difference between the sample and standard ratios that is of interest in the isotope ratio calculation.

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