CHAPTER 2

Sources of variation in the stable isotopic composition of plants*

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Introduction

The use of stable isotopes of carbon, nitrogen, oxygen, and hydrogen to study physiological processes has increased exponentially in the past three decades. When Harmon Craig (1953, 1954), a geochemist and early pioneer of natural abundance stable isotopes, first measured isotopic values of plant materials, he found that plants tended to have a fairly narrow $\delta^{13}C$ range of −25 to −35‰. In these initial surveys, he was unable to find large taxonomic or environmental effects on these values. Since that time ecologists have identified clear isotopic signatures based not only on different photosynthetic pathways, but also on ecophysiological differences, such as photosynthetic water-use efficiency (WUE) and sources of water and nitrogen used. As large empirical databases have accumulated and our theoretical understanding of isotopic composition has improved, scientists have continued to discover mismatches between theoretical and observed values, as well as confounding effects from sources and factors not previously considered. In the best tradition of science, these discoveries have led to important new insights into physiological or ecological processes, as well as new uses of stable isotopes in plant ecophysiology. This chapter reviews the most common applications of stable isotope analysis in plant ecophysiology.

Carbon isotopes

Photosynthetic pathways

Plants contain less ^{13}C than the atmospheric CO_2 on which they rely for photosynthesis. They are therefore "depleted" of ^{13}C relative to the atmosphere. This depletion is caused by enzymatic and physical processes that discriminate against ^{13}C in favor of ^{12}C. Discrimination varies among plants using different photosynthetic pathways. The Calvin cycle (C3), Hatch–Slack

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cycle (C4) and Crassulacean acid metabolism (CAM) photosynthetic pathways differ so profoundly and so consistently (O'Leary 1981, 1988) that ecologists have used isotopic signatures to distinguish them in large-scale surveys of plant species (Teeri & Stowe 1976; Sage & Monson 1999).

The C3 pathway begins with the diffusion of CO_2 from the atmosphere into the air-filled spaces within the leaf. This diffusion occurs through the still air occupying stomatal pores. Such diffusion has an apparent fractionation ($\Delta\delta$) of ~4.4‰ due to the slower motion of the heavier ^{13}C-containing CO_2 molecules. Within the leaf, the carboxylating enzyme ribulose bisphosphate carboxylase/oxygenase (rubisco) discriminates further against the ^{13}C, with a $\Delta\delta$ of about 29‰. If atmospheric diffusion were the sole limitation for CO_2 uptake, i.e., if rubisco did not discriminate against ^{13}C, then we would expect to see only the fractionation of 4.4‰. This 4.4‰ would be subtracted from the $\delta^{13}C$ value for CO_2 in the atmosphere, which is about −8‰, yielding a $\delta^{13}C$ of −12.4‰. At the opposite extreme, if enzyme activity were the sole limitation for CO_2 uptake, i.e., if diffusion did not discriminate, then only the rubisco fractionation would be observed. These conditions would yield a predicted leaf $\delta^{13}C$ value of about −37‰. In fact, $\delta^{13}C$ values for C3 plants lie between these extremes, with a median of about −27‰. Variation about this median depends on the balance between diffusive supply and enzymatic demand for CO_2. Estimates of this balance point have been exploited by ecologists as an index of water-use efficiency (see following section).

Isotopic composition of C4 plants differs substantially from that of C3 plants. The initial step in C4 photosynthesis is the same: the diffusion of CO_2 from the atmosphere into the leaf via stomata. However, C4 photosynthesis is catalyzed by a different enzyme, phosphoenolpyruvate (PEP) carboxylase, which has a different discrimination, approximately −6‰, for the fixation of CO_2 (Farquhar 1983). If this enzymatic fractionation were fully and exclusively expressed relative to atmospheric CO_2, it would yield tissue values around −2‰. Diffusion-limited uptake would be the same as that for C3 plants, namely −12.4‰. One might expect real C4 plants to lie between these extremes, analogous to C3 plants. In fact, measured $\delta^{13}C$ values for C4 plants lie below this range, clustering around −14‰.

These surprisingly negative values result from the unique physiology of C4 photosynthesis. The C4 compounds produced by PEP carboxylase are transported into the bundle sheath, which is the cylinder of vascular tissue enclosed in the center of the leaf. Inside the bundle sheath, the C4 compounds are catabolized to C3 compounds, releasing CO_2, which accumulates to high concentrations. The released CO_2 is then refixed by rubisco, the same enzyme used by C3 photosynthesis. The surprisingly negative $\delta^{13}C$ values are caused by a slow leak of enriched CO_2 from the bundle sheath. The leaking CO_2 pool is enriched in ^{13}C by the preference of rubisco for the light isotope. As the enriched CO_2 leaks out, it depletes the $\delta^{13}C$ of the CO_2 left behind B1

(Ehleringer & Pearcy 1983; Berry 1989). Support for this mechanism comes from evidence that C4 plants with the most leaky bundle sheaths tend to exhibit the most negative $\delta^{13}C$ (Hattersley 1982; Henderson et al. 1992; Sandquist & Ehleringer 1995). These interacting controls over C4 isotope fractionation serve as an example of the sometimes complex metabolic feedbacks influencing stable isotope composition.

Plants using the CAM photosynthetic pathway rely on the same carboxylating enzymes as C4 plants and in the same sequence. However, they segregate the activities of the enzymes between night and day. Initially, CO_2 is fixed by PEP carboxylase into C4 acids at night; the acids are stored in the vacuoles of the bulky leaves and stems (e.g., in cacti). When the sun comes up, the carbon is released from the C4 acids and refixed by rubisco. Because the enzyme sequence is similar, obligate-CAM plants discriminate against ^{13}C much as C4 plants do (Farquhar 1983), though perhaps with less leakage. Their $\delta^{13}C$ values cluster around −11‰. Some species use the CAM pathway facultatively, conducting C3 photosynthesis when conditions are favorable, switching to the CAM sequence when drought strikes. Facultative-CAM plants have $\delta^{13}C$ values intermediate between −11‰ and those of obligate-C3 plants (−27‰). These intermediate carbon isotope signatures can be used to estimate the proportion of CAM vs. C3 photosynthesis during the period when the tissue was produced (Osmond et al. 1976; Teeri & Gurevitch 1984; Kalisz & Teeri 1986; Smith et al. 1986; Mooney et al. 1989; Kluge et al. 1991).

Carbon isotopes cannot effectively distinguish CAM from C4 plants because of their similarity. However, empirical evidence suggests that CAM plants are considerably enriched in deuterium relative to source water, providing yet another means of distinguishing them from C4 plants (Figure 2.1; Ziegler et al. 1976; Sternberg & DeNiro 1983; Sternberg et al. 1984a,b,c; Sternberg 1989). Traditionally the distinction between C4 and CAM has relied on anatomical methods, specifically succulence (CAM) vs. Kranz anatomy (C4).

Plant water-use efficiency

Ecologists also use carbon isotope ratios to infer photosynthetic water-use efficiency (WUE). Traditionally WUE has been defined as the ratio of net photosynthesis to transpiration (A/E). Farquhar et al. (1982) demonstrated that $\delta^{13}C$ in C3 plant tissues provides a reliable index of water-use efficiency because both WUE and $\delta^{13}C$ are controlled by intercellular CO_2 levels. The relationship can be described as:

$$\delta^{13}C_{leaf} = \delta^{13}C_{atm} - a - (b - a)\, c_i/c_a \qquad (2.1)$$

where $\delta^{13}C_{atm}$ is −8.1‰ at this writing (http://cdiac.esd.ornl.gov/trends/co2/iso-sio/data/iso-data.html); it continues to fall as fossil-fuel sources of C are

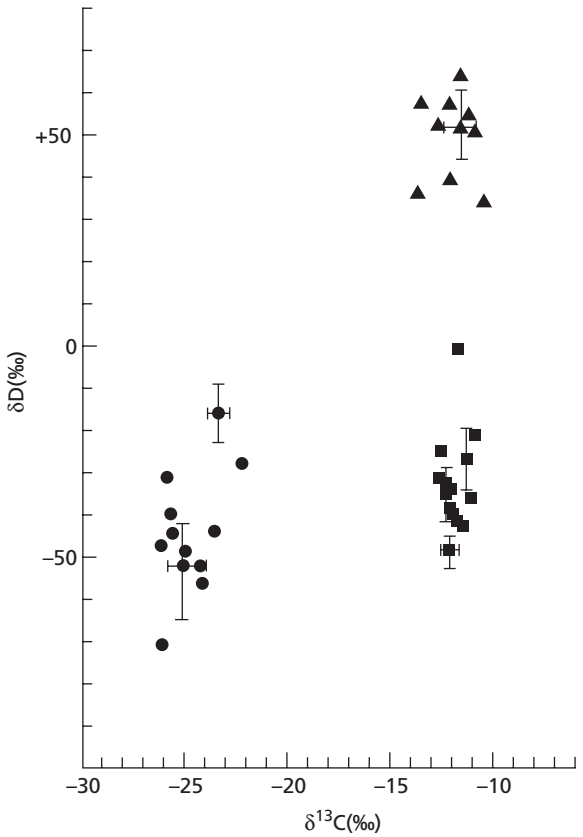

Figure 2.1 Hydrogen vs. carbon isotope ratios of plant cellulose nitrate for plants having different photosynthetic pathways. Squares represent C4, circles C3, and triangles Crassulacean acid metabolism. (From Sternberg et al. 1984a.)

added to the atmosphere. Term a is the fractionation caused by diffusion (4.4‰), b is the fractionation associated with carbon dioxide fixation (27‰), and c_i/c_a is the ratio of intercellular to ambient concentrations of CO_2. This form of the equation has been simplified to enhance utility; it is easily parameterized and adequate for many applications. We discuss its limitations and present a more complex form of the equation below.

It was noted earlier that $\delta^{13}C$ is also related to WUE. Using traditional symbols to describe photosynthetic gas exchange, we describe these relationships as:

$$A = (c_a - c_i)g/1.53 \quad (2.2)$$

$$E = g(\text{LAVD}) \quad (2.3)$$

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$$\text{WUE} = A/E = (c_a - c_i)/[1.53(\text{LAVD})] \quad (2.4)$$

where A is net photosynthetic rate, E is transpiration rate, g is stomatal conductance to water vapor, 1.53 is the ratio of diffusivities of water vapor and CO_2 in air (Campbell & Norman 1998), and leaf-to-air vapor difference (LAVD) is the difference in water vapor concentration between the interior of the leaf and the surrounding atmosphere (Farquhar & Richards 1984). Because c_a is nearly constant in the global troposphere within a given year, WUE varies primarily with c_i and LAVD. If LAVD can be assumed constant among the plants being considered at a given site, then plant $\delta^{13}C$ will be linearly correlated with WUE. However, LAVD is not always constant, especially when one compares across leaves that differ in morphology and function (e.g., Goldstein et al. 1989). When LAVD cannot be assumed constant or is unknown, many ecologists instead infer A/g, termed the intrinsic water-use efficiency, from stable isotope data:

$$A/g = (c_a - c_i)/1.53 \quad (2.5)$$

where the terms have already been defined above and the result is no longer dependent on assumed LAVD.

The relationship between $\delta^{13}C$ and WUE can be understood intuitively if one considers the behavior of CO_2 molecules as they enter a photosynthesizing leaf. The CO_2 molecules diffuse down a concentration gradient into the leaf. The CO_2 is diffusing against a countervailing diffusive flux of water vapor out of the leaf due to transpiration. Partial closure of the stomata reduces stomatal conductance, which reduces both gas fluxes – but not equally. The reduction in transpiration is proportional to the reduction in stomatal conductance because the water vapor concentration gradient is unaffected by stomatal closure. The air within the leaf remains saturated, the air outside the leaf remains at ambient, and the only change is in the ability of the water molecules to diffuse down this gradient. Net photosynthetic rates also decline, but the decline is less than proportional. This smaller decline occurs because the photosynthetic consumption of CO_2 within the leaf continues at a high rate even as the diffusive transport of CO_2 into the leaf is reduced. The resulting shift in the balance between diffusive supply and biochemical demand reduces c_i, offsetting some of the loss in photosynthesis that would otherwise have resulted from stomatal closure. Thus, the decline in net photosynthesis is less than the decline in transpiration, and water-use efficiency increases. Such a reduction in c_i would also modify the isotopic composition of photosynthate. As c_i falls, the $\delta^{13}C$ of the CO_2 inside the leaf is progressively enriched and the photosynthate produced is likewise enriched. The observed correlation between $\delta^{13}C$ and WUE thus results from independent, but

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correlated responses of these variables to falling c_i. As C4 plants show no evidence of variation in the balance between enzymatic assimilation and diffusion through the stomata, their $\delta^{13}C$ cannot be interpreted in terms of water-use efficiency.

A particular advantage of using $\delta^{13}C$ to estimate WUE is its long integration time. Closed-system gas-exchange techniques generally measure A/E on an instantaneous (15–30 s) basis, and on a few leaves. They can be programmed to integrate over a longer time course (Field et al. 1989), but the measurement itself represents a period of seconds. Carbon isotope analysis provides an estimate of WUE integrated over the time during which carbon in the plant was fixed, often weeks to months, and can do so for large numbers of independent samples.

Several complications have arisen related to integration time. The integration time depends on the time course of carbon emplacement, which varies during a tissue's lifetime. Meinzer et al. (1992) found that the integration heavily favors the period during which the leaf expanded. Another complication is that early stages of leaf construction are sometimes fueled by "heterotrophic" photosynthate (produced elsewhere in the plant). This imported photosynthate would contaminate the "autotrophic" carbon signal, and would need to be corrected to estimate WUE (Terwilliger et al. 2001). Finally, in evergreen leaves, small amounts of carbon continue to be added in the years after the leaves are first produced, which may similarly interfere with the interpretation of the isotopic signal (Hobbie et al. 2002).

Integration time may also vary on a shorter time scale, as where diel gas-exchange patterns differ. Net photosynthesis might sample different LAVD, e.g., if a leaf were active only during cool portions of the day, when LAVD is low. This would reduce leaf temperature (and therefore LAVD) during periods of carbon gain, increasing WUE. Such a pattern has been observed in a comparison of coastal to continental genotypes of Douglas-fir (Figure 2.2; Zhang et al. 1993). Inland genotypes maintained high conductance and high transpiration in mid-afternoon, despite high LAVD. In contrast, coastal genotypes closed their stomata almost completely in mid-afternoon, reducing gas exchange almost to zero. Average LAVD, weighted by net photosynthetic rates, was therefore different, as was WUE. These differences in diurnal pattern would change WUE, but would not be reflected in $\delta^{13}C$.

These caveats related to integration time are usually minor and are often viewed as advantages of the isotopic technique. They are presented here only to ensure that isotopic data are used knowledgeably. The use of $\delta^{13}C$ analysis to estimate long-term WUE has now been routine for more than a decade (Ehleringer 1989, 1991; Ehleringer & Osmond 1989; Farquhar et al. 1989a,b).

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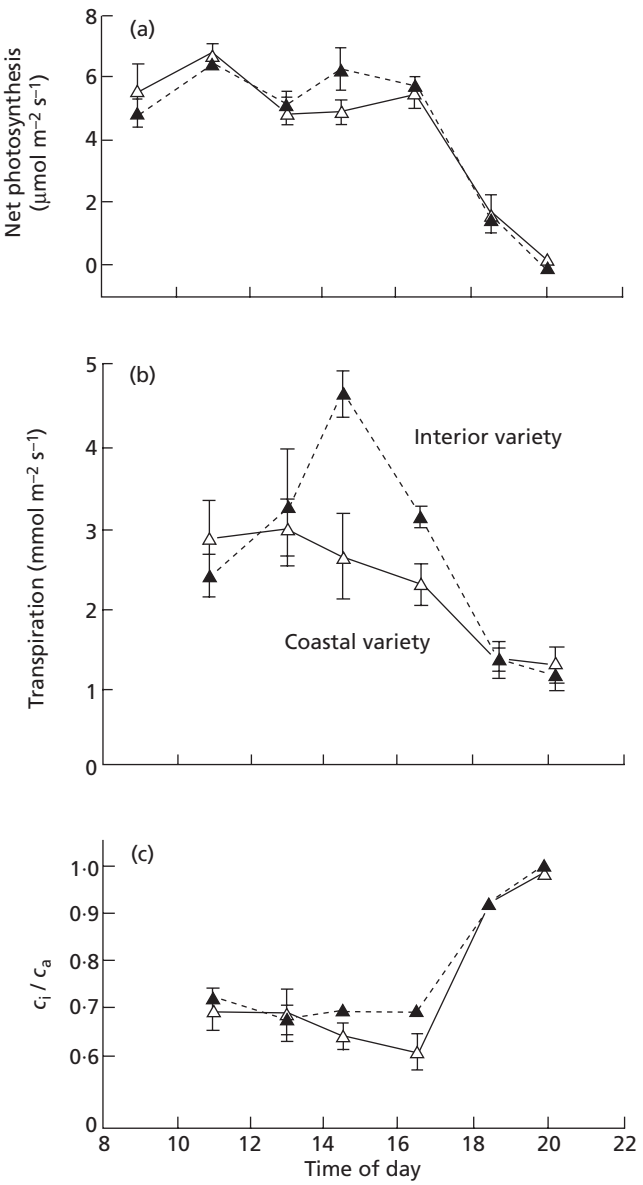

Figure 2.2 Diel patterns of (a) net photosynthesis (A), (b) transpiration (E), and (c) c_i/c_a from an experiment comparing the coastal and interior varieties of Douglas-fir in a common garden. (From Zhang et al. 1993.)

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Landscape and population patterns of plant $\delta^{13}C$

Soon after the isotopic method of estimating WUE became available, ecologists began to gain important insights from surveys of carbon isotope ratios in natural ecosystems. Much of this research focused on water gradients in desert ecosystems and altitude gradients in montane ecosystems. A survey of co-occurring species found that short-lived annual or herbaceous species had significantly lower $\delta^{13}C$ values than long-lived perennial species (Smedley et al. 1991). Species active during the wetter, more favorable months discriminated against ^{13}C more than did species that persisted over dry seasons, reflecting a lower WUE in the less drought-tolerant species. Desert plants that spanned a gradient from wash (an intermittent streambed) to a drier upland slope expressed higher WUE in the drier upland habitat (Ehleringer & Cooper 1988) than in the wash. This same pattern was observed in a survey of forest tree species in the mesic Appalachian Mountains (Garten & Taylor 1992).

In global surveys of $\delta^{13}C$ over altitudinal gradients, Körner et al. (1988, 1991) found that $\delta^{13}C$ increased with altitude. Körner et al. (1991) suggested that decreased oxygen partial pressures inhibited photorespiration, decreasing c_i. Morecroft & Woodward (1990) attributed the altitudinal gradient primarily to temperature effects on gas exchange, based on extrapolations from controlled-environment studies. Similarly consistent altitude gradients were observed in the Rocky Mountainst (Marshall & Zhang 1994). Because of shifts in $\delta^{13}C$ and LAVD, WUE increased threefold over 2000 m of altitude (Figure 2.3).

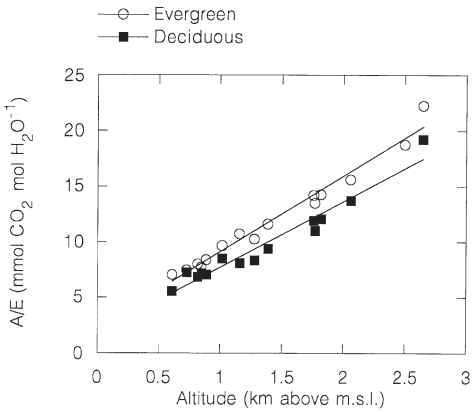

Figure 2.3 Water-use efficiency (A/E) of evergreen and deciduous species occurring along an altitude gradient in the northern Rocky Mountains. (From Marshall & Zhang 1994.)

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Some of the variation observed in these surveys has a genetic basis. At the species level, there are consistent differences at a site that can be related to leaf morphology (Marshall & Zhang 1994). Deciduous species are generally more depleted in ^{13}C (have more negative $\delta^{13}C$) than evergreens. Among evergreens, scale-leaved species (e.g., members of the Cupressaceae) are most enriched (less negative $\delta^{13}C$).

Further genetic variation is observed within species. Populations within a species often vary in $\delta^{13}C$ when planted into common environments. Such population differences have been studied especially in dryland crops, rangeland grasses, and forest trees. In most cases, the isotope data have compared well with other methods of estimating WUE and the results have been remarkably consistent across environments (Farquhar & Richards 1984; Condon et al. 1987; Hubick et al. 1988; Ehleringer et al. 1990; Johnson et al. 1990; Ehdaie et al. 1991; Read et al. 1991; Zhang et al. 1993, 1994). Because these measurements are made with a single species in a common environment, variation in temperature and LAVD are minimized and the isotopic differences are readily interpreted as WUE differences. Reported heritabilities are often high (Hubick et al. 1988; Farquhar et al. 1989b; Hall et al. 1990; Ehdaie et al. 1991), suggesting that WUE is a trait with strong genetic control, and for which breeding programs could readily be designed. The application of molecular genetic techniques to map and analyze the genetic basis of variation in WUE provides opportunities for deeper insights into this trait.

These surveys sometimes found variation that was not explained by the simple model presented earlier (equation 2.1). For example, Vitousek et al. (1990) found that foliar $\delta^{13}C$ of *Metrosideros polymorpha* increased with elevation on wet lava flows but not dry lava flows on the Mauna Loa volcano. But these patterns were not correlated with c_i, as measured by leaf gas-exchange. They were, however, correlated with specific leaf weight, or leaf mass per area, a measure of leaf thickness and density. Specific leaf weight increased significantly with elevation. The authors hypothesized that this discrepancy might be attributed to high internal resistance to CO_2 diffusion within the leaves. This resistance might explain the discrepancy because gas-exchange techniques estimate c_i in the substomatal cavities, just beneath the leaf surface. In contrast, stable carbon isotope ratios are determined by the CO_2 concentration in the chloroplasts (c_{ch}), deep within the leaves. In the thick *Metrosideros* leaves at the high elevation sites, c_{ch} was substantially lower than c_i and therefore WUE and $\delta^{13}C$ were decoupled. This observation has subsequently been confirmed several times, particularly in thick-leaved species (Lloyd et al. 1992; Hultine & Marshall 2000; Warren et al. 2003) . This decoupling, expressed as a varying offset from the equation relating $\delta^{13}C$ to WUE, might be expected in any species with high leaf mass per area.

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A detailed model of isotope fractionation in plants

In their detailed model, Farquhar et al. (1982) divided the path of photosynthetic CO_2 transfer into four distinct parts (boundary layer, stomata, transfer from stomata to chloroplast, and chloroplast) and used the following equation to describe each:

$$\delta^{13}C_{leaf} = \delta^{13}C_{atm} - a_b(p_a - p_s)/p_a - a(p_s - p_i)/p_a - (e_s + a_i)(p_i - p_c)/p_a - b(p_c/p_a) + (eR_d/k + f\Gamma^*)/p_a \quad (2.6)$$

where p represents partial pressures, which are closely related to concentrations, and subscripts s, i, and c designate measurements made at the leaf surface, in the intercellular space within the leaf, and within the chloroplast, respectively. The term a_b is the fractionation caused by the mixture of diffusion and convection in the boundary layer (2.9‰); e_s and a_i are the fractionations due to dissolution into and diffusion through water, respectively; e and f are the fractionations due to dark respiration and photorespiration, respectively; R_d is the dark respiration rate, k is the carboxylation efficiency, and Γ^* is the the CO_2 compensation point in the absence of dark respiration. The simplified form of the equation presented earlier includes only the first, third, and fifth terms in this equation. The second term, $a_b(p_a - p_s)/p_a$, accounts for the discrimination as CO_2 moves across the leaf boundary layer. When the boundary layer is thin, as at high windspeeds, in rough canopies, or over narrow leaves, this term is negligible. The fourth term, $(e_s + a_i)(p_i - p_c)/p_a$, accounts for the fractionation that occurs as gas-phase CO_2 dissolves in the cell wall and then diffuses through liquid water to the chloroplast. Because this discrimination is small and relatively constant (<1%) it is often neglected as well.

The sixth term has been the focus of intense research since the model was first published. The fractionation due to respiration (e) has been addressed in a series of recent papers describing the difference between respiratory substrates and the CO_2 produced. Protoplasts extracted from plant tissue and forced to deplete a substrate *in vitro* show no fractionation (Lin & Ehleringer 1997). However, intact plants nearly always fractionate, with CO_2 produced in respiration enriched by 2–7‰ relative to their substrate (Duranceau et al. 1999; Ghashghaie et al. 2001; Tcherkez et al. 2002; Ocheltree & Marshall 2004). If total respiration consumes on the order of 50 percent of photosynthate, this release of ^{13}C-enriched CO_2 would significantly deplete the $\delta^{13}C$ of the tissue produced. In fact, plant tissue is often depleted in ^{13}C relative to the carbohydrate pools from which it is produced (Park & Epstein 1961; Ghashghaie et al. 2003; Ocheltree & Marshall 2004).

Photorespiratory fractionation (f) has been difficult to measure, but may be significant; estimates range from 0 to 9‰, with most estimates on the higher end of this range (Gillon & Griffiths 1997; Ghashghaie et al. 2003). It

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is straightforward to eliminate photorespiration experimentally by lowering the oxygen concentration. The difficulty is in measuring the fractionation; photorespiratory CO_2 is produced within the chloroplast; where much of the CO_2 is immediately recycled in photosynthesis rather than leaving the leaf. Likewise, the fractionated dihydroxyacetone phosphate (DHAP) is recycled into ribulose and then consumed in photosynthesis. If such recycling were complete, no fractionation would be observed (Ghashghaie et al. 2003). However, photorespiration leaves its signature in another unique pattern; the C4 of the glucose molecule would be expected to vary with photorespiratory activity (Hobbie & Werner 2004). This insight may help to constrain estimates of apparent photorespiratory discrimination in the future.

There are many opportunities for respired CO_2 to be refixed in chlorophyllous tissues other than leaves. These tissues include fruits, floral parts, twigs, and in some cases, large-diameter stems (Aschan & Pfanz 2003). Refixed C can be quite depleted because it begins at the $\delta^{13}C$ of photosynthate and is further depleted by the fractionation due to rubisco (Cernusak et al. 2001). Although refixation does not constitute a net gain of C, it can eliminate as much as 100 percent of the diffusive loss from respiring tissues. Shading experiments show that the refixed C is incorporated into biosynthetic pathways, decreasing the $\delta^{13}C$ of the tissues in which it occurs (Cernusak et al. 2001).

A remaining source of variation in gas exchange is the transfer conductance, sometimes referred to as the mesophyll conductance, which was described earlier. The existence of a diffusive resistance across the leaf was recognized in the fourth term of equation 2.6 (Farquhar et al., 1982). In the simplified form of the equation, c_i is used to predict $\delta^{13}C$, though c_i is not at the end of the gaseous diffusion path across the leaf. The transfer conductance, which describes the remainder of the diffusion path to the chloroplast, has been related to chloroplast surface area (Evans et al. 1994). In the leaves of Douglas-fir, the transfer conductance is similar in magnitude to stomatal conductance, and must therefore be accounted for. Fortunately, it is uniform throughout the canopy (Warren et al. 2003).

One last source of variation is related to the fixation of CO_2 by enzymes other than rubisco. Even C3 plants fix small amounts of CO_2 using PEP carboxylase, the enzyme we associate with the first steps in C4 and CAM photosynthesis. In C3 plants, PEP carboxylase activity is usually small in comparison with rubisco activity, but the isotopic fractionation is so different (29‰ vs. −6‰) that a small amount of PEP carboxylase activity could significantly influence $\delta^{13}C$. Brugnoli et al. (1988) speculated that variation in b (equation 2.1) might be caused by differences in the proportion of CO_2 fixed by these two enzymes.

The observed correlation between – gas-exchange measurements derived from an infrared gas analyzer (IRGA) and carbon isotope ratios was in part the basis upon which Farquhar et al. (1982) constructed their theory. The

term *b* in equation 2.1, which describes photosynthetic discrimination, was parameterized by fitting a regression equation. If one were to construct the theory from its mechanistic basis upward, the term *b* would account for discrimination due to dissolution of CO_2 in water, diffusion of CO_2 through water, and rubisco; its value would be around 29–30‰ (Roeske & O'Leary 1984; Guy et al. 1987). In the simplified form of the equation presented above the empirically derived *b*-value is about 27‰. This difference is due to the numerous minor influences on carbon isotope ratios discussed above (Farquhar et al. 1982). The variable magnitudes of these influences would lead to variation in this parameter.

Source of carbon

This chapter has focused so far on physiological processes that modify $\delta^{13}C$. Another source of variation in plant $\delta^{13}C$ is the source of CO_2 that they use for photosynthesis, which we have designated $\delta^{13}C_{atm}$, the first term in equation 2.1. These issues were first raised in dense, closed-canopy forests, where foliar $\delta^{13}C$ often increases with canopy height (Vogel 1978; Medina & Minchin 1980; Medina et al. 1986, 1991; Sternberg et al. 1989). Such a vertical gradient in foliar $\delta^{13}C$ might be due to changes in $\delta^{13}C_{atm}$. In fact, several authors have found only limited vertical gradients in the isotopic composition of air from the canopy to the forest floor during the daytime when surface heating supports rapid air mixing. In these systems, the refixation of soil CO_2 is limited to a narrow zone near the soil surface. Respired CO_2 represented 18 percent of total CO_2 at 0.5 m above the forest floor in a tropical forest (Sternberg et al. 1989) and was substrate for 5–6 percent of total canopy photosynthesis in a boreal conifer forest (Brooks et al. 1997). This suggests that even in dense closed-canopy forests, estimates of WUE from foliar $\delta^{13}C$ do not need to be corrected for vertical changes in $\delta^{13}C_{atm}$, except perhaps for leaves produced near the soil surface.

More recently, source CO_2 has become particularly important in studies of CO_2 enrichment. Here the effects are often pronounced. Experimental enrichment of CO_2 is generally accomplished by adding fossil-fuel-derived carbon dioxide, which is depleted in ^{13}C, to ambient carbon dioxide, which is relatively enriched in ^{13}C. The CO_2 enrichment significantly leads to ^{13}C depletion relative to ambient (Ellsworth 1999). Few researchers have had the foresight and the funding to determine $\delta^{13}C$ of atmospheric CO_2 as frequently as necessary to characterize it well in these experiments. Several solutions have been developed. Ellsworth (1999) inferred $\delta^{13}C$ from CO_2 concentration. An alternate has been the deployment of C4 plants within the experimental atmosphere (Marino & McElroy 1991; Beerling 1999).

In long-term studies, variation in atmospheric $\delta^{13}C$ must be accounted for. The atmospheric concentration of CO_2 has increased by 30 percent over the past 100 years, due primarily to fossil fuel combustion (Figure 2.4). This rapid

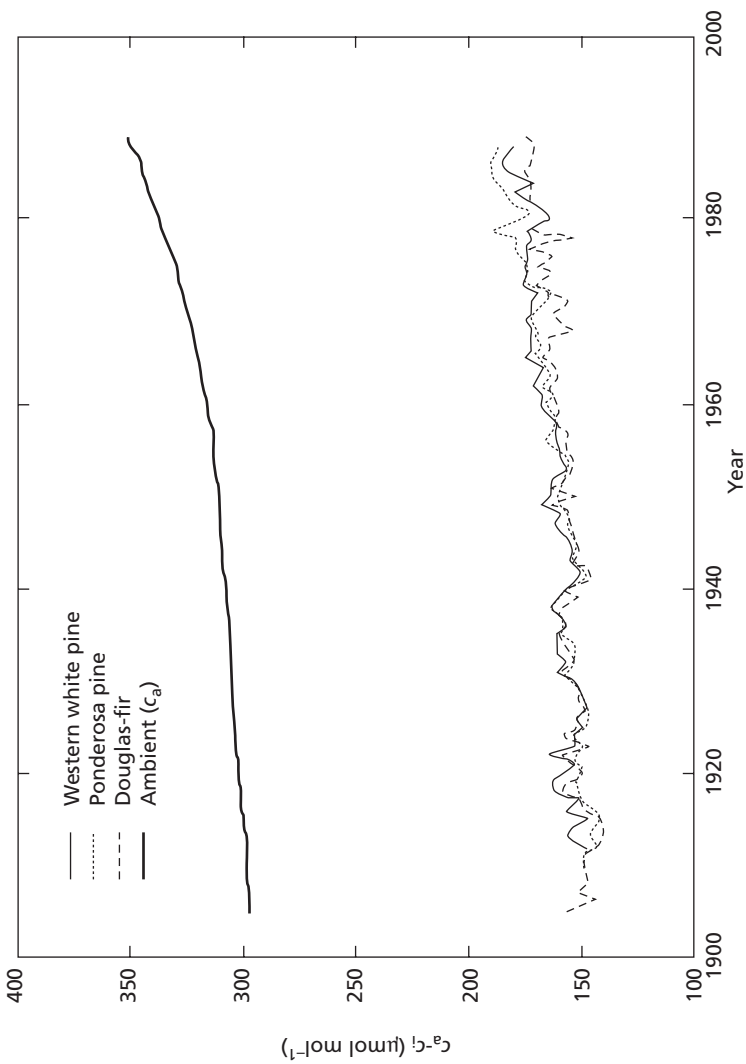

Figure 2.4 Ambient CO_2 concentration (c_a) and time series for internal leaf concentration (c_i) by species. The difference $c_a - c_i$ is proportional to intrinsic water-use efficiency. Estimates are based on $\delta^{13}C$ measured in ice cores and tree rings, respectively. (From Monserud & Marshall 2001.)

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change must be accounted for in multi-decade tree-ring studies (Freyer 1979; Marshall & Monserud 1996; Feng 1998; Monserud & Marshall 2001). The atmosphere is so well mixed that global averages can often be used to infer $\delta^{13}C_{atm}$ (http://cdiac.esd.ornl.gov/trends/co2/iso-sio/data/iso-data.html).

Variation among tissues and compounds

Stable carbon isotope composition also varies among plant tissues (O'Leary 1981). Some of this variation is due to differences among the chemical components of plant tissue. Lipids can be as much as 10‰ lighter than the whole tissue (O'Leary 1981; Badeck et al. 2005). In contrast, cellulose and other carbohydrates are typically 1–2‰ heavier than whole tissue (e.g., Leavitt & Long 1986) and lignin is typically 1–2‰ lighter. In detailed work, sample variation can be reduced by extracting a single compound or class of compounds. Because cellulose is the most abundant single compound in plant tissue and its structure is homogeneous, it is frequently used where such control of variation is necessary (Park & Epstein 1961; Freyer 1979).

Isotopic composition of tree rings is often enriched by 1.5–2‰ relative to foliage (Leavitt & Long 1986). This difference is important because 2‰ would represent a shift of over 25 percent if one were to use it to infer water-use efficiency of most C3 plants. It is presently unclear which tissue better reflects the $\delta^{13}C$ of canopy photosynthate or how these differences come about. Several possible mechanisms have been proposed to explain the discrepancy, including fractionation of the phloem contents with vertical distance (Damesin & LeLarge 2003) and enrichment of cellulose due to the production of lignin (Hobbie & Werner 2004). This mechanism needs to be identified before stable carbon isotope data from tree rings can be used to generate reliable gas-exchange inferences. Once resolved, it should be possible to use stable carbon isotope data to parameterize gas-exchange algorithms in ecosystem models (Katul et al. 2000).

Nitrogen isotopes

Although the use of natural abundance ratios of N in plants is not as well established as that of C isotope ratios, there is a great deal to be learned from a comparison of $\delta^{15}N$ among plants within an ecosystem, between plants and their source of N, and among plant components. Many studies have assumed that plant $\delta^{15}N$ reflected the bulk N source, but this assumption has not been universally supported. Differences between N source and plant isotope signatures has been an effective tool in understanding plant nutrient physiology. The physiological mechanisms that influence plant N isotopic signatures have been most recently reviewed by Evans (2001), and earlier by Högberg (1997) and Handley & Raven (1992). Some key applications using $\delta^{15}N$ in plant

tissues include assessing contributions of various N sources to plant N uptake in the field, including symbiotic nitrogen fixation and atmospheric deposition, the role of mycorrhizal infection, uptake of dissolved N, and the interpretation of $\delta^{15}N$ profiles in soils. Two other chapters in this book discuss specific applications of the use of $\delta^{15}N$ natural abundance measurements in ecology and environmental science (see Garten et al., pp. 00–00 and Evans, pp. 00–00); we will limit the discussion here to natural abundance measurements in plant tissues.

Sources of plant nitrogen

Although a number of pot culture studies have reported significant discrimination between plant tissues and the N in solution, there is general agreement that discrimination is only observed when plant N demand is low compared with N supply (Evans et al. 1996; Högberg et al. 1999). Although nitrate reductase (the enzyme responsible for nitrate assimilation and transformation to ammonium) and the glutamine-synthetase–glutamate-synthetase pathway (responsible for assimilation of ammonium) both discriminate against ^{15}N, this discrimination will only be observed if there is a pool of enriched inorganic N that can leak from plant roots after uptake, which is unlikely if plant N demand is high relative to N supply (Evans et al. 1996). Because N demand frequently exceeds N supply in natural systems, this suggests that plant $\delta^{15}N$ is a good approximation of $\delta^{15}N$ of the available N source(s), under most field conditions.

With this as a baseline assumption, much can be discerned from a comparison of measured ecosystem N pools and plant $\delta^{15}N$. However, we offer three precautions. First, it should be noted that $\delta^{15}N$ of either bulk soil or soil organic matter cannot be used as an indicator of source N to plants. Most N in soil is highly recalcitrant and unavailable to plants, the dissolved labile N pool is small, transient, and may have a significantly different isotopic composition than bulk soil (Bergersen et al. 1990). Secondly, plants may take up either NO_3^-, NH_4^+, or dissolved organic nitrogen (DON), with many (but not all) plants showing distinct preferences. Because nitrification has a fairly large isotope effect (α) (Shearer et al. 1974; Delwiche & Steyn 1970), NH_4^+ in soils with significant nitrification will be enriched in ^{15}N compared with NO_3^-. Thus a simple analysis of soil solution, or extractable N, will not conclusively identify the source of N to a specific plant, although Hobbie et al. (1998) used isotopic differences between NH_4^+ and NO_3^- to infer differences in plant preference for NH_4^+ vs. NO_3^-. Plants can change their preference for NO_3^- vs. NH_4^+ with environmental conditions; factors such as forest harvest (e.g., Pardo et al. 2002) that increase nitrification, or the application of fertilizer, can shift the relative uptake rates of NH_4^+ and NO_3^- (Högberg 1997). Taken together, these factors may be the reason that different studies have reported both depletions (e.g., Virginia & Delwiche 1982; Vitousek et al. 1989; Gebauer &

Schulze 1991) and enrichments in ^{15}N with plant uptake of soil N in the field.

A third precaution when comparing $\delta^{15}N$ values of plants and presumed sources of N is that foliar N cannot always be used as a proxy of whole plant N (Kolb & Evans 2002), which can be problematic for studies of large woody species. Different organic compounds can have quite different $\delta^{15}N$ values, dependent on their biosynthetic pathways (reviewed in Werner & Schmidt 2002). Due to fractionation associated with glutamate, proteins are generally ^{15}N-enriched relative to the bulk $\delta^{15}N$ of the plant cell, while secondary products such as chlorophyll, lipids, amino sugars and alkaloids are depleted in ^{15}N.

Nitrogen fixation

Within these sideboards on the interpretation of plant isotopic signatures, many authors have used $\delta^{15}N$ data to draw inferences regarding N sources. The degree of N fixation has been assessed in a number of different ecosystems and species, using techniques first developed by Shearer and colleagues (Shearer et al. 1983; Shearer & Kohl 1986). Their technique relied on finding local reference species that would integrate the signal from available soil N that could then be compared with the signature of the presumed fixing species. Since the atmospheric signal is defined as 0, and they assumed no fractionation, a simple two-component mixing model could determine the percent contribution by N_2 fixation This technique has been extensively reviewed, used, modified, and criticized (Peoples et al. 1991; Binkley et al. 1985; Lajtha & Schlesinger 1986; Pate et al. 1993; Bowman et al. 1996; Spriggs et al. 2003), with the general conclusion that this technique should be used with great caution, if at all. Handley et al. (1994) point out that plant available N cannot be treated as a single source at any one site, and that large variations in $\delta^{15}N$ can be found in reference plants due to variations in rooting depth, timing and preference of NH_4^+ vs. NO_3^- uptake, and possibly mycorrhizal status, making comparisons between fixing and non-fixing species difficult at best. Still, authors have used plant $\delta^{15}N$ as a qualitative indication of diazotrophy (Hobbie et al. 1998).

Atmospheric sources of nitrogen

One recent application of the $\delta^{15}N$ of plant tissues has been to assess the contribution of atmospheric sources of N to plants. The ability of plants to take up N directly via foliage has been recognized for some time through experiments using ^{15}N in various gas or liquid sources as tracers (Boyce et al. 1996; Wilson & Tiley 1998) or from leaf-chamber input–output budgets (Sparks et al. 2001, 2003). However, quantifying direct N uptake from atmospheric sources vs. soil sources in the field has proven difficult. Although

published $\delta^{15}N$ of specific pollutant N sources vary widely from +24.9‰ for NH_3 in barnyard samples to −13‰ for NO_x in automobile exhaust (Heaton 1986, 1990; Moore 1977), mixing models using soil and atmospheric sources can be confounded by multiple fractionations upon uptake or metabolism (Gebauer et al. 1994).

Although it might be difficult to quantify the importance of atmospheric sources of N to plants using natural abundance $\delta^{15}N$ values, general inferences can be made. For example, Evans & Ehleringer (1994) showed that relatively depleted N in marine fog was not a significant source of N to plants in the fog zone of the Atacama Desert of Chile, as $\delta^{15}N$ values of plants in the fog zone were significantly more positive than the fog. In contrast, Tozer et al. (2005) argued that the extremely depleted isotopic signatures found in lithophytes near Rotorua, New Zealand, were due both to significant fractionation upon volatilization of marine ammonia (NH_3) into the air and fractionation upon assimilation of NH_3 into plants. Because the volatilization of NH_3 to the atmosphere is accompanied by a very large fractionation, it should be easy to trace this source of N to plants where NH_3 might be significant, such as near intensive animal husbandry, guano deposits, or grazed land. Indeed, Erskine et al. (1998) suggested that the wide variation in plant $\delta^{15}N$ on subantarctic Macquarie Island reflected locations near either highly enriched scavenger excrement or significantly depleted NH_3 volatilized from penguin guano. Similarly, Frank et al. (2004) used isotope data to show that shoots of grassland plants in Yellowstone National Park directly absorbed ^{15}N – depleted NH_3–N that was volatilized from urine patches. Tozer et al. (2005) hypothesized that the uptake of highly depleted NH_3 is more widespread than currently thought and may be most significant in ecosystems that are highly N-limited; this hypothesis certainly deserves to be explored with additional measurements.

Mycorrhizal status

The idea that mycorrhizal status can affect the relationship between plant $\delta^{15}N$ and plant N source has been debated by many authors. Differences between mycorrhizal plants and non-mycorrhizal plants have been observed in numerous studies (Bardin et al. 1977; Högberg 1990; Chang & Handley 2000), due either to differences in N source (such as organic N) or due to differences in isotopic fractionation during N uptake. Since ectomycorrhizal fungi can directly use small organic compounds, the mineralization/nitrification pathway can be "short-circuited" (Trudell et al. 2004) and different factors can control isotopic abundance of this fungal-derived N. Many studies have shown that $\delta^{15}N$ in ectomycorrhizal fungi is generally greater than that of plant foliage and of their substrates in soil (Gebauer & Dietrich 1993; Högberg et al. 1999; Henn & Chapela 2001; Spriggs et al. 2003), but the exact mechanism of this difference is not clear, and could relate either to

discrimination within fungi or upon transfer to the host plant, or else due to differences in substrate accessibility.

Hobbie et al. (1998, 1999, 2000) described a range of sites representing different post-deglaciation ages at Glacier Bay, Alaska. They hypothesized that the low $\delta^{15}N$ values in foliage from plants with a high dependence on mycorrhizal fungi were due to a large isotopic fractionation within the fungi. The fungi produced isotopically depleted amino acids, which were subsequently passed on to plant symbionts. Thus, the observed difference between soil mineral nitrogen $\delta^{15}N$ and foliar $\delta^{15}N$ in later succession could be a consequence of greater reliance on mycorrhizae under N-limited conditions. Spriggs et al. (2003) also observed a large discrimination against ^{15}N during transfer of N from the fungus to the host plant, leaving the latter with a more negative $\delta^{15}N$ value. However; as Högberg (1990) points out, these studies have not excluded uptake of organic N as a cause of the differences. Many of these studies have found that the fungal rhizomorphs were enriched in ^{15}N, yet Högberg et al. (1999) calculated that the limited biomass of the fungus could cause only a marginal decrease in $\delta^{15}N$ of the N passing from the substrate through the fungus to the host. This conclusion was supported by the high transfer efficiency of N between the fungus and the plant. Clearly, more mechanistic studies are needed to resolve this conflict.

Hydrogen and oxygen isotopes

Hydrogen and oxygen atoms in plant tissues predominantly come from water, thus processes that affect the isotopic ratio of water will influence the hydrogen and oxygen isotope ratios in plants. The main sources of isotopic variation in plant water come from isotopic variation in precipitation inputs and enrichment of the heavy isotope (both ^{18}O and D) in water from evaporation from the soil surface, and evaporation from the leaf surface during transpiration.

Isotopic fractionation in water

As mentioned in McGuire & McDonnell (this volume, pp. 334–374), the isotopic variation in water is predominantly due to fractionation associated with phase changes from solid to liquid to vapor and *vice versa* (equilibrium fractionation), and to the diffusion processes of water vapor (kinetic fractionation). Both kinetic and equilibrium fractionation are important depending on where and how the phase changes occur.

Kinetic fractionation of hydrogen and oxygen isotopes is exactly analogous to that of carbon, and can be attributed to the faster diffusion of molecules containing the lighter isotopes. Kinetic fractionation factors for H_2O/DHO and $H_2{}^{16}O/H_2{}^{18}O$ are 1.016 and 1.032, respectively (Cappa et al. 2003); these

factors describe the 2–3 percent faster diffusion of light water relative to heavy water.

When the liquid and vapor phases of water are in equilibrium, the equilibrium fractionation factor describes the isotopic difference between the two phases. In the liquid phase, the heavier isotope is bound more tightly through hydrogen bonding than the lighter isotope, thus the lighter isotope is more readily released to the vapor phase. Equilibrium fractionation factors increase with decreasing temperature. For example at 25°C, fractionation values are 1.076 and 1.0092 for H_2O/DHO and $H_2{}^{16}O/H_2{}^{18}O$, respectively (Majoube 1971); these values describe the isotope ratio of the vapor divided by that of the liquid phase. Thus, at 25°C isotope ratios of water vapor would be 76‰ and 9.2‰ more negative than those of liquid water at equilibrium for δD and $\delta^{18}O$, respectively. However at 0°C fractionation values are 1.108 and 1.011 for H_2O/DHO and $H_2{}^{16}O/H_2{}^{18}O$, respectively, and the vapor would be 108‰ and 11.0‰ more negative than the liquid water.

Isotopic composition of water available for plant uptake

Isotopic ratios of hydrogen and oxygen in precipitation worldwide are closely related, lying on a single line known as the global meteoric water line (GMWL), where $\delta D = 8\ \delta^{18}O + 10$ (Craig 1961; Gat et al. 2001) (Figure 2.5). One end of the GMWL lies near the origin where the isotopic values of precipitation are similar to mean ocean water (V-SMOW, the isotopic standard for δD and $\delta^{18}O$ in water). Most of the line lies below the origin, reflecting the depletion of heavy isotopes in the vapor phase upon evaporation (see grey shaded arrow in Figure 2.5). The specific slope of the GMWL results because condensation in rain clouds occurs under equilibrium conditions, and kinetic fractionation is generally not a factor. The slope is simply the ratio of the equilibrium fractionation factors for the two atoms (δD and $\delta^{18}O$), which has the value of 8.

If evaporation from the ocean occurred under equilibrium conditions (RH = 100%) the intercept of the GMWL would be zero. However, the relative humidity (RH) is generally lower, thus kinetic fractionation through diffusion plays a role in evaporation of ocean waters. Kinetic fractionation is greater for $\delta^{18}O$ compared with equilibrium fractionation (1.032 vs. 1.0092 at 25°C, respectively), but the fractionation difference for δD is the opposite (1.016 vs. 1.076). The degree to which kinetic fractionation affects evaporation depends on relative humidity. This kinetic fractionation during evaporation causes the evaporation slope to be less than 8 and the slope decreases with increasing kinetic effect (lower RH). For example, in Figure 2.5, if vapor was formed completely under equilibrium conditions at 25°C, its isotopic ratios would be −76 and −9.2‰ for δD and $\delta^{18}O$, respectively (black solid point in Figure 2.5). However, since relative humidity is less than 100 percent (approximately 85% over the ocean) kinetic fractionation is

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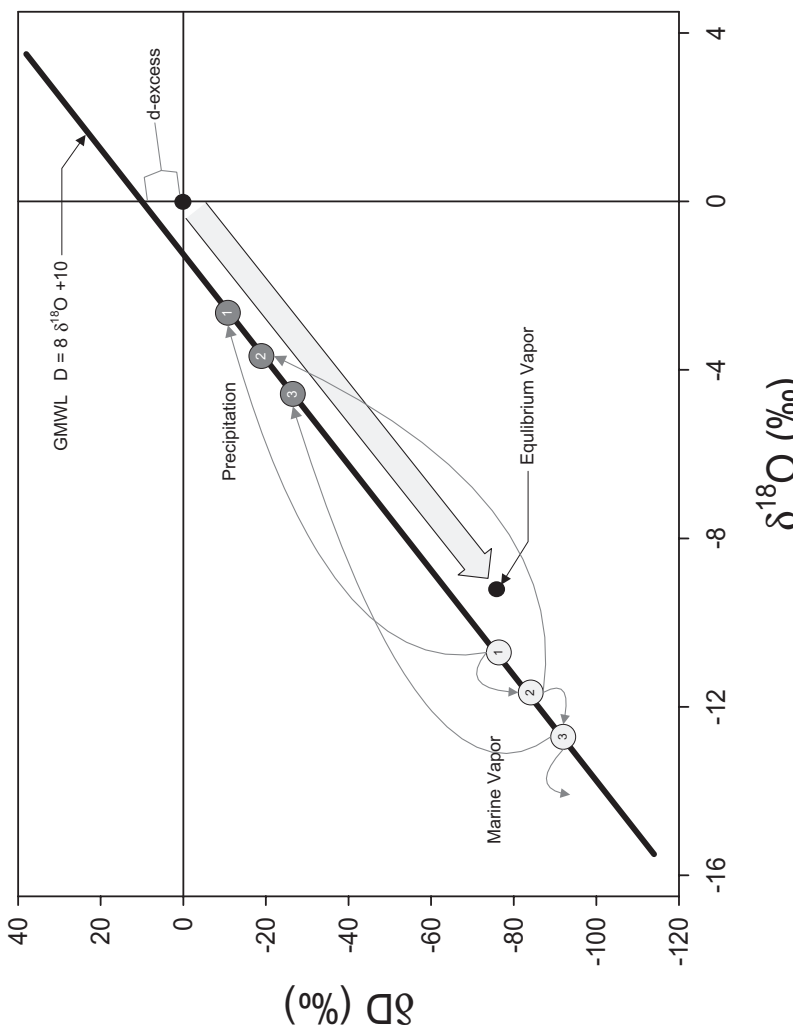

Figure 2.5 Schematic representation of the GMWL from evaporation from the ocean. The black dot at 0,0 represents ocean water, and the grey shaded arrow represents evaporation under equilibrium conditions (100% RH). The grey circles are the actual isotopic value of marine vapor since ocean evaporation occurs at RH lower than 100 percent (~85%), which leads to a d-excess of approximately 10. The numbered circles (1–3) for marine vapour (light circles) and precipitation (dark circles) illustrate the increasing depletion as precipitation forms under equilibrium conditions and falls from the cloud (rainout effect). (After Gat et al. 2001.)

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involved, and the $\delta^{18}O$ value is more depleted than for the equilibrium case (see Figure 2.5 Marine Vapor 1). Note that the δD is about the same value in both cases. When precipitation is formed from this vapor (under equilibrium conditions because rain cloud RH = 100%, so the slope will again be 8), the resulting precipitation will have an excess of deuterium (d-excess in Figure 2.5) that causes the intercept of the GMWL to be around 10‰ (Clark & Fritz 1997).

If precipitation falls through relatively dry air and evaporates as it falls, kinetic fractionation again influences the respective evaporation rates of DHO and $H_2{}^{18}O$, drawing the water off the global meteoric water line. The deviations give rise to local meteoric water lines (LMWL) that usually have slopes less than 8. The slope of a LMWL can be used to determine the extent of evaporation after raindrops are formed (Gat et al. 2001).

The isotopic ratio of precipitation will change along the GMWL because of temperature effects on fractionation, and the depletion of precipitation with continued rainfall. Since clouds and precipitation form under equilibrium conditions, heavier isotopes accumulate in the liquid phase and will fall first in the precipitation. Consequently, rain clouds will be come isotopically lighter over time. This "rainout" depletion of rain clouds follows a Rayleigh distillation process (see McGuire & McDonnell, this volume, pp. 334–374, for more details). These temperature and rainout effects underlie pronounced geographical and seasonal variation in the isotope ratios of precipitation that can be summarized as follows (influencing variables noted in parentheses):

Latitude effect	more negative with increasing latitude (temperature and rainout)
Elevation effect	more negative with increasing altitude (temperature and rainout)
Continental effect	more negative as an air mass moves inland (rainout)
Seasonal effect	more negative in winter, less negative in summer (temperature)
Amount effect	more negative when more precipitation falls (rainout and decreased evaporation of rain due to higher relative humidity with more rain)

Within a particular ecosystem, the isotopic ratios of precipitation vary from storm to storm depending on the origin of the storm (high vs. low latitudes) and the cloud temperature during condensation. Generally, more depleted isotopic values of precipitation are found during the winter and enriched precipitation during the summer. For example, values for δD in one study of precipitation in Austria range from −180‰ in December to −5‰ in May (Libby et al. 1976). Similar data from Utah range from −220‰ for April snow to −15‰ from a summer thundershower (Dawson & Ehleringer, 1991). Precipitation also interacts with plant canopies as it falls, which can alter the isotopic signal of precipitation reaching the soil through evaporation of

intercepted water (see McGuire & McDonnell, this volume, pp. 334–374). This variation in precipitation isotopic values along with precipitation amount can create unique isotopic patterns within the soil profile from which plants obtain their moisture.

Generally, deep soil water or ground water has been reported as approximately the average of mean annual precipitation (Clark & Fritz 1997). However, the actual value may vary from this mean depending on seasonal patterns of water inputs (precipitation) and water losses (evapotranspiration). For example, evapotranspiration is usually less during the winter dormant season so winter precipitation has a greater chance of percolating to deeper soil layers rather than being evaporated or transpired compared with summer precipitation, leading to more lighter isotopic values deeper within the soil profile. Summer precipitation tends to be enriched isotopically, leading to heavier isotopic signals in the upper soil during the active growing season. Evaporation can also occur from the soil surface, further enriching the surface soil water. However, evaporation from the soil surface generally occurs within the top 10 to 20 cm, and not much deeper.

Water uptake by plants

Water is not isotopically fractionated when taken up by the plant (Dawson & Ehleringer 1993), except perhaps under exceedingly unusual conditions such as salt-water uptake by salt-excluding halophytes (Lin & Sternberg 1993). As a result, water in plant tissues carries the same isotopic signal as the source water – until it reaches the sites of evaporation, generally in the leaves. Therefore, the isotope ratio of xylem water can be used as a measure of the isotopic signature of the soil-water being utilized. This measurement can be particularly useful, provided the various water sources are sufficiently distinct in their isotopic composition, e.g., surface water vs. ground water vs. fog. For two sources, a simple two-ended mixing model is used with the two sources being the end members, and the plant-water value being somewhere in between. With more isotopically distinct sources, the precision for percent water use from each source will increase (Phillips & Gregg 2001). With two isotopes for water (δD and δ^{18}O), theoretically, three sources could be separated using a simple mixing model; however, as mentioned above, δD and δ^{18}O are highly correlated (GMWL), so in reality two sources can be separated using water isotopes (Phillips & Gregg 2001). Phillips & Gregg (2003) have also worked out techniques to deal with more sources than can be uniquely solved for, so it is possible to work with more than two sources of water. In addition, other information can be used to help constrain possibilities such as information on soil-water potential. For example, for a particular soil layer, if soil-water potential was lower than leaf-water potential, that soil layer could not be a source for water. One problem with the two-ended mixing model technique is that isotopic profiles in soils are a

continuum rather than two discrete isotopic values (Allison et al. 1983; Thorburn & Ehleringer 1995; Meinzer et al. 1999; Plamboeck et al. 1999; Moreira et al. 2000).

In situations where sources are isotopically distinct, the natural abundance of isotopes in water has proved extremely useful for understanding patterns of plant water uptake. For example, this technique was used to demonstrate that co-existing plant life forms with varying rooting morphologies can utilize different sources of water (Ehleringer et al. 1991). Williams & Ehleringer (2000) used this technique to separate surface and deep soil water usage for three tree species over 2 years along a summer monsoonal gradient. They found that one species (*Quercus gambelii*) did not use summer precipitation where summer precipitation was not a reliable resource. However, the two conifer species (*Juniperus osteosperma* and *Pinus edulis*) did utilize surface summer rains when available along the entire gradient. In a classic study, Dawson & Ehleringer (1991) demonstrated that riparian trees do not necessarily use stream water, but instead may rely on deeper aquifer water. Stable isotopes of stem water have also been useful for documenting seasonal use of fog water in a redwood forest (Dawson 1998), and in epiphytic plants (Field & Dawson 1998). Meinzer et al. (1999) reported that smaller tropical trees relied more on deep water than did larger co-located trees. Thorburn & Ehleringer (1995) also sampled roots with depth, and found that roots in a particular soil layer did not always take up water from that layer. Dawson & Pate (1996) found that the isotopic ratio in surface lateral roots shifted seasonally, with water in lateral roots matching the isotopic value in deep sinker roots during the dry season rather than the isotopic value of water in the surrounding soil, which indicated that during the dry season, the sinker roots were providing water to the surface roots as well as the tree. In cases where natural abundance differences within the soil are not sufficient for determining source utilization, using an isotope label can also be highly effective to examine source utilization (Plamboeck et al. 1999; Moreira et al. 2000; Brooks et al. 2002, 2006), but tracer enrichment studies are beyond the scope of this book.

Isotope ratios of leaf water

As mentioned earlier, transpiration from plant leaves leads to fractionation of the xylem water. As a result, leaf water is often considerably enriched in the heavier isotopes. The problem of describing leaf-water enrichment is an elaboration of the more general problem of water evaporation; the seminal work in this area was conducted by Craig & Gordon (1965). Flanagan et al. (1991) expanded on this framework by incorporating leaf boundary layer effects, resulting in the following Craig–Gordon model to describe the isotopic composition of water in a transpiring leaf at steady state:

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$$R_{wl} = \alpha^* \left[\alpha_k R_{wx} \left(\frac{e_i - e_s}{e_i} \right) + \alpha_{kb} R_{wx} \left(\frac{e_s - e_a}{e_i} \right) + R_a \left(\frac{e_a}{e_i} \right) \right] \quad (2.7)$$

where R is the molar ratio of the heavy to light isotope, e is partial pressure of water vapor and the subscripts "wl", "wx", "i" "s" and "a" refer to leaf water, xylem water, intercellular spaces, leaf surface and bulk air, respectively; α^* is the equilibrium fractionation factor; α_k is the kinetic fractionation factor; α_{kb} is the kinetic fractionation factor associated with diffusion through a turbulent boundary layer (α_k to the 2/3 power). This model can also be written in terms of enrichment above the source water (Farquhar et al. 1989b; Farquhar & Lloyd 1993; Farquhar & Gan 2003):

$$\Delta_e = \alpha^* + \alpha_k + (\Delta_v - \alpha_k) \frac{e_a}{e_i} \quad (2.8)$$

where Δ_v is the oxygen isotope composition of the atmospheric water relative to the xylem water source. If isotopic composition of the xylem and atmospheric water and leaf temperature are assumed constant, which would hold α^*, e_s and e_i constant, then R_{wl} and Δ_e would be linearly related to e_a/e_i, which is the relative humidity of the atmosphere. Because leaf water is the source for oxygen and hydrogen in leaf organic matter, leaf organic matter can contain information about relative humidity during the leaf's lifespan.

If bulk leaf water comprised a single homogeneous, equilibrated pool, one would expect the isotope ratio of leaf water to match predictions based on the equations above. However, bulk leaf water does not match this simple model (Yakir et al. 1989; Flanagan et al. 1991) for several reasons. First, it takes a matter of hours for leaves to approach isotopic steady state, the point at which the isotopic composition of the water entering the leaf is equal to that of the water diffusing out (White 1989; Cernusak et al. 2002). Second, leaves often vary in water volume on a diel basis, and third, the various pools of water within a leaf are seldom well-mixed, resulting in isotopic heterogeneity within the leaf (Yakir 1998; Barbour et al. 2000). One reason for heterogeneous leaf water is that gradients of enriched water can result from convection of unenriched xylem water towards the site of evaporation and the opposing diffusion of enriched water away from the site of evaporation (Farquhar & Lloyd 1993), which is known as the Péclet effect ($\wp$). The influence of the Péclet effect on the isotopic composition of bulk leaf water can be estimated with the following equations:

$$\Delta_L = \frac{\Delta_e \left(1 - e^{-\wp}\right)}{\wp} \quad (2.9)$$

$$\wp = \frac{LE}{CD} \quad (2.10)$$

where Δ_L is the isotopic composition of bulk leaf water relative to source water, L is the effective path length, E is transpiration, C is the molar density

of water, and D is the diffusivity of $H_2{}^{18}O$ (Farquhar & Lloyd 1993; Barbour et al. 2004). The Péclet effect varies with the rate of transpiration from the leaf, with enrichment being relatively lower with higher transpiration rates. This can affect the $\delta^{18}O$ of sucrose because sucrose is not synthesized at the site of evaporation within a leaf but in the chloroplast, which will have a different leaf-water signature than the site of evaporation (Barbour et al. 2000).

For a broad range of environmental conditions, the basic Craig–Gordon model, which does not account for the Péclet effect, provides a useful interpretation of the $\delta^{18}O$ and δD of leaf water and the organic matter derived from leaf water (Roden & Ehleringer 1999b). However, depending on the particular application, accounting for the Péclet effect may improve estimates of transpiration rates (Wang & Yakir 2000; Barbour & Farquhar 2003; Barbour et al. 2004). More elaborate models have recently been developed to predict bulk leaf water while accounting for the Péclet effect (Barbour & Farquhar 2003; Farquhar & Gan 2003) and for non-steady state conditions (Cernusak et al. 2002; Farquhar & Cernusak 2005). As always, more elaborate models are more difficult to parameterize. The optimal modeling approach depends on the objectives of the user.

How are leaf water enrichment data used? First, as mentioned above, the degree of enrichment reflects the relative humidity of the environment and may allow inference of transpiration rates. These isotopic signals are preserved in organic matter and so may provide long-lived records of the distant past. In addition to the climate signal, $\delta^{18}O$ in leaf organic matter might provide insights as to whether changes in $\delta^{13}C$ were caused by differences in stomatal conductance or photosynthetic capacity (Scheidegger et al. 2000). Recall that changes in $\delta^{13}C$ can be through changes in stomatal supply or photosynthetic demand. Knowing the relative humidity can help one deduce which variable was more likely responsible for variation in $\delta^{13}C$, and thus, c_i. Second, CO_2 entering the leaf exchanges oxygen with enriched leaf water; a portion of this CO_2 then diffuses back out of the leaf, affecting the $\delta^{18}O$ of atmospheric CO_2 (Farquhar et al. 1993). Oxygen evolved during photosynthesis also contains the leaf enrichment signature. This isotopic information in atmospheric gases is used by global modelers to separate terrestrial and marine productivity.

Hydrogen and oxygen isotopes in plant tissues

The primary interest in $\delta^{18}O$ and δD in plant tissue has been to obtain climate information. The above discussion elaborates on how leaf-water isotopic signatures are influenced by relative humidity. Recall that xylem water has the same isotopic composition as the soil water it is derived from. The $\delta^{18}O$ and δD of plant tissue is influenced by both these pools of water (leaf water and soil/xylem water). Several authors have noted correlations between

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treering cellulose isotope data and temperature (Gray & Thompson 1976; Yapp & Epstein 1982), relative humidity (Yapp & Epstein 1982; Edwards & Fritz 1986, 1988), or precipitation amount (Lawrence & White 1984; Krishnamurthy & Epstein 1985; White et al. 1994; Saurer et al. 1997b); however, these correlations differ in the relative influence of each factor. A number of investigators began to realize that two water sources were important for determining the $\delta^{18}O$ and δD of plant tissue: xylem water and atmospheric water vapor. Thus plant tissue samples could contain information on both temperature and relative humidit (Luo & Sternberg 1992; White et al. 1994; Saurer et al. 1997a).

To determine past climate signals, it is important for the tissue to maintain the isotopic signature over time. Some authors have argued that plant tissues need to be extracted to yield α-cellulose for oxygen and nitrated cellulose for hydrogen to remove all oxygen and hydrogen atoms not bound to carbon (Leavitt & Danzer 1993; Loader et al. 1997). Atoms not bound to carbon continue to exchange in the presence of water, thus losing the $\delta^{18}O$ or δD signal at the time the cellulose was formed. However, a recent study has indicated that whole wood contains isotopic data of sufficient quality to analyze environmental signals (Barbour et al. 2001), thus for some purposes extraction may not be necessary, but further research into the necessity of extraction is needed. A larger problem exists in separating the temperature and relative humidity signals contained in $\delta^{18}O$ and δD of tree rings for climate reconstruction.

In a landmark series of papers, Roden, Ehleringer and others (Roden & Ehleringer 1999a, 1999b, 2000; Roden et al. 2000) elucidated the relative influence of leaf water and source water on tree ring $\delta^{18}O$ and δD composition as follows:

$$\begin{aligned} \delta D_{cx} &= f_H(\delta D_{wx} + \varepsilon_{HH}) + (1 - f_H)(\delta D_{wl} + \varepsilon_{HA}) \\ \delta^{18}O_{cx} &= f_o(\delta^{18}O_{wx} + \varepsilon_o) + (1 - f_o)(\delta^{18}O_{wl} + \varepsilon_o) \end{aligned} \tag{2.11}$$

where f_H and f_o refer to the fraction of carbon-bound hydrogen and oxygen, respectively, that undergo exchange with the water source during cellulose formation. Leaf water is subscripted 'wl' and xylem water is subscripted 'wx'. These f values were estimated to be 0.36 and 0.42, respectively. Large biochemical fractionation factors are associated with the formation of sucrose and cellulose, and are different for oxygen and hydrogen isotopes. The hydrogen isotope fractionation factor associated with autotrophic carbohydrate metabolism (ε_{HA}) was determined to be between −120 and −171‰ (Yakir & DeNiro 1990), and for heterotrophic carbohydrate metabolism (ε_{HH}), between +144 and +166‰ (Yakir & DeNiro 1990; Luo & Sternberg 1992). The oxygen isotope ratio has just one fractionation factor (ε_o) of +27‰ associated with the water/carbonyl interactions (Sternberg 1989; Yakir & DeNiro 1990). In some situations, the water in which cellulose is being

formed may be a mixture of xylem water and enriched leaf water (Barbour & Farquhar 2000), and thus an equation using xylem water values would not be correct. For oxygen, Barbour & Farquhar (2000) proposed the following model:

$$\Delta_{cx} = \Delta_L(1 - p_{ex}p_x) + \varepsilon_o \quad (2.12)$$

where p_{ex} is the proportional exchange (equivalent to f_o) and p_x is the proportion of unenriched water (xylem water) at the site of cellulose formation. Sternberg et al. (2003) proposed a method for separating leaf and xylem water signals by examining only oxygens in cellulose that completely exchange with xylem water at the time of cellulose formation; however, the authors express the need for more work on this method before it will be useful in tree-ring studies.

Separating the influence of humidity (enriched leaf water) and temperature (xylem water) will be a challenge; however, significant and interesting environmental trends have been found in tree-ring chronologies of $\delta^{18}O$ or δD. Saurer et al. (2000) found that the $\delta^{18}O$ in a latewood tree-ring chronology of *Abies* in Switzerland was highly correlated with $\delta^{18}O$ in June/July precipitation. Saurer et al. (1997b) noted similar results for *Fagus* trees in Switzerland. However, in both studies the correlation between temperature and $\delta^{18}O$ in tree rings was weak. Subdividing rings is a promising approach for examining seasonal dynamics. Barbour et al. (2002) documented both site and seasonal differences related to relative humidity in $\delta^{18}O$ and $\delta^{13}C$ chronologies of *Pinus radiata* that were subdivided. White et al. (1994) also found seasonal changes in δD on subdivided rings with increasing enrichment through the season. Tree rings are not the only material that can be used to study climate. Helliker & Ehleringer (2002) demonstrated that the $\delta^{18}O$ in sections of grass blades could record shifts in relative humidity during leaf expansion. Jäggi et al. (2003) noted that short-term climate change was more strongly reflected in the $\delta^{18}O$ of needle tissue compared with either early or late wood. These studies and others indicate the potential power of $\delta^{18}O$ and δD in plant tissues to record environmental information (McCarroll & Loader 2004).

Separating evapotranspiration using stable isotopes

Another emerging area using $\delta^{18}O$ and δD in water are studies which have attempted to quantify evaporation from soil relative to transpiration from leaves (Moreira et al. 1997; Wang & Yakir 2000; Yepez et al. 2003; Williams et al. 2004). Water evaporated from leaves and from soil have different isotopic signatures. Leaf water is closer to steady state and enriched to a greater degree than soil water. By definition, water vapor diffusing from a leaf at steady state must have the same isotopic signature as the source xylem water (Yakir et al. 1993), whereas water vapor from soil is more depleted. By measuring the isotopic ratio of soil water where evaporation is taking place and

using the Craig–Gordon model (equation 2.7), the isotopic composition of soil-water vapor can be estimated. The third source of water vapor is from the atmosphere. Separating evaporated and transpired water relies on both a mixing model approach and a technique known as Keeling plots (Pataki et al. 2003). The Keeling-plot approach will determine the signature of evapotranspired water, and the mixing model will determine the relative proportion of evaporated and transpired water. In the Amazon forest, transpiration was the dominant source of water vapor (Moreira et al. 1997). Similarly, Wang & Yakir (2000) found that soil evaporation was only 1.5–3.5 percent of the evapotranspiration flux from crops in a desert environment. Williams et al. (2004) observed that soil evaporation changed from 0 percent in an olive orchard prior to irrigation to 14–31 percent for the 5 days following irrigation. This approach is still in its infancy and is also complicated by non-steady state transpiration. Lai et al. (2006) found it necessary to include a non-steady state model for transpiration to tease appart diurnal patterns in the isoflux (isotope signal of water and the water flux) from a 400-year-old coniferous forest.

Conclusions

The use of natural abundance stable isotopes to elucidate physiological processes in plants is one of the most common, and one of the oldest, applications of isotope analysis in ecology. Plants display particularly strong isotopic signals because they construct their tissues from such small molecules. These molecules, e.g., CO_2, NO_3^-, NH_4^+, H_2O, are small enough that the presence of an extra neutron in a heavy atom affects the behavior of the whole molecule. These isotopic effects are often less pronounced in animals, which tend to use bigger molecules as substrates, and therefore tend to retain the signals imposed on them by the plants they consume.

Isotope analysis is now a well-established tool used to determine carbon fixation pathways of plant species, plant water-use efficiency, and source of water used; new uses are rapidly being developed. Understanding the physiological processes behind stable isotope signatures of primary producers has given researchers new tools to analyze animal paleodiets (see Evans, this volume, pp. 83–98), to trace food webs in both marine (see Montoya, this volume, pp. 176–201) and terrestrial ecosystems, and to trace carbon sources in estuarine systems (see Finlay & Kendall, this volume, pp. 283–333), among other applications.

As with any other research tool, it is critical to understand the assumptions and where they might be violated. In this chapter we have described the theoretical assumptions that underlie the interpretation of stable isotope signatures in plant ecophysiology and the types of empirical data that are commonly collected. We have described several cases where these assumptions

might be violated. These caveats notwithstanding, we fully expect that the potential of these powerful new isotope techniques will continue to expand in the future. It is our intention to encourage the use of these techniques by ensuring that users are knowledgeable. As work continues to identify the detailed mechanisms underlying isotopic composition, the range of applications is sure to expand.

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