

# 12

## Assessing Ecosystem-Level Water Relations Through Stable Isotope Ratio Analyses

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### Introduction

Virtually all elements of biological interest have multiple stable isotopic forms and the fractionation events associated with biological and physical processes help to create spatial and temporal variations in isotopic abundance that can be used to understand the dynamics of ecological systems. Stable isotope ratio analyses at natural abundance levels can provide integrated information on ecosystem functioning, such as variations in water-use activities by different elements within an ecosystem (Ehleringer et al. 1993; Dawson and Ehleringer 1998). Stable isotope ratio analyses do not provide information on water flux rates through the ecosystem, but instead they help constrain the analysis of flux data, such as through identifying those specific soil layers that are the source of current moisture use by the vegetation or the ratio of carbon dioxide-to-water ( $\text{CO}_2$ -to- $\text{H}_2\text{O}$ ) flux.

### Stable Isotopes: Natural Abundances and $\delta$ Notation

Most elements are present in multiple stable forms, although one isotope is usually far more abundant than the others. Rather than consider the abundances of different stable isotopes in absolute terms, it is much more common and convenient to describe the abundance of stable isotopes as the molar ratios of the heavy-to-light forms ( $R$ ) in a sample ( $R_{\text{sample}}$ ) relative to that of an international standard ( $R_{\text{standard}}$ ). This is the so called delta notation ( $\delta$ ) where

$$\delta = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \cdot 1000\text{‰} \quad (12.1)$$

and the  $\delta$  value has units of parts per thousand, or per mil (‰).

With regard to water relations functions within ecosystems, carbon (C), hydrogen (H), and oxygen (O) isotopes are most relevant. Table 12.1 presents the typical background value for different isotopic forms as well as the range of  $\delta$  values that one might encounter when investigating different ecosystem components on a global basis.

### Isotope Ratio Mass Spectrometry

In isotope ratio mass spectrometry, light gases are introduced into a chamber maintained at a very low pressure ( $<10^{-6}$  torr), in which there is an electron source. The gases (in our case  $\text{H}_2$  or  $\text{CO}_2$ ) are bombarded by this electron source, resulting in ion formation in approximately 1 out of every 1000–4000 molecules. The most likely molecular possibilities that are ionized include  $^1\text{H}^1\text{H}$  and  $^2\text{H}^1\text{H}$  for  $\text{H}_2$  and  $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ ,  $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ , and  $^{12}\text{C}^{18}\text{O}^{16}\text{O}$  for  $\text{CO}_2$ , although other rarer combinations most certainly occur. These charged ions are then accelerated and pass along a flight tube with an even lower pressure ( $10^{-8}$  torr). During this flight, their trajectory is deflected by a magnet located at the center point. The extent of an ion's magnetic deflection depends on its  $m/e$  (i.e., mass to charge) ratio. Thus,  $^1\text{H}^1\text{H}^+$  is deflected more than  $^2\text{H}^1\text{H}^+$  and likewise  $^{12}\text{C}^{16}\text{O}^{16}\text{O}^+$  more than  $^{12}\text{C}^{18}\text{O}^{16}\text{O}^+$ . At the end of the flight tube are a

TABLE 12.1. The stable isotopes that are of primary interest for addressing water relations issues within ecosystems.  $R_{\text{standard}}$  is the molar abundance ratio of the heavy to light isotopic forms of the international standard. The range of expected variation is presented in delta notation with units of ‰. The reference standards are Standard Mean Ocean Water (SMOW) and Pee Dee Belemnite (PDB). Standards values are from Hayes (1983).

Element	Isotopic abundance (%)	$R_{\text{standard}}$	Typical range of $\delta$ values for different ecosystem components (‰)
Hydrogen	$^1\text{H}$ 99.985	$^2\text{H}/^1\text{H} = 0.00015576$ (SMOW)	-200 to +50
	$^2\text{H}$ 0.015		
Carbon	$^{12}\text{C}$ 98.89	$^{13}\text{C}/^{12}\text{C} = 0.0112372$ (PDB)	-80 to +15
	$^{13}\text{C}$ 1.11		
Oxygen	$^{16}\text{O}$ 99.759	$^{18}\text{O}/^{16}\text{O} = 0.0020671$ (SMOW)	-30 to +40
	$^{17}\text{O}$ 0.037		
	$^{18}\text{O}$ 0.204		

series of ion current counters that measure the intensity of different mass signals that strike Faraday cups positioned to measure specific m/e ratios. From these current measurements, the relative ratio of the different masses can be determined and the isotope ratios of the sample calculated relative to that of the working and international standards.

Samples are introduced into the mass spectrometer by one of two conventional means. The first and more traditional approach is called the dual inlet mode, because it involves introducing the clean sample into one variable volume bellows and the standard into a second variable volume bellows. Both samples are then alternately introduced 6 to 12 times into the mass spectrometer from their bellows via a capillary connection to the source chamber. The isotope ratio calculation is based on the average of these 6 to 12 individual, short-term observations. This approach yields the highest precision measurement and typically the precision is  $\pm 0.006\text{‰}$ ,  $\pm 0.25\text{‰}$ , and  $\pm 0.012\text{‰}$  for carbon, hydrogen, and oxygen isotope ratios, respectively (Platzner et al. 1997). The second approach for introducing the sample into the chamber is called the continuous flow mode. In this approach, the sample and standard gases are carried into the mass spectrometer by a helium (He) (or argon [Ar]) carrier stream. The mass spectrometer gets a single, but longer time interval to measure the sample or standard signal. The stated precision of the continuous flow approach by different instrument manufacturers is  $\pm 0.3\text{‰}$  and  $\pm 0.3\text{‰}$  for carbon and oxygen iso-

tope ratios, respectively (continuous flow hydrogen analysis is not routinely available). This lower precision arises because of the single versus multiple observation constraint and because of a reduction in ionization efficiency introduced by the presence of the He carrier. However, in practice, the precision can be much higher ( $< 0.1\text{‰}$ ) for carbon and oxygen isotope ratios of  $\text{CO}_2$  gas samples (Ehleringer and Cook 1998) and  $\pm 0.1\text{‰}$  and  $\pm 0.2\text{‰}$  for carbon and oxygen isotope ratios of  $\text{CO}_2$  from organic samples (Saurer et al., 1998).

Historically, the conversion of sample material from either water or organic matter into the gases that are introduced into the mass spectrometer was done off-line and the sample gases were introduced into the mass spectrometer using the dual inlet approach. Today, the continuous flow approach is becoming much more frequent because of its ease of use, which then allows peripheral instruments to be directly linked to the mass spectrometer for an on-line sample preparation and analysis (Brand 1996). It is now common to find that elemental analyzers or gas chromatographs are combined with isotope ratio mass spectrometers. For organic samples, the furnace of the elemental analyzer is used for a flash combustion reaction that ultimately allows for the sequential measurement of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios from the same sample (Platzner et al. 1997). If the combustion reaction is instead replaced by a pyrolysis reaction, measurements of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  ratios are possible from the same sample (Saurer et al. 1998).

## Meteoric Water Line

The isotopic composition of ocean water is approximately  $\delta D = 0$  and  $\delta^{18}O = 0$ . Since the bonding is stronger when heavier isotopes are involved, the evaporating water vapor is depleted in the heavier isotopes relative to its water source. Under equilibrium conditions and at 25°C, the water vapor is depleted by approximately  $-74\%$  and  $-9.2\%$  for  $\delta D$  and  $\delta^{18}O$ , respectively, relative to the water source (Gat and Gonfiantini 1981). When this moisture condenses from clouds during precipitation events, the precipitation is slightly enriched in the heavier isotopes relative to the vapor remaining in the clouds. As a consequence, subsequent precipitation events from this cloud produce precipitation that is progressively more depleted in the heavier isotopes. The slope of the relationship of  $\delta D$  and  $\delta^{18}O$  in these precipitation events should follow vapor pressure differences (i.e.,  $\Delta\delta D/\Delta\delta^{18}O \approx 74/9.2 \approx 8$ ).

The observed relationship between  $\delta D$  and  $\delta^{18}O$  of precipitation collected from stations throughout the world generally follows a slope of 8 (Fig. 12.1). This regression is known as the meteoric water line (MWL). While local variations do exist in this relationship, the MWL serves as a foundation for predicting the isotopic composition of precipitation in

different locations and allowing measurement of one isotope without always requiring the second isotopic measurement. Because the isotopic composition of subsequent precipitation events along a storm trajectory will follow Rayleigh distillation kinetics, the  $\delta D$  and  $\delta^{18}O$  of precipitation will become progressively more negative as the storm moves inland (Mazor 1991). The isotopic depletion of water from inland regions serves as a valuable signal that can be recorded in both plants and animals. The  $\delta D$  and  $\delta^{18}O$  of precipitation are related to temperature (Gat and Gonfiantini 1981). Summer precipitation events have higher  $\delta D$  and  $\delta^{18}O$  values than winter precipitation events, which provides a basic mechanism for creating isotopic variation throughout the soil moisture profile (Mazor 1991; Gat 1996).

## Evaporative Enrichment

While the isotopic composition of incoming precipitation follows the MWL, evaporating bodies (e.g., lakes, leaves, soils) within the ecosystem tend to exhibit a  $\Delta\delta D/\Delta\delta^{18}O$  slope that is substantially less than 8 (see Fig. 12.1). Both the upper soil layers and leaves exhibit a strong evaporative enrichment relative to the input source water under nat-

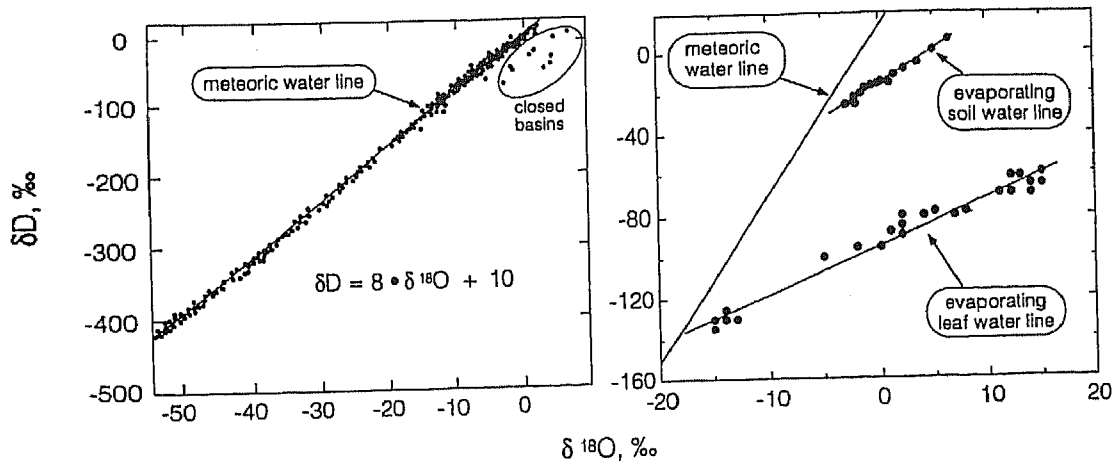


FIGURE 12.1. Left, A plot of the relationships between hydrogen isotope and oxygen isotope ratios for precipitation globally. Right, A plot of the relationships between hydrogen isotope and oxygen isotope ratios for an evaporating soil and leaf water values of different leaves in the same environment. (Data from Allison et al. [1983], Craig [1961], and Flanagan et al. [1991a].)

ural conditions. Craig and Gordon (1965), Allison et al. (1983), and Flanagan et al. (1991b) provide the quantitative models necessary to predict the isotopic composition of open waters, drying soils, and leaf water, respectively, from environmental parameters. Under strict conditions, it is possible to extrapolate evaporatively enriched water from either drying soils or leaves back to the isotopic composition of the original, unevaporated source waters (see Moreira et al. 1997).

## Methods for Water Sampling, Extraction, and Analysis

### Water Sample Collection and Storage

Representative sampling and prevention of evaporative enrichment are the primary concerns when collecting samples for determining the stable isotopes in water. When collecting water samples from an open water surface (e.g., lake), do not sample from a still surface, which may be enriched relative to deeper water. Stirring up the sample area can help to ensure a representative aliquot. Collect stream water from the site of interest, since evaporative enrichment will occur as water moves down the length of a river. Sampling from well water is often a good proxy for ground water, but do not keep the first water coming out of the pipe since it may be enriched relative to the source. Fill the vials and seal the lid with parafilm. It is desirable to store water samples at cold temperatures (5°C), but short-term storage at room temperature is adequate if the vials are well sealed.

Similar concerns apply to sampling leaves for extraction of water (i.e., sealed in a vial immediately). Remember when sampling for leaf water that the degree of evaporative enrichment in leaf water will be a function of local humidity and leaf temperature (Flanagan et al. 1991a). Thus, it is essential that there be consistency in sampling leaves that are experiencing the same microclimatological patterns (e.g., sun versus shade leaves). The portions of the leaf that are subject to evaporative enrichment are those regions away from the major veins (Yakir et al. 1989). Therefore, some investigators prefer to use a punch to cut out those interveinal regions for sampling. Samples should be

stored in a freezer as soon as possible after sampling and kept there until the water is extracted.

Since there is no fractionation during water uptake, sampling xylem waters becomes a convenient and reliable approach for determining the current water source used by a plant (Ehleringer and Dawson 1992). Stems or other suberized tissues are a convenient means of sampling xylem water in order to determine plant water sources from the soil. Emphasis should be placed on sampling organs with a rapid water turnover. For example, sampling of small suberized stems is better than sampling a tree trunk, where external and internal portions may be turning over water at different rates. Use only suberized stem materials, as green stem tissues are subject to evaporative enrichment (Dawson and Ehleringer 1993).

Collection of precipitation from open areas should be done immediately after the storm to avoid evaporation. On the other hand, within a forest, some evaporative enrichment will occur as throughfall makes its way into the soil, in which case, it makes more sense to sample the throughfall since this is what actually enters the soil (Dawson 1998). Soil samples must be collected across the range of possible rooting depths since water in surface soils is exposed to evaporative enrichment. Soil samples are usually stored in the same type of glass vials used to store leaf or stem samples.

If precipitation input and transpiration activity occur at roughly the same time, then it is likely that the atmospheric water vapor will be in isotopic equilibrium with that of the input precipitation. However, these input and loss processes are often temporally offset and may be offset by as much as several months in regions where precipitation occurs primarily in the winter and growth occurs later in the spring and summer. In most cases, it is essential that atmospheric water vapor be collected, since its isotopic value has such a strong influence in determining the isotopic composition of leaf water. Atmospheric vapor is collected by using a pump to flow air through a condensing-loop trap. This requires a very cold trap (dry ice in an ethanol slush) to ensure complete vapor extraction, since any water vapor that escapes would be isotopically altered. For later data interpretation, it is best to also measure air temperature and humidity at the time of vapor collection.

### Soil, Leaf, and Stem Water Extraction

Water is extracted from the bulk sample by cryogenic extraction. The entire frozen leaf, stem, or soil sample along with the vial is placed into the sample holder shown in Figure 12.2, which is then immersed in liquid nitrogen. Once the sample is frozen, the sample and holder are evacuated. Once the sample is under vacuum, it is warmed by substituting boiling water for the liquid nitrogen. The collection tube is then immersed into liquid nitrogen to draw the moisture from the sample into this collection tube. [Hint: The frozen tissue still retains some atmospheric gases. Once thawed, these gasses will also migrate to the collection tube. Prevent bubbles from forming within the ice, which will cause problems later when trying to seal the collection tube.] In order to eliminate fractionation, all of the water must be moved from the sample into the collection tube. Continue the cryogenic extraction procedure until no further condensation occurs on the vacuum line walls; this process normally requires 45 to 90 minutes. Thaw the ice and collect

the water for isotopic analysis. [Hint: Precut stems into small sections prior to extraction in order to reduce the time required for complete water extraction.]

### $\delta D$ Analysis of Water

Perhaps the most common method for obtaining  $\delta D$  of water involves reducing the H in  $H_2O$  to  $H_2$  using a zinc (Zn) catalyst (Coleman et al. 1982). A 2- $\mu l$  subsample of water (using a capillary) is placed in a Pyrex tube preloaded with 100 mg of Zn contaminated with a small amount of alloy and backfilled with nitrogen (the most reliable Zn is obtained from the Geology Department at Indiana University). The tube is then frozen with liquid nitrogen, evacuated, and sealed. The samples can then be batch processed. The samples are heated to 500°C for 60 minutes. The resulting  $H_2$  gas (sole gas within the sealed tube) can then be directly introduced into the mass spectrometer for analysis. The overall precision of this approach is  $\pm 1\%$ .

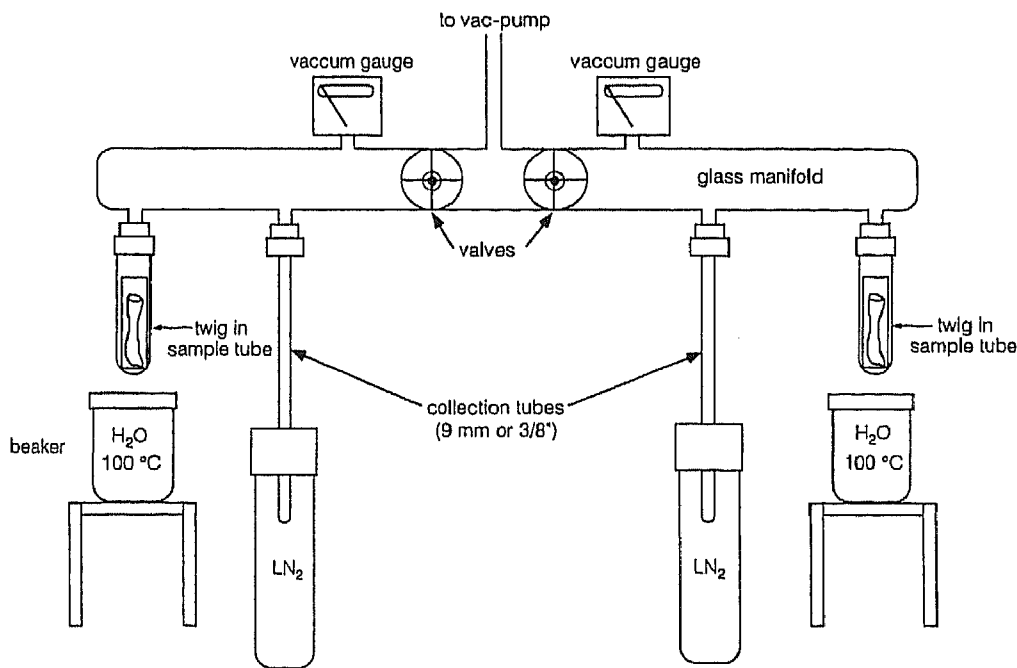


FIGURE 12.2. A cryogenic vacuum line for extracting water from leaves, stems, and soils. Samples are introduced into a chamber and placed under vacuum. The chamber is then heated with boiling water to drive all of the water to a collection tube, which is surrounded by liquid nitrogen ( $-196^{\circ}C$ ).

A promising new, on-line method for determination of  $\delta D$  in water uses a chromium reaction furnace (Gehre et al. 1996). The reaction unit, which is directly coupled to the mass spectrometer inlet, is filled with chromium powder (<0.3 mm) and set to 900°C. Very small sample sizes (1  $\mu$ l) are injected through a layered septum into a pre-evacuated reaction furnace, and are subject to flash evaporation and reduction to  $H_2$  upon contact with the hot chromium. The  $H_2$  gas flows directly into the bellows of the sample side in the dual inlet mode, because of pressure gradients. One advantage of this approach is that no special sample preparation is needed since the hot chromium reduces water while simultaneously binding other elements in the sample (C, N, O, sulfur [S], and halogens) to thermally stable compounds (Gehre et al. 1996). The overall precision of this approach is  $\pm 1\%$ .

### $\delta^{18}O$ Analysis of Water

For relatively large volumes of water (0.2 to 0.5 ml), the  $^{18}O/^{16}O$  composition is determined by equilibration with  $CO_2$  of known isotopic composition (Dugan et al. 1985; Compston and Epstein 1958). A volume of  $CO_2$  adequate for analysis on the mass spectrometer is placed along with the water sample in a pre-evacuated tube. After equilibration in a constant temperature water bath for at least 24 hr, the  $CO_2$  is extracted cryogenically using liquid nitrogen along with dry ice/ethanol traps to prevent water vapor and other gases from entering the collection tube. The  $CO_2$  collected will have the  $^{18}O/^{16}O$  signature of the water since the oxygen in the water should completely exchange with the much smaller mole fraction of oxygen in the  $CO_2$ . This method is easily batched, but requires large volumes of water and is time consuming. This method has a precision better than  $\pm 0.2\%$ .

Equilibration techniques have also been developed for much smaller sample sizes (40 to 50  $\mu$ l) (Kishima and Sakai 1980; Socki et al. 1992). These methods add the sample using a micropipet into the bottom of 9-mm Pyrex tube that was previously evacuated and backfilled with dry nitrogen or argon. The sample is frozen with liquid nitrogen, evacuated, and then 250  $\mu$ l of  $CO_2$  is injected through a septa port. The tube is sealed under vacuum and equilibrated in a constant temperature water bath for at least 24 hr. After equilibration, the

$CO_2$  is extracted as discussed above and ready for the mass spectrometer. An external liquid nitrogen trapping device (similar to a cold finger) has proven useful for enhancing the mass spectrometer precision for the small volumes of  $CO_2$  utilized. A variation on this method can be found in Scrimgeour (1995).

Even smaller volumes (5 to 10  $\mu$ l) of water can be analyzed by reacting with guanidine hydrochloride to produce  $CO_2$  (Dugan et al. 1985; Wong et al. 1987). The water and guanidine hydrochloride are heated in an evacuated sealed tube at 260°C for 16 hr. The two gases formed in this reaction are ammonia ( $NH_3$ ) and  $CO_2$ . Upon cooling, the  $CO_2$  combines with  $NH_3$  to form an ammonium carbamate. The  $CO_2$  is released from the ammonium carbamate by reacting it with phosphoric acid and trapped at liquid nitrogen temperatures. The method is most useful when very small sample sizes are required, otherwise the method is more time consuming than the  $H_2O-CO_2$  equilibration methods. The precisions of both techniques are similar, 0.2%.

### Methods for Leaf and Stem Organic Matter Sampling, Extraction, and Analysis

#### Total Tissue Versus Cellulose Analysis

Most isotopic studies on water relations utilize cellulose since it has very slow turnover rates (Lea and Leegood 1993) and represents the water signature at the time of fixation. The study of various components of organic matter can yield information regarding the dynamics of isotopic fractionation during metabolism. However, for considerations of water relations, the differences in  $\delta D$  between various components (e.g., lipids and carbohydrates, Sternberg et al. 1984) make whole tissue samples more difficult to interpret. Although the carbon-bound H is considered nonexchangeable, post-photosynthetic heterotrophic metabolism has been shown to modify the  $\delta D$  of plant carbohydrates as they are exposed to isotopically different water fractions (Yakir 1992). Even though the O of organic matter tends to be less exchangeable than H, the use of cellulose rather than total tissue is particularly important for  $\delta^{18}O$  studies due to the com-

plications caused by nitric oxides formed upon combustion when nitrogenous compounds are present. Whole tissue samples are often used for measurements of time-averaged water use efficiency even though the  $\delta^{13}\text{C}$  of various metabolites can vary considerably. The choice of what fraction to measure depends greatly on the purpose of the study, but in general  $\alpha$ -cellulose is a good choice for information on water relations.

### Leaf Sampling Considerations

The isotopic composition within a plant varies due to differences in source water, environmental effects, genetic differences, biochemical composition, phenology, etc., and a sampling plan must take all these factors into consideration. Sampling should be done at the same time of the day as diurnal changes in isotopic composition can occur. Samples should be collected from the same canopy position, since differences in microenvironment will affect the extent of evaporative enrichment in the leaves. Since incident light levels can be quite variable throughout a canopy, it is important to be consistent in sampling only sunlit or shaded leaves from all four cardinal directions. While leaves have been commonly used in the past, there is now increasing interest in other tissues and plant parts (Farquhar et al. 1989). The number of plants sampled is dependent on the research question being addressed, and will be affected by the amount of environmental and genetic heterogeneity expected. It is appealing to bulk samples to save time and money, but there is likely some information on population-level genetic variation in isotopic composition that then will be lost.

After harvesting, plant samples should be dried immediately to avoid changes in chemical composition associated with slow drying of living material. If that is not possible, then freezing the tissue at time of collection (dry ice works well) and drying later is adequate. Dried material can be stored at room temperature for extended periods. Dried tissues are ground to pass a 40-mesh screen or in a mortar and pestle for analysis of the isotopic composition of organic matter. Finely ground material burns more uniformly as well as enhances the efficiency of extraction procedures (e.g., cellulose purification). Since the amount of tissue required for analysis is so small (<3 mg or <0.1 mg with a

cold finger) care must be taken to ensure sample homogeneity. Often there will be greater variation in the repeated analysis of the same "bulk sample" than in repeated analysis of an individual sample through the mass spectrometer.

### Tree Ring Separation and Cellulose Purification

Most approaches to tree ring separation involve simply cutting along visibly delineated rings. With the aid of a dissecting microscope or a microtome, it is possible to subdivide annual rings into finer temporal resolution in order to quantify seasonal variation in  $\delta$  values (Loader et al. 1995). Care must be taken to ensure that false rings and other anomalies are avoided (Stokes and Smiley 1996). Although destructive, the best way to get at tree rings is by cutting out an entire cross-section of the bole. Preliminary sampling should be done when using increment cores to determine how many cores will be required to obtain enough material for isotopic analysis from a typical annual ring. Due to potential variation along the stem, it is best to sample increment cores from all four cardinal directions (Leavitt and Long 1986).

There has been a tendency for long-term, multiple-year studies to focus sampling only on  $\alpha$ -cellulose from organic matter. To obtain  $\alpha$ -cellulose from a bulk plant sample (either leaf or tree ring), various modifications of the Wise (1944) method have been used. The approach used by Leavitt and Danzer (1993) allows one to batch process many samples at once. Basically, the preparation of plant material into  $\alpha$ -cellulose involves a delipification step (using a Soxhlet apparatus), followed by repeated boiling (to remove any water-soluble compounds, such as sugars), bleaching with sodium chlorite and acetic acid (to remove lignins and proteins), and washing of the sample to produce cellulose ( $\alpha$ -cellulose and hemicellulose). Since hemicelluloses have exchangeable O atoms, it is important to remove them in a strong sodium hydroxide solution followed by an acetic acid wash. The resulting  $\alpha$ -cellulose is then ready for  $\delta^{18}\text{O}$  analysis. For  $\delta\text{D}$  analysis, the  $\alpha$ -cellulose must first be nitrated in order to remove the exchangeable H atoms (see below).

### $\delta^{13}\text{C}$ Analysis of Organic Matter

The off-line analysis of the  $\delta^{13}\text{C}$  in organic matter involves an in-tube combustion with cupric oxide and a silver foil catalyst (Buchanan and Corcoran 1959). Quartz tubes are loaded with 2 to 3 mg dried organic matter, 1 g cupric oxide, and silver foil (if halogens or S is present), which are then sealed under vacuum. The materials are combusted at  $850^\circ\text{C}$  for 4 hr and then allowed to slowly cool. The resulting gases,  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , and  $\text{N}_2$ , are separated using a utility vacuum line (Fig. 12.3). In such a line, the sample gases pass through a series of traps to freeze  $\text{H}_2\text{O}$  (ethanol/dry ice) (trap 1) and then  $\text{CO}_2$  (liquid nitrogen) (trap 2). The  $\text{N}_2$  is pumped away by the vacuum pump. The  $\text{CO}_2$  is then isolated by closing valves in the vacuum line and is then cryogenically moved from trap 2 into a collection tube, which can then be sealed so that the sample can be later analyzed on the mass spectrometer operating in a dual inlet mode. Organic samples should be cleaned up soon after combustion, because  $\text{H}_2\text{O}$  and  $\text{CO}_2$  will slowly interact with the copper present to form copper carbonate, potentially affecting the isotopic composition of the  $\text{CO}_2$ . The overall  $\delta^{13}\text{C}$  precision of this off-line preparation and analysis is  $<0.1\%$ .

The development of the continuous flow mode and linking an elemental analyzer to the mass spec-

trometer have greatly simplified the measurement of  $\delta^{13}\text{C}$  in organic matter, but does so by providing an analysis of somewhat lower precision (precision varies between  $\pm 0.15$  to  $\pm 0.3\%$ ). For on-line analysis, dried organic matter (1 to 2 mg) is placed into a tin cup, which is then dropped into a hot ( $1060^\circ\text{C}$ ) quartz combustion furnace flooded with oxygen-enriched helium. As the tin cup ignites (flash combustion) the temperature rises to  $1800^\circ\text{C}$ . A helium carrier stream carries the sample products from the combustion furnace through to the mass spectrometer. The combustion products (primarily  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , and  $\text{N}_2$ ) first flow through an oxidation column (containing chromium oxide), which facilitates complete oxidation, and then through a reduction column (reduced copper to remove excess O and reduce nitrogen oxides to  $\text{N}_2$ ). Water is then removed using magnesium perchlorate, and the remaining gases ( $\text{CO}_2$  and  $\text{N}_2$ ) are separated by gas chromatography before they are sent into the mass spectrometer for analysis. Both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  can thus be obtained from a single analysis (Platzner et al. 1997). If the initial sample is weighed precisely, then %N and %C contents can be derived by measurement of the peak area. A typical cycle time for this analysis is approximately 12 minutes and by using an autosampler attached to the elemental analyzer, larger numbers of samples can be run with ease.

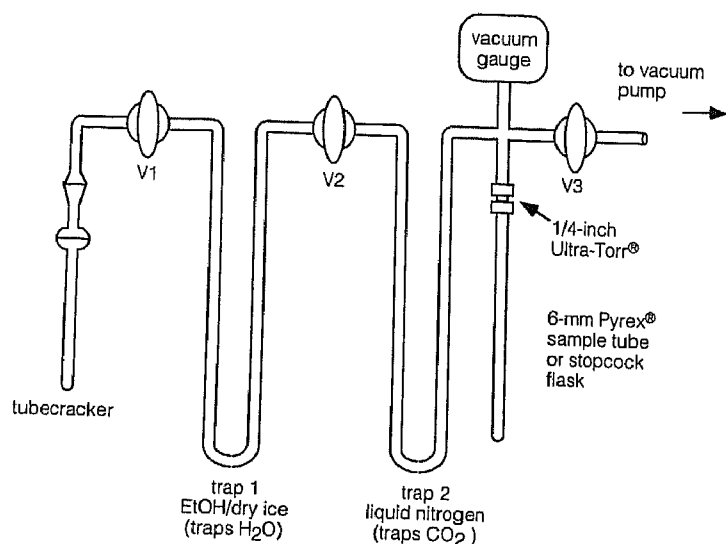


FIGURE 12.3. A general vacuum line for the separation of water (ethanol slush trap at  $-78^\circ\text{C}$ ) and  $\text{CO}_2$  (liquid nitrogen trap at  $-196^\circ\text{C}$ ) from other gases.



### $\delta D$ Analysis of Organic Matter

All carbohydrates contain hydroxyl groups that can exchange their hydrogens with those in water, and thus in order to determine the  $\delta D$  of organic matter at the time of formation, only nonexchangeable hydrogens (C-H bonds) can be analyzed for isotopic composition. The most common process is nitration in which each hydroxyl group is exchanged for a nitrate ( $NO_3$ ). The purified organic material ( $\alpha$ -cellulose, see above) is placed in a flask with a solution of nitric acid and acetic anhydride on ice due to the exothermic nature of the reactions (Bennett and Timell 1955; DeNiro 1981; Yapp and Epstein 1982). The material is then washed, dissolved in acetone and then precipitated in water to obtain purified tri-nitrated cellulose (all three hydroxyls per glucose are nitrated). The material is then freeze-dried for storage.

The nitrated material (9 to 14 mg) is then placed into a tube with cupric oxide (1 g), evacuated, sealed, and combusted for 3 hr at 520°C. Following combustion, the gases are separated on the same type of vacuum line (see Figure 12.3) as used for organic  $\delta^{13}C$ , only instead of trapping  $CO_2$ , water vapor is trapped by using the ethanol/dry ice slush to control the water movement while evacuating the other gases. The water is then transferred to a tube containing a Zn catalyst (see section on  $\delta D$  analysis of water above) for reduction to diatomic hydrogen and input into the mass spectrometer. Although this procedure yields good precision (<2‰), it is also labor intensive and requires relatively large sample sizes for the nitration.

### $\delta^{18}O$ Analysis of Organic Matter

Conversion of organic matter to  $CO_2$  for  $\delta^{18}O$  analysis involves a pyrolytic reaction. For off-line conversion, the  $\alpha$ -cellulose (see above) is placed in a tube with mercuric chloride, evacuated, sealed, and then heated for 6 hr at 550°C to form carbon monoxide (CO),  $CO_2$ , and hydrochloric acid (HCl) gases. The CO is converted to  $CO_2$  in an ionization chamber and the HCl is reacted with Zn. After a distillation step, the  $CO_2$  is then ready for the mass spectrometer. This procedure, especially the conversion of CO to  $CO_2$  in the spark chamber, is time consuming and along with the toxicity of mercuric chloride makes this technique much less appealing than newer on-line techniques.

Similar to on-line analysis of  $\delta^{13}C$  (see above), the use of continuous flow and an elemental analyzer as an automated pyrolysis device has greatly simplified the estimation of  $\delta^{18}O$  in organic matter (Saurer et al. 1998). Dried  $\alpha$ -cellulose (1 to 2 mg) contained in a silver cup is dropped into a hot (1100°C) quartz combustion furnace containing glassy carbon catalyst in a stream of He. The excess C from the catalyst facilitates the conversion at high temperatures to the thermodynamically favorable products of CO and  $N_2$ . These two gases are then separated by a chromatographic column and sent into the mass spectrometer for analysis. A typical cycle time is around 20 minutes and many samples can be run by using an autosampler. If care is taken to reduce memory effects (repeated sampling is recommended) then this method has proven to be robust and precise (<0.4‰).

### Short-Term, Ecosystem Process-Level Applications

Our understanding of ecosystem-level water use on a short-term basis has been significantly advanced with the application of stable isotope methods. This is particularly true if the isotopic methods and data collected are linked with other methods at the plant, catchment, and atmospheric levels (Dawson and Ehleringer 1998). If chemical and/or physical reactions result in differential representation of the heavy and light isotopes (Figure 12.4), the isotopic distributions of the reactants and products can inform us about "processes" (i.e., reaction conditions) (Peterson and Fry 1987). The different distributions of stable isotopes, in water for example, can also help to identify and then trace the origin of different water resources in the ecosystem as well (Ehleringer and Dawson 1992). The isotopic values (signatures) of source pools then provide a baseline with which to assess process-level information by looking for changes in the isotopic signature along a reaction pathway (termed isotope fractionation or discrimination) (Peterson and Fry 1987; Hoefs 1987; Lajtha and Michener 1994).

### Partitioning of Water Resources Among Plants Within Ecosystems

There are now a number of very good examples of how different plant species may partition soil water resources that have used H or O stable isotope anal-

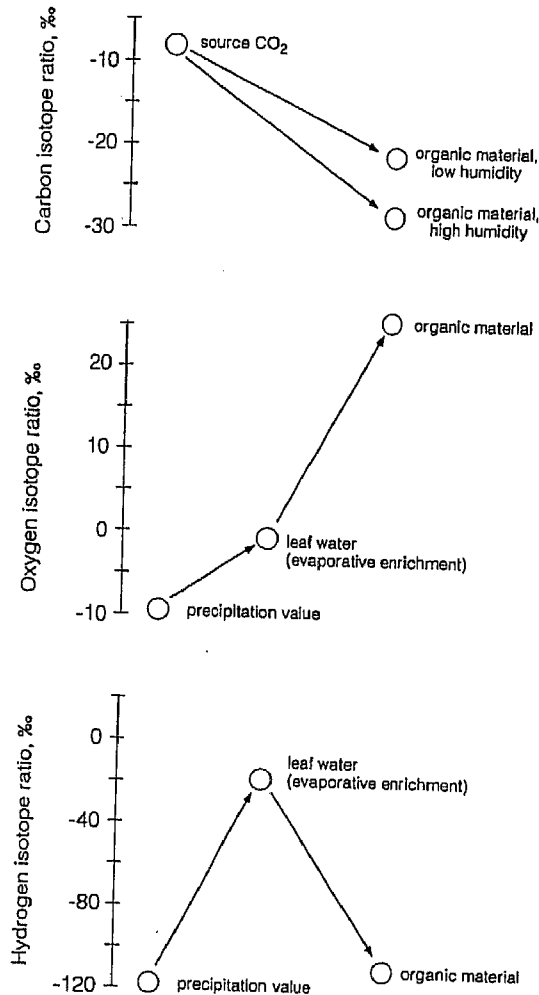


FIGURE 12.4. Stylized relationships between the carbon isotope ratios of organic matter relative to source  $\text{CO}_2$  as a function of humidity growth conditions (top) and the oxygen and hydrogen isotope ratios of source water, leaf water, and cellulose (middle and bottom).

yses of both source and plant water (reviewed in Ehleringer and Dawson 1992; Dawson 1993a; Dawson and Ehleringer 1998). Because there is no isotopic fractionation during water uptake by terrestrial plants (Wershaw et al. 1966; but see Lin and Sternberg 1993), if one carefully samples the different potential sources of water used by the vegetation (e.g., soil water, ground water, recent precipitation, fog drip, etc.) as well as the water within the plants themselves (xylem sap), it is possible to

qualitatively assess which water sources are being used by coexisting species within a particular community. In the case in which two different water sources can be clearly identified, a simple two-end-member mixing model can be used to estimate the proportional use of each water source (e.g., shallow vs. deep soil water) (see White 198; Brunel et al. 1995; Dawson 1993b) being utilized by different species (Flanagan et al. 1992; Jackson et al. 1995; Ehleringer et al. 1998), in relation to plant distribution along natural gradients of water availability (Dawson and Ehleringer 1991; Sternberg and Swart 1987; Thorburn et al. 1993; Mensforth et al. 1994), over time between, say, wet and dry seasons (Ehleringer et al. 1991; Dawson and Pate 1996; Dawson 1998), or in relation to life history stages or strategies (Ehleringer et al. 1991; Feild and Dawson 1998), overall plant size (Dawson 1996), or in proximity to neighbors (Dawson 1993b). Such information can inform us about the degree of water resource partitioning among plants within and among communities or ecosystems, providing important information which contributes to our understanding of patterns.

When more than two different water sources are present, assessing the proportional use of each becomes more problematic. Recent quantitative models have been developed, however, and are providing a means to assess where the water being used by particular plants comes from when roots at several different zones of the soil profile are active (see Cramer et al. 1999) or at different times of the growing season (Dawson et al., unpublished manuscript). While these sorts of models require more detailed information about the spatial and temporal variation of water resources within an ecosystem, they may be the only way to quantitatively assess resource use and partitioning among vegetation elements within an ecosystem; in some instances, that may also be the only way to more powerfully interpret a host of different data sets in cases in which simple two-source models cannot or should not be applied (Thorburn and Dawson in prep.). In ecosystems in which only two water sources (shallow and deep) can be identified (e.g., arid and semiarid systems), water pulses can be used to better understand patterns of plant water use and the possible partitioning of water resources among species (Schwinning and Ehleringer 2000 and below).

### Using $\delta D$ and $\delta^{18}O$ Water Pulses and Interpretation of Mixing Models

Even though the soil forms a continuum of moisture availabilities, it is often appropriate to distinguish two distinct soil water sources for plants (see above and Dawson and Ehleringer 1998; Dawson et al. 1998). One, the rain-fed shallow soil layer is dominated by pulse dynamics, where generally low soil moisture availability is punctuated by short episodes of high soil moisture availability. These pulses may occur naturally or artificially by augmenting the irrigation water with either  $^2H$ - or  $^{18}O$ -enriched water (both readily available commercially). Second, the deeper soil layer is not or is very little affected by single rainfall events and is dominated instead by seasonal dynamics in which soil moisture is depleted during spring and summer and replenished during fall and winter. In many ecosystems, such as grasslands and deserts, the division of soil space and its resources among plants is central to explaining competitive relationships among plants and the community consequences of a changing climate. Stable isotope methods can help quantify the absolute and relative division of

shallow and deep soil moisture sources among plant species.

Shallow and deep soil water pools often have distinct isotopic composition, particularly in temperate ecosystems and ecosystems receiving bimodal precipitation. If minimal isotopic differences exist within the soil profile, they can also be enhanced, creating artificial moisture pulses with  $^2H$ - or  $^{18}O$ -enriched irrigation water. As stated above, plants do not discriminate between isotopically different forms of water (Ehleringer and Dawson 1992). Thus, the isotopic composition of xylem water reveals the relative contribution from two distinct water sources. The question becomes how much ecologically relevant information can we obtain from the xylem signal?

There are several levels of sophistication in interpreting the xylem water signal. At the simplest level, the composition of xylem water merely informs whether one or two water sources are available to the plant at the time of measurement (Fig. 12.5). For example, if xylem water is indistinguishable from one of the two water sources this would suggest that the plant uses only one water source,

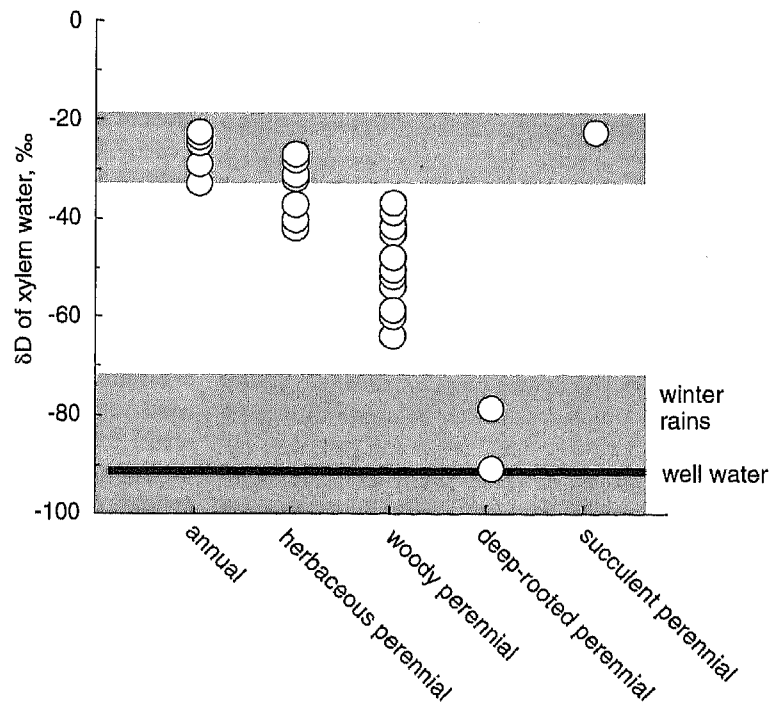


FIGURE 12.5. Hydrogen isotope ratios of xylem water during the summer monsoon period for different species, co-occurring within a desert ecosystem. (Data from Ehleringer et al. [1991].)

while an intermediate isotopic composition would suggest that both water sources are being used. A more precise quantification of the relative or absolute utilization of the two water sources is more difficult and requires additional measurements, such as of water flow rate through the plant and the exchangeable water volume. This is particularly important when one of the two water sources becomes available as a pulse.

This can be shown in a simple dynamic mixing model, where we consider a constant transpiration flow ( $E$ ) through a well-mixed constant volume ( $V$ ), fed in part ( $f$ ) by a labeled source and in part ( $1 - f$ ) by an unlabeled source. The concentration of label in the fixed volume depends not only on the relative contribution,  $f$ , of the labeled source to the uptake, but also on the flow-to-volume ratio,  $E/V$ . When this ratio is low, the concentration of label lags behind the true fraction,  $f$ , of labeled source in the total flow,  $E$ . Thus, the true utilization fraction of a pulsed source is likely to be underestimated, unless the dynamics of xylem water composition, as affected by  $E/V$ , are accounted for. Actual pulse label dynamics are often more complicated, because water uptake rates and ratios may not remain constant during or after a pulse. Thus, any one-time estimation of the fraction of water from a pulsed source in the transpiration stream would give incomplete information on overall pulse utilization. In this case, pulse utilization can be quantified precisely only when xylem composition and transpiration rates are monitored during and after the pulse. Then, total pulse utilization can be estimated by integrating, over time, the fraction of pulsed resource in the xylem and the whole plant transpiration rate. Since such intensive data collection is often not possible, methods have been developed to estimate integrated pulse utilization based on substantially less information.

In studies in which the utilization of single pulses is the focus, simplified models of pulse utilization dynamics have been used to fill the gaps left by limited data collection. Schwinning and Ehleringer (2000) suggested that relative pulse utilization by plants with contrasting life history strategies can be estimated within an accuracy of 10 to 20% based on only four data points: the time and magnitude of the maximal fraction of pulsed source water in the xylem, and two estimates of the whole-plant  $E/V$  ratios during and after the pulse. Schwinning

and Ehleringer (2000) suggest that seasonally integrated pulse utilization can be estimated by randomly sampling xylem water composition and whole-plant transpiration rates throughout the season, regardless of time since last pulse. This method makes no assumption on pulse dynamics, but simply represents a statistical estimate of the seasonally integrated pulse-fed transpiration rate, based on randomly selected subsamples.

### Water-Use Efficiency

If water sources are differentially available to plants with either different rooting depths or requirements for water, this could influence their water use behavior and thereby the efficiency with which they fix carbon. Differences in water use behavior have been assessed with the use of carbon isotope analyses of plant leaves (Farquhar et al., 1989) or tree ring cellulose (see Livingston and Spittlehouse, 1993). In plants with the  $C_3$  photosynthetic pathway, variation in the leaf carbon isotope ratio ( $\delta^{13}C$ ) can be correlated with the long-term efficiency with which the water resource is used (see Ehleringer 1991). This is because, during the photosynthetic process,  $C_3$  leaves discriminate differentially against  $^{13}CO_2$  more than  $^{12}CO_2$ , particularly when the leaf stomata are fully opened (Farquhar et al. 1989). Therefore, if plants within an ecosystem are using different water sources and experiencing different levels of water stress that lead to differences among species or over time in the water use behaviors, this could be "recorded" in the leaf or wood carbon being synthesized at that time. More conservative water use behavior should therefore, for example, in shallowly rooted species that experience more water stress, lead to a different leaf or wood  $\delta^{13}C$  than more deeply rooted species that are using deeper, more stable water sources. This sort of pattern has been documented in arid land systems by Flanagan et al. (1992). Assessments of the variation in  $\delta^{13}C$  have also been used to estimate evapotranspiration components over simple plant canopies (see Bariac et al. 1989; Ebdon et al. 1998). This sort of approach has the potential to help link water sources and water use behavior to the total system water flux in ecosystem-level investigations.

## Short-Term, Regional Process-Level Applications Across Ecosystems

### Recycling of Water Among and Between Ecosystems

The terrestrial biosphere influences global cycles of CO<sub>2</sub> and water. Stable isotopes can be used to probe these cycles since biological fractionation and subsequent exchange with the atmosphere alters ambient isotopic signatures of CO<sub>2</sub> and water vapor (Hsieh et al., 1998). For example, Gat and Matsui (1991) used the  $\delta D$  and  $\delta^{18}O$  of precipitation across a longitudinal gradient to quantify the relative contribution of evaporation and transpiration to the recycling of water vapor in the Amazon basin. There is a predictable isotopic depletion of water in precipitation as storm fronts progress (the continental effect) due to Rayleigh distillation effects, and deviations imply that a source of isotopically enriched vapor must be coming from the biosphere. The source can be further delineated since vapor from evaporation processes (lakes, rainfall, stemflow, soil water, etc.) should be isotopically depleted relative to that from transpiration (Gat and Matsui 1991; Martinelli et al. 1996). Rather than sampling precipitation, Moreira et al. (1997) used flasks located on towers and balloons to sample atmospheric vapor directly and, using simple isotope mixing models, demonstrated that canopy transpiration can be a major source of water vapor recycling in Amazonia. These kinds of studies can also elucidate the effects of land use changes on ecosystem water cycles.

### $\delta^{18}O$ of Atmospheric Carbon Dioxide

The biosphere can also have a significant influence on the oxygen isotope ratio of atmospheric CO<sub>2</sub> (Francey and Tans 1987; Farquhar et al. 1993; Flanagan et al. 1997). The equilibrium isotope effect that occurs between oxygen in CO<sub>2</sub> and oxygen in water may be the primary factor influencing the  $\delta^{18}O$  of respired CO<sub>2</sub> (Farquhar and Lloyd 1993). However, discrimination against C<sup>18</sup>O<sup>16</sup>O during photosynthesis acts to enrich atmospheric CO<sub>2</sub> in <sup>18</sup>O, while respiration has the opposite effect by releasing CO<sub>2</sub> depleted in <sup>18</sup>O. Thus, ecosystem

respiration flux can be partitioned into soil and plant components due to the differences in the  $\delta^{18}O$  of medium water (Flanagan et al. 1997; Ehleringer and Cook 1998; Flanagan et al. 1999). In addition, global-scale partitioning of CO<sub>2</sub> fluxes can be studied since the  $\delta^{18}O$  of water differs widely between marine and terrestrial sources (even between different biomes and latitudes) helping to resolve conflicts over the relative role of the oceans in net CO<sub>2</sub> uptake (Farquhar et al. 1993).

## Long-Term, Temporal Scaling of Ecosystem Processes

### Decadal-to-Century: Tree Rings

Tree rings have been frequently used as a record of long-term climatic fluctuations. The hydrogen and oxygen isotopic composition of tree ring cellulose reflects, to a first approximation, precipitation inputs (Yapp and Epstein 1982; Lawrence and White 1984; White et al. 1994), and many studies have used the variations in  $\delta D$  and  $\delta^{18}O$  in tree rings for temperature reconstruction (Gray and Thompson 1976; Epstein and Yapp 1977). However, White et al. (1985) observed that pine trees from wet and dry microsites, that presumably receive similar precipitation inputs, exhibited differences in their cellulose  $\delta D$  values, suggesting that water stress and/or humidity differences may alter leaf water  $\delta D$  and thus tree ring cellulose  $\delta D$ . There has been substantial controversy over exactly what environmental information is recorded in the  $\delta D$  and  $\delta^{18}O$  of tree ring cellulose (e.g., DeNiro and Cooper 1989; Edwards 1990). However, Roden et al. (1999) have recently developed and tested a mechanistic model for the interpretation of hydrogen and oxygen isotope ratios in cellulose and have concluded that both water source and humidity information are recorded in tree rings.

The  $\delta^{13}C$  of tree ring cellulose has also been used to study temperature and humidity signals (Loader et al. 1995; Lipp et al. 1996), climate change (Leavitt and Long 1991), water use efficiency (Duquesnay et al. 1998), soil moisture availability (Saurer et al. 1997), atmospheric  $\delta^{13}C$ , and the global CO<sub>2</sub> cycle (Switsur and Waterhouse 1998) over century-long time scales. These relationships are derived from the Farquhar et al. (1982) model for carbon

isotope discrimination and environmental influences on  $c_i/c_a$ . Some studies have found it useful to measure both  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  to separate out changes in precipitation input and local environmental conditions (Lipp et al. 1996; Saurer et al. 1997). Although tree rings are most often used to study long-term changes in the water relations of ecosystems, they can also be subdivided and provide fine-scale seasonal climatic information (Loader et al. [1995] subdivided an annual ring into 20 units).

### Millennial: Caliche

Pedogenic carbonates are formed from soil-respired  $\text{CO}_2$  and are therefore long-term indicators of the vegetation within an ecosystem. The variation in paleosol carbonate  $\delta^{13}\text{C}$  signals at depth have been used to indicate shifts in  $\text{C}_3$  and  $\text{C}_4$  vegetation (Cole and Monger 1994; Quade and Cerling 1995; Liu et al. 1996). However, the  $\delta^{18}\text{O}$  in the  $\text{CO}_2$  evolved (using phosphoric acid) can also provide climatic information. For example, the lack of a change in  $\delta^{18}\text{O}$  with changes in  $\delta^{13}\text{C}$  implies that shifts in photosynthetic pathway may be due more to shifts in  $\text{CO}_2$  concentration than climate (Cole and Monger 1994; Quade and Cerling 1995). Variation in the  $\delta^{18}\text{O}$  of pedogenic carbonates may be caused by a number of climatic factors, including temperature change, shift in the amount of precipitation, shifts in precipitation patterns (summer vs. winter), and shifts in regional moisture sources (Quade and Cerling 1995), and are therefore a valuable long-term record of ecosystem water relations.

## Animals

### Short-Term Indicators of Water Source

Stable isotope ratio analyses can provide direct evidence of short-term animal movements across the landscape. Often, more than 60% of an animal's mass is water. The isotopic composition of water in animals reflects that of their drinking source modified by the isotopic effects of evaporative enrichment, in much the same way that leaf water reflects both a source water component and an evaporative enrichment component. Longinelli (1984) first showed that a strong linear relationship existed between the  $\delta^{18}\text{O}$  values of blood and an animal's source water (Fig. 12.6). He further provided evidence for seasonality in the  $\delta^{18}\text{O}$  values of blood, reflecting the seasonal changes in the  $\delta^{18}\text{O}$  values of precipitation. Because different animals vary in overall water turnover rates and have different relative water rates of water loss through evaporation versus excretion, the slope of the  $\delta^{18}\text{O}$  of source water and blood are different across species. Only microliter quantities are required for these analyses. Consequently, analyses of the  $\delta^{18}\text{O}$  values of blood is a viable and less expensive alternative to radiotransmitters for tracing the recent movement patterns of animals between ecosystems and across the landscape.

### Long-Term Indicators of Water Source

The  $\delta^{18}\text{O}$  values of water in blood are recorded in bone phosphate (see Figure 12.6). Longinelli (1984) first showed that a linear 1:1 relationship

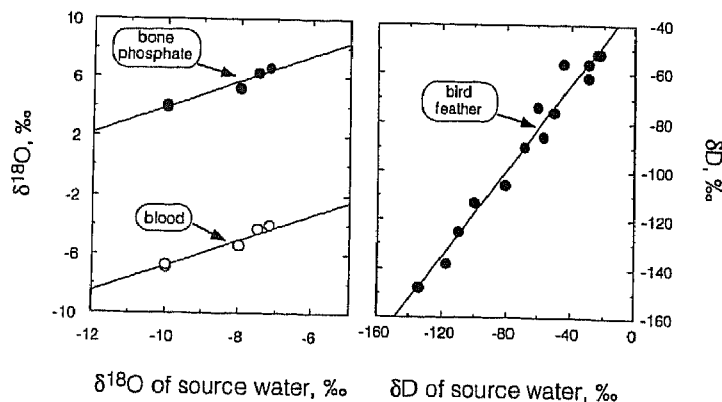


FIGURE 12.6. Relationships between source water and oxygen isotope ratios of blood and bone phosphate (left) (Longinelli 1984), and hydrogen isotope ratios of bird feathers (right) (Hobson and Wassenaar 1997).

existed between the  $\delta^{18}\text{O}$  values of source water and bone phosphate. Ecological applications have included studies of the distribution of white-tailed deer across North America (Luz et al. 1990). Similar approaches have been used to show that the  $\delta\text{D}$  values of bone phosphate and bone gelatin also reflect source waters (Cormie et al. 1994). More recently, Hobson and Wassenaar (1997) have shown that the  $\delta\text{D}$  and  $\delta^{18}\text{O}$  values of bird feathers also reflect that of their source water, which has far reaching implications for tracing long-distance bird migrations (see Figure 12.6). While many migratory animals, such as insects, lack bones, they too record long-term information about the source water of the ecosystem in which they were reared. Hobson et al. (1999) have shown that it is feasible to trace the migratory origins of monarch butterflies from the  $\delta\text{D}$  and  $\delta^{18}\text{O}$  values of the organic matter in their wings. Further exploration of this approach is likely to have widespread ecosystem-level applications in cases in which animals move back and forth between adjacent ecosystems.

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