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## The use of stable isotopes to study ecosystem gas exchange

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**Abstract** Stable isotopes are a powerful research tool in environmental sciences and their use in ecosystem research is increasing. In this review we introduce and discuss the relevant details underlying the use of carbon and oxygen isotopic compositions in ecosystem gas exchange research. The current use and potential developments of stable isotope measurements together with concentration and flux measurements of CO<sub>2</sub> and water vapor are emphasized. For these applications it is critical to know the isotopic identity of specific ecosystem components such as the isotopic composition of CO<sub>2</sub>, organic matter, liquid water, and water vapor, as well as the associated isotopic fractionations, in the soil-plant-atmosphere system. Combining stable isotopes and concentration measurements is very effective through the use of “Keeling plots.” This approach allows the identification of the isotopic composition and the contribution of ecosystem, or ecosystem components, to the exchange fluxes with the atmosphere. It also allows the estimation of net ecosystem discrimination and soil disequilibrium effects. Recent modifications of the Keeling plot approach permit examination of CO<sub>2</sub> recycling in ecosystems. Combining stable isotopes with dynamic flux measurements requires precision in isotopic sampling and analysis, which is currently at the limit of detection. Combined with the micrometeorological gradient approach (applicable mostly in grasslands and crop fields), stable isotope measurements allow separation of net CO<sub>2</sub> exchange into photosynthetic and soil respiration components, and the evapotranspiration flux into soil evaporation and leaf transpiration. Similar applications in conjunction with eddy correlation techniques (applicable to

forests, in addition to grasslands and crop fields) are more demanding, but can potentially be applied in combination with the Keeling plot relationship. The advance and potential in using stable isotope measurements should make their use a standard component in the limited arsenal of ecosystem-scale research tools.

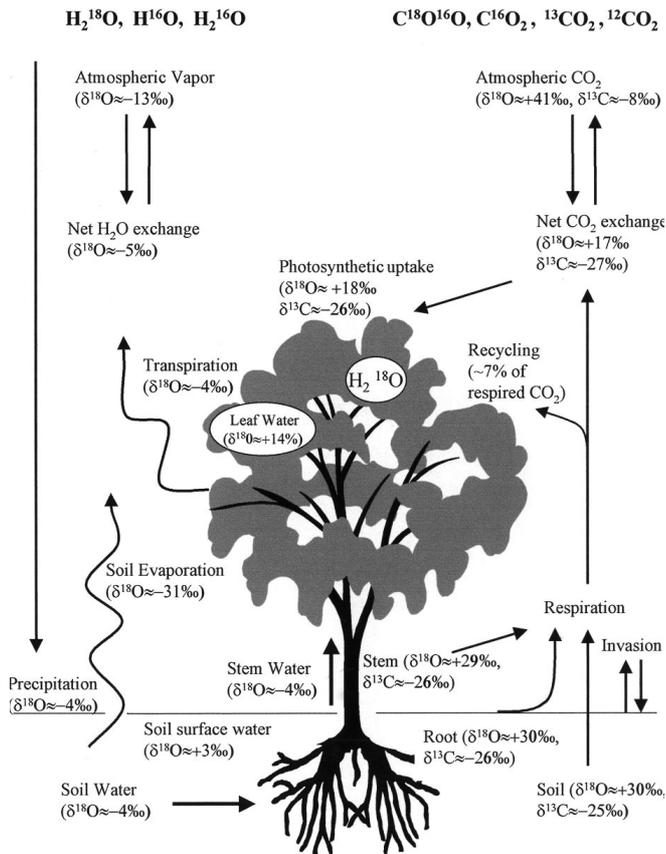
**Key words** Ecosystem CO<sub>2</sub> exchange · Ecosystem discrimination · Oxygen-18 · Carbon-13 · Evapotranspiration

### Introduction

The terrestrial biosphere is thought to play an important role in the observed interannual and seasonal variability of atmospheric CO<sub>2</sub> concentrations, (i.e., the strength of the biological carbon sink; Conway et al. 1994; Schimel 1995). Isotopic measurements also reflect seasonal and interannual variability and indicate terrestrial uptake and release of CO<sub>2</sub> as the source of the observed variation (Ciais et al. 1995, 1997). Recent evidence suggests that climate, through its differential effects on photosynthesis and respiration in terrestrial ecosystems, can have a significant effect on the concentration and isotopic signature of atmospheric CO<sub>2</sub> (Keeling et al. 1996). At the ecosystem scale, flux measurements such as reported by Goulden et al. (1996a, 1996b) and isotopic measurements such as reported by Flanagan et al. (1996, 1997) have revealed interannual changes in productivity and respiration driven by variations in temperature, soil moisture, and other factors. Such measurements provide empirical means for understanding the processes that govern patterns of carbon storage and release in terrestrial ecosystems, but our ability to understand processes at the prognostic level, and make predictions about distinctive ecosystems is still limited. There is therefore a further need for information on ecosystem functioning in different environments and across different time scales. Such needs have motivated the development of better techniques for canopy-scale flux measurements (Baldocchi et al. 1988)

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**Fig. 1** Isotopic compositions of the major components and isotopic fractionations important for the use of stable isotopes in the investigation of ecosystem water and CO<sub>2</sub> exchange. The values are rough approximations and can vary greatly with geographical location and environmental conditions (given for demonstration purposes only and based mostly on data from Israel). The main advantages of the isotopic approach lie in the unique labeling of flux components: photosynthesis (depleted uptake) tends to enrich the atmosphere, while respiration (depleted release) tends to deplete the atmosphere in <sup>18</sup>O and <sup>13</sup>C; leaf transpiration and soil evaporation are isotopically very different fluxes; root and soil respiration can have distinct <sup>13</sup>C labeling (values are on the SMOW and PDB scales for δ<sup>18</sup>O and δ<sup>13</sup>C values, respectively)

and the establishment of measurement sites in major ecosystems around the world (Goulden et al. 1996a; Grace et al. 1996; Baldocchi 1997).

In most cases, “net” fluxes of water vapor, CO<sub>2</sub>, and other trace gases exchanged between ecosystems and the atmosphere are measured. These net fluxes, however, reflect the balance between different components. In the case of CO<sub>2</sub>, two opposing fluxes contribute to the net flux, uptake during photosynthesis and release during respiration. For water vapor, leaf transpiration and soil evaporation are the major contributors to the combined net flux. Studies of these fluxes are complicated by CO<sub>2</sub> recycling within canopies (i.e., refixation of respiratory CO<sub>2</sub> before it leaves the system), and redistribution of water among ecosystem reservoirs. Distinguishing among these components is critical to obtain insights into the processes underlying ecosystem responses to cli-

mate forcing. This is because environmental parameters, such as temperature and soil moisture, affect biological activities differentially. Observing a net annual increase in CO<sub>2</sub> uptake of a forest, for example, is not sufficient to determine whether this is due to an increase in ecosystem photosynthesis or to a decrease in ecosystem respiration.

Oxygen and carbon stable isotope compositions of different ecosystem components provide a powerful tool towards quantifying the contribution of different components to the ecosystem exchange. When this tool is used in conjunction with concentration or flux measurements, even more information is derived. Deciphering the individual fluxes of an ecosystem using stable isotopes requires knowledge of the isotopic identity of the major ecosystem components as summarized in a simplified way in Fig. 1. Determining these isotopic signatures requires, in turn, understanding the isotopic fractionations associated with specific ecosystem processes and their interactions during gas exchange. We briefly review here our current knowledge of the isotope identity of different ecosystem components and the relevant details underlying its application to the study of ecosystem functioning. A more comprehensive perspective of the stable isotope methodology and its applications to a wide range of environmental studies can be obtained from compilations of review articles in three recent edited volumes (Rundel et al. 1989; Ehleringer et al. 1992; Griffiths 1998), and from other reviews (Farquhar et al. 1989; Yakir 1992; Flanagan and Ehleringer 1998; Bouton et al. 1999).

## Isotopic identity of different ecosystem gas exchange components

### Isotopic composition of water

Soil water and leaf water are the sources of the evapotranspiration that transfer large quantities of water and energy (as latent heat) between the land surface and the atmosphere. Evaporation also modifies the isotopic composition of water creating a natural isotopic signal for different water reservoirs and fluxes (Gat 1996). The isotopic identity of leaf water and soil water affect the oxygen isotopic signature of ambient carbon dioxide through extensive oxygen exchange between these two constituents.

The principles underlying variations in the isotopic composition of water vapor, δ<sub>E</sub>, and the water surface undergoing evaporation, δ<sub>L</sub>, were first described by Craig and Gordon (1965):

$$\delta_E = [\alpha_{eq} \delta_L - h \delta_a - \epsilon_{eq} - (1-h) \epsilon_k] / [(1-h) + (1-h) \epsilon_k / 1000] \quad (1)$$

where δ(‰) = [(R<sub>sample</sub>/R<sub>standard</sub>) - 1] 1000, R is the ratio of heavy to light isotope of the element of interest, subscripts E, L, and a stand for evaporating water vapor, liquid water body, and ambient air, respectively; α<sub>eq</sub> is the equilibrium fractionation factor (α = R<sub>p</sub>/R<sub>r</sub>, where R<sub>p</sub> and

$R_r$  are the ratios in different phases of a substance), which can also be expressed as  $\epsilon_{eq}$  [i.e.,  $\epsilon=(1-\alpha)1000$ ;  $\epsilon_{eq}=9.8\text{‰}$  for  $\delta^{18}\text{O}$  at  $20^\circ\text{C}$ ; Majoube 1971]; similarly,  $\epsilon_K$  is the kinetic fractionation factor (15–30‰, with high values for diffusive and low values for turbulent boundary layers; Merlivat 1978); and  $h$  traditionally represents the relative humidity of the ambient air normalized to the temperature of the water surface. Eq. 1 indicates that the vapor is greatly depleted in  $^{18}\text{O}$  relative to the evaporating water body. The extent of this depletion is influenced by the isotopic composition of the atmospheric vapor, the relative humidity, and a fractionation associated with the diffusivity of water molecules across the boundary layer.

Good agreement has been observed between theoretical predictions using the Craig-Gordon model and experimental results for soil water undergoing evaporation (Zimmermann et al. 1966, 1967; Allison and Leaney 1982; Allison and Barnes 1983; Barnes and Allison 1988; Walker and Brunel 1990; Mathieu and Bariac 1996a, 1996b). Soil water becomes gradually enriched in heavy isotopes of  $^{18}\text{O}$ , and a highly enriched “evaporation front” usually develops at 0.1–0.5 m below the dry soil surface (Barnes and Allison 1988). Below such fronts, the isotopic enrichment decreases, exponentially, with depth to the value of the source water in the system (Zimmermann et al. 1966; Allison and Barnes 1983; Walker and Brunel 1990). The time required for the development of a steady-state isotopic profile can be long (e.g., several months; Allison and Barnes 1983; Mathieu and Bariac 1996a).

Leaves are usually treated as thin, well-mixed and isotopically uniform water pools to which the Craig and Gordon model (Eq. 1) can be applied (e.g., Farris and Strain 1978; Förstel 1978). Due to the expected large ratio of evaporation flux over water volume in leaves, leaf water was generally assumed to be always near isotopic steady state with respect to ambient environmental conditions. Steady-state conditions, in turn, imply equality of the isotopic compositions of the water entering,  $\delta_s$ , and leaving,  $\delta_E$ , the leaf. By substituting  $\delta_s$  for  $\delta_E$  in Eq. 1, it can be solved for  $\delta_L$ , termed  $\delta_{ss}$ , for leaf water at steady state. This is often approximated by:

$$\delta_{ss}=\delta_s+\epsilon_{eq}+\epsilon_k+h(\delta_a-\epsilon_k-\delta_s) \quad (2)$$

where  $h$ , relative humidity at the leaf surface temperature, can be replaced with the ratio of the partial vapor pressures in the ambient air and inside the leaf ( $e_a/e_i$ ), and  $\epsilon_K$  should represent the weighted mean of the kinetic fractionation in the laminar leaf boundary layer and the stagnant substomatal cavity (Flanagan et al. 1991). In deriving Eq. 2,  $\epsilon_K/1000$  is assumed to be small.

Numerous studies have noted that the observed isotopic composition of bulk leaf water is usually less enriched than that predicted by Eq. 2 (Dongmann et al. 1974; Leaney et al. 1985; Bariac et al. 1989; Walker et al. 1989; Yakir et al. 1989, 1990; Flanagan and Ehleringer 1991; Yakir 1992; Farquhar and Lloyd 1993; Roden and

Ehleringer 1999). Such differences were first proposed to reflect the fact that Eq. 2 represents only the isotopic composition at the evaporating cell wall surfaces within the leaves, and not that of the mixed bulk leaf water. The latter can be highly heterogeneous and involve slow mixing between internal pools and the influence of vein water (Leaney et al. 1985; Bariac et al. 1989; Walker et al. 1989; Yakir et al. 1989; Luo and Sternberg 1992). Perhaps a more realistic approach was proposed by Farquhar and Lloyd (1993) based on a one-dimensional advection-diffusion model that directly incorporates the rate of transpiration ( $T$ ) and an effective mixing length ( $L$ ):

$$\delta_{LW}=\delta_s+(\delta_{ss}-\delta_s)(1-e^{-\rho})/\rho \quad (3)$$

where  $\rho=(TL)/(CD)$  is a Peclet number,  $C$  represents molar concentration of water ( $5.56\times 10^4 \text{ mol m}^{-3}$ ),  $D$  the diffusivity of  $\text{H}_2^{18}\text{O}$  in water ( $2.66\times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ ) (Wang 1954),  $T$  is the rate of transpiration and  $L$  is the effective mixing length in the leaf. This approach, similar to that of Zimmermann et al. (1967) applied to soils (see above), considers a continuous isotopic gradient within the leaf. This gradient is the result of the shifting balance between the convective evaporation flux of unfractionated water through the leaf, and the back diffusion of isotopically enriched water away from the evaporating sites. Note that Eq. 3 predicts an inverse correlation between  $\delta_{LW}$  (for bulk leaf water) and the rate of leaf transpiration,  $T$ , and that the difference between  $\delta_{LW}$  and  $\delta_{ss}$  will increase with  $T$  (Bariac et al. 1989; Flanagan et al. 1991; and see below). Roden and Ehleringer (1999; cf. Leaney et al. 1985) suggested that the isotopic composition of bulk leaf water can be corrected by an empirical, species-dependent factor to obtain estimates of isotopic composition of the water at the evaporating surfaces that are in good agreement with predicted  $\delta_{ss}$  values (Eqs. 2, 3).

If plants are always near isotopic steady state (Flanagan et al. 1991; Wang and Yakir 1995), then the isotopic composition of the transpiration water vapor flux will simply be that of the source water utilized by the plants (i.e., xylem water derived from local soil water at a certain depth; Dawson 1993; Bouton et al. 1999), irrespective of the isotopic enrichment of leaf water. This leads to a significant difference between the isotopic composition of the highly fractionated evaporation flux from the soil ( $\delta_E$ , Eq. 1) and the unfractionated transpiration flux through leaves ( $\delta_s$ , when Eqs. 2 or 3 apply). This, usually large, difference is the basis for using stable isotopes in tracing the soil evaporation and leaf transpiration fluxes separately (Moreira et al. 1997; Wang and Yakir, in press). Note that although the transpiration flux through the leaf is often assumed to be unfractionated with respect to xylem water, the  $\delta^{18}\text{O}$  value of this water can vary significantly (Wang et al. 1998). Sources of such variations include soil depth profiles (Mathieu and Bariac 1996a), enrichment in stems (Dawson and Ehleringer 1993) and seasonal effects (Dawson and Pate 1996; White et al. 1985).

Carbon isotope ratios of CO<sub>2</sub>

The carbon isotope ratio of ambient CO<sub>2</sub> is determined by the δ<sup>13</sup>C values of respiratory CO<sub>2</sub>, the discrimination by photosynthesis, and the rates of these processes relative to turbulent mixing with the atmospheric CO<sub>2</sub> pool (Sternberg 1989; Lloyd et al. 1996). The general assumption is that there is little or no isotopic fractionation associated with respiration processes (Lin and Ehleringer 1997; but see Duranceau et al. 1999; Gillon and Griffiths 1997), and the δ<sup>13</sup>C values of respired CO<sub>2</sub> are mostly determined by the mean isotopic composition of plant biomass and soil organic carbon (Hungate et al. 1997; Rochette and Flanagan 1997; Lin et al. 1999; Rochette et al. 1999). Note that due to diffusional fractionation (ε<sub>CO<sub>2</sub>-diff</sub> = -4.4‰), soil respired CO<sub>2</sub> is depleted in <sup>13</sup>C relative to soil CO<sub>2</sub>. This is not apparent under steady-state conditions, in which case, soil CO<sub>2</sub> becomes enriched by +4.4‰ and the δ<sup>13</sup>C of soil-respired CO<sub>2</sub> approaches that of the source organic material (Dörr and Münich 1987; Cerling et al. 1991; Davidson 1995).

The relative proportions of C3 plants having average δ<sup>13</sup>C values of about -26‰ to C4 plants having average δ<sup>13</sup>C values of about -12‰ in ecosystems has important influence on the isotopic identity of respired CO<sub>2</sub>. Ecosystems, such as savannas or grasslands, which have a high proportion of C4 plants, are expected to have respired CO<sub>2</sub> with a less negative δ<sup>13</sup>C value than those with a greater proportion of C3 plants such as temperate and tropical forests. However, numerous complications can confound these expectations. Different photosynthetic types may dominate some ecosystems, such as croplands, tropical flood plains, and deforested areas, at different periods of time (Victoria et al. 1992; Buchmann and Ehleringer 1998). Soil organic carbon may retain the isotopic signal of the previous vegetation for several years contributing to differences between the isotopic composition of respired CO<sub>2</sub> and that of the biomass present at the time of sampling (Buchmann and Ehleringer 1998). This phenomenon is usually referred to as isotopic disequilibrium. Disequilibrium can also arise when soil organic matter is derived from photosynthesis that occurred at a time when the carbon isotopic ratio of atmospheric CO<sub>2</sub> differed from that of the present day (Enting et al. 1995; Fung et al. 1997). The continuous increase in the concentration of atmospheric CO<sub>2</sub> over the past hundred years is due to addition of <sup>13</sup>C-depleted CO<sub>2</sub> from organic sources (fossil fuels and biomass burning), a process that has progressively changed the isotopic composition of the atmosphere (Troiler et al. 1996) and all biomass produced from atmospheric CO<sub>2</sub>. This, in turn, results in a small difference between the mean δ<sup>13</sup>C values of current biomass production and of soil respiration that is derived from older biomass (Enting et al. 1995; Fung et al. 1997).

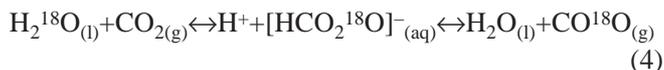
Ecosystems with variations in the isotopic composition of biomass across spatial gradients also present a problem in deciphering the isotopic composition of respired CO<sub>2</sub>. For example, tropical forests exhibit heterogeneous isotopic signatures throughout their vertical pro-

files with upper canopy tissue δ<sup>13</sup>C values in the range of -28 to -26‰, and understory tissue exhibiting δ<sup>13</sup>C values in the range of -36 to -32‰ (Medina and Minchin 1980; Sternberg et al. 1989; Medina et al. 1991). Since determining the respiratory contribution by each component of these ecosystems is nearly impossible, the isotopic signature of respired CO<sub>2</sub> is determined by "Keeling-type" plots of isotopic and concentration measurements carried out at night (discussed below).

The imprint in the δ<sup>13</sup>C values of ambient CO<sub>2</sub> by photosynthesis occurs because photosynthesis discriminates against <sup>13</sup>C; therefore, the remaining atmospheric CO<sub>2</sub> pool will be enriched in <sup>13</sup>C. The extent of this imprint will also be influenced by the proportion of C3 and C4 plants, since the former discriminates more against <sup>13</sup>C than the latter. Carbon isotope ratios may be above and concentration of CO<sub>2</sub> below the atmospheric values if the rate of photosynthesis is high relative to turbulent mixing. This has been observed in tropical forests (Quay et al. 1989) and in agricultural crops (Yakir and Wang 1996; Buchmann and Ehleringer 1998). Because photosynthetic discrimination is subject to diurnal and seasonal environmental influences, the photosynthetic imprint by the ecosystem is also expected to vary on these time scales.

Oxygen isotope ratios of CO<sub>2</sub>

The <sup>18</sup>O of CO<sub>2</sub> from both root respiration and soil decomposition is strongly influenced by the oxygen isotopic composition of the water with which it is in contact (Hesterberg and Siegenthaler 1991; Amundson et al. 1998; Tans 1998; Stern et al. 1999). CO<sub>2</sub> isotopically equilibrates with water according to:



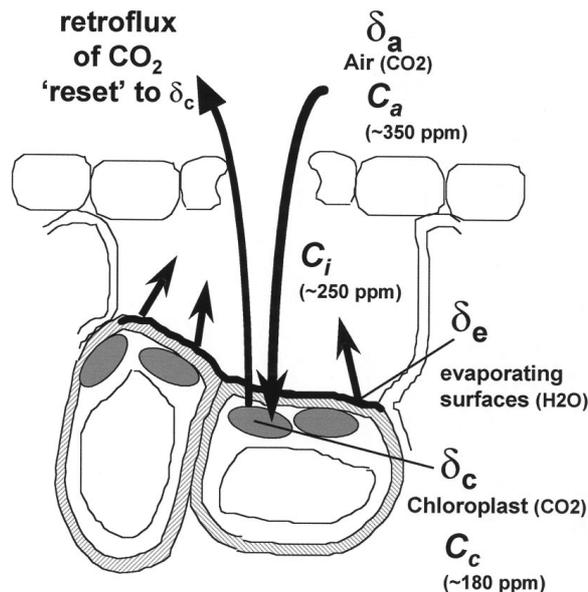
The temperature-dependent value for the equilibrium fractionation ε<sub>eq-CO<sub>2</sub></sub> between the oxygen in the CO<sub>2</sub> and water is ε<sub>eq-CO<sub>2</sub></sub>(T) = 17604/T - 17.93, where dε<sub>eq-CO<sub>2</sub></sub>/dT = -0.20‰/°C, so that at 25°C, ε<sub>eq-CO<sub>2</sub></sub> = 41.11‰ (Brenninkmeijer et al. 1983). Water must be in the liquid phase for the hydration reaction to occur. The rate constant for the isotopic reaction is two-thirds the rate of the chemical reaction because of the three oxygens present in the intermediate species (Mills and Urey 1940). With the presence of carbonic anhydrase (CA), ubiquitous in leaves and micro-organisms, equilibrium in Eq. 4 is reached nearly instantaneously. The quantity of water usually involved in Eq. 4 is many orders of magnitude greater than that of the CO<sub>2</sub> present, so isotopically equilibrated CO<sub>2</sub> will take on the oxygen isotopic ratio of the water in which it is dissolved (modified by the temperature-dependent equilibrium fractionation) regardless of its initial δ<sup>18</sup>O value. In the simplest case, δ<sup>18</sup>O of rainwater directly translates to the δ<sup>18</sup>O of soil water, δ<sub>sw</sub>, and, after equilibrium (Eq. 4 above) and ki-

netic fractionation ( $\epsilon_{\text{eff-soil}}$ ) are taken into account,  $\delta^{18}\text{O}$  of  $\text{CO}_2$  production by respiration ( $\delta_p$ ) is given by:

$$\delta_p = \delta_{\text{sw}} + \epsilon_{\text{eq-co}_2} + \epsilon_{\text{eff-soil}} \quad (5)$$

The diffusional, kinetic, fractionation of  $\text{CO}_2$  is expected to be 8.8‰ based on the binary diffusivities of the isotopic species of  $\text{CO}_2$  in air. Near the soil surface, however, the rate of  $\text{CO}_2$ - $\text{H}_2\text{O}$  equilibrium is often too slow compared to the rate of  $\text{CO}_2$  escape from the soil (Stern et al. 1999). As a result, some  $^{18}\text{O}$  enrichment of soil  $\text{CO}_2$  occurs which partially offsets the kinetic fractionation with respect to the relevant soil water  $\delta^{18}\text{O}$  value. Global isotopic mass balance approaches have been used to solve for the effective kinetic fractionation of  $\text{CO}_2$  diffusing out of soil,  $\epsilon_{\text{eff-soil}}$ , assuming that all other terms were well known. These studies arrived at estimates of 5.00 (Ciais et al. 1997) and 7.6‰ (Farquhar et al. 1993). Miller et al. (1999) used direct, small-scale measurements to suggest a value of  $\epsilon_{\text{eff-soil}} = 7.0$ ‰. Irrespective of respiratory activity, the  $^{18}\text{O}$  of atmospheric  $\text{CO}_2$  is also influenced by invasion into soils. In this process (Tans 1998; Miller et al. 1999),  $\text{CO}_2$  diffuses into, and back out of, the soil (no net flux) allowing for isotopic equilibration of ambient  $\text{CO}_2$  with soil water. Invasion may be most important when soil moisture near the surface is high and there is a stagnant boundary layer above the soil, such as in forest during the night, or if there is any enhancement of isotopic equilibrium (e.g., by CA activity in soils, for which there is currently no direct evidence). Consideration of invasion is critical in soil chamber experiments that include  $^{18}\text{O}$  measurements.

While the simplest treatment of Eq. 5 above was by assuming soil water  $\delta^{18}\text{O}$  to be constant and equivalent to rainwater, in fact large variation in the  $\delta^{18}\text{O}$  of soil water is observed. Soil water may become enriched near the surface relative to water deeper in the soil profile by evaporation (Allison and Barnes 1983; Mathieu and Bariac 1996a). The extent to which this enriched water affects the  $^{18}\text{O}$  of respired  $\text{CO}_2$  diffusing out of the soil may be relatively small because of the slow rate of  $\text{CO}_2$ - $\text{H}_2\text{O}$  equilibration relative to the rate of  $\text{CO}_2$  escape out of the soil (Stern et al. 1999). Miller et al. (1999) observed that while the largest soil water enrichment occurred in the top 5 cm of the soil, the effective depths that influenced the  $\delta^{18}\text{O}$  value of soil respired  $\text{CO}_2$  were 5–15 cm. However, moisture above the soil surface such as that in moss or surface roots exposed to evaporation can re-equilibrate with soil-respired  $\text{CO}_2$ , causing a deviation in the isotopic signature of respired  $\text{CO}_2$  from that equilibrated with bulk soil water (Flanagan et al. 1997; Sternberg et al. 1998). Local soil water,  $\delta_{\text{sw}}$ , may also change seasonally because of changes in precipitation or groundwater sources, or vegetative cover. For example, Flanagan et al. (1997) observed a large effect of moss cover in a spruce forest, which changed seasonally with the extent of evaporative enrichment. On the other hand Sternberg et al. (1998) observed seasonal changes in the



**Fig. 2** Major steps involved in the  $\text{CO}_2$  exchange between a C3 leaf and the atmosphere, and the isotopic labeling at these steps. Note that the values for  $\text{CO}_2$  concentrations are a rough approximation and can vary among species and conditions, and that the difference between the diffusion influx and the retroflux of  $\text{CO}_2$  constitutes the photosynthetic  $\text{CO}_2$  uptake

$\delta^{18}\text{O}$  values of respired  $\text{CO}_2$  in a seasonal tropical forest in eastern Amazon, yet no differences were observed in the  $\delta^{18}\text{O}$  values of surface water. These authors hypothesized that the difference in  $\delta^{18}\text{O}$  values of respired  $\text{CO}_2$  between seasons was related to seasonal changes in the depth of root activity. Miller et al. (1999) observed a change of 5‰ in the  $\delta^{18}\text{O}$  of soil water due to input of snow-melt water in Colorado and a concomitant change of 4‰ in the  $\delta^{18}\text{O}$  value of soil respired  $\text{CO}_2$ . In addition to actual changes in the  $\delta^{18}\text{O}$  of the source water, the soil, or soil cover, water content influences the effective hydration rate, and seasonal and spatial changes in temperature will affect both  $\epsilon_{\text{eq-co}_2}$  and the hydration rate constant,  $k_h$  (Eq. 4; Stern et al. 1999). Clearly, some knowledge of the isotopic composition of the local hydrologic cycle is necessary to predict  $\delta_{\text{sw}}$  and  $\delta_p$ . Global sampling networks such as IAEA and GNIP, as well as efforts to model soil water such as Jouzel et al. (1987) and Mathieu and Bariac (1996b), may provide much of the needed information.

$\text{CO}_2$  diffusing into leaves during photosynthesis dissolves and rapidly (catalyzed by CA) exchanges its oxygen with water in the chloroplasts (C3 leaves) or mesophyll (C4 leaves). About one-third of the  $\text{CO}_2$  diffusing into the leaves is fixed in C3 photosynthesis (more in C4). The remaining two-thirds diffuses back to the atmosphere after isotopically equilibrating with leaf water. The retrodiffused  $\text{CO}_2$  (Fig. 2) is enriched in  $^{18}\text{O}$  relative to the soil water that feeds the plant because of the evaporative enrichment of leaf water (Eqs. 2, 3). Photosynthetic  $\text{CO}_2$  uptake by leaves is therefore associated with  $^{18}\text{O}$  enrichment of atmospheric  $\text{CO}_2$  (Francey and Tans

1987). Alternatively, this process can be viewed as apparent discrimination,  $^{18}\Delta$ , against  $^{18}\text{O}$  by leaves, which was quantitatively described by Farquhar and Lloyd (1993) as:

$$^{18}\Delta = [c_c / (c_a - c_c)] (\delta_c - \delta_a) + \bar{a} \quad (6)$$

where  $^{18}\Delta = R_a / R_A$ ,  $R_a$  and  $R_A$  are the oxygen isotope ratios ( $R = ^{18}\text{O}/^{16}\text{O}$ ) of  $\text{CO}_2$  in the air and in the  $\text{CO}_2$  flux into the leaf chloroplast and in the atmosphere,  $\bar{a}$  is the weighted average fractionation during diffusion of  $\text{CO}_2$  from the atmosphere to the chloroplast,  $c_a$  and  $c_c$  are the  $\text{CO}_2$  mixing ratios in the atmosphere and chloroplast, respectively, and  $\delta_a$  and  $\delta_c$  are the  $\delta^{18}\text{O}$  values of  $\text{CO}_2$  in the atmosphere and in equilibrium with chloroplast water, respectively. Equation 6 was developed for C3 plants in which CA is concentrated mainly in the chloroplast and  $c_c$  can be estimated from  $^{13}\Delta$  as noted above. In C4 plants, CA and, therefore,  $^{18}\text{O}$  exchange occur mainly in the mesophyll cells and  $c_c$  and  $\delta_c$  in using Eq. 6, would refer to this site. Furthermore, the  $\text{CO}_2$  concentration at the site of  $^{18}\text{O}$  exchange cannot be accurately estimated in C4 plants.

The discrimination values,  $^{18}\Delta$ , are highly variable among plant species (both C3 and C4) and environmental conditions, and modeled discrimination terms for different biomes can range from  $-20\%$  to  $+32\%$  (Farquhar et al. 1993). Negative or positive  $^{18}\Delta$  values stem from its reference to  $\delta^{18}\text{O}$  of atmospheric  $\text{CO}_2$ , and the geographical variations in  $\delta^{18}\text{O}$  values of leaf water. Comparison of modeled and measured ecosystem-scale  $^{18}\Delta$ , however, indicate large discrepancies in the Amazon basin and further comparisons with other ecosystems are necessary (Sternberg et al. 1998).

Much of the variation observed in  $^{18}\Delta$  is related to the variation in  $\delta^{18}\text{O}$  values of chloroplast water (often estimated with Eq. 2; but see also Yakir et al. 1994; Gillon and Yakir, in press), and the species-dependent retrodiffusion  $\text{CO}_2$  flux [ $c_c / (c_a - c_c)$ ]. Values of  $c_c$  are difficult to estimate. Leaf-scale measurements indicated that the  $\text{CO}_2$  draw-down from the substomata cavities ( $c_i$ , readily estimated in leaf-scale measurements) to the chloroplast is generally of the order of  $(c_i - c_c) / c_a \approx 0.2$  and  $c_c / c_a \approx 0.55$ , (Fig. 2; Caemmerer and Evans 1991; Lloyd et al. 1992; Loreto et al. 1992; Epron et al. 1995). Note, however, that in C4 plants, internal  $\text{CO}_2$  concentrations are much lower ( $c_i / c_a$  is typically  $\sim 0.3$ – $0.4$  with further draw-down to the mesophyll cells where  $\text{CO}_2$  is fixed by PEP-carboxylase).

There are two other factors that can influence estimates of  $^{18}\Delta$  with Eq. 6. First,  $c_c$  represents the  $\text{CO}_2$  mixing ratio at the site of Rubisco (the primary photosynthetic enzyme in the chloroplast), but the  $\text{CO}_2$ –water equilibration occurs at the chloroplast or cellular membranes. Due to large internal resistances to  $\text{CO}_2$  diffusion,  $\text{CO}_2$  mixing ratios at these sites can be significantly different from  $c_c$ , influencing predictions of  $^{18}\text{O}$  discrimination. Recently, the  $\text{CO}_2$  concentration at the site of  $\text{CO}_2$ – $\text{H}_2\text{O}$  equilibrium was defined as  $c_{cs}$  and was

estimated to be about midway between  $c_i$  and  $c_c$ ,  $(c_i - c_{cs}) / c_a \approx 0.1$  (Wang et al. 1998; Yakir 1998; Gillon and Yakir, in press). Second, Eq. 6 assumes full isotopic equilibrium between  $\text{CO}_2$  and chloroplast water, due to the presence of CA. In leaf-scale studies, data agreed better with predictions when only partial equilibration was assumed (Williams et al. 1996). Studies on a range of C3 and C4 species indicated a large range of CA activities, and that full isotopic equilibrium is likely achieved in dicot C3 species only at low rates of assimilation, and not for monocot C3 or C4 species (J.S. Gillon and D. Yakir, unpublished data). In monocot C3 and C4, oxygen isotopic exchange reached only about 80% of equilibrium, and the appropriate correction to Eq. 6 was applied. A direct estimate of the extent of isotopic equilibrium ( $\theta_{\text{eq}}$ ) can be obtained from the ratio of the observed  $^{18}\text{O}$  discrimination during on-line  $\text{CO}_2$  measurements ( $^{18}\Delta_{\text{ca}}$ ) and the potential discrimination assuming full equilibration with measured chloroplast water  $\delta^{18}\text{O}$  ( $^{18}\Delta_{\text{ca}}$ ; i.e.,  $\theta_{\text{eq}} \approx ^{18}\Delta_{\text{ca}} / ^{18}\Delta_{\text{ea}}$ ) (Gillon and Yakir, in press). Farquhar and Lloyd (1993) also proposed an indirect correction to Eq. 6 in which the extent of equilibrium is related to the ratio,  $\rho$ , of Rubisco to CA activities.

Some uncertainties are still associated with estimating the  $^{18}\text{O}$  signal of  $\text{CO}_2$  exchange with soil and leaves. For example, it is not clear if enhancement of the  $\text{CO}_2$ – $\text{H}_2\text{O}$  exchange can occur as a result of CA activity, or other factors, in soil. Also unclear is which internal  $\text{CO}_2$  concentrations should be used in estimating  $\Delta^{18}\text{O}$ . Resolution of these uncertainties will provide the essential fine-tuning of the system. Even with the uncertainties, however, large differences can be expected between soil-respired  $\text{CO}_2$  and leaf exchange flux at most locations. The soil-respired  $\text{CO}_2$  will largely reflect  $^{18}\text{O}$ -depleted soil water, while the leaf  $\text{CO}_2$  exchange flux will reflect the large  $^{18}\text{O}$  enrichment of leaf water. This difference allows identification of  $\text{CO}_2$  sources and sinks in the ecosystem and may allow actual estimates of the individual photosynthetic and respiratory fluxes (see below).

### Combining isotopic composition and concentration measurements

Stable isotope signatures of  $\text{CO}_2$  along with  $\text{CO}_2$  concentration measurements provide the basis for “Keeling-type” plots (Keeling 1958, 1961). The equation used in the Keeling plot is derived from the basic assumption that the atmospheric concentration of a substance in an ecosystem reflects the combination of some background amount of the substance that is already present in the atmosphere and some amount of substance that is added or removed by sources or sinks in the ecosystem:

$$C_E = C_a + C_s \quad (7)$$

where  $C_E$ ,  $C_a$ , and  $C_s$  are the concentrations of the substance in the ecosystem, in the atmosphere, and that con-

tributed by ecosystem sources, respectively. Isotope ratios of these different components can be expressed by a simple mass balance equation:

$$C_E \delta_E = C_a \delta_a + C_s \delta_s \quad (8)$$

where  $\delta_E$ ,  $\delta_a$ , and  $\delta_s$  represent the isotopic composition of the substance in the ecosystem, in the atmosphere, and of the sources, respectively. Combining Eqs. 7 and 8:

$$\delta_E = C_a (\delta_a - \delta_s) / (1/C_E) + \delta_s \quad (9)$$

This is a linear relationship with a slope of  $C_a (\delta_a - \delta_s)$  and an intercept at the  $\delta_s$  value of the net sources/sinks in the ecosystem. Note that even if the ecosystem source/sink is composed of several different subsources/sinks, the Keeling-type plot can still be used as long as the relative contribution of each of these subcomponents remains fixed.

This relationship was first used by Keeling (1958, 1961) to interpret carbon isotope ratios of ambient  $\text{CO}_2$  and to identify the sources that contribute to increases in atmospheric  $\text{CO}_2$  concentrations on a regional basis. Later, researchers used this expression to identify the isotopic composition of respired  $\text{CO}_2$  in forest ecosystems (Quay et al. 1989; Sternberg et al. 1989, 1997; Buchmann et al. 1997a, 1997b; Harwood et al. 1999). More recently, the derivation of the isotopic composition of respired  $\text{CO}_2$  has been used to determine ecosystem carbon isotope fractionation ( $\Delta_e$ ) by the following equation:

$$\Delta_e = (\delta_{\text{trop}} - \delta_{\text{resp}}) / (1 + \delta_{\text{resp}}) \quad (10)$$

where  $\delta_{\text{trop}}$  and  $\delta_{\text{resp}}$  represent the  $\delta^{13}\text{C}$  values of tropospheric and respired  $\text{CO}_2$ , respectively (Flanagan et al. 1996; Buchmann et al. 1998). A modified equation ( $\Delta_e = \delta_{\text{atm}} - \delta_{\text{resp}}$ ) was also used by Bakwin et al. (1998) to estimate regional-scale biological discrimination. Note that in these equations, the traditional use of  $\delta_{\text{resp}}$  (Keeling 1961) is applied for both nighttime and daytime measurements. This term would be better represented by  $\delta_s$  (Eq. 9), which includes effects of both respiration and photosynthesis and will be equal to  $\delta_{\text{resp}}$  only for nighttime measurements. Whole-ecosystem discrimination was also assessed by relating the isotopic composition of  $\text{CO}_2$  in the convective boundary layer (CBL), which integrates the effects of photosynthesis, respiration, and turbulent transport, to the isotopic composition of tropospheric  $\text{CO}_2$  (Lloyd et al. 1996; Nakazawa et al. 1997). Whole-ecosystem discrimination and CBL measurements can increase the window of observation to landscape or regional scale. This approach can help, first, in quantifying the contribution of C3 or C4 vegetation to productivity such as in tropical areas (Lloyd et al. 1996; Miranda et al. 1997) and, second, in providing the landscape- to regional-scale discrimination parameters required as inputs for global-scale studies (e.g., Fung et al. 1997).

One of the problems with the Keeling-type plot is that the extrapolated intercept from the linear regression is

many units away from the actual measurements. Therefore, small errors in measuring either the isotope ratios or concentration of the component of interest can lead to large errors in the extrapolation (such problems apply to both water and  $\text{CO}_2$  measurements). Recycling of respired  $\text{CO}_2$  can be another possible factor that contributes to errors in estimating the  $\delta$  values of respired  $\text{CO}_2$  (Sternberg 1989).

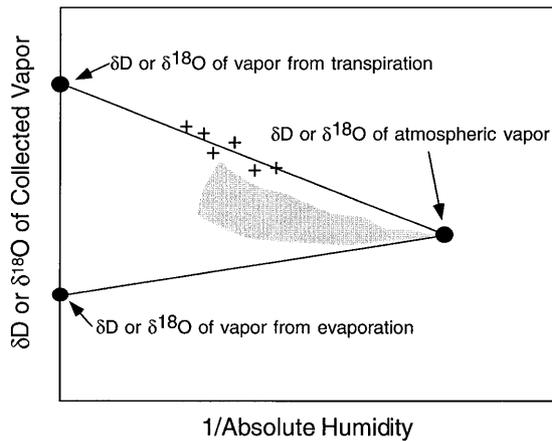
Very little is known about  $\text{CO}_2$  recycling in ecosystems and it can be defined in different ways. Sternberg (1989) defined a recycling index,  $\phi_S$ , as the proportion of respired  $\text{CO}_2$  refixed by photosynthesis relative to the total respiration flux. Consequently, a modified Keeling equation that takes this recycling index into account was developed (Sternberg 1989):

$$\delta_F = \{ (\delta_a - \delta_R) [\text{CO}_2]_a (1 - \phi_S) / [\text{CO}_2]_F \} + \delta_R + \phi_S \Delta \quad (11)$$

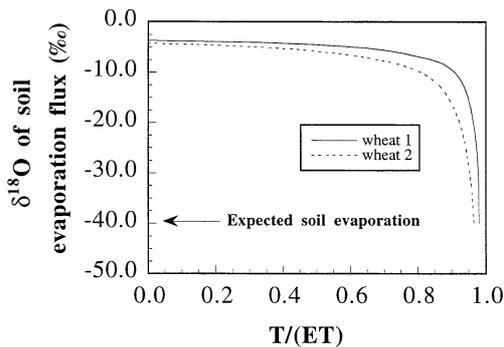
where  $\delta_F$ ,  $\delta_a$ , and  $\delta_R$  are the  $\delta^{13}\text{C}$  value of ambient (forest), atmospheric, and respired  $\text{CO}_2$ , respectively,  $[\text{CO}_2]_a$  and  $[\text{CO}_2]_F$  are the concentrations of tropospheric and ambient  $\text{CO}_2$ , respectively, and  $\Delta$  is fractionation in photosynthesis. When there is no recycling (i.e.,  $\phi_S = 0$ ), Eq. 11 reduces to Eq. 9, the simpler Keeling-type equation. Using this equation, recycling rates ranging from 7% to 40% have been estimated (Broadmeadow and Griffiths 1993; Sternberg et al. 1997). Further study of respiratory  $\text{CO}_2$  recycling in different ecosystems and conditions is clearly needed. Such studies should also consider that recycling cannot be derived by pooling data from several layers in the forest profile and deriving recycling from slopes and intercepts of the regression equation (Lloyd et al. 1997; Sternberg 1997). Differences in fractionation and photosynthetic rates at different levels of the forest could lead to spurious slopes and intercepts.

Lloyd et al. (1996) proposed a different recycling index ( $\Phi_L$ ) derived from micrometeorological flux measurements and that measures the relative proportion of respired  $\text{CO}_2$  fixed by photosynthesis relative to the total  $\text{CO}_2$  assimilated. As noted by Sternberg (1997), the two recycling indexes provide estimates of different quantities and replacement of one by another is not justified. The different definition of  $[\text{CO}_2]_a$  and  $\delta_a$  (Eq. 11) in the two approaches should, however, be noted. Lloyd et al. (1996) reasoned that because there are substantial differences in the concentration and isotopic composition between  $\text{CO}_2$  in the CBL and the troposphere, the CBL values, representing the actual air that enters the canopy, should be used in this equation. Sternberg (1997) argued that differences between  $\text{CO}_2$  in the CBL and the troposphere are brought about by the interaction of the very processes under study: respiration, turbulent mixing, and photosynthesis. The concentration and isotopic composition of tropospheric  $\text{CO}_2$  should therefore better represent the true end-member of the mixing model described by Eq. 11.

The Keeling expression has also been used to identify the source of water vapor to the evapotranspiration flux



**Fig. 3** Possible relationships between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of ambient vapor versus the inverse of its concentration in a forest. The *upper line* represents a situation where evapotranspiration is solely generated from transpiration, the *bottom line* represents a situation where evapotranspiration is solely generated from evaporation, and the *shaded area* represents a case where evapotranspiration is generated from both transpiration and evaporation



**Fig. 4** Relationships between the ratio of leaf transpiration ( $T$ ) over total evapotranspiration ( $ET$ ) and estimates of the  $\delta^{18}\text{O}$  values of the soil evaporation flux in a mature wheat field on two different dates. The  $\delta^{18}\text{O}$  values of leaf transpiration and soil evaporation were estimated directly from measured values of stem water, or calculated from stem water values and Eq. 6, respectively. The  $\delta^{18}\text{O}$  value of the total  $ET$  flux was estimated with a “Keeling plot”

of ecosystems (Bariac et al. 1989; Brunel et al. 1992; Yakir and Wang 1996; Moreira et al. 1997; Harwood et al. 1999). When using the Keeling expression for this purpose two assumptions must be made: (1) there is no loss of water vapor by the ecosystem other than that lost by turbulent mixing with the atmospheric pool (for example, no vapor can condense out of the ecosystem during measurements); (2) there are no more than two sources having distinct isotopic composition for the evapotranspired water vapor. To simplify the analysis, a useful assumption is that transpiring plants are under isotopic steady state, thus making the isotopic composition of transpired water vapor the same as stem water (cf. Eqs. 1, and 2 above). Harwood et al. (1998) demonstrated that direct measurement of transpired water vapor from leaves in the field is possible, eliminating the re-

quirements for a steady-state assumption. This is significant because both Wang and Yakir (1995), using leaf-scale studies, and Harwood et al. (1998), in a canopy-scale study, showed that the steady-state assumption is only an approximation. Leaf water may lag behind the meteorologically derived isotopic steady state (Eq. 2). As a result, the  $\delta^{18}\text{O}$  value of the transpired water vapor is somewhat lower in the morning and higher in the afternoon than the stem water  $\delta^{18}\text{O}$  value expected at steady state. Note, however, that on diel and longer time-scales, leaf water must be at steady state, as plants do not progressively accumulate any of the isotopes. Using the Keeling approach in a wheat field (Yakir and Wang 1996), Amazonian forest (Moreira et al. 1997), and European oak forest (Harwood et al. 1998), the isotopic composition of vapor along a height gradient was regressed on the inverse of the water vapor concentration to derive the isotopic composition of the evapotranspiration source (the intercept of the best-fit line to the data; Fig 3). In these studies, the calculated  $\delta^{18}\text{O}$  value of the evapotranspired vapor closely matched the  $\delta^{18}\text{O}$  value of plant stem water. Considering assumption 2 above led to the conclusion that most, if not all, of the evapotranspired water vapor in these study sites was generated by transpiration. In a latter study (Moreira 1998), the isotopic composition of the respective sources (transpiration/evaporation, from stem water and soil evaporation; Eq. 1) together with the estimate of the isotopic composition of the evapotranspired water vapor (from a Keeling plot) were used to calculate the percent contribution by transpiration:

$$F_T(\%) = (\delta_{ET} - \delta_E) / (\delta_T - \delta_E) \quad (12)$$

where  $F_T(\%)$  is the fractional contribution by transpiration to the evapotranspiration flux, and  $\delta_{ET}$ ,  $\delta_E$ , and  $\delta_T$  are the isotopic compositions of evapotranspiration vapor, evaporative and transpiration vapor sources, respectively. Estimates of  $\delta_T$  were obtained from measurements of stem water and of  $\delta_E$  from Eq. 1 using measurements of soil water. Values of  $F_T(\%)$  ranging from 76 to 100% were observed in two different forests in the Amazon basin.

Wang and Yakir (in press) used a similar approach with data from wheat fields to test the sensitivity of the isotopic partitioning of  $ET$  into soil evaporation and leaf transpiration to estimates of  $\delta_E$ . Here too,  $\delta_{ET}$  was obtained by a Keeling plot ( $-4.2\text{‰}$ ), and  $\delta_T$  was estimated for stem water based on the steady-state assumption ( $-2.9\text{‰}$ ). Measurements of stem water were also used as estimates of soil water and  $\delta_E$  was calculated to be  $-39.1\text{‰}$  with Eq. 1. These data yielded estimates of  $F_T(\%)$  for the wheat fields in the range of 96–98% and the sensitivity of these ratios to uncertainties in estimating  $\delta_E$  was tested as shown in Fig. 4. Evidently, estimates of  $F_T(\%)$  rely on the large difference between  $\delta_T$  and  $\delta_E$  that are normally observed (e.g.,  $-2.9$  vs  $-39.1\text{‰}$ ). Such differences can diminish if large isotopic enrichment due to evaporation occurs near the soil sur-

face. Figure 4 demonstrates, however, that only when the soil becomes exceedingly dry and soil water approaches isotopic steady state, does sensitivity increase. This is encouraging in light of the results of Mathieu and Bariac (1996a, 1996b) that demonstrate the slow approach to isotopic steady state of soil water. Such slow approaches to steady state can take several months and are often not attained between rain (or irrigation) events.

### Combining isotopic composition and flux measurements

The first applications of stable isotopes to canopy-scale flux measurements used a flux-gradient approach, with the isotopic composition and concentration gradients of atmospheric moisture. Such studies, mostly in soil and similar media, provided important reassurances with respect to the applicability of the isotope mass balance approach at the canopy and field scales (Zimmermann et al. 1966, 1967; Allison and Leaney 1982; Allison and Barnes 1983; Barnes and Allison 1988; Walker and Brunel 1990). Brunel et al. (1992) used the isotopic composition of atmospheric water vapor (collected over 6–7 h) above a rice field to show that it provides a faithful tracer of ET.

The dynamics of canopy-scale net fluxes of water and  $\text{CO}_2$  exchanged between vegetation and the atmosphere are routinely measured with micrometeorological methods (e.g., with eddy covariance; Baldocchi et al. 1988). Combining these methods with isotopic measurements should allow partitioning of a net flux into its gross flux components. To understand this idea, assume  $F_N$  is a net flux of a chemical constituent (e.g.,  $\text{CO}_2$  or water vapor) which is composed of two gross flux components,  $F_1$  and  $F_2$  (e.g., photosynthetic  $\text{CO}_2$  uptake and soil respiration, or leaf transpiration and soil evaporation, respectively), such that  $F_N = F_1 + F_2$ . Each flux carries a unique isotopic identity,  $\delta_1$ ,  $\delta_2$ , and  $\delta_N$  (e.g., the  $\delta$  values of  $\text{CO}_2$  taken up in photosynthesis, released in soil respiration, and of the combined net flux, respectively) and isotopic mass balance takes the form:

$$F_N \delta_N = F_1 \delta_1 + F_2 \delta_2 \quad (13)$$

$$F_1 = [F_N \delta_N - (F_N - F_1) \delta_2] / \delta_1 \quad (14)$$

$$F_2 = [F_N \delta_N - (F_N - F_2) \delta_1] / \delta_2 \quad (15)$$

By rearrangement we obtain estimates of the component gross fluxes:

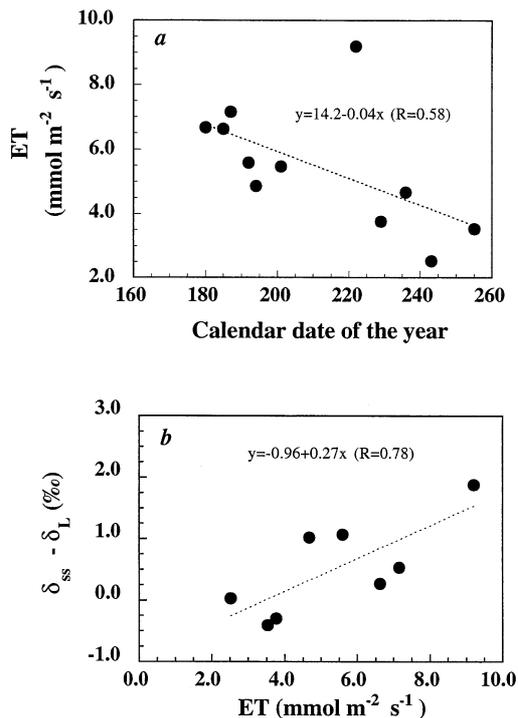
$$F_1 = F_N \phi_1; F_2 = F_N \phi_2 \quad (16)$$

where  $\phi_1 = (\delta_N - \delta_2) / (\delta_1 - \delta_2)$  and  $\phi_2 = (\delta_1 - \delta_N) / (\delta_1 - \delta_2)$ . Thus, knowledge of the net flux,  $F_N$ , and the isotopic signatures  $\delta_N$ ,  $\delta_1$ , and  $\delta_2$  allows us to derive an estimate of the component gross fluxes.  $F_N$  and  $\delta_N$  may be directly estimated by micrometeorological techniques; values of  $\delta_N$

can also be estimated from Keeling-type plots. But the specific isotopic signatures,  $\delta_1$  and  $\delta_2$ , must be independently estimated from plant and soil samples of organic material or water, or from leaf-scale gas exchange measurements. Sampling and estimates of  $\delta_1$  and  $\delta_2$  must also consider heterogeneity in the ecosystem and be representative of the relevant scale (determined by the type of micrometeorological measurement).

There are now several methods for estimating canopy-scale net fluxes ( $F_N$ ) and continuous progress in stable isotope research provides optimism both in resolving  $\delta_N$  in canopy-scale measurements and in estimating the isotopic identity of ecosystem components ( $\delta_1$ ,  $\delta_2$ ). Partitioning of net fluxes by adding the isotopic analyses should, therefore, be limited mostly by technical and instrumental limitations. Mass spectrometric analysis has also progressed over the last decade with smaller and more robust instruments and a variety of associated automation. The best precision in isotopic measurements of  $\text{CO}_2$  is currently about  $\pm 0.03\%$  for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  (J.W.C. White, personal communication; cf. Troiler et al. 1996) and at least 0.1% for  $^{18}\text{O}$  in water (e.g., Yakir and Wang 1996). Observed gradients in isotopic composition in canopy boundary layers are of the order of 0.3‰/m for  $^{13}\text{C}$  and  $^{18}\text{O}$  in  $\text{CO}_2$ , or typically 0.02–0.1‰/ppm  $\text{CO}_2$ , for both  $^{18}\text{O}$  and  $^{13}\text{C}$ , and of the order of 0.6‰/m for  $^{18}\text{O}$  in water (e.g., Lloyd et al. 1996; Yakir and Wang 1996; Moreira et al. 1997; Nakazawa et al. 1997; Buchmann and Ehleringer 1998; Harwood et al. 1998). The larger isotopic gradients are observed, however, only above vegetation with high photosynthetic rates and under optimal conditions. Furthermore, it is difficult to sample  $\text{CO}_2$  and water vapor rapidly without fractionation; therefore, measurements of isotopic signals in conjunction with dynamic flux measurements are at the current detection limits.

While the techniques discussed above are currently very demanding, a simple alternative to evaluate canopy-scale ET in arid conditions based on leaf sampling and standard meteorological monitoring has been proposed by Wang and Yakir (in press). This technique is based on the discrepancy (discussed above) between the observed and predicted isotopic enrichments of leaf water (Eq. 2). Notably, Eqs. 2 and 3 show that the extent of the difference between modeled ( $\delta_{ss}$ ) and observed ( $\delta_{LW}$ ) isotopic compositions of leaf water ( $\delta_{ss} - \delta_{LW}$ ) is related to the rate of leaf transpiration (cf. Flanagan et al. 1991). Seasonal variation in  $\delta_{ss} - \delta_{LW}$ , obtained from leaf and stem samples (for  $\delta_{LW}$ ), and from a local meteorological station (for  $\delta_{ss}$ , Eq. 2), was observed in a desert agroforest system of *Acacia seligna* in the central Negev region, Israel. Periodic measurements of ET fluxes from the same acacia plots were carried out by the Bowen ratio method (W. Zhao and P.R. Berliner, unpublished data). A pronounced decrease was observed in the ET flux during the season (Fig. 5). Note that in this desert environment, the contribution of soil evaporation to total ET flux is negligible except for shortly after the rainy season. The linear correlation observed between ET and  $\delta_{ss} - \delta_{LW}$



**Fig. 5** Seasonal changes in the rate of evapotranspiration (ET) measured by micrometeorological methods (Bowen ratio) in a desert agroforest system in the Negev desert (**a**), and relationships between the seasonal trend in ET and in the isotopic difference ( $\delta_{ss} - \delta_L$ ) between the predicted (based on local meteorological conditions) and measured mean  $\delta^{18}\text{O}$  values of leaf water (**b**). While both isotopic values are influenced by meteorological conditions (primarily relative humidity), the difference is expected to be inversely proportional to the rate of transpiration through the leaves

(Fig. 5) demonstrate the usefulness of the isotopic composition of leaf water as an indicator of seasonal trends in ET. Furthermore, the simplified relationships between ET and  $\delta_{ss} - \delta_{LW}$  in arid locations (i.e., where soil evaporation is negligible) shows the promise of this approach for estimating canopy-scale leaf transpiration in non-arid environments (i.e., where soil evaporation is significant). When combined with micrometeorological flux measurements of ET, such isotope approaches may allow estimation of the transpiration flux and  $F_T$  (%).

Micrometeorological measurements of trace gas exchange are commonly made using the flux-gradient or eddy correlation techniques. The flux-gradient technique combines an eddy diffusivity and concentration gradient with height to calculate the flux (Baldochi et al. 1988). The main advantages of the gradient approach are that large gradients can be observed over several meters between sampling heights, and it does not require fast-responding instruments. Measurements inside plant canopies, however, are not possible because of possible counter-gradient transport (Baldochi et al. 1988). This flux-gradient technique can be readily adapted to include isotopic measurements. Air samples, or air moisture samples, are collected at the same heights, over the same time intervals and shipped to a stable isotope laboratory.

The concentration and isotopic data can then be used to produce a Keeling plot, which may give an estimate of the isotopic identity of the net flux,  $\delta_N$  (cf.  $\delta_s$  in Eq. 9). Estimation of  $\delta_N$  in this method, as pointed out previously, may be subject to errors caused by the heterogeneity of the vegetation and the fact that small errors in measurements can lead to large errors in the intercept ( $\delta_N$ ). Sampling of soil, stem and leaf water, and soil organics representative of the underlying vegetation can provide the necessary information to estimate the relevant isotopic signatures of the soil and leaf exchange flux ( $\delta_1$  and  $\delta_2$  for either water or  $\text{CO}_2$ ). More directly, some of these signatures can be obtained from leaf-scale gas exchange and/or isotopic measurements carried out concomitantly in the field (e.g., Harwood et al. 1998; Wang et al. 1998), or by Keeling plots for data obtained within the canopies to determine isotopic identities of soil fluxes. Yakir and Wang (1996) have successfully used the gradient approach to partition net ecosystem exchange of  $\text{CO}_2$  into photosynthetic assimilation and respiration for several crop species. Such studies provide confidence in the feasibility of the flux-isotope approach, which is clearly supported also by other canopy-scale isotopic studies (Flanagan et al. 1997; Moreira et al. 1997; Buchmann and Ehleringer 1998).

In using the gradient/isotope approach to partition net fluxes, at least two approximations should be noted. First, we assume that the two flux components originate at the same place and ignore small (relative to the precision of measurement) differences in the concentration/isotopic profiles when they differentially originate from the soil and canopy levels. Second, a sample of the background atmosphere is usually taken upwind of the sampling site, or above the canopy boundary layer, and is assumed to be constant (within the measurement precision) across the concentration/isotope gradient profile measured.

In the above discussion, no distinction is made between the use of  $^{18}\text{O}$  or  $^{13}\text{C}$  in  $\text{CO}_2$  as the same principles apply in both cases. The preferred choice can vary among locations and with the advancement of methodology. Important prerequisites for the application of the isotopic approach are the existence of the differences between  $\delta_1$  and  $\delta_2$  and between  $\delta_N$  and  $\delta_a$ . Differences between  $\delta_1$  and  $\delta_2$  in  $^{13}\text{C}$  may be small in established ecosystems where the  $\delta^{13}\text{C}$  values of decomposing and newly fixed organic matter are very similar. But diurnal (Harwood et al. 1998) and seasonal (Flanagan et al. 1996; 1997; Buchmann et al. 1997a, 1997b) variations in photosynthetic discrimination, as well as possible disequilibrium due to slow turnover of soil carbon (Enting et al. 1995; Fung et al. 1997) are likely to help in this respect. In contrast, considerable differences in  $^{18}\text{O}$  between  $\delta_1$  and  $\delta_2$  can be generally expected because of the large  $^{18}\text{O}$  enrichment in leaf water. The larger signal in  $^{18}\text{O}$  is clearly observed in the  $^{18}\text{O}/\text{CO}_2$  gradients in tropospheric samples, which are about twice as large as for  $^{13}\text{C}/\text{CO}_2$  (Nakazawa et al. 1997). Similar differences are also observed in the sea-

sonal amplitude in  $^{18}\text{O}$  or  $^{13}\text{C}$  of atmospheric  $\text{CO}_2$  (Troiler et al. 1996). Such differences may provide a significant advantage for  $^{18}\text{O}$  over  $^{13}\text{C}$  as tracer for partitioning leaf and soil  $\text{CO}_2$  exchange. This advantage may be restricted, however, in locations where geographical variations in source water impose similar  $\delta_{\text{N}}$  and  $\delta_{\text{a}}$  values, in locations where very high relative humidity (e.g., tropics) reduces leaf water enrichments, or by the larger scatter in  $^{18}\text{O}$  data (e.g., Sternberg et al. 1998; Bowling et al. 1999b).

The flux-profile relationships utilized above crop fields and grasslands (cf. Cellier and Brunet 1992) may not be valid, and are therefore not generally accepted, above forests. In forests, the eddy correlation method offers a more direct approach to flux measurements (Baldocchi et al. 1988, 1996; Wofsy et al. 1993). In this case, a fast-responding sonic anemometer is used to indicate the velocity and direction of the vertical air movement component (up or down eddies). A fast-responding analyzer records the concentration of the trace gas of interest in conjunction with anemometer measurements. Once data are collected over an appropriate time interval (e.g., 30 min), the concentration measurements can be separated to those related to eddies moving up from the canopy or down into the canopy and the net eddy flux calculated.

Fast-responding isotope analyzers with the required precision are not available at present and the eddy correlation technique cannot be directly combined with in situ stable isotope measurements to estimate  $\delta_{\text{N}}$  (Eqs. 13–16). Alternatively, independent methods, such as the Keeling plot, with certain caveats, can be used to estimate  $\delta_{\text{N}}$ . This requires that the data used to construct the Keeling plot represent the same time interval and footprint as the eddy correlation system. Isotopic data for Keeling plot estimates can be obtained by slow flask sampling and laboratory analysis. Bowling et al. (1999b) have recently argued that in the range and precision of data obtained for Keeling plots, the relationships between  $\text{CO}_2$  concentration and  $\delta^{18}\text{O}$  or  $\delta^{13}\text{C}$  are practically linear such that the equation  $\delta = mc + b$  (where  $\delta$  and  $c$  are the measured isotopic and concentration values) can be used to predict the isotopic composition based on the component concentration. If the data are representative, such a relationship can be used, with conventional eddy correlation equations (Baldocchi et al. 1988, 1996; Wofsy et al. 1993), to produce the isotopic flux of  $^{13}\text{CO}_2$ ,  $F_{13}$ , or similarly for  $^{18}\text{O}$ ,  $F_{18}$ :

$$F_{13} \text{ (or } F_{18}) = \overline{\rho \omega [c(mc + b)]'} \quad (17)$$

where  $\rho$  is the density of dry air,  $\omega$  is the vertical wind velocity component (positive and negative values correspond to up/down) and  $c$  is the mixing ratio or mole fraction of the chemical constituent with respect to dry air. An overbar indicates a time-averaged quantity and a prime indicates deviation from the mean.  $c$  is measured by the eddy correlation system and the constants  $m$  and  $b$  are derived from a plot of  $\delta$  vs  $c$  based on concurrent

flask sampling. Assuming the  $^{13}\text{C}$  signature of the biological system,  $\delta_{\text{N}}$ , is a constant at the measurement scale,  $F_{13}$  and  $F_{18}$  correspond also to  $F_{\text{N}}\delta_{\text{N}}$ .

To date, difficulties still exist in precise estimates of the components of Eq. 17. Recent studies have addressed the differences between the Keeling relationship as determined from slow, whole-air flask sampling, and that of eddy covariance sampling (Bowling et al. 1999a). Additionally, isotopic analyses are incorporated into studies using conditional sampling techniques (relaxed eddy accumulation, REA, where fast-responding analytical instruments are replaced with a fast-responding valve system and an accumulator that collect separate air samples from “updraft” and “down-draft” eddies over a time interval; Businger and Oncley 1990; Bowling et al. 1998, 1999a). This approach should allow independent validation of Eq. 17 by directly measuring  $F_{13}$  and  $F_{18}$ , but has not yet yielded clear results (D.Y. Hollinger, personal communication; D.R. Bowling, personal communication). Once further studies, currently underway, provide validation of Eq. 17 or overcome difficulties in REA techniques, then considerable promise exists for coupling stable isotope analysis to eddy covariance techniques, and estimating the independent contributions of gross photosynthetic and respiratory fluxes to net ecosystem  $\text{CO}_2$  exchange.

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## Global implications

The isotopic signal of  $\text{CO}_2$  exchanged between ecosystems and the atmosphere has important implications for global-scale studies of the carbon cycle and the atmospheric budget of  $\text{CO}_2$ . Incorporation of  $^{13}\text{C}$  analysis of  $\text{CO}_2$  enabled the distinction between  $\text{CO}_2$  exchange with the ocean or with the land biosphere (e.g., Tans et al. 1993; Ciais et al. 1995; Francey et al. 1995). It is now anticipated that  $^{18}\text{O}$  analyses of  $\text{CO}_2$  will enable the partitioning of global-scale net  $\text{CO}_2$  exchange fluxes on land into its photosynthetic (gross primary productivity, GPP) and respiration (ecosystem respiration,  $R$ ) components. The potential in this approach was first noted by Francey and Tans (1987) followed by more recent studies (Farquhar et al. 1993; Ciais et al. 1997; Peylin et al. 1999). These studies have shown, first, that the  $^{18}\text{O}$  signature of biospheric activities is clearly observed in the global atmosphere (as latitudinal gradients and seasonal variations) and, second, that for a first approximation, the global-scale  $^{18}\text{O}$  mass balance of atmospheric  $\text{CO}_2$  is consistent with ecosystem processes. These studies also clearly highlighted the critical need to reduce uncertainties associated with the isotopic signatures of leaves and soil. The burden in reducing these uncertainties lies mostly with process-based studies at the laboratory and ecosystems, such as those discussed in this review.

In global-scale studies, information on whole-ecosystem discrimination is integrated for the entire globe and on an

annual scale, as in a CO<sub>2</sub> δ<sup>18</sup>O budget (Farquhar et al. 1993; Ciais et al. 1977; Miller et al. 1999):

$$\frac{d\delta_a}{dt} = \frac{1}{C_a} [F_{oa}(\delta_o - \delta_a) + \varepsilon_w(F_{oa} - F_{ao}) + F_{ff}(\delta_{ff} - \delta_a) + F_{bb}(\delta_{bb} - \delta_a) + F_A(\delta_l - \delta_a + \varepsilon_l) + F_{sa}(\delta_{eq} - \delta_a + \varepsilon_{eff}) + F_l(\delta_{eq} - \delta_a)] \quad (18)$$

where

$$F_A = \frac{C_l}{C_a - C_l} A, \quad \text{and} \quad A = F_{la} - F_{al} \quad (19)$$

*c* refers to concentrations, *F* to fluxes,  $\delta$  to isotopic ratios, and *A* to net photosynthetic assimilation (equivalent to GPP). Subscripts represent the reservoirs from which CO<sub>2</sub> leaves or enters: *a* for atmospheres, *o* for oceans, *ff* for fossil fuels, *bb* for biomass burning, *l* for leaves, *s* for soil; *eq* refers to soil CO<sub>2</sub> in equilibrium with soil water, and *I* refers to invasion of atmospheric CO<sub>2</sub> into soils (Miller et al. 1999).  $\varepsilon$  refers to the kinetic fractionation associated with fluxes between reservoirs.  $\delta_a$  and *C<sub>a</sub>* are known accurately by measurement (e.g., Troiler et al. 1996). The fossil fuel flux is well known and its isotopic composition is assumed to be that of atmospheric O<sub>2</sub>. Although the oceanic exchange flux (*F<sub>oa</sub>*) is large, the isotopic disequilibrium between the ocean and atmosphere is relatively small, so its influence on the atmospheric isotopic value is also small. The biomass burning flux is relatively small and its isotopic signature is also assumed to be that of atmospheric O<sub>2</sub>. It is the soil and leaf components that dominate the uncertainty in Eq. 18. Soil and leaf isotopic fluxes each contribute roughly five times more to the atmospheric signal of δ<sup>18</sup>O than either the oceanic or fossil fuel components, which are the next most significant terms. Combining estimates of net ecosystem productivity (from <sup>13</sup>C or O<sub>2</sub>) with accurate measurements and modeling of the <sup>18</sup>O signals of soils and leaves should allow the use of Eq. 18 to better estimate *F<sub>sa</sub>* and *F<sub>A</sub>* and consequently GPP and *R*.

A similar isotopic mass balance is also used with <sup>13</sup>C (e.g., Fung et al. 1997). As indicated above, such approaches allow for the distinction between oceanic and terrestrial CO<sub>2</sub> fluxes. But it also critically depends on quantitative estimates of photosynthetic discrimination which must come from ecosystem-scale studies such as those discussed above. Note that as the scale of observation increases and the systems become more heterogeneous, they become increasingly difficult to characterize isotopically. Lloyd and Farquhar (1994) derived biome-scale <sup>13</sup>C discrimination by combining global vegetation maps, and modeling leaf-scale physiological and Δ (<sup>13</sup>C discrimination) responses to climatic conditions across the globe. Buchmann and Ehleringer (1998) suggested that whole-ecosystem discrimination (Δ<sub>e</sub>) should be derived from comparing tropospheric CO<sub>2</sub> concentration and isotopic composition, and the <sup>13</sup>C signature of total ecosystem respiration (Eq. 10). As discussed in detail above, the latter could be obtained from Keeling-type plots based on air sampling above or within the plant

canopies over the diel cycle (Buchmann and Ehleringer 1998), or from aircraft measurements (e.g., Nakazawa et al. 1997). Lloyd et al. (1996) estimated whole-ecosystem discrimination by relating the isotopic composition of CO<sub>2</sub> in the CBL to the isotopic composition of tropospheric CO<sub>2</sub>.

As noted above, ecosystem and regional-scale studies are also important for estimating the contributions of C3 and C4 vegetation to global-scale GPP (Lloyd and Farquhar 1994; Fung et al. 1997; cf. Ehleringer et al. 1997; Bakwin et al. 1998). The use of <sup>13</sup>C to estimate C3/C4 contribution to GPP is quite obvious, based on the large differences in discrimination between the two photosynthetic modes (but note the complications due to disequilibrium effects discussed above). That there should be large C3/C4 differences also in <sup>18</sup>O discrimination is becoming evident. This is first due to the lower internal CO<sub>2</sub> concentration (*C<sub>i</sub>*) in C4 leaves and the expected effect of *C<sub>i</sub>* on <sup>18</sup>O discrimination (Farquhar and Lloyd 1993), but also to the low CA activity in these plants (J.S. Gillon and D. Yakir, unpublished data). In the future, regional- and global-scale measurements of both <sup>13</sup>C and <sup>18</sup>O in atmospheric CO<sub>2</sub> should provide additional constraints on estimates of C3/C4 contributions to global productivity on land, and variations across seasonal and longer time scales.

## Conclusions

We have shown that the application of stable isotope analysis provides great potential to the investigation of individual fluxes responsible for the net exchange of CO<sub>2</sub> and water in an ecosystem. Additional research is required in several areas. There is still no way of determining the isotopic identity of CO<sub>2</sub> and water vapor sources other than by the extrapolation using Keeling-type plots, which are subject to errors. Furthermore, we need to better understand the source of respired CO<sub>2</sub> among root respiration, organic matter decay, and aboveground respiration in natural systems. The <sup>18</sup>O labeling of respiratory CO<sub>2</sub> is not yet well understood and can involve equilibration with water in different depths, enhancement of the equilibrium by CA or other soil components, substantial effects of invasion of atmospheric CO<sub>2</sub> into the soil and equilibration of respired CO<sub>2</sub> with aboveground moisture. <sup>18</sup>O discrimination by leaves, and differences in discrimination between C3 and C4 plants need to be better characterized. Keeling-type plots should be further developed to account for problems such as condensation and recycling. Recycling of CO<sub>2</sub> in ecosystems is an important component of its CO<sub>2</sub> budget and current models need to be tested further. Merging of isotope ratio analysis with dynamic flux measurements has only recently been made, and clearly indicates the need for improved rapid sampling techniques. This research agenda is feasible and worthwhile in light of the potential benefits and the very few, if any, alternative research tools for investigating ecosystem gas exchange.

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