Stable Isotopes and Carbon Cycle Processes in Forests and Grasslands

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Abstract: Scaling and partitioning are frequently two difficult challenges facing ecology today. With regard to ecosystem carbon balance studies, ecologists and atmospheric scientists are often interested in asking how fluxes of carbon dioxide scale across the landscape, region and continent. Yet at the same time, physiological ecologists and ecosystem ecologists are interested in dissecting the net ecosystem CO_2 exchange between the biosphere and the atmosphere to achieve a better understanding of the balance between photosynthesis and respiration within a forest. In both of these multiple-scale ecological questions, stable isotope analyses of carbon dioxide can play a central role in influencing our understanding of the extent to which terrestrial ecosystems are carbon sinks. In this synthesis, we review the theory and present field evidence to address isotopic scaling of CO_2 fluxes. We first show that the ^{13}C isotopic signal which ecosystems impart to the atmosphere does not remain constant over time at either temporal or spatial scales. The relative balances of different biological activities and plant responses to stress result in dynamic changes in the ^{13}C isotopic exchange between the biosphere and atmosphere, with both seasonal and stand-age factors playing major roles influencing the ^{13}C biosphere-atmosphere exchange. We then examine how stable isotopes are used to partition net ecosystem exchange fluxes in order to calculate shifts in the balance of photosynthesis and respiration. Lastly, we explore how fundamental differences in the ^{18}O isotopic gas exchange of forest and grassland ecosystems can be used to further partition terrestrial fluxes.

Key words: Carbon isotope, oxygen isotope, stable isotope, carbon cycle.

Introduction

Stable isotopes can be used as tracers of regional and global carbon fluxes. The concentration and isotopic composition (δ^{13}C, δ^{18}O) of atmospheric CO_2 are key variables used in the analysis of the global carbon cycle by inversion of atmospheric transport models (Ciais et al., 1995[9]; Enting et al., 1995[15]; Fung et al., 1997 [26]; Keeling et al., 1995[36], 1996[35]; Lloyd et al., 1996[39]; Tans et al., 1990[50], 1993[49], 1996[48]). Carbon isotopes are used in inverse calculations to distinguish oceanic from terrestrial exchange processes and in evaluating the magnitude of the terrestrial carbon sink (Ciais et al., 1999[7]; Enting et al., 1995[15]; Fung et al., 1997[26]; Keeling et al., 1995[36], 1996[35]; Tans et al., 1993[49]). These tracers have tremendous potential to improve our understanding of the terrestrial carbon cycle. However, there are limited data available regarding the isotope effects on CO_2 exchange between terrestrial ecosystems and the atmosphere and how these isotope effects might vary on seasonal and interannual bases. Most ^{13}C data available are for leaves of individual species and not canopy-scale CO_2-exchange measures (Lloyd and Farquhar, 1994[38]).

Establishing the ^{13}C variation in ecosystem-level gas exchange processes is critical, since assumptions about a terrestrial biosphere with a fixed versus a dynamic ^{13}C signal will necessarily influence the magnitude of any regional terrestrial carbon sink (Fung et al., 1997[26]). The interannual partitioning of anthropogenic carbon dioxide uptake between ocean and land ecosystems is still a matter of debate (Bousquet et al., 2000[3]). We must improve this knowledge because carbon dioxide taken up by land and ocean can have very different fates and consequences for future atmospheric increase. Calculations of ocean-land partitioning for the net uptake of anthropogenic CO_2 emissions are based on information from annual changes in atmospheric CO_2 concentration and the δ^{13}C composition of atmospheric CO_2. A major assumption in most global partitioning exercises is that isotopic discrimination during land photosynthesis remains constant on an annual basis.

This assumption allows the global mass balance equations to be simplified by using net land and net ocean fluxes, rather than the gross, one-way photosynthesis and respiration fluxes, introducing some uncertainties in the global partitioning exercise.

Photosynthetic discrimination can change substantially with seasonal and annual changes in weather – environmental changes that also influence gross primary productivity (GPP) in land ecosystems. Even in tropical rainforests of the Amazon Basin, interannual changes in terrestrial GPP and net ecosystem exchange are predicted to occur in response to El Niño/La Niña events that dramatically alter precipitation patterns in the Amazon Basin (Tian et al., 1998[51]). It has been predicted that interannual changes in GPP and photosynthetic discrimi-

nation could alter atmospheric $^{13}C/^{12}C$ composition with significant effects on the ocean-land partitioning (Randerson et al., 2000[44]). Additional uncertainties in the ocean-land partitioning arise because of limited information on the magnitude and isotopic composition of the deforestation flux at some latitudes. In particular the isotopic composition of the flux associated with deforestation can change substantially in tropical regions following the conversion of C_3 forest vegetation to C_4 pasture ecosystems. Currently, the isotopic composition of the biosphere-to-atmosphere flux is assumed to remain constant with no interannual variability. Below we show the results of recent measurements made in the temperate evergreen forests of North America and tropical forests of South America that illustrate significant seasonal and interannual variation in photosynthetic discrimination. In addition, we have documented important temporal variation in the isotopic composition of respired CO_2 in pasture ecosystems, ^{13}C variation associated with woody vegetation encroachment and ^{13}C variation associated with the timing of pasture management practices (burning).

We know that carbon isotope discrimination by C_3 and C_4 plants varies with physiological and meteorological factors (Ehleringer et al., 1993[14]; Farquhar et al., 1989[17]), leading to both spatial and temporal changes on a regional basis.

Furthermore, C_4 species account for a significant fraction of terrestrial productivity, some 16–30% of total terrestrial photosynthesis (Collatz et al., 1998[10]; Fung et al., 1997[26]; Lloyd and Farquhar, 1994[38]). Many natural grassland ecosystems contain a mixture of C_3 and C_4 species, and the proportion can vary seasonally, as well as interannually, with climatic fluctuations. The reliability of atmospheric inversions to estimate locations and magnitudes of terrestrial carbon sinks would be improved if terrestrial $^{13}CO_2$ exchange data reflecting these spatial and temporal dynamics were available for inclusion.

Interpreting the ^{13}C Signal of Terrestrial Photosynthesis at Leaf Versus Ecosystem Scales

Photosynthesis discriminates against $^{13}CO_2$, resulting in organic matter ($\delta^{13}C_p$) that is ^{13}C depleted relative to the atmosphere. For C_3 plants, the overall relation was originally described as

$$\delta^{13}C_p = \delta^{13}C_a - a - (b - a) \cdot c_i/c_a \tag{1}$$

where $\delta^{13}C_a$ is the carbon isotope ratio of atmospheric CO_2 surrounding a leaf and c_a and c_i are the atmospheric and intercellular CO_2 concentrations, respectively, a = 4.4‰ (diffusional fractionation against $^{13}CO_2$), and b = 27‰ (net fractionation during carboxylation) (Farquhar et al., 1989[17]; Farquhar et al., 1982[19]; Lloyd and Farquhar, 1994[38]). For C_4 plants, c_i is concentrated by a biochemical mechanism so that the effective value of b is substantially lower in value. In C_3 plants, the c_i/c_a ratio is subject to change (0.5–0.8), mostly as a function of changes in the stomatal conductance relative to CO_2 assimilation capacity. Farquhar and Richards (1984[20]) modified this theory to eliminate the effects of changing $\delta^{13}C_a$ values in the isotopic expression by describing a photosynthetic carbon isotope discrimination (Δ), where

$$\Delta = (\delta^{13}C_a - \delta^{13}C_p)/(1 + \delta^{13}C_p) \tag{2}$$
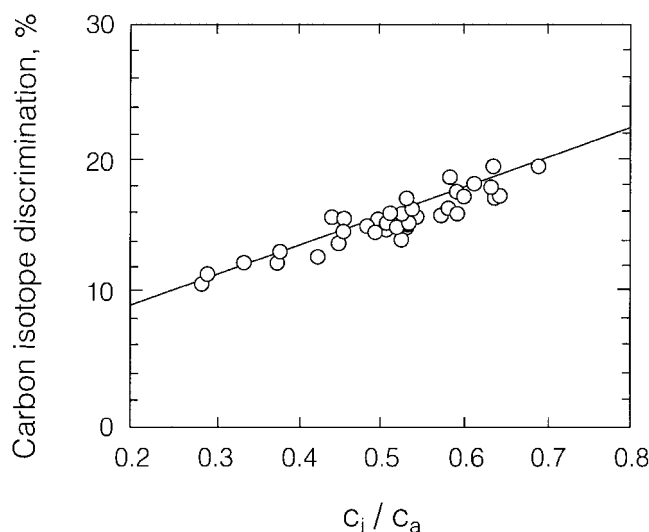

Fig. 1 A plot of the relationship between photosynthetic carbon isotope discrimination (Δ) and the ratio of intercellular to ambient CO_2 concentrations. Adapted from Ehleringer et al. (1993[14]).

In most cases, there is strong agreement between the theoretically expected photosynthetic discrimination and actual physiological measurements (Fig. **1**). Increases in the c_i/c_a ratio result in linear increases in carbon isotope discrimination against ^{13}C as predicted by Equation 1. One consequence of this $^{13}CO_2$ discrimination is that the atmosphere surrounding leaves is isotopically enriched or depleted depending on whether the leaf is photosynthesizing or respiring. Net photosynthetic processes result in an overall increase in the $\delta^{13}C_a$ value of the bulk atmospheric CO_2, whereas respiratory processes result in a decrease in $\delta^{13}C_a$.

The impact of terrestrial photosynthesis and the eventual release of CO_2 during respiration on atmospheric $\delta^{13}C$ and $[CO_2]$ can be viewed at both the leaf and ecosystem scales. Measurements of $[CO_2]$ and isotope ratios are important observations in efforts to bridge the gap between global inversions and ecosystemscale CO_2 exchange studies. Keeling (1958[34]) determined that the carbon isotope ratio of CO_2 respired from ecosystems ($\delta^{13}C_R$) could be measured based on changes in the concentration ($[CO_2]_{a-i}$) and isotope ratio of atmospheric CO_2 ($\delta^{13}C_{a-i}$) within the area of interest. Keeling (1958[34]) showed that by plotting the mixing relationship between $\delta^{13}C_{a-i}$ and $1/[CO_2]_{a-i}$ of atmospheric CO_2, a linear relationship was obtained and the intercept of this relationship was the isotope ratio of the respired CO_2 input into the canopy air space.

$$\delta^{13}C_{a-i} = \frac{[CO_2]_{a-o}}{[CO_2]_{a-i}} \times (\delta^{13}C_{a-o} - \delta^{13}C_R) + \delta^{13}C_R \tag{3}$$

where the subscripts $a–i$ and $a–o$ represent the atmosphere inside a canopy and the atmosphere above (outside) the forest, respectively. It can be seen from Equation 3 that a plot of $1/[CO_2]_{a-i}$ versus $\delta^{13}C_{a-i}$ gives a straight line relationship with an intercept, $\delta^{13}C_R$. At the ecosystem (forest canopy) scale, $\delta^{13}C_R$ represents a spatially integrated measure of the $\delta^{13}C$ of CO_2 respired from both vegetation and soil components. In a
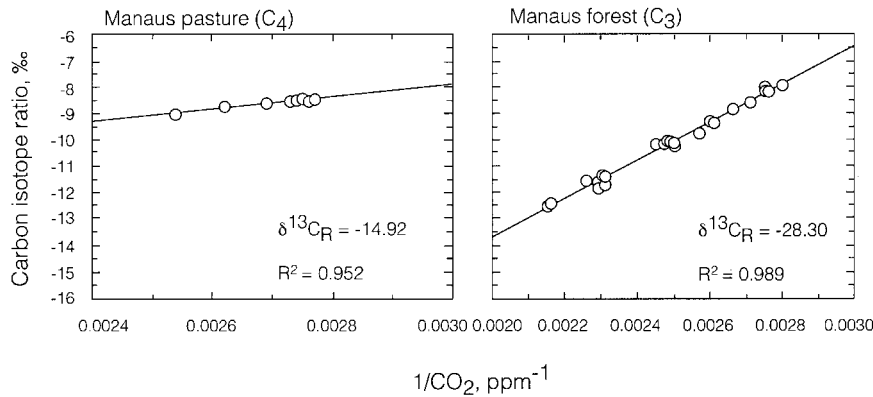
Fig. 2 Keeling plot diagrams of the relationship between the inverse of atmospheric CO_2 concentration within a canopy and the carbon isotope ratio of the CO_2. Figure illustrates the variations in Keeling-plot relationships observed for primary rainforest (C_3) and converted pasture (C_4) ecosystems near Santarem, Brazil, in central Amazonia. J. P. H. B. Ometto et al. (unpublished).

comparison of air sampled at both C_3 rainforest and C_4 pasture sites in Amazonia, we see the huge impact that vegetation has on the isotopic composition of atmospheric CO_2 and the large differences in these effects between C_3 (forest) and C_4 (pasture) ecosystems (Fig. **2**).

$\delta^{13}C_p$ and $\delta^{13}C_R$ Values Vary in Both Space and Time

It is well-known that average $\delta^{13}C_p$ values of plants from different ecosystems vary across geographic scales (Collatz et al., 1998[10]; Ehleringer et al., 1993[14]; Farquhar et al., 1989[17]; Lloyd and Farquhar, 1994[38]), reflecting in some cases changes in c_i/c_a values of C_3 plants and in other cases shifts in the relative abundances of C_3/C_4 taxa. While it is convenient for modeling purposes to assume a constant $\delta^{13}C_p$ value, the evidence is quite strong that for plants producing multiple leaf cohorts during the year, that variations in $\delta^{13}C_p$ values are associated with changes in site water balance (Ehleringer and Cooper, 1988[12]; Ehleringer et al., 1993[14]; Farquhar et al., 1989[17]; Guy et al., 1980[31]). Even in plants typically producing only a single annual flush of photosynthetic tissues, there can be a muted seasonal shift in $\delta^{13}C_p$ values. For example, when Douglas fir needles from throughout a canopy profile are sampled at both the beginning and end of the growing season (Fig. **3**), we see that there is a large spatial variation associated with canopy depth and a small but significant increase in the $\delta^{13}C_p$ value, reflecting the progressive reduction in c_i/c_a, values associated with the prolonged summer droughts of the Pacific Northwest (Fessenden and Ehleringer, 2002 a[21]).
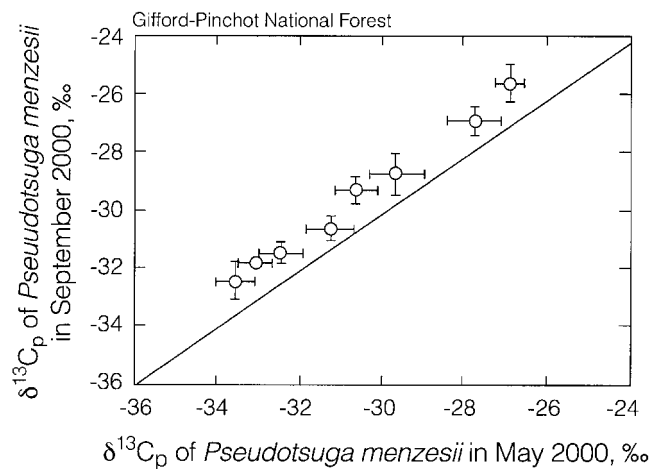

Fig. 3 A plot of the correlation between needle carbon isotope ratios sampled in May and again in September from dominant trees growing under natural condition in southern Washington, USA. Adapted from Fessenden and Ehleringer (2002 a[21]).

The $\delta^{13}C_R$ values within an ecosystem also change, possibly reflecting the consequences of changes in environmental conditions on photosynthetic processes. What is perhaps unexpected is that the amplitude of the annual fluctuations in $\delta^{13}C_R$ exceed those of $\delta^{13}C_p$ values. Consider recent observations we have collected at a Douglas fir ecosystem in southern Washington during dry and wet years (Fig. **4**). The $\delta^{13}C_R$ values
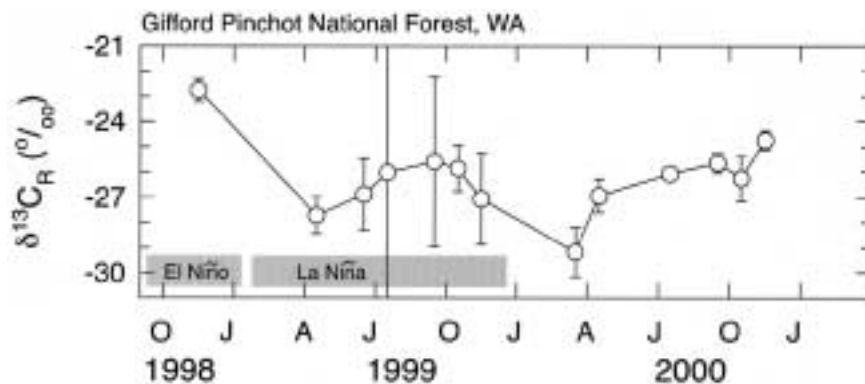

Fig. 4 A time sequence of the carbon isotope ratio of ecosystem respiration from an old-growth coniferous forest at the T. T. Munger Research Natural Area in southern Washington, USA. Adapted from Fessenden and Ehleringer (2002 b[22]).

responded to seasonal and interannual differences in climate, changing by more than 5‰ correlated with shifts between El Niño (dry) and La Niña (wet) events (Fessenden and Ehleringer, 2002 b[22]). We believe that a significant fraction of the seasonal changes in $\delta^{13}C_R$ reflect shifts in c_i/c_a values by photosynthetic tissues in response to changes in soil moisture availability and/or hydraulic conductivity. Undoubtedly though, some fraction of the $\delta^{13}C_R$ signal must indicate a component of older soil carbon being respired by the ecosystem. This respired carbon would be expected to have a more or less constant value, reflecting a long-term average ^{13}C content of the soil organic matter. While there has been tremendous progress in understanding processes at the leaf-level (summarized in Farquhar et al., 1989[17] and Ehleringer et al., 1993[14]), much of the current work underway is addressing the scaling potential of leaf-level observations to larger ecosystem and regional scales (Ciais and Meijer, 1998[8]; Gillon and Yakir, 2001[29]).

If the nature of the turnover of carbon associated with $\delta^{13}C_R$ and $\delta^{13}C_p$ components are not similar, then we would expect to see that short- and long-term estimates of c_i/c_a values based on these two isotopic measures yield different c_i/c_a values. In fact, this is indeed observed. Winner et al. (2001[53]) have shown that c_i/c_a values estimated by photosynthetic gas exchange and from $\delta^{13}C_p$ observations differ when measured at the end of the growing season (Fig. **5**). The magnitude of the deviations from between c_i/c_a values predicted from short- and long-term measures is a function of plant height, being greatest in the highest portions of the canopy. This is not surprising, since photosynthetic tissues in the upper reaches of the canopy are the ones most likely to be exposed to water stress and the highest vapor pressure deficits. What is perhaps surprising is the consistency of the response across species. All three tree species within this evergreen forest responded in an identical manner, suggesting that c_i/c_a relationships may be similar and scalable across species in the forest.

Partitioning of NEE into Photosynthesis and Respiration Components

The balance of photosynthesis and respiration in many ecosystems may be altered by climate change, and the terrestrial sink could eventually slow or cease altogether. The processes that contribute to biosphere-atmosphere CO_2 exchange must be understood if we are to make informed predictions about the future of the global carbon cycle. At the organismal level, photosynthesis and respiration can easily be studied independently, but this is more difficult at larger scales. The net ecosystem exchange of CO_2 (NEE) is now monitored in a wide variety of global biomes and locations (Baldocchi et al., 1996[2]; Goulden et al., 1996[30]; Wofsy et al., 1993[54]; Falge et al., 2001[16]). NEE is the net result of photosynthetic uptake of CO_2 and respiratory release:

$$NEE = F_A + F_R \qquad (4)$$

where F_A and F_R represent the total assimilatory (photosynthetic) and respiratory fluxes, respectively, and they differ in sign. NEE is analogous to the net assimilation of CO_2 by a leaf (gross photosynthetic uptake -leaf respiration), but in this case the respiratory flux includes respiration by all ecosystem components, both autotrophic and heterotrophic. NEE is the composite of small differences in the two major fluxes. It is fairly
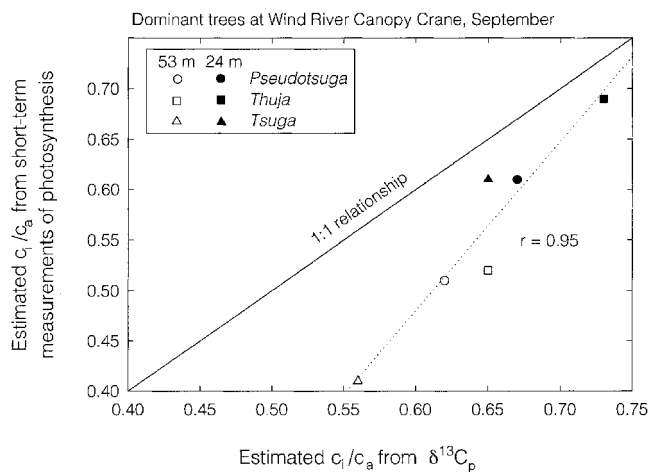

Fig. 5 The correlation between the observed and predicted c_i/c_a ratios of needles from *Pseudotsuga menziesii*, *Tsuga heterophylla* and *Thuja plicata*. The short-term c_i/c_a ratio was determined from *in situ* photosynthesis gas-exchange measurements, while the long-term estimate was based on $\delta^{13}C_p$ measurements. Adapted from Winner et al. (2001[53]).

straightforward to measure, but does not provide information about F_A and F_R individually. Ecologically, it is important to separate the relative influences of changes in photosynthesis versus changes in respiration on NEE. These important processes are subject to different environmental constraints and show differences based on land-use history. A recent study by Valentini et al. (2000[52]) suggests that changes in ecosystem respiration, and not photosynthesis, are the main determinant of the carbon balance of European forests.

Yakir and Wang (1996[55]) described a mass balance approach that combined ecosystem-scale flux measurements with isotopic analyses to provide isotopic estimates of F_A and F_R in agricultural ecosystems. Bowling et al. (2001[4]) modified the mass balance approach to yield,

$$isoflux = (\delta^{13}C_R)(F_R) + (\delta^{13}C_{a-o} - \Delta_{canopy})(F_A) \qquad (5)$$

where the *isoflux* term represents the net ecosystem exchange for $^{13}CO_2$. Conceptually, this equation simply states that the *isoflux* has two basic components: the total $^{13}CO_2$ added to atmosphere $(\delta^{13}C_R)(F_R)$ by respiration, and the total $^{13}CO_2$ removed from the atmosphere by net photosynthesis $(\delta^{13}C_{a-o} - \Delta_{canopy})(F_A)$. Δ_{canopy} refers to the mean carbon isotope discrimination by the canopy, in contrast to Δ, which is the carbon isotope discrimination value for any individual leaf within the canopy. As there is no fractionation associated with mitochondrial respiration (Lin and Ehleringer, 1997[37]), the isotope ratio of respired CO_2 should reflect that of photoassimilated CO_2, with appropriate time lags between the time carbon is assimilated and respired, and allowing for small potential differences in the specific chemical components being respired by heterotrophs.

Provided the *isoflux* and Δ_{canopy} terms can be determined, the two equations (4 and 5) may be solved for the two fluxes, F_A and F_R. Bowling et al. (2001[4]) applied this approach to determine photosynthesis and respiration fluxes for a single time period in the growing season of a deciduous forest (Fig. **6**).
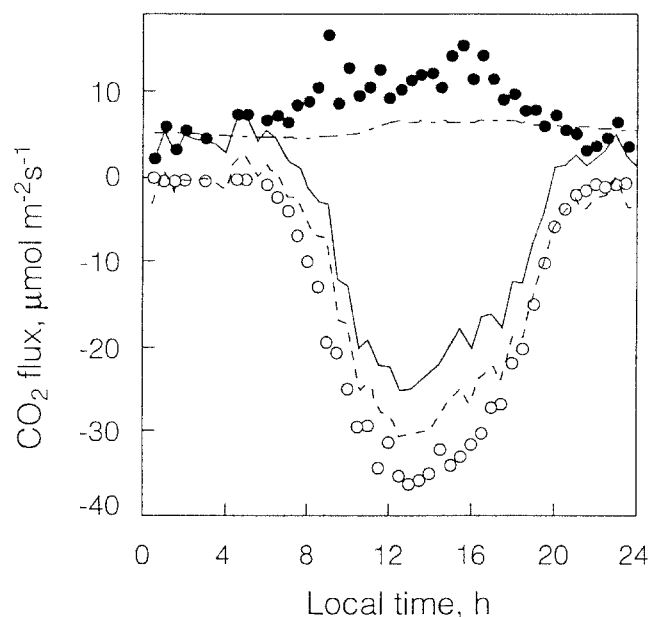
Fig. 6 A plot of the daily course of partitioning net ecosystem exchange into its photosynthetic and respiratory components using stable isotope approaches. The solid line represents the diurnal course of NEE, with the dashed lines representing the partitioning of NEE into A and R components using micrometeorological approaches. In contrast, the open and closed circles represent the partioning of A ($\bigcirc$) and R ($\bullet$), based on the isoflux partitioning approach. Adapted from Bowling et al. (2001[4]).

Presently, their method has not yet been used to examine if there are changing temporal patterns in the balance of F_A and F_R in different ecosystems or to examine how F_A and F_R might differ in contrasting ecosystems (C_3/C_4 and evergreen/deciduous). Yet the Valentini et al. (2000[52]) results for European forests suggest that inclusion of carbon partitioning studies is critical for improving our understanding of how critical terrestrial forests may be as current and/or future carbon sinks.

Both Disturbance and Stand Age Influence
^{13}C Biosphere-Atmosphere Isotopic Exchange

Few forest ecosystems remain in a pristine state. Selected timber harvesting, increased fire frequency, abandonment and afforestation, and clear cutting followed by plantation growth have resulted in forest stands that have an age frequency distribution that is different today (Casperson et al., 2000[6]). Shifts in $\delta^{13}C_R$ are not only responsive to changes in climate, but it appears that land-use history also plays a significant role. Fig. 7 shows data for adjacent coniferous stands of different ages in southern Washington, USA. Aspects of ^{13}C exchange between the biosphere and atmosphere exhibit changes with stand-age development. These changes may in part reflect aspects of canopy development, such as shifts from a forest with a single dominant tree species to a forest stand with significant overstory and understory components, differing in their ^{13}C values. Ehleringer et al. (1986[13]) and subsequent studies have documented that leaf δ^{13}C values are a function of light levels within a canopy, which would be consistent with overstory/understory shifts contributing to changes in $\delta^{13}C_R$. Alternatively, shifts in $\delta^{13}C_R$ with canopy age may also reflect reductions in the hydraulic conductivity of older trees (Hubbard et al.,
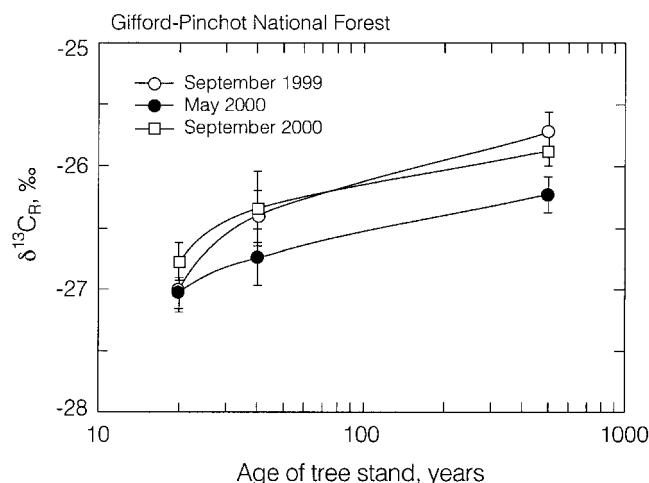

Fig. 7 Age-dependent variation of the carbon isotope ratio of ecosystem respiration ($\delta^{13}C_R$) is shown for the three forests. A comparison is made between water-limited conditions, September 1999 and 2000, and water saturated conditions, May 2000. Adapted from Fessenden and Ehleringer (2002a[21]).

1998[33]; McDowell et al., 2001[41]; Ryan et al., 1997[46]). There is growing evidence that hydraulic constraints limit stomatal conductance, resulting in higher ^{13}C values in older trees (Yoder et al., 1994[56]). At the moment, we do not know how extensive this forest-age history pattern is or how extensive it might be in different forest types. Yet the > 1‰ shift in $\delta^{13}C_R$ at the ecosystem scale (Fig. 7) is of similar magnitude to the $\delta^{13}C_p$ shifts documented for coniferous trees of different ages in the Pacific Northwest (McDowell et al., 2001[41]; Panek, 1996[42]). To the extent that global carbon cycle models capitalize on $\delta^{13}C_R$ and Δ values as inherent constraints on source-sink relations, these models should also incorporate the known differential age distributions of forests, particularly coniferous forests, where the evidence of age-dependent shifts in δ^{13}C is most evident.

Interpreting the ^{18}O Signal of Terrestrial Photosynthesis at Leaf Versus Ecosystem Scales

Throughout the world, land-use changes are resulting in large-scale ecosystem conversions from forest to pastures (tropics) and grasslands to forest and/or cultivated grass crops (temperate regions) (Ramankutty and Foley, 1999[43]). These conversions can have effects on $C^{18}O^{16}O$ discrimination at the ecosystem, regional and potentially even the global levels. These large-scale effects are facilitated primarily by three whole-plant level factors: leaf water enrichment, carbonic anhydrase activity and the c_i/c_a ratio. As CO_2 diffuses into a leaf, the oxygen atoms in CO_2 exchange with the oxygen atoms in leaf water, taking on the δ^{18}O value of leaf water with the appropriate equilibration fractionation. The completeness of this exchange is determined by carbonic anhydrase activity in the leaf. A certain fraction of this equilibrated CO_2 will then diffuse back out of the leaf (retrodiffusion), and that fraction is determined ultimately by the c_i/c_a ratio. In this section we examine these effects theoretically and develop predictions for $C^{18}O^{16}O$ discrimination (hereafter $\Delta_{C^{18}O^{16}O}$) in forest-to-grassland conversions in tropical and temperate regions.

The isotopic composition of source water that enters plants is unenriched until the water reaches the leaves, where the evaporative enrichment of ^{18}O is explained by the Craig-Gordon model (Craig and Gordon, 1965[11]; Flanagan et al., 1991[24]):

$$R_l = \alpha^* \left[\alpha_k R_s \left(\frac{e_i - e_s}{e_i} \right) + \alpha_{kb} R_s \left(\frac{e_s - e_a}{e_i} \right) + R_a \left(\frac{e_a}{e_i} \right) \right] \qquad (6)$$

where R_l, R_s and R_a represent the molar isotope ratios of $^{18}O/^{16}O$ of leaf water, plant source water and atmospheric water vapor, respectively. The equilibrium fractionation factor α^* is temperature dependent as described by equations of Majoube (1971[40]). The kinetic fractionation factor is α_k and for the leaf boundary layer, α_{kb}. The water vapor pressure of the leaf, boundary layer and atmosphere is represented by e_i, e_s and e_a, respectively. This model has been tested extensively at the leaf level and shown to be robust in a variety of dicotyledonous species (Flanagan et al., 1991[24]; Roden and Ehleringer, 1999[45]). The ^{18}O content of leaves is expected to be higher during the daytime period when vapor pressure deficits and temperatures are highest.

Helliker and Ehleringer (2000[32]), showed that the Craig-Gordon model does not always accurately predict the ^{18}O-enrichment in grasses, particularly C_4 grasses. It was observed that C_4 grasses were as much as 7‰ more enriched than predictions from the Craig-Gordon model. This observation is associated with progressive enrichment along parallel veins in grasses, analogous to the "string of pools" model developed by Gat and Bowser (1991[27]) which can account for variability in evaporative flux along the string. As such, deviations of grass leaf-water from Craig-Gordon predictions are affected by the evaporative flux profile along the leaf, which can change with plant water availability. Hence, the leaf water composition of grasslands can be considerably above or even below Craig-Gordon predictions and, as a corollary, C_3 forests. An additional important factor to consider which also influences leaf water enrichment is energy balance. Converted grasslands are typically warmer than adjacent forests. Grasslands receiving the same solar radiation and thermal forcing inputs as a nearby forest will typically experience higher leaf temperatures. This trend alone will increase evaporative ^{18}O-enrichment in grasslands over forests. In turn, that should influence the $C^{18}O^{16}O$ isotopic composition of the CO_2 retrodiffusing from leaves in these ecosystems.

The isotopic composition of leaf water is an important determinant of the discrimination against $C^{18}O^{16}O$ as described by Farquhar et al. (1993[18]) and Gillon and Yakir (2001[29]),

$$\Delta_{C^{18}OO} = \frac{\bar{a} + \varepsilon [\theta_{eq} \Delta_{ea} - (1 - \theta_{eq}) \Delta_{ca0}]}{1 - \varepsilon [\theta_{eq} \Delta_{ea} - (1 - \theta_{eq}) \Delta_{ca0}]} \qquad (7)$$

where $\bar{a}$ is the weighted mean fractionation of CO_2 diffusion through the gas and aqueous phase to the site of equilibrium. ε is defined as $C_{cs}/(C_a - C_{cs})$, where C_{cs} is the CO_2 concentration at the chloroplast surface (the limit of $H_2O:CO_2$ equilibration) and C_a is ambient CO_2 concentration. C_{cs} is related to the more commonly used c_i values by $c_i - A/g_w$, where A is assimilation and g_w is cell wall conductance to CO_2. Δ_{ea} is $R_c/R_a - 1$ and R_c and R_a represent the molar $^{18}O/^{16}O$ ratio of CO_2 at the site of
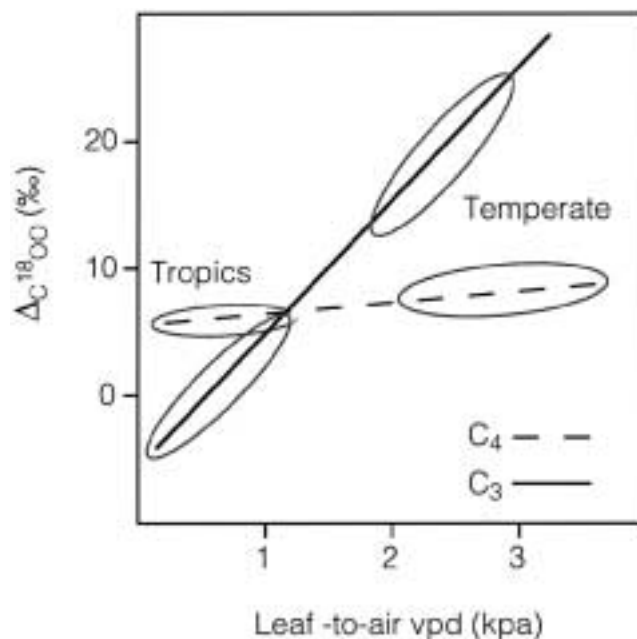

Fig. 8 A plot of the predicted oxygen isotope discrimination for CO_2 diffusing out of C_3 and C_4 vegetation as a function of the vapor pressure deficit. $\delta^{18}O_{atmwv} = -20‰$, $\delta^{18}O_{atmco2} = -1.5‰$, $\delta^{18}O_{s.water} = -8‰$, $T_{leaf} = 30$, $33 °C$ for forest and pasture, respectively, $C_{cs}/C_a = 0.66$, 00.30 and $\theta = 1$, 0.3 for forest and pasture, respectively.

equilibrium and ambient CO_2, respectively. R_c is obtained from R_l above. $\Delta_{ca0} = a/(1 + \varepsilon)$. θ is a variable representing the extent of equilibrium during the discrimination process, the value of which falls between 0 and 1. Leaf level carbonic anhydrase activity is the primary determinant of equilibration, and hence θ, of a plant.

In equation 7, the overall effect of the $\delta^{18}O$ of leaf water on the $\delta^{18}O$ of retro-diffused CO_2 is determined firstly by the C_{cs}/C_a ratio (via ε), because this ratio determines the size of the retro-diffused CO_2 flux. For example, assume identical inputs into equation 7, except for C_{cs} values characteristic of C_3 and C_4 plants. The effective discrimination by C_3 plants will be much greater than C_4 plants as more CO_2 is labeled by leaf water $\delta^{18}O$ and retrodiffused. On this basis alone, C_4 grasslands should have markedly different $\Delta_{C^{18}O^{16}O}$ than C_3 forests.

A multiplicative factor separating $\Delta_{C^{18}O^{16}O}$ of C_4 grasslands and C_3 forest is highlighted by recent work of Gillon and Yakir (2000[28], 2001[29]), where they showed that θ for C_4 grasses was, on average, 38% ($\theta = 0.38$) of C_3 dicot values (which include the overwhelming majority of forest species). This difference arises because carbonic anhydrase activity, which determines the level of equilibration of $H_2O:CO_2$, is significantly lower in C_4 grasses.

When we take into account all of the factors that affect $\Delta_{C^{18}O^{16}O}$ in forest to grassland conversions (leaf water enrichment, C_{cs}/C_a ratios, and carbonic anhydrase activity), we can develop a plot of predicted $\Delta_{C^{18}O^{16}O}$ discrimination as a function of the environmental parameters of the geographical locations where the conversions occur. The major assumption is that, in a given locale, the isotopic inputs into the system do not change. Con-

sidering that the geographical location itself is the primary determinant of the isotopic inputs, this is a safe assumption. Fig. **8** represents one such plot in which the only factors that differ between C_3 forest and C_4 grasslands are microclimate differences (warmer leaf temperatures in C_4 grasslands and hence greater vapor pressure deficits [vpd]), C_{cs} values and θ (note that within an ecosystem type, these values are held constant in all predictions). The first thing that becomes apparent in such a plot is the small C_4 response to increasing vpd. As vpd increases, leaf water enrichment of C_4 plants also increases, but because of the low C_{cs}/C_a ratios and low carbonic anhydrase activity, the increased enrichment has little effect upon $\Delta_{C^{18}O^{16}O}$. Because of the low C_{cs}/C_a and low carbonic anhydrase activity, the predicted $\Delta_{C^{18}O^{16}O}$ of C_4 grasslands and C_3 forest do not converge at a vpd of zero, where there would be no evaporative enrichment of leaf water. The warmer temperatures in grasslands will further increase the magnitude of the vapor pressure deficit between grassland and adjacent forest sites. Note that in the tropics, conversion from forest to pasture is predicted to increase $\Delta_{C^{18}O^{16}O}$ which, in theory, reduces the likelihood that the C_3 forest to C_4 pasture conversion are contributing to the decreasing global trend in $\delta^{18}O$ of atmospheric CO_2 as suggested by Gillon and Yakir (2001[29]).

The ^{18}O enrichment provides an additional signal with which to isolate and identify grassland source/sink inputs into the global carbon cycle (Fig. **8**). Isotopically, the ^{13}C discrimination by C_4 ecosystems is small and of similar magnitude to oceanic sources (Fung et al., 1997[26]). Thus, knowing the additional information provided by ^{18}O analysis provides an additional constraints for partitioning oceanic/terrestrial carbon sink strength in those tropical and monsoonal regions where C_4 ecosystems dominate (Sage and Monson, 1999[47]).

Summary

To really understand the magnitude and distribution of any terrestrial sink, we need a better knowledge of how $\delta^{13}C_R$ and photosynthetic carbon isotope discrimination vary in response to climatic factors, geographical gradients and land-use history. Some ecosystems are likely to exhibit limited variations in $\delta^{13}C_R$ (e. g., boreal conifers, Flanagan et al., 1996[23]), whereas others are likely to be dynamic and responsive as shown in Figs. **3 – 5**. Fung et al. (1997[26]) stressed the importance of constraining the degrees of freedom in global carbon cycle inversions by improving our understanding of the ^{13}C exchanges between the biosphere and the atmosphere.

A number of terrestrial studies are already beginning to address measurements of the ^{13}C exchanges between the biosphere and the atmosphere (Bakwin et al., 1998[1]; Buchmann et al., 1997[5]; Flanagan et al., 1996[23]). There is additional information to be gained at every eddy covariance site by including $^{13}CO_2$ measurements (Flanagan and Ehleringer, 1997[25]; Fung et al., 1997[26]). There is also significant value to be added by capitalizing on an existing network which spans isotope measurements across eddy covariance sites. The Biosphere–Atmosphere Stable Isotope Network (BASIN) is a Global Change and Terrestrial Ecosystem (GCTE-IGBP) effort at integrating isotope and flux studies and to bridging the gap between ecosystems-scale and global-scale carbon cycle studies (http://GCTE-focus1.org/BASIN.html). The objectives of this international network are to develop a uniform framework for isotope sampling, promote cross-site comparisons and data exchange, and engage in common experiments at our respective sites that will improve our understanding of ^{13}C exchange between the biosphere and the atmosphere. Future global inversion studies should benefit from the mechanistic insights to be derived from this international effort and by the larger-scale data sets with which to test the global inversion models.

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Section Editor: C. B. Osmond