Tansley review

Carbon isotopes in terrestrial ecosystem pools and CO₂ fluxes

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Summary
Stable carbon isotopes are used extensively to examine physiological, ecological, and biogeochemical processes related to ecosystem, regional, and global carbon cycles and provide information at a variety of temporal and spatial scales. Much is known about the processes that regulate the carbon isotopic composition (δ¹³C) of leaf, plant, and ecosystem carbon pools and of photosynthetic and respiratory carbon dioxide (CO₂) fluxes. In this review, systematic patterns and mechanisms underlying variation in δ¹³C of plant and ecosystem carbon pools and fluxes are described. We examine the hypothesis that the δ¹³C of leaf biomass can be used as a reference point for other carbon pools and fluxes, which differ from the leaf in δ¹³C in a systematic fashion. Plant organs are typically enriched in ¹³C relative to leaves, and most ecosystem pools and respiratory fluxes are enriched relative to sun leaves of dominant plants, with the notable exception of root respiration. Analysis of the chemical and isotopic composition of leaves and leaf respiration suggests that growth respiration has the potential to contribute substantially to the observed offset between the δ¹³C values of ecosystem respiration and the bulk leaf. We discuss the implications of systematic variations in δ¹³C of ecosystem pools and CO₂ fluxes for studies of carbon cycling within ecosystems, as well as for studies that use the δ¹³C of atmospheric CO₂ to diagnose changes in the terrestrial biosphere over annual to millennial time scales.

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I. Introduction

Differences in the stable carbon (C) isotopic composition ($\delta^{13}C$) of organic and inorganic compounds are useful for studying processes that control C cycling within and between plants, animals, and ecosystems, and exchanges between these and other reservoirs and the atmosphere and hydrosphere. $\delta^{13}C$ is defined by the equation $\delta^{13}C = (R_{\text{sample}}/R_{\text{VPDB}} - 1) \times 1000\%$, where $R$ is the molar ratio of $^{13}C/^{12}C$ and VPDB is the Vienna Pee Dee Belemnite laboratory standard. For a review of isotope terminology, see Farquhar et al. (1989). Here we use $\delta^{13}C$ to refer to isotopic composition in general, and $\delta^{13}CO_2$ to refer to the isotopic composition of CO$_2$.

Plants are isotopically 'lighter' than CO$_2$ in air, meaning that they contain less $^{13}C$ relative to $^{12}C$ in their tissues than atmospheric CO$_2$. This is a result of differences in diffusion rates of $^{12}CO_2$ and $^{13}CO_2$ into leaves, and especially differences in reaction rates with Rubisco during carboxylation. There is a rich understanding of the processes that control the C isotope discrimination ($\Delta$) associated with photosynthesis, and C isotope studies have made important contributions to much of our understanding of plant physiology and ecosystem ecology. Isotope discrimination is defined as the isotope effect in the conversion from substrate to product: $\Delta = (\delta_{\text{substrate}} - \delta_{\text{product}})/(1 + \delta_{\text{product}}/1000)$. For photosynthesis, the substrate is CO$_2$ in air, and the product is either bulk leaf biomass or photosynthetic sugars. For respiration, the substrate is harder to define, and the product (respiratory CO$_2$) is usually compared with possible substrates for respiration (glucose, starch, lipids, etc.). For this reason, respiratory discrimination is usually referred to as apparent respiratory discrimination. In this paper, we use $\Delta_A$ to refer to discrimination associated with photosynthesis.

A host of processes influence the $\delta^{13}C$ of bulk leaf material, including light, photosynthetic capacity, water availability, atmospheric humidity, and others, which have been thoroughly reviewed (Farquhar et al., 1989; Brugnoli & Farquhar, 2000; Dawson et al., 2002). Here we explore the hypothesis that bulk leaf $\delta^{13}C$, which is controlled by physiological and ecological factors, can be used as a reference point from which there are predictable differences in the $\delta^{13}C$ of plant and ecosystem C pools and fluxes. Such differences can be utilized to make inferences about ecosystem C cycling and related effects on the isotopic composition of atmospheric CO$_2$. This hypothesis has strong support when comparing the isotopic composition of leaf metabolites to that of the bulk leaf, as noted by others (e.g. Boutton, 1996). In this review, we will show that it also appears valid when comparing bulk leaf biomass to specific plant tissue classes (e.g. phloem sap organics, wood, or roots), to ecosystem C pools (e.g. bulk soil organic matter or fungal biomass) and to respiratory fluxes (from leaves, roots, soil, and whole forests). While the concepts developed here may have applicability to C$_4$ systems, we have restricted our analysis to vascular plants and associated ecosystems that are dominated by the C$_3$ photosynthetic pathway.

Our choice of bulk leaf tissue as the isotopic reference point is one of pragmatism. One could argue that triose phosphates or leaf sugars would be a better choice, as they are more directly linked to photosynthetic discrimination than the bulk leaf tissue. Alternatively, leaf or wood cellulose might be appropriate because of its stability. While appealing, these are not commonly measured in the majority of studies relevant to this review. The most commonly measured organic component $\delta^{13}C$ in plant and ecosystem studies is the bulk leaf tissue, and for this reason we use it as our common unit for comparison.

A conceptual example helps to illustrate our central theme (Fig. 1). The isotopic composition of atmospheric CO$_2$ (which we denote $\delta^{13}CO_2$) currently averages c. –8‰, while the $\delta^{13}C$ of C$_3$ plants ranges from –20 to –35‰ because of variability in photosynthetic discrimination (Dawson et al., 2002). As a consequence, the C that returns to the atmosphere through release of respiratory CO$_2$ from plants and animals is depleted in $^{13}CO_2$ relative to the atmosphere, and therefore the $\delta^{13}C$ of whole-forest respiration in C$_3$ ecosystems ranges from –24 to –30‰. (‘Enriched’ and ‘depleted’ refer to the relative amount of each isotope in one sample relative to another. Sample A is enriched (or depleted) relative to sample B if $^{13}C/^{12}C$ for sample A is larger (or smaller) than for sample B. In $\delta$ notation, sample A is enriched (or depleted) relative to sample B if $\delta^{13}C_A$ is less (or more) negative than $\delta^{13}C_B$.) Several studies have shown that $\delta^{13}C$ of leaves varies across broad gradients of precipitation, with the general pattern that plants in wetter ecosystems tend...
to have more negative $\delta^{13}C$ in their leaves than those in drier regions (Read & Farquhar, 1991; Stewart et al., 1995; but see Schulze et al., 1998). As we will show, there is substantial post-photosynthetic discrimination against $^{13}C$ during plant tissue synthesis, and the relationship between respiration and bulk leaf composition shown in Fig. 1 follows as a result. A similar pattern in $\delta^{13}C$ of respiration has been observed across a wide range of biomes; for sites that ranged in mean annual precipitation from 200 to 2300 mm, the $\delta^{13}C$ of ecosystem respiration ranged from $-24$ to $-30\%$ (Fig. 1, and Pataki et al., 2003). Therefore, ecological factors (precipitation in this example) influence the $\delta^{13}C$ of plant tissues, plant respiration, and ecosystem respiration. We present evidence in this paper that many C pools and fluxes show systematic offsets from bulk leaf $\delta^{13}C$.

Isotopic mass balance at the leaf, plant, and ecosystem scales is unattainable at present because of gaps in our understanding of several processes. Ideally, to fully utilize the information contained in the $\delta^{13}C$ of terrestrial ecosystems, an understanding of all the processes that influence the $\delta^{13}C$ of C pools and fluxes is necessary. In this review we summarize the current understanding of the factors that influence the C isotopic composition of plant and ecosystem C pools and fluxes, with an emphasis on systematic patterns of the $\delta^{13}C$ of pools and fluxes relative to bulk leaf organic matter. The first section is a discussion of the isotopic composition of plant metabolites and leaf respiration, followed by a review of observed patterns in $\delta^{13}C$ of plant and ecosystem C pools. Next, we examine the isotope content of assimilation and respiration fluxes, and then discuss these fluxes at various spatial scales, with an emphasis on interpreting the atmospheric record of $^{13}CO_2$. We conclude with a discussion of important areas for future research.

II. Isotopic composition of plant metabolites and autotrophic respiration

1. Variability in $\delta^{13}C$ of plant metabolites

The factors that affect photosynthetic discrimination and the isotopic composition of bulk leaf biomass and organic compounds within leaves and other plant organs have been reviewed in detail (O’Leary, 1981; Farquhar et al., 1989; Dawson et al., 2002; Ghishgaie et al., 2003; Hobbie & Werner, 2004). Photosynthetic discrimination is often estimated with the simplified model $\Delta A = a + (b - a)i/c$, where $a$ is diffusive fractionation ($4.4\%$), $b$ is enzymatic fractionation, primarily Rubisco ($27\%$), and $i/c$ is the ratio of $CO_2$ in the intercellular air space to that outside the leaf. This model (or the more detailed form) is commonly called the Farquhar et al. model after a highly influential paper by Graham Farquhar, Marion O’Leary, and Joe Berry (Farquhar et al., 1982). Hence, discrimination varies in response to factors that influence the balance between photosynthetic drawdown of $CO_2$ in the substomatal cavity and the diffusion of $CO_2$ into the leaf, and therefore in response to $i/c$. For example, the influence of light availability on photosynthetic capacity leads to variation in the $\delta^{13}C$ of leaves at different heights within a forest, with most negative values of $\delta^{13}C$ in leaves near the ground and least negative values at the top of the canopy (e.g. Martinelli et al., 1998; Pate & Arthur, 1998). Environmental conditions that result in stomatal closure such as drought, low atmospheric humidity, or nutrient stress generally decrease $\Delta A$, causing plant biomass and leaf sugars to be relatively enriched under these conditions (e.g. Brugnoli et al., 1988; Madhavan et al., 1991; Gaudillere et al., 2002).

Within a plant, the $\delta^{13}C$ of organic compounds varies (e.g. Gleixner et al., 1998), as a result primarily of isotope effects expressed at branch points in biochemical pathways. No fractionation is observed if all of a particular substrate is converted to a product. Biosynthesis of reduced compounds from photosynthetic sucrose involves numerous biochemical pathways in which enzymatic and positional isotope effects may be expressed, resulting in fairly systematic, compound-specific values of $\delta^{13}C$ for plant organic compounds (Fig. 2). For example, sucrose and starch are isotopically enriched relative to bulk leaves, while lipids and lignin are generally depleted (Fig. 2). The depletion of lipids appears to originate in the oxidation of pyruvate to acetyl CoA by the pyruvate dehydrogenase complex (DeNiro & Epstein, 1977). All metabolic products derived from acetyl CoA are expected to be isotopically depleted as a result of enzymatic fractionation.

2. Isotope effects influencing leaf respiration

The isotope effects addressed above for biosynthetic pathways may also directly influence the $\delta^{13}C$ of respiratory $CO_2$, as the biochemical pathways are interrelated. Positional isotope effects refer to nonrandom, intramolecular distributions of heavy and light isotopes. For example, C at position 4 of glucose tends to be enriched relative to the whole molecule, while position 6 tends to be depleted (Rossmann et al., 1991). In addition, there may be kinetic effects in respiratory enzymes such as pyruvate dehydrogenase, which are temperature and substrate dependent (DeNiro & Epstein, 1977; Melzer & Schmidt, 1987). The net result of these effects is that isotopic depletion or enrichment of biosynthetic products must be balanced by opposing effects elsewhere in the plant, including evolved $CO_2$. In the case of acetyl-CoA synthesis, oxidation of pyruvate releases $CO_2$ that is enriched relative to the pyruvate substrate, as the acetyl CoA is generally depleted (DeNiro & Epstein, 1977; Melzer & Schmidt, 1987).

In reviews of the early literature on the $\delta^{13}C$ of dark respiration, O’Leary (1981) and Farquhar et al. (1982) noted that comparisons of respiratory $CO_2$ and plant biomass yielded inconsistent differences, with respiration sometimes more enriched or more depleted than leaves (Park & Epstein, 1961; Smith, 1971; Troughton et al., 1974). Therefore, it was assumed...
that the δ¹³C of respiratory CO₂ is not changed by biochemical reactions of the Krebs cycle, and thus should reflect the respiratory substrate. This differs fundamentally from photosynthetic discrimination, where a biochemical fractionation dominates. Previous literature showing fractionation in dark respiration of up to 8‰ (Park & Epstein, 1961) was attributed to differences in respiratory substrates, such as oxidation of glucose vs lipids (Lin & Ehleringer, 1997).

Several studies have subsequently tested the effects of respiratory substrate by measuring the isotopic composition of CO₂ evolved during dark respiration and comparing it to that of plant compounds such as sugars, starch, and lipids (see review by Ghisghaie et al., 2003). Duranceau et al. (1999, 2001) found that respiratory CO₂ was enriched by 2–6‰ relative to sucrose in Phaseolus vulgaris and Nicotiana sylvesteris, while Ghisghaie et al. (2001) found enrichment of respiratory CO₂ in N. sylvesteris and Helianthus annuus relative to all measured carbohydrates, organic acids, and lipids.

Tcherkez et al. (2003) further assessed the role of respiratory substrate with simultaneous laboratory measurements of δ¹³C of dark respiration (leaves in the dark for 2 h or longer) and the respiratory quotient (RQ), the ratio of CO₂ produced to O₂ consumed during respiration. Oxidation of carbohydrates results in RQ values close to 1, while oxidation of highly reduced compounds results in RQ < 1. At low leaf temperature, the δ¹³C of respiration in Phaseolus vulgaris was 4‰ enriched relative to sucrose, while RQ was close to 1 (Fig. 3). RQ declined with increasing leaf temperature, suggesting that, as an increasing proportion of reduced respiratory substrates was consumed, the δ¹³C of respiration became more depleted. These results suggest that the δ¹³C of dark respiration varies with respiratory substrate, but they also indicate that the δ¹³C of the CO₂ evolved in respiration is offset from the δ¹³C of the substrate. The mostly evident explanation is fractionation at metabolic branch points between oxidation of pyruvate to CO₂ in the Krebs cycle and biosynthetic pathways such as fatty acid synthesis from acetyl CoA. Indeed, the only known process that can account for large enrichments of respiratory CO₂ of 6‰ or greater relative to sucrose is the synthesis of equally depleted compounds in lipid production.

Field experiments with Quercus ilex, Quercus cerris, and Pinus elliottii have found a strong pattern of diurnal enrichment in δ¹³C of CO₂ evolved by leaves that were removed from the light and placed in the dark for several minutes (Hymus et al., 2005; Prater et al., 2006; also see Wingate et al., 2007). Respiratory CO₂ near the end of the daily photoperiod was enriched by up to 7‰ relative to predawn, and always enriched relative to possible respiratory substrates. These diel changes could not be reconciled with changes in the δ¹³C of possible respiratory substrates within the leaf, or with environmental changes in ΔΔ throughout the day (Hymus et al., 2005). There may be differences in the diel pattern of respiratory enrichment in woody vs herbaceous plants. A recent study with Ricinus communis showed that some of the respiratory...
from a large number of studies suggests that leaf dark respiration is usually enriched relative to bulk leaf tissue (Fig. 4), whether the leaves are in the dark for a few minutes or for several hours or days. Other categories in Fig. 4 will be discussed as they are introduced.

3. Relative importance of growth and maintenance respiration

Allocation of C to autotrophic respiration in different ecosystems and under varying environmental conditions remains a source of major uncertainty in the terrestrial C cycle. Autotrophic respiration involves many possible biochemical pathways for generating ATP, NAD(P)H, and biosynthetic intermediates, and is therefore more complex to represent in plant and ecosystem models than photosynthesis (Cannell & Thornley, 2000). A useful concept for modeling autotrophic respiration has been the distinction between growth and maintenance respiration. Growth respiration provides energy, reducing power, and C skeletons for biosynthesis of plant compounds, while maintenance respiration supports existing biomass through replacement of proteins, membranes, and ion gradients (see reviews by Amthor, 2000; Thornley & Cannell, 2000; Gifford, 2003). Although the metabolic pathways for growth and maintenance respiration are the same, growth respiration is theoretically fixed for a given set of plant compounds because of their inherent construction costs, while maintenance respiration varies with environmental conditions such as temperature (Amthor, 1984; Ryan, 1991). Temporal and spatial variability in the $\delta^{13}C$ of autotrophic respiration may provide important information about mechanisms underlying variation in growth and maintenance respiration.

Bulk leaf tissue is slightly depleted relative to the sugar substrates from which leaf compounds are produced (Fig. 2). Mass balance dictates that this depletion must be opposed by an enriched pool of C which may leave the leaf via respiration (as discussed) or phloem export. Phloem sap sugars tend to be more enriched than bulk leaf tissue and leaf sugars by a few ‰ (Fig. 4), possibly as a result of fractionation during phloem loading, unloading, or transport (Damesin & Lelarge, 2003; Scartazza et al., 2004), although the mechanisms are not known. In addition, while starch, proteins, and cellulose tend to be enriched relative to bulk leaf material, lignin and lipids can be depleted by several ‰ (Fig. 2). Lignin and lipids constitute c. 10% of the biomass of herbaceous leaves on average, and 20% of the biomass of leaves of woody plants, which tend to have high lignin content (Poorter & Villar, 1997). While these fractions are not large, the synthesis of lipids evolves more CO$_2$ per gram than other compounds. For example, synthesis of 1 g of cellulose evolves c. 3 mmol CO$_2$ in comparison to 36 mmol for 1 g of lipids (Poorter & Villar, 1997). Hence, a small isotopic enrichment relative to sucrose is expected for respiratory CO$_2$ associated with growth.
Fig. 4 A comparison of the isotopic composition of plant and ecosystem carbon pools and respiratory fluxes, expressed relative to bulk leaf biomass. Where studies reported sun and shade leaf carbon isotopic composition (δ¹³C), only sun leaf values were used. The boxes encompass the upper and lower quartiles of the data, the line shows the median, the symbol shows the arithmetic mean, and the error bars show the upper and lower 10th percentiles of the data. The vertical dashed line is included for easy reference to the δ¹³C of leaf sugars. Horizontal lines separate (a) plant pools, (b) ecosystem pools, and (c) respiratory fluxes. This figure is not meant to be an exhaustive compilation of all relevant studies, rather a compilation large enough to indicate general patterns. No study has examined all of these categories at a single site. Other studies are relevant, but bulk leaf δ¹³C is not always reported. (d) Number of samples (n) and references used for each category. As some studies reported means and others reported individual sample values, the values of n shown are minima and the actual number of samples is much larger in most cases. SOM, soil organic matter.
The isotopic composition of amino acids is widely variable (Abelson & Hoering, 1961; Smallwood et al., 2003), reflecting multiple biosynthetic pathways in photosynthesis, glycolysis, and the Krebs cycle (Lea & Ireland, 1999). In higher plants, individual amino acids have been found to be both enriched and depleted relative to the bulk leaf by 6‰ or more (Whelan et al., 1970; Smallwood et al., 2003); an isotopic depletion similar to that of lipids may be expected for amino acids synthesized from Krebs cycle intermediates. Protein turnover may involve recycling of amino acids in addition to the synthesis of new ones (Amthor, 2000), and the effects of both processes on C isotope fractionation have not been well examined. There is a great potential to gain a process-level understanding of maintenance respiration and its variations through evaluation of natural abundance C isotope effects in plant respiration and biosynthetic fractions. Detailed examination of positional isotope effects in various compounds, including amino acids which have a pronounced enrichment in carboxyl groups, may prove particularly useful in the future (Brenna, 2001; Schmidt, 2003; Savidge & Blair, 2004).

A role for maintenance respiration in isotopic enrichment of respiratory CO₂ is supported by Ocheltree & Marshall (2004), who found a negative correlation between respiratory enrichment and relative growth rate (RGR). Helianthus annuus growing in low light with low RGR showed enrichment of respiratory CO₂ of 6‰ relative to soluble carbohydrates, while plants in high light with high RGR showed enrichment of only 2‰. Maintenance respiration is a larger proportion of total respiratory CO₂ in slow-growing as opposed to fast-growing plants (Amthor, 1984), which may explain this pattern. Furthermore, the protein-turnover component of maintenance respiration has been correlated with protein content (Penning de Vries, 1975). The isotopic composition of amino acids is widely variable (Abelson & Hoering, 1961; Smallwood et al., 2003), reflecting multiple biosynthetic pathways in photosynthesis, glycolysis, and the Krebs cycle (Lea & Ireland, 1999). In higher plants, individual amino acids have been found to be both enriched and depleted relative to the bulk leaf by 6‰ or more (Whelan et al., 1970; Smallwood et al., 2003); an isotopic depletion similar to that of lipids may be expected for amino acids synthesized from Krebs cycle intermediates. Protein turnover may involve recycling of amino acids in addition to the synthesis of new ones (Amthor, 2000), and the effects of both processes on C isotope fractionation have not been well examined. There is a great potential to gain a process-level understanding of maintenance respiration and its variations through evaluation of natural abundance C isotope effects in plant respiration and biosynthetic fractions. Detailed examination of positional isotope effects in various compounds, including amino acids which have a pronounced enrichment in carboxyl groups, may prove particularly useful in the future (Brenna, 2001; Schmidt, 2003; Savidge & Blair, 2004).

Using data on the chemical composition of leaves and their construction costs compiled by Poorter & Villar (1997), the C content of different plant compounds from Randerson et al. (2006), and the data in Fig. 2, the isotopic composition of growth respiratory CO₂ for an average leaf can be calculated (Table 1). This involves the simplifying assumptions that phloem transport can be neglected and that the mass of each isotope in the compounds created and in the respiratory flux is conserved. Undoubtedly, the first of these assumptions is incorrect, as sugar export is certain to be significant and metabolic and biosynthetic pathways are intricately linked. Nevertheless, this exercise is informative. Starting with photosynthetic sugars with an isotopic composition of –26.0‰, for example, an herbaceous leaf may be expected to have an isotope ratio of –26.9‰, with individual components varying widely, including the fairly depleted lipids (–31.4‰) and lignin (–23.5‰) for an herbaceous leaf and –20.9‰ for a woody plant leaf, or 2.5 and 5.1‰ enriched, respectively, relative to leaf sugar. Relative to the bulk leaf these differences are even larger (3.4 and 6.8‰) and imply that growth respiration has the potential to contribute substantially to the offset observed between the δ¹³C values of ecosystem respiration and the bulk leaf (Figs 1, 4).

In practice, larger enrichments of respiratory CO₂ relative to sucrose have been reported, with values as high as 6‰ (Duranceau et al., 1999; Ghoshghaie et al., 2001; Ghoshghaie et al., 2003). This implies a large flux of respiratory CO₂ from synthesis of depleted compounds, given the relatively small pool of lipids and lignin in most leaves. The largest component of maintenance respiration is protein turnover, which can account for 60% of maintenance costs (Penning de Vries, 1975). The isotopic composition of amino acids is widely variable (Abelson & Hoering, 1961; Smallwood et al., 2003), reflecting multiple biosynthetic pathways in photosynthesis, glycolysis, and the Krebs cycle (Lea & Ireland, 1999). In higher plants, individual amino acids have been found to be both enriched and depleted relative to the bulk leaf by 6‰ or more (Whelan et al., 1970; Smallwood et al., 2003); an isotopic depletion similar to that of lipids may be expected for amino acids synthesized from Krebs cycle intermediates. Protein turnover may involve recycling of amino acids in addition to the synthesis of new ones (Amthor, 2000), and the effects of both processes on C isotope fractionation have not been well examined. There is a great potential to gain a process-level understanding of maintenance respiration and its variations through evaluation of natural abundance C isotope effects in plant respiration and biosynthetic fractions. Detailed examination of positional isotope effects in various compounds, including amino acids which have a pronounced enrichment in carboxyl groups, may prove particularly useful in the future (Brenna, 2001; Schmidt, 2003; Savidge & Blair, 2004).

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Table 1 Values used in the calculation of the isotope ratio of growth respiration: the average concentration of compounds in herbaceous and woody leaves, the per cent carbon in each compound, the CO₂ produced in the synthesis of each compound, the average carbon isotope ratio (δ¹³C) of each compound relative to leaf sugars, and the δ¹³C of growth respiration relative to leaf sugars

<table>
<thead>
<tr>
<th>Compound</th>
<th>Herbaceous concentration¹ (mg g⁻¹)</th>
<th>Woody concentration¹ (mg g⁻¹)</th>
<th>Per cent carbon in compound²</th>
<th>CO₂ produced¹ (mmol g⁻¹)</th>
<th>δ¹³C relative to sugar (‰)³</th>
<th>δ¹³C of growth respiration relative to sugar (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipids</td>
<td>45</td>
<td>56</td>
<td>77.3</td>
<td>36.5</td>
<td>−5.4</td>
<td>10</td>
</tr>
<tr>
<td>Protein</td>
<td>222</td>
<td>131</td>
<td>46.8</td>
<td>37.9</td>
<td>−0.6</td>
<td>1</td>
</tr>
<tr>
<td>Lignin</td>
<td>44</td>
<td>113</td>
<td>66.6</td>
<td>13.1</td>
<td>−4.2</td>
<td>18</td>
</tr>
<tr>
<td>TSC</td>
<td>253</td>
<td>189</td>
<td>40.0</td>
<td>2.8</td>
<td>0.6</td>
<td>−7</td>
</tr>
<tr>
<td>TNC</td>
<td>176</td>
<td>131</td>
<td>40.0</td>
<td>1.8</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Organic acids</td>
<td>83</td>
<td>51</td>
<td>41.9</td>
<td>−1.0</td>
<td>−0.8</td>
<td>−28</td>
</tr>
</tbody>
</table>

TSC, total structural carbohydrates; TNC, total nonstructural carbohydrates.
¹From Poorter & Villar (1997).
²From Randerson et al. (2006).
³From Fig. 2.
have a high ion uptake maintenance cost (Amthor, 1984). Klumpp et al. (2005) found that, while the respiratory CO$_2$ of shoots was enriched relative to shoot biomass, root respiratory CO$_2$ was depleted relative to root biomass in H. annuus, Medicago sativa, and Lolium perenne. These findings support a role for maintenance respiration in isotopic enrichment of shoot respiratory CO$_2$; in addition, there are interesting implications for consideration of the isotopic composition of whole-plant respiration. The opposing isotope effects in root and shoot respiration were nearly balanced, so the difference in $\delta^{13}$C between whole-plant respiration and whole-plant biomass was small or negligible (Klumpp et al., 2005). The generality of these results remains to be seen, particularly as the allocation of C to aboveground vs belowground biomass and metabolism may differ for other taxa.

### III. Isotopic composition of plant and ecosystem carbon pools

#### 1. Plant carbon pools

Plant tissues exhibit systematic differences in their C isotopic composition relative to the leaf (Boutton, 1996; Hobbie & Werner, 2004; Badeck et al., 2005). As mentioned, phloem sap sugars and bulk phloem sap are generally enriched relative to bulk leaf material (Fig. 4) for unknown reasons. Additionally, in a Pinus sylvestris forest phloem sap in boles was more enriched (by 1.1‰) than phloem sap in twigs, a difference that persisted in both summer and winter (Brandes et al., 2007).

The $\delta^{13}$C of phloem sap also varies in response to environmental influences on photosynthetic C isotope discrimination. Pate & Arthur (1998) showed seasonal variability of up to 8% in the $\delta^{13}$C of phloem sap of Eucalyptus globulus that matched the seasonal pattern of plant water stress (more enriched during months of low precipitation). Gessler et al. (2001) found that the relationship between leaf water potential and the $\delta^{13}$C of phloem sap was additionally modified by light availability, both in the short term and with aspect relative to the sun. The $\delta^{13}$C of phloem sap has also been related to sap flux-derived canopy conductance (Keitel et al., 2003; Gessler et al., 2004), and to $c_{i}/c_{a}$ from leaf gas exchange (Brandes et al., 2006), further indication that $\Delta_A$ influences short-term variability. Diel variability in the $\delta^{13}$C of water-soluble C in leaves and phloem sap in twigs and boles of P. sylvestris has been observed (Brandes et al., 2006), with peak enrichment occurring during the night/early morning. Correlations have been found between the $\delta^{13}$C of phloem sap and the seasonal pattern of the $\delta^{13}$C of total ecosystem respiration (Scartazza et al., 2004), although the linkages appear quite complex and sometimes are not observed (Barbour et al., 2005).

The systematic offset between the $\delta^{13}$C values of wood and leaf tissue has long been known (Leavitt & Long, 1982). This is apparent as a mean enrichment in woody tissue of boles of 3.2‰, and in twigs of 1.3‰ (Fig. 4). Note, however, that the $\delta^{13}$C of new wood growth is not constant over time. Environmental variation in $\Delta_A$ is transferred to tree rings (e.g. Pate & Arthur, 1998), providing one basis for paleo-ecological environmental studies. There are consistent systematic patterns in the $\delta^{13}$C of wood within annual rings that are independent of photosynthetic fractionation and are likely related to the relative use of stored C reserves vs new photosynthesize (Helle & Schleser, 2004). Root tissues are generally enriched relative to leaf tissue, by 2.3‰ on average (Fig. 4). The difference in enrichment among twigs, boles, and roots is not well understood, although it appears fairly robust based on the sample sizes in Fig. 4. Cernusak et al. (2005) reported that newly formed xylem tissue in E. globulus was isotopically similar to phloem sap, suggesting that the enrichment of phloem sap is not associated with wood formation.

#### 2. Ecosystem carbon pools

After aboveground woody tissue senesces and begins decomposing as coarse woody debris, there may be additional enrichment of the dead woody tissue (Fig. 4). Possible reasons for this enrichment are unknown. Laboratory incubation of beech (Fagus crenata) wood with the saprotrophic fungus Trametes versicolor resulted in no change in the $\delta^{13}$C of the remaining wood despite a mass loss of 69% and a 2‰ enrichment in the $\delta^{13}$C of fungal respiration over time (Kohzu et al., 1999). Decomposition studies with various plant materials have shown that the $\delta^{13}$C of the remaining undecomposed tissues becomes more (not less) negative as mass is lost, at least in part as a result of the higher relative abundance of isotopically light lignin (Fernandez et al., 2003; Preston et al., 2006).

Among the categories listed in Fig. 4, the $\delta^{13}$C of C from soil organic matter (SOM) into the bulk matrix was the cause of enrichment over time. In general, SOM within the top 10 cm of mineral soil, or the A-horizon, is enriched relative to leaves by c. 2% (Fig. 4). This enrichment varies with depth in the soil profile; possible reasons for this have been recently reviewed (Ehleringer et al., 2000).

The biomass of soil microbes, invertebrates, and other heterotrophic organisms, at least the smallest ones, are a small component of the bulk SOM values reported in Fig. 4. It is difficult to estimate the isotopic composition of the soil microbial biomass pool separately from that of the dead organic material in the soil, but the microbial biomass pool is probably enriched by 1–2.5‰ relative to total SOM (Santruckova et al., 2000; Dijkstra et al., 2006). In general, the $\delta^{13}$C of...
animals reflects the $\delta^{13}C$ of their food source. The $\delta^{13}C$ of larger soil organisms such as earthworms can be enriched relative to litter by a few % (Uchida et al., 2004). Fungivorous microarthropods, consuming enriched hyphae of both mycorrhizal and saprotrophic fungi, can be enriched above leaves by more than 10% (Hobbie et al., 2007).

The isotopic composition of sporocarps (mushrooms) of soil-dwelling fungi has been examined extensively. Fungi that form mycorrhizal symbioses with host plants are enriched relative to bulk leaves by c. 3‰, and saprotrophic fungi are even more enriched, by nearly 6‰ above the leaves of the dominant plants in the forests (Fig. 4). Fungal mycelia, however, are not as enriched as the sporocarps (Wallander et al., 2004; Bostrom et al., 2007). Similar patterns of enrichment are found in nitrogen (N) isotopes, and C and N isotopes are often used together to distinguish the trophic status of fungal species (Högberg et al., 1999; Hobbie et al., 2001). There are poorly understood but consistent differences between fungal taxa at the genus and species levels (Taylor et al., 2003; Trudell et al., 2004). Although there is some evidence for fractionation upon fungal uptake of sucrose in culture (Henn & Chapela, 2000; Henn et al., 2002), it is likely that the difference in the $\delta^{13}C$ values of mycorrhizal and saprotrophic fungi reflects a difference in C sources (Henn & Chapela, 2001). The mycorrhizal fungi use C provided by the roots, and the decomposers utilize more enriched soil organic compounds (Fig. 4). The isotopic composition of the root compounds that are provided to the mycorrhizal fungi is unknown but is likely to reflect recent photosynthate that is hours to days old (Högberg et al., 2001; Johnson et al., 2002). The isotopic composition of recent photosynthate is difficult to measure, but studies of phloem isotopic composition (e.g. Brandes et al., 2006) provide a reasonable estimate.

A final C pool that deserves attention is the dissolved organic carbon (DOC) in the soil solution. Carbon isotopes are used widely by the limnological community to infer the origin and transport of organic materials from terrestrial to aquatic ecosystems (e.g. Hood et al., 2005), but there have been few studies of the $\delta^{13}C$ of DOC within the soil solution in terrestrial ecosystems (Ludwig et al., 2000). One recent study compared the isotopic compositions of leaves, soil, soil solution, and stream water in catchments with deciduous and coniferous vegetation (Amiotte-suchet et al., 2007). SOM was enriched relative to leaves as shown in Fig. 4. The $\delta^{13}C$ of the soil solution was c. 1‰ depleted relative to SOM under deciduous vegetation, and c. 1‰ enriched relative to SOM in coniferous forests. Variation with depth in the relationship between the $\delta^{13}C$ values of SOM and DOC appears to be important (Ludwig et al., 2000); see Tu & Dawson (2005) for further discussion. We cannot yet provide a range for the isotopic composition of the soil DOC pool, but it may be important in determining overall isotopic mass balance in terrestrial ecosystems (especially for those with high precipitation).

### IV. Isotopic composition of assimilation and respiration fluxes

With the exception of studies of the leaf level photosynthetic discrimination of net assimilation (leaf uptake – leaf respiration), now routinely measured in the laboratory via online discrimination (Evans et al., 1986), the $\delta^{13}C$ of the assimilation flux is difficult to measure. Direct measurements of $\Delta_A$ at the leaf or branch level in the field are very rare (Harwood et al., 1998; Wingate et al., 2007), and to our knowledge there has been only one attempt to scale such field measurements to an entire vegetation canopy (Tissue et al., 2006). More typically in the field, gas exchange measurements (without isotopes) are used to measure $c_i/c_a$, and then $\Delta_A$ is calculated using the Farquhar et al. model or a variant (e.g. Hymus et al., 2005). Researchers have used tower-based water vapor flux measurements and a Penman–Monteith inversion to calculate a canopy conductance and the Farquhar et al. model or a variant to compute $\Delta_A$ for a whole forest (Bowling et al., 2001; Ogée et al., 2003; Knöhl & Buchmann, 2005). This approach tends to underestimate $\Delta_A$ relative to estimates that come from multi-layer biophysical models (e.g. compare the results of Bowling et al., 2001 and Baldocchi & Bowling, 2003 for the same forest).

Other studies have used the $\delta^{13}C$ of nocturnal whole-ecosystem respiration as a proxy from which to derive $\Delta_A$, either directly or indirectly (e.g. Flanagan et al., 1996; Bowling et al., 2002; Alstad et al., 2007). While reasonable, results obtained using this approach differ fundamentally from those obtained using a variety of other measurement and modeling methods that examined $c_i/c_a$ at different scales in a temperate rainforest (Tissue et al., 2006). The difference may be attributable to a systematic offset in $\delta^{13}C$ between assimilation and respiration fluxes, including the expected enrichment from plant growth respiration described in Section II ‘Isotopic composition of plant metabolites and autotrophic respiration’, or to the fact that the $\delta^{13}C$ of the assimilation flux is poorly quantified at present at spatial scales larger than a single leaf. Ultimately, the $\delta^{13}C$ of the assimilation flux is likely to be more closely related to the isotopic composition of leaf sugars (Fig. 2) or the organics exported from the leaf in the phloem (Fig. 4) than the $\delta^{13}C$ of bulk leaves or ecosystem respiration, because of the fractionations associated with leaf respiration, biosynthesis and C allocation discussed above.

Although the biochemical process of mitochondrial respiration does not appear to lead to direct fractionation (Lin & Ehleringer, 1997), the $\delta^{13}C$ of respiration differs from that of the bulk organic material that is respiring (such as a leaf or stem) or being decomposed. Theoretical calculations suggest that there may be inherent biochemical fractionations in the Krebs cycle (Tcherkez & Farquhar, 2005). As discussed, leaf respiration is enriched on average above bulk leaf composition by more than 3‰ (Fig. 4).
The isotopic composition of root respiration fluxes is difficult to measure and, to our knowledge, measurements of the $\delta^{13}C$ of respiration from intact roots in the field have not been reported. Tu & Dawson (2005) reported a $\delta^{13}C$ of root respiration from detached roots that was mildly enriched relative to sun leaves at the top of a Sequoia sempervirens canopy, a result that differs markedly from those of laboratory studies. Klumpp et al. (2005) and Schnyder & Lattanzi (2005) used a special gas exchange system to measure the $\delta^{13}C$ of respiration from whole-shoot and whole-root tissues in herbaceous annuals and perennials. They found that root respiration was in general depleted in $\delta^{13}C$ relative to bulk leaf (whole shoot) biomass. In partial agreement with these laboratory studies, Badeck et al. (2005) reported that the $\delta^{13}C$ of root respiration in P. vulgaris was depleted relative to root sucrose, but not relative to bulk leaf material. Root respiration is the only quantity shown in Fig. 4 that is consistently more negative in $\delta^{13}C$ than leaves; a mechanism to explain this trend is lacking. Our understanding of the $\delta^{13}C$ of root respiration is limited at present and more studies are needed to determine whether root respiratory CO$_2$ is consistently depleted under field conditions.

The isotopic composition of respiration from stems and boles of living plants is also poorly understood. While woody tissue respiration represents only a few per cent of total ecosystem respiration (e.g. Law et al., 1999), there is evidence to suggest that the CO$_2$ evolved may originate not only from the living cells within the stem, but also from respiration elsewhere (presumably roots) the products of which are transported in the xylem stream (Teskey & McGuire, 2007). Studies that have reported the $\delta^{13}C$ of respiration of woody tissue have shown an enrichment relative to the associated bulk leaf tissue (Fig. 4, Cernusak et al., 2001; Damesin & Lelarge, 2003; Brandes et al., 2006). A recent study with Q. petraea identified pronounced seasonal and diurnal variability in the $\delta^{13}C$ of respiration of woody tissue (Maunoury et al., 2007). Seasonal changes were attributed to phenology and the relative contributions of growth and maintenance respiration. Diurnal changes were greatest in winter when there were no leaves on the trees.

In general, the $\delta^{13}C$ of heterotrophic respiration by microbes and other soil organisms cannot be measured in the field without experimental manipulations such as isotopic labeling, stem girdling, or trenching or other means of root exclusion. Incubation studies with decomposing organic materials generally show a $\delta^{13}C$ of microbial respiration that is initially depleted (relative to the material being decomposed), then becomes enriched, and eventually stabilizes over many months as the labile C is consumed (Andrews et al., 1999; Schweizer et al., 1999; Fernandez & Cadisch, 2003; Fernandez et al., 2003; Crow et al., 2006). A similar pattern of enrichment in respired CO$_2$ over time has been found with CO$_2$ evolved as fungi decompose wood (Kohzu et al., 1999). Changes in $\delta^{13}C$ over time during incubations are potentially quite useful in identifying the $\delta^{13}C$ of the labile vs recalcitrant C pools in the soil (Andrews et al., 1999), but their applicability to understanding the $\delta^{13}C$ of respiratory fluxes in the field may be limited as the connection to the rapidly cycling C pool (Högberg et al., 2001) has been lost.

In general, the soil respiration flux (measured from chambers on the soil surface) is enriched relative to leaves of the dominant trees in a forest by an average of 2.6‰ (Fig. 4). Variability in the $\delta^{13}C$ of soil respiration has been linked to environmental conditions, presumably through meteorological influences on the $\Delta_\lambda$ of whole forests (Ekblad & Högberg, 2001; Ekblad et al., 2005; Mortazavi et al., 2005). Linkages with weather involve time lags of a few days; that is, a particular weather event influences the $\delta^{13}C$ of soil respiration a few days later. Reasons for the time lags are not well understood, but presumably involve the time necessary for C transport belowground and C exchange with root symbionts. There is evidence that there are several pools of respiratory metabolites with differing turnover times in plants (Nogues et al., 2004, 2006).

The isotopic composition of whole-ecosystem respiration (measured with the Keeling-plot technique; Pataki et al., 2003) is enriched relative to bulk leaf tissue across a wide variety of biomes, by an average of 1.7‰ (Fig. 4). As mentioned, factors such as mean annual precipitation strongly influence the $\delta^{13}C$ of leaves, and the $\delta^{13}C$ of ecosystem respiration corresponds to mean annual precipitation as a result (Fig. 1). The variability in the $\delta^{13}C$ of ecosystem respiration at a particular site, however, shows important variation on a time scale of days to months that has been linked to a variety of factors. These include soil moisture (Lai et al., 2005), atmospheric humidity and radiation (Bowling et al., 2002; Knohl et al., 2005), and air or soil temperatures (Bowling et al., 2002; McDowell et al., 2004b). A more complete listing of studies that have shown these linkages and related discussion can be found in Schaeffer et al. (in press).

A lack of complete understanding of the isotopic composition of C pools and fluxes in terrestrial ecosystems is apparent in the trends shown in Fig. 4. While no study has yet measured the $\delta^{13}C$ values of leaf, root, wood, soil, and ecosystem respiration simultaneously, a few have measured several of these fluxes at once (Bowling et al., 2003; McDowell et al., 2004a; Mortazavi et al., 2005) and do not usually achieve mass balance. Root respiration makes up a significant component of soil respiration (Subke et al., 2006), and as root respiration is fairly depleted in $\delta^{13}C$ (c. −2‰ relative to leaves; Fig. 4) an unidentified but enriched source of CO$_2$ is required to explain the enriched values of soil respiration shown in Fig. 4 (a +2.6‰ enrichment relative to leaves). CO$_2$ originating from decomposition of SOM is unlikely to be enriched relative to SOM; such a fractionation could not be sustained over long periods of time without gradual isotopic depletion of the soil C pool. The enriched source may come from fungal respiration, as the biomass of fungi is enriched relative to other ecosystem components (Fig. 4), but to our knowledge the $\delta^{13}C$ of fungal respiration has not been measured in the field. This
mass balance dilemma extends to the whole ecosystem as well. How can total ecosystem respiration be 1.7‰ enriched relative to leaves on average if both leaf respiration and soil respiration are more enriched than ecosystem respiration (Fig. 4)? This problem does not result from the compilation of disparate studies in Fig. 4 – it has been observed in individual ecosystems (Bowling et al., 2003; McDowell et al., 2004a).

Many studies have examined the δ13C of bulk soil surface respiration, using a wide variety of methods (see references in Fig. 4). The relatively small variability in the δ13C of soil respiration (Fig. 4) is encouraging given the various methods used, but it is quite possible that they all are in error. Because of diffusive fractionation, CO2 in the soil gas is enriched relative to the flux leaving the soil by 4.4‰ (Cerling et al., 1991). If enriched CO2 was inadvertently advected from the soil during flux measurements, the resulting δ13C of measured soil surface flux would be too enriched (Bowling et al., 2003; McDowell et al., 2004a). Subtracting 4.4‰ from the soil respiration value in Fig. 4 places it much closer to the δ13C of root respiration, although root respiration studies have been relatively few and should be compared with other ecosystem fluxes in a greater variety of ecosystems and environmental conditions.

**V. Ecosystem to global scale issues – isotopic composition of gross primary production, net primary production, and net ecosystem production**

Variations in the δ13C of ecosystem C pools and fluxes (Fig. 4) provide a context for understanding how the isotopic compositions of gross primary production (GPP), net primary production (NPP), and net ecosystem production (NEP) are likely to differ. They are also critical for interpreting temporal and spatial variability in the isotopic composition of atmospheric CO2. The isotopic composition of NPP begins with the δ13C of assimilates from GPP, including the sensitivity of Δg to environmental conditions, but depends additionally on post-assimilation fractionation during tissue synthesis. As a result, plant-to-plant and ecosystem-to-ecosystem differences in C allocation and tissue chemistry are likely to simultaneously influence the δ13C of plant respiration and the biomass increment that ultimately provides the substrate for the heterotrophic community. The δ13C variations caused by allocation are not necessarily small compared with those arising from environmental regulation of GPP: the mean difference between bole and bulk leaf δ13C values is 3.2‰ (Fig. 4) and is of comparable magnitude to variability in the δ13C of whole-forest respiration (Bowling et al., 2002; Lai et al., 2005). The isotopic composition of NEP starts with the δ13C of C flow from NPP but depends additionally on the variable decomposition rates of different plant tissues (Parton et al., 2007), and on the biosynthesis of new compounds by the soil microbial community. In terms of the net effect of terrestrial ecosystems on atmospheric (and oceanic) δ13C, other fluxes including fire, leaching of dissolved organic C compounds, and erosion can be important in some instances and are a part of the net ecosystem C balance.

The use of atmospheric δ13CO2 and CO2 measurements to partition land and ocean C sinks takes advantage of large differences in discrimination associated with C uptake by terrestrial ecosystems and by the oceans (Quay et al., 1992; Tans et al., 1993; Francey et al., 1995; Keeling et al., 1995; Heimann & Maier-Reimer, 1996). In past work, GPP-weighted estimates of ΔA have been used as estimates of discrimination associated with the net terrestrial C sink (Ciais et al., 1995), with recent work accounting for interannual variability in climate (Randerson et al., 2002a; Scholze et al., 2003; Suits et al., 2005) and time-varying contributions from C3 and C4 ecosystems (Townsend et al., 2002; Still et al., 2003). GPP-weighted estimates of ΔA are only appropriate for use with the net terrestrial sink when fractionations during plant tissue synthesis, C allocation and transport, and decomposition are negligible and when C storage is proportional to GPP. In light of the evidence provided here for substantial discrimination processes in terrestrial C cycling, manifested as systematic differences in δ13C of the net C record (net storage or release) of C from terrestrial ecosystems. The challenge is that the C pools that contribute to net storage vary considerably with the time scale of the net exchange – with consequences for δ13C of the net exchange. The discussion that follows is diagrammed conceptually in Fig. 5. For a fully developed forest canopy at midday over a period of a few minutes, for example, net C gain is probably dominated by photosynthesis and so the discrimination associated with net storage is near Δx. On this time scale, most of the C storage is associated with expanding pools of sucrose and starch in leaves. During the night, by contrast, the discrimination inferred from ecosystem respiration (nighttime Keeling plots; Fig. 5) may be smaller than the actual ΔA during the day, as a result of contributions from growth respiration associated with the construction of more reduced plant compounds (Section II, ‘Isotopic composition of plant metabolites and autotrophic respiration’). This explanation may resolve the contradiction in c/ε estimates using different methods identified by Tissue et al. (2006).

Over seasonal time scales, the contribution of plant respiration to the atmospheric record probably becomes less important because of its rapid turnover. Several lines of evidence show that plant respiration is composed mostly of recent assimilates that have a turnover time of days to a few weeks (Ekblad & Högbom, 2001; Högborg et al., 2001; Bowling et al., 2002; Brandes et al., 2006; Carbone et al., 2007). As a
result, plant respiration probably contributes only negligibly to the seasonal cycle of $\delta^{13}$CO$_2$ in the northern hemisphere, as the minimum in late spring (typically April or May) is separated from the maximum (typically August or September) by ~3–4 months (Trolier et al., 1996), a time interval one order of magnitude larger than the turnover time of the C pool associated with plant respiration. Instead, the seasonal atmospheric enrichment (and concurrent drawdown of CO$_2$) has to be driven primarily by growth from assimilates that have been transformed into longer-lived compounds associated with new plant tissues such as leaves, roots, or stems. Leaf and fine-root components of NPP are probably the most important contributors to the seasonal exchange because their combined growth is often 60–80% of total NPP (Saugier et al., 2001). As a result, discrimination inferred from a seasonal cycle inversion based on flask measurements of the seasonal cycle of $\delta^{13}$CO$_2$ and CO$_2$ (e.g. Randerson et al., 2002b) may be larger than GPP-weighted $\Delta_A$ (Fig. 5). In fact, it may be substantially larger than that inferred from nighttime Keeling plots (Fig. 5) or approaches that take advantage of synoptic CO$_2$ variability, including the departure of CO$_2$ and $\delta^{13}$CO$_2$ from a mean seasonal cycle (Bakwin et al., 1998; Miller et al., 2003), because sucrose and other recent assimilates contribute proportionally more to atmospheric CO$_2$ variability on these shorter time scales and are enriched relative to bulk leaf and litter biomass pools (Figs 2, 4).

While leaf and fine-root dynamics contribute substantially to seasonal exchange, they are less important for C storage on decadal time scales because leaf and fine-root litter pools decompose relatively rapidly and thus have long-term storage capacity. On decadal time scales, woody biomass and coarse woody debris pools play important roles in C storage of many terrestrial ecosystems. A primary mechanism contributing to the northern hemisphere terrestrial sink at the end of the 20th century, for example, was the accumulation of woody biomass in forest ecosystems as a result of decreasing fires and other forms of disturbance (Goodale et al., 2002; Hurtt et al., 2002). In the tropics, a primary contemporary loss pathway is deforestation, with the largest flux originating from the aboveground woody biomass pool (Houghton et al., 2000; Achard et al., 2002; Saatchi et al., 2007). Thus, even though changes in the wood pool have a relatively small effect on the $\delta^{13}$C of ecosystem respiration or on seasonal exchange, because of their role in long-term C storage the isotopic composition of the wood pool may be the most appropriate to assign to the net terrestrial C sink in partitioning studies that assess variability on interannual to decadal timescales.

On centennial to millennial timescales, C storage in wood is eclipsed by C storage in soils, and so for interpretation of Holocene or glacial-interglacial changes in the C cycle using ice core records (Trudinger et al., 1999; Kohler et al., 2006), a weighted combination of the $\delta^{13}$C of the wood and soil pools may be the most appropriate to assign to the net terrestrial flux. More generally, the isotopic composition of the terrestrial flux is likely to vary with its temporal scale and the measurement techniques used to diagnose it (Fig. 5).

VI. Conclusions and suggestions for further research

We have provided evidence to support the hypothesis that bulk leaf $\delta^{13}$C acts as an isotopic reference point from which there are predictable differences in the $\delta^{13}$C of plant and ecosystem C pools and fluxes. From this work, we have identified several questions or topics that merit additional research. These topics are compelling enough to move our understanding of C cycling forward substantially, and detailed investigations of these issues will likely lead to an enhanced understanding of plant and ecosystem C cycling and allocation patterns.

• What are the factors controlling the $\delta^{13}$C of respiration in plants?
• Why is the $\delta^{13}$C of phloem sap enriched relative to leaves? What factors control this enrichment?
• How does growth respiration contribute to the enrichment of ecosystem respiration?
• Where does assimilation fit on Fig. 4, at whole-forest, regional, and global scales?
• How does the $\delta^{13}C$ of whole-forest respiration compare with that of whole-forest assimilation, and how does the $\delta^{13}C$ of these fluxes change seasonally or interannually?
• Is root respiration generally as depleted as in Fig. 4? Are soil chamber isotope methods artifact-free, and if so, what is the source of enriched CO$_2$ that balances the depleted root respiration?

New and complementary tools for studying ecosystem C cycling will provide additional insight into the factors that cause isotopic variability in C pools and fluxes. Bomb (and experimentally added) radiocarbon is useful to study C cycling, particularly in longer-lived reservoirs such as wood and soils, but also in respiratory fluxes (Trumbore, 2000; Hahn et al., 2006; Schuur & Trumbore, 2006). Combined studies with $^{13}$C and $^{14}$C will yield very complementary information about C cycle processes (Carbone & Trumbore, 2007). Isotope labels can be applied to whole trees or whole ecosystems over periods from hours to years (Steinmann et al., 2004; Taneva et al., 2006; Carbone et al., 2007), and can be applied in very small amounts to investigate particular ecosystem processes (Ekblad et al., 2002; Czimczik et al., 2005). Real-time measurements of $\delta^{13}C$ of CO$_2$ are now possible in air (Bowling et al., 2005), and isotopic flux measurements can be made in real time using chambers (Barbour et al., 2007). Combinations of these methods with more traditional isotope sampling approaches are likely to be especially fruitful.

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