Fine-root turnover patterns and their relationship to root diameter and soil depth in a $^{14}$C-labeled hardwood forest

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Summary

• Characterization of turnover times of fine roots is essential to understanding patterns of carbon allocation in plants and describing forest C cycling. We used the rate of decline in the ratio of $^{14}$C to $^{12}$C in a mature hardwood forest, enriched by an inadvertent $^{14}$C pulse, to investigate fine-root turnover and its relationship with fine-root diameter and soil depth.

• Biomass and $\Delta^{14}$C values were determined for fine roots collected during three consecutive winters from four sites, by depth, diameter size classes (< 0.5 or 0.5–2 mm), and live-or-dead status.

• Live-root pools retained significant $^{14}$C enrichment over 3 yr, demonstrating a mean turnover time on the order of years. However, elevated $\Delta^{14}$C values in dead-root pools within 18 months of the pulse indicated an additional component of live roots with short turnover times (months). Our results challenge assumptions of a single live fine-root pool with a unimodal and normal age distribution.

• Live fine roots < 0.5 mm and those near the surface, especially those in the O horizon, had more rapid turnover than 0.5–2 mm roots and deeper roots, respectively.

Key words: $^{14}$C, carbon cycling, fine-root lifespan, fine-root turnover, live fine-root turnover time, root biomass, root diameter, root mortality.

Introduction

The ability to quantify the amount of carbon plants allocate to fine roots and its below-ground residence time is a major missing link in efforts to quantify and describe forest C cycles. Our ability to estimate below-ground C allocation through the most dynamic portion of the root system, typically assumed to be roots < 2 mm in diameter (‘fine roots’; Pregitzer et al., 2002), is still poor (Gower et al., 1996; Vogt et al., 1996). The large amount of work required to obtain representative samples of fine roots from soil necessary to calculate C fluxes has led to sampling and analysis strategies that use arbitrary, yet rather easily applied criteria (such as diameter) to categorize roots. These approaches assume normally distributed ages within fine-root populations. Recent work using isotopes and minirhizotrons, however, shows that these older approaches may not accurately represent complexity in the life-and-death cycles of fine roots (Gaudinski et al., 2001; Tierney & Fahay, 2002; Luo, 2003; Matamala et al., 2003; Trumbore & Gaudinski, 2003).

Most published literature on fine-root dynamics is based on a variety of methods for measuring fine-root turnover, all of which have major sources of error and bias (McClaugherty et al., 1982; Joslin & Henderson, 1982, 1987; Santantonio & Grace, 1987; Hendricks et al., 2006), but until recently have been our best sources of information on fine-root turnover. In a review of 59 forest studies, Gill & Jackson (2000) found estimates of mean turnover time of fine roots (defined variously
as roots < 1 to < 10 mm diameter) ranging from several months to 10 yr. For the finest roots (< 2 mm), turnover times ranged from 5 months to 2 yr, averaging 10 months.

Isotopic approaches measure the $^{13}$C or $^{14}$C content of root tissues in sites where the atmospheric record of $^{13}$CO$_2$ or $^{14}$CO$_2$ is known and has changed over time. Results with these approaches have shown that a significant portion of the fine-root population lives considerably longer than the 10-month average reported by Gill & Jackson (2000). Gaudinski et al. (2001) used the amount of ‘bomb’ $^{14}$C in fine roots to estimate the range of fine-root turnover time (FRTT) for trees as 3–18 yr, with a best estimate of 6–8 yr. Matamala et al. (2003), using a continuous $^{13}$C label from a Free-Air CO$_2$ Enrichment experiment, found that C in live woody fine roots (< 2 mm) had average turnover times of 1.2–5.7 yr.

Differences in estimated live FRTT as a function of method probably arise because different approaches are biased towards different ends of the FRTT spectrum (Gaudinski et al., 2001; Tierney & Fahey, 2002; Luo, 2003; Trumbore & Gaudinski, 2003). Accurate quantification of root-tissue C fluxes requires methods of characterizing turnover time distributions within a fine-root population, and of separating fine roots into pools that differ in their average turnover times.

But what criteria can be used to develop these distributions and make such separations? Over the past three decades, most scientists have sorted roots by diameter class and assumed that diameter was strongly correlated with live FRTT (Gill & Jackson, 2000; Pregitzer et al., 2002). There is evidence linking diameter to turnover time for live fine roots (Gaudinski et al., 2001; Wells & Eisenstat, 2001; Tierney & Fahey, 2002; Wells et al., 2002; Matamala et al., 2003; Baddeley & Watson, 2005). However, given the large number of studies that calculate root C fluxes based on this assumption, the strength of this relationship needs further evaluation and comparison with other root parameters, especially in mature forest species.

Another variable that may have an important relationship with live FRTT is soil depth. Increases in FRTT with depth have been seen in minihizotron studies of sugar maple (Hendrick & Pregitzer, 1996) and in fruit trees (Wells et al., 2002; Baddeley & Watson, 2005). Moreover, recent isotopic data also suggest that turnover time increases with depth, but are not conclusive (Gaudinski et al., 2001).

The goal of this study was to take advantage of an unplanned atmospheric release of $^{14}$CO$_2$ near a mature mixed-species forest surrounding Oak Ridge National Laboratory, TN, USA (Trumbore et al., 2002) to assess turnover in fine-root pools and to examine whether fine-root diameter size class and soil depth relate to live FRTT. Modifying the approaches of Caldwell & Campbell (1974) and Trumbore et al. (2002), we have used the rate at which $^{14}$C enrichment (the $^{14}$C: $^{13}$C ratio) declines over time in live root material to assess FRTT and to study its distribution with depth. The more rapidly fine roots turn over, the more quickly the $^{14}$C label in root tissues will decline as it is replaced (diluted) by new root-growth C not enriched in $^{14}$C. We also quantified live and dead root biomass at three annual intervals to estimate C storage in fine-root pools, its variability over time and space, and its distribution by depth.

Materials and Methods

Enriched Background Isotope Study (EBIS) sites

In 1999 and 2000, large inadvertent releases of $^{14}$CO$_2$, apparently from a local hazardous waste incinerator, labeled a wide area of the mature deciduous forests in the Department of Energy Oak Ridge Reservation (ORR; 36°N, 84°W) near Oak Ridge, TN, USA. Soil gas sampling at ORR detected elevated levels of atmospheric $^{14}$CO$_2$ in June 1999 (Trumbore et al., 2002; Gaudinski & Trumbore, 2003). The forest nearest the source (West ORR) received a much larger $^{14}$C label than the forest several miles distant (East ORR).

The EBIS project is studying the cycling and fate of ecosystem C using the $^{14}$C in the litter, roots and soil at the two strongly labeled West ORR sites and the two weakly labeled East ORR sites (Trumbore et al., 2002; Hanson et al., 2005). The West ORR sites are named Pine Ridge (PR) and Tennessee Valley Authority (TVA). The East ORR sites are Walker Branch Watershed (WB) and Haw Ridge (HR). All sites are located on ridges and upper slopes. The TVA and WB sites have cherty dolomitic limestone parent material, and the soils are typic Paleudults. The PR and HR sites have shale and/or sandstone parent material, and the soils are chiefly inceptic Hapluudults mixed with some typical Dystrudepts (Table 1).

Based on $^{14}$C in tree sapwood cellulose, East ORR received a pulse of $^{14}$CO$_2$ in 1999 and West ORR had slightly elevated tree core $^{14}$C content beginning in 1995 with a strong peak in 1999–2000 (Trumbore et al., 2002; http://ebis.ornl.gov/pretreat.html). After 2000, $^{14}$C levels returned to near-background (Trumbore et al., 2002; http://ebis.ornl.gov/pretreat.html). The elevation in $^{14}$C incorporated by plants over this period (1999 for East ORR; 1999–2000 for West ORR) will henceforth be referred to as ‘the enrichment’.

Continuous air sampling, initiated in September 2000, did not detect any growing season $^{14}$CO$_2$ pulses during the period of root sampling in this study. To estimate the $^{14}$C value of new photosynthate at ORR after the pulse, we analysed tree-ring cellulose (Hua et al., 1999). For 2001 and 2002, respectively, average tree ring $^{14}$C values for the two East ORR sites were 107 ± 3 and 95 ± 4%, and for West ORR sites 213 ± 28 and 187 ± 4% (C. Swanston, unpublished data). Tree-ring cellulose was used instead of the continuous air-sampling data for these estimates, because the latter included night-time air influenced by soil and plant respiration, and was therefore not representative of air taken up by foliage during the day. For reference, background northern hemisphere atmospheric $^{14}$CO$_2$ in 2001 and 2002 was 81 ± 2 and 75 ± 2%, respectively (Levin & Kromer, 2004). EBIS tree-ring cellulose $^{14}$C values are
slightly higher than atmospheric background in the northern hemisphere, probably because of local low-level atmospheric contamination, use of re-respired 14CO2 and possible use of stored C fixed in 1999. However, they are an order of magnitude lower than values observed during the enrichment (Trumbore et al., 2002; http://ebis.ornl.gov/pretreat.html).

Mean annual precipitation for the reservation is 1358 mm and mean annual temperature is 14.1°C (Johnson & Van Hook, 1989). All sites are upland-oak forest, with white oak (Quercus alba L.), chestnut oak (Quercus prinus L.), scarlet oak (Quercus coccinea Muenchh.), southern red oak (Quercus falcata Michx.) and northern red oak (Quercus rubra L.) constituting 70–83% of the total basal area at all sites except PR (P.J.H., personal communication; Table 1). At PR, oaks make up 46% of the basal area, while red maple (Acer rubrum L.) comprises a considerable portion (27%). Hickory species (Carya spp.), yellow poplar (Liriodendron tulipifera L.), black gum (Nyssa sylvatica Marsh.) and sourwood (Oxydendron arboreum L.) are minor overstory and understory components at all the sites. Total basal area ranged from 31 to 33 m2 ha−1 across the four sites in 2003. Further details on research projects and site locations and characteristics are given by Trumbore et al. (2002) and Hanson et al. (2005). Below-ground net primary productivity has been estimated to be 105 g C m−2 yr−1 for roots < 2 mm diameter at a nearby upland oak site [the Throughfall Displacement Experiment (TDE) site; Hanson et al., 2003] on the same watershed as the WB site (Joslin & Wolfe, 2003). Total net primary productivity at TDE has been reported to be 729 ± 69 g C m−2 yr−1 (Hanson et al., 2003).

Use of the ORR 14C dilution over time in fine-root tissues

Four processes result in the turnover of live fine-root C: new growth, mortality, respiration and exudation. The effect of respiration on root 14C is significant. However, the effect of respiration and exudation on the depletion of root 14C has been minimized in this study by collecting roots at least 12 months after the atmospheric 14C pulse. Allowing an entire growing season to elapse before sampling ensured that practically all the pulse-derived 14C in labile root C pools was respired, and the remaining pulse-derived 14C resided in the structural component of live fine roots (Milchunas et al., 1985; Milchunas & Lauenroth, 1992). Therefore subsequent annual sampling events measured changes in root 14C content that had resulted almost entirely from mortality losses and replacement by new growth. To be sure, many short-lived roots died during the 12–18-month period before sampling began. Insight into the mortality patterns of these short-lived roots came from observations of 14C concentrations in the dead-root pool.

The data from our three annual sample points allowed us to examine turnover in nonlabile fine-root C (structural tissue C) in these populations. Young live roots within a population with a short average turnover time (months) would quickly incorporate a large amount of 14CO2 from recent photosynthate. The Δ14C content of a portion of these roots would initially approach that of the atmospheric maximum. Subsequently, as atmospheric levels return to near background, the Δ14C content of this population would decline rapidly as the roots with high 14C content were replaced by roots formed from plant photosynthate that is less enriched in atmospheric 14CO2. By contrast, long average turnover times (years) would result in peak root Δ14C values substantially below the atmospheric maximum, and a longer time to return to background levels.

This study measured the 14C content of bulk live and dead fine-root tissues. Structural root components (primarily cellulose) do not exchange C after creation, thus the 14C content will remain stable after initial fixation (Farquhar et al., 1998; Barbour et al., 2004). Nonstructural carbohydrates (NSC) supply root growth, respiration and exudation. Despite both structural and nonstructural components being present in bulk root tissues, our measured Δ14C values largely represent the structural C components, for several reasons.

Table 1 Characteristics of the four Enriched Background Isotope Study (EBIS) sites with respect to 14C enrichment, parent material, soil classification, basal area and vegetation

<table>
<thead>
<tr>
<th>Site</th>
<th>Site location</th>
<th>Relative 14C enrichment</th>
<th>Soil subgroups and series</th>
<th>Basal area (m2 ha−1)*</th>
<th>Dominant overstory vegetation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tennessee Valley Authority (TVA)</td>
<td>West ORR</td>
<td>High</td>
<td>Typic Paleudults: Fullerton series, Bodine series</td>
<td>31.3</td>
<td>Chestnut oak, scarlet oak, white oak, southern red oak</td>
</tr>
<tr>
<td>Pine Ridge (PR)</td>
<td>West ORR</td>
<td>High</td>
<td>Inceptic Hapludult: Armuchee Typic Dystrudept: Lehw series</td>
<td>30.8</td>
<td>Chestnut oak, scarlet oak, red maple, white oak, yellow poplar</td>
</tr>
<tr>
<td>Walker Branch (WB)</td>
<td>East ORR</td>
<td>Low</td>
<td>Typic Paleudult: Fullerton series</td>
<td>33.3</td>
<td>Chestnut oak, white oak, scarlet oak, southern red oak, red maple</td>
</tr>
<tr>
<td>Haw Ridge (HR)</td>
<td>East ORR</td>
<td>Low</td>
<td>Inceptic Hapludult: Armuchee Typic Dystrudepts: Lehw &amp; Muskingum series</td>
<td>32.4</td>
<td>Chestnut oak, white oak, scarlet oak, southern red oak</td>
</tr>
</tbody>
</table>

*Basal area measurements taken in 2003.
First, the nonstructural components are a small fraction of the total mass. NSC in roots < 10 mm diameter comprise 3–13% of tissue dry weight in the upland oak forests on the ORR, depending on the time sampled, with a mean annual value of 9% (starch + sugar; McLaughlin et al., 1980). The size class of the McLaughlin et al. (1980) roots < 10 mm in diameter includes roots larger than our <2-mm-diameter roots; however, two other studies on similar species show either a decrease or little change in NSC content as a function of diameter (McClaugherty et al., 1982; Barbaroux et al., 2003). Additionally, low NSC content (<4%) has been shown for fine roots (<5 mm diameter) in white oak (Joslin, 1983) and other forest species (Wruth et al., 2005).

Second, our time-series sampling began over a year after the enrichment pulse (in January–February 2001), so virtually all the 14C-labeled labile carbohydrate pools were already respired or converted to structural tissue (Milchunas et al., 1985; Milchunas & Lauenroth, 1992). It is, however, possible that labile C from stored reserves containing elevated levels of 14C label was used in the construction of new roots in years following the initial 14C pulse. To quantify such use, we sampled the Δ14C values of newly grown roots using ingrowth screens. These new root Δ14C values decreased quickly after the period of enrichment (mean of 179% in the 2000 growing season at East ORR), to stable levels (means of 117 and 112% for the 2001 and 2002 growing seasons, respectively, at East ORR; J.B. Gaudinski and co-workers, unpublished data). This decrease after the enrichment episode indicates that new growth was primarily from newly fixed carbohydrates with a near-background level of 14C (Horwath et al., 1994). In summary, the small NSC mass relative to the total sample mass; lack of enriched NSC remaining in the sample because of respiration or conversion to structural tissues; and the fact that any new growth is from recently fixed nonenriched NSC sources all argue that the changes in Δ14C signature we measured in root populations over time primarily represent patterns of change for structural C forms.

Soil coring and subsampling
To measure their biomass and Δ14C values, roots were collected from organic horizons and mineral soil from eight plots at each of the four sites. Soil cores were collected in January and/or February 2001, 2002 and 2003. For each collection we determined (a) the mass of live and dead fine roots for two size classes at five depth intervals; and (b) the Δ14C value of fine-root subsamples from each category of roots. Only approx. 5% of all annual root growth occurs during the winter months in this ecosystem (Joslin et al., 2001). By sampling in January and February, we sampled at the time of lowest root growth. At each site, three cores were collected from each of eight plots with a gasoline-powered post-hole digger equipped with a detachable cylindrical steel core (10 cm diameter, 30 cm length). A hole-saw blade at the bottom of the corer sliced cleanly through all roots. Cores were divided into four mineral soil-depth intervals (0–15, 15–30, 30–60, 60–90 cm). Samples from three cores at each depth for each plot were composited to create a total of eight replicate mineral samples per site per depth interval (32 mineral soil samples per site) each year.

Soil rockiness or high bulk density frequently restricted coring depth. Coring to 30 cm was always successful and coring to 60 cm was usually achieved: In 2001, a depth of 53 cm or more was reached in 72% of the cores, and 50% of the cores could be sampled below 75 cm. The average depth of coring was greater in 2002 and 2003. Calculations of fine-root density for each depth interval were based on actual volume sampled. Cores were frozen until processed.

Three replicate samples per plot of the organic horizon (Oe + Oa subhorizons) were collected using a sampling ring. Ring diameter was 56.4 cm in 2001 and 28 cm in 2002 and 2003. In 2001, organic horizon samples were oven-dried at 65°C before subsampling and further processing. The drying that year made it impossible to perform the live and dead separations on roots from these samples. Therefore in 2001 only, we estimated the fraction of total live and dead roots, and their 14C-signatures, in the O horizon from O-horizon-like material containing fresh roots collected from dense root mats growing in the uppermost 2–3 cm of mineral soil in 0–15-cm cores. In these soils, dense mats of roots typically grow at the interface between the O horizon and mineral soil. In 2002 and 2003, the three O-horizon core samples from each plot were composited and refrigerated at 4°C. Within 5 d, each organic sample was subsampled (10 ± 2% by moist weight). The subsamples were refrigerated at 4°C and shipped within 5 d (overnight on ice) to the University of California, Santa Cruz for processing.

Separation, cleaning and sorting of roots from soil cores
Roots were separated from mineral soil following the outline shown in Fig. 1. Mineral soil samples were passed through a 12.5-mm-mesh sieve to remove large roots, rocks and coarse organic matter. Roots collected on top of the coarse screen were separated by hand from rocks and other organic matter and washed on a 2-mm sieve. Roots >2 mm diameter were measured using calipers for later determinations of oven-dry mass by diameter size class (2–5; 5–10; >10 mm). Roots <2 mm diameter were refrozen and added to samples of <2 mm roots that passed through the 12.5-mm sieve.

Material passing through the 12.5-mm sieve was washed through sieves of 2.00, 0.50 and 0.25 mm. All roots >2 mm diameter collected on the 2-mm screen were separated by hand using forceps and calipers, labeled, and refrozen for later determinations of oven-dry mass. All remaining roots (<2 mm diameter) from each sample collected on the 2-mm sieve were meticulously separated from rocks and nonroot material in trays of water by hand, using forceps. These collections
included all root fragments > 4 mm in length. Very fine root (< 0.5 mm diameter) fragments < 4 mm length were operationally defined as ‘soil organic matter’ and omitted because of the very long time required for a very small percentage of the total fine-root biomass.

Organic horizon samples were processed entirely by hand using forceps. Roots were separated into < 2- and > 2-mm classes, and the > 2-mm roots were dried and weighed. Similarly to the mineral soils, roots < 2 mm in diameter and very fine root fragments > 4 mm in length were separated from non-root material in trays of water.

All roots < 2 mm were further sorted into two size classes, < 0.5- and 0.5–2-mm diameter. The < 0.5-mm roots are considered the most dynamic and active in absorption processes for a number of tree species (Pregitzer et al., 1997, 1998, 2002). Our exploratory examination of samples sorted into more finely divided size classes (< 0.3, 0.3–0.5, 0.5–0.8, 0.8–1.1 and 1.1–2 mm) showed either no significant difference in \( \Delta^{14}C \) among live root diameter size classes (WB site), or changes in \( ^{14}C \) values at 0.5 mm (TVA site, live and dead roots) or 0.8 mm (PR site, live and dead roots; data not shown).

The amount of fine roots retained on the two smallest sieves (0.50 and 0.25 mm) was estimated by subsampling. Approximately 10% of the total fresh mass of all material (roots plus soil and organic matter) retained on the two smallest sieves from each sample was removed, weighed, and the fraction of the total fresh mass subsampled was recorded. Roots from these subsamples were meticulously removed from soil and organic matter with forceps to include all root fragments > 4 mm in length. All the 0.50-mm-sieved samples were subsampled and roots collected, whereas only a subset (12%) of samples collected on the 0.25-mm sieves was subsampled, as virtually no root material was found on this finest sieve.

### Sorting live from dead roots

Root samples < 2 mm diameter from three depth intervals (O horizon, 0–15 cm and 30–60 cm) were sorted into live and dead categories. Live roots were distinguished from dead roots based on tensile strength, integrity and color of vascular tissue (Vogt & Persson, 1991). Live and dead separations for the subsamples from the smallest two sieves (0.50 and 0.25 mm) were performed only for 2001 roots. In total there were 384 live or dead samples from the mineral soil [four sites \( \times \) eight plots \( \times \) two size classes \( \times \) two states (live and dead roots) \( \times \) three depths]. Each of these samples was oven-dried at 50°C for a minimum of 48 h and weighed.

### Sampling for \( \Delta^{14}C \) values

The 384 samples were composited in pairs from within the same size class, live or dead category, depth and plot to reduce the number of samples to be analysed; therefore the number analysed was reduced from 384 to 192. Compositing was achieved using the cone-and-quarter method, which consists of combining the samples to be composited and cutting the sample in half perpendicular to the long direction of the roots. One split per composite was ground in a Spex Certprep 8000M Mixer Mill (SPER SamplePrep LLC, Metuchen, NJ, USA) until a fine homogeneous powder was achieved (5–15 min). Samples were sent to Lawrence Livermore National Laboratory (LLNL) for \( ^{14}C \) analysis. The second split per composite was archived in a glass vial. In addition, a subset of 113 samples was also analysed for \( ^{13}C \) at LLNL.

Composited root subsamples were converted to graphite by hydrogen reduction (Vogel et al., 1987) and measured for \( ^{14}C \) content on an accelerator mass spectrometer at the Center for Accelerator Mass Spectrometry, LLNL. Radiocarbon results are expressed as \( \Delta^{14}C \), which includes a \( ^{13}C \) rectification to correct...
for the effects of mass-dependent isotopic fractionation, such as discrimination against atmospheric $^{14}$C during photosynthesis (Stuiver & Polach, 1977). For root samples from mineral soil cores, we determined $^{13}$C values of 113 samples (out of 576 total samples analysed for $^{14}$C) and used the sample-specific $^{13}$C value. For the remaining 463 samples we used the mean of these 113 (28.1‰) to make the $^{13}$C correction.

Determination of root mass per unit area by category

Oven-dried root mass was calculated for each category of roots from each plot and year as described above, for four screen-mesh sizes: 12.5 mm, 2 mm, subsamples of 0.50 mm, and subsamples of a subset of 0.25 mm (Fig. 1). These data were combined to calculate total root mass per area sampled for (1) living < 0.5 mm roots; (2) living 0.5–2 mm roots; (3) dead < 0.5-mm roots; and (4) dead 0.5–2-mm roots by depth interval, site and year. As described above, values were adjusted for (a) samples where only a portion of the intended depth interval was obtained by coring; (b) subsampling of material from the two smallest sieve sizes; and (c) subsampling of the O horizon material.

For two depth intervals (15–30 and 60–90 cm), live and dead roots were not separated. The 15–30-cm live and dead fractions were interpolated from the two adjacent depths and the 60–90-cm fractions were assumed to be the same as the depth above. Live and dead fractions were multiplied by their respective total root mass to estimate values of live and dead root masses for these depth intervals.

Calculation of fine-root turnover time index

We have used the decrease in live root $^{14}$C enrichment (the $^{14}$C : $^{12}$C ratio) over time to estimate an equivalent one-pool index of live fine-root turnover time, $T$ (yr), which we refer to here as the ‘FRTT index’. As we contend that our data suggest at least a two-pool system, $T$ should not be construed as a mean estimate of FRTT. Rather, we treat $T$ as an index useful to judge relative differences in turnover rates at a given size class and depth for these roots that have already survived at least 12–18 months.

Our approach to estimating $T$ is analogous to the approach described by Trumbore et al. (2002) to estimate turnover time. We assume the $^{13}$C and $^{14}$C fluxes out of a given root pool are first-order with respect to mass within the pool. A mass balance for $^{14}$C, assuming that the root pool $^{12}$C content is in steady state, gives:

$$\frac{d\Delta}{dt} = \Delta_p - \Delta - \lambda \Delta$$  \quad \text{Eqn 1}$$

Here $\Delta$ is the $^{14}$C value (‰) of the root pool at time $t$ (yr) and $\Delta_p$ is the $^{14}$C value (‰) of new photosynthate. $\Delta$ was determined from tree-ring cellulose data as described previously, and was found to be virtually constant over the 2 yr after the pulse, but different for East ORR (101‰) and West ORR (200‰).

In equation 1, the impact of radioactive decay on the $^{14}$C mass balance ($\lambda$) is negligible compared with the other terms, and is ignored. Solving equation 1 for $\Delta$, as a function of time by integration, and rearranging, gives:

$$T = -\Delta / \ln[(\Delta_t - \Delta) / (\Delta_0 - \Delta_p)]$$  \quad \text{Eqn 2}$$

where $\Delta_t$ is 2 yr and $\Delta_0$ is the $^{14}$C value of the root pool in 2001. Equation 2 differs from the analogous relationship developed in Trumbore et al. (2002) by taking into account the $^{14}$C content of new photosynthate. For a given site (East ORR or West ORR), size class and depth interval, $T$ was calculated using the best fit to all data points measured in 2001, 2002 and 2003.

Statistical analysis

Differences in biomass or $\Delta^{14}$C values among sites, years, depths and size classes were analysed statistically using univariate ANOVA with Tukey's post hoc analysis to determine which means across a single variable were significantly different from each other. Differences in mean values between East ORR sites (HR and WB data combined) and West ORR sites (TVA and PR data combined) as a function of year, depth and size class were analysed separately using simple t-tests. Results are reported as significant if $P < 0.05$. Analyses were performed with the programs SAS (SAS Institute, 1989) and SYSTAT 10.2.

Results

Characterization of fine-root mass across sites, size classes, depth and time

The total mass of live and dead fine roots < 2 mm, averaged across 3 yr for all sites, was 788 ± 24 g m$^{-2}$ (± SEM; Fig. 2). The 3-yr averages for each of the four sites were within 14% of this mean. Nevertheless, there were statistically significant differences among sites in live and dead root mass (Fig. 2). Haw Ridge had the largest mass of both live and dead roots, and TVA had the smallest.

There were almost even proportions of live and dead roots across all sites, size classes and depths. Total live root biomass (418 ± 13 g m$^{-2}$) averaged only slightly higher (53% of total mass) than total dead root mass (370 ± 12 g m$^{-2}$; Figs 2, 3). In the O horizon, however, > 70% of root mass was live roots in each size class. Of the total (live + dead) root mass, 0.5–2.0-mm roots comprised, on average across sites, 56% of the total (mean = 445 ± 26 compared with 343 ± 26 g m$^{-2}$ for the < 0.5-mm root class).

Averaging across all sites, approx. 50% of the total profile fine-root mass was in the 0–15-cm layer of the mineral soil (Fig. 3a,b). The 15–30-cm depth interval contained 16% of
total root mass. The thicker 30–60-cm interval contained 17%, while 60–90 cm had approx. 10%. The O horizon (1–5 cm thick) contained 7%. There were significant differences in the size-class composition with depth. In the O horizon, 65% of the root mass was in the < 0.5-mm diameter class, whereas in the mineral soil only 34–50% of the mass was < 0.5 mm (Fig. 3a,b). There were no statistically significant changes in live root biomass between the three annual samplings (data not shown).

Fine-root Δ14C values over time

General patterns There were no statistically significant differences in Δ14C values between the two West ORR sites (PR and TVA). Similarly, there were no statistically significant differences in Δ14C values between the two East ORR sites (WB and HR) in 35 of 36 comparisons. Therefore we felt justified in simplifying our analysis by averaging values for the two East ORR sites and for the two West ORR sites. West ORR values were consistently 100–225‰ higher than East ORR values for roots of similar size class, depth, or live-or-dead status, providing further justification for this simplification. The Δ14C values across the various root categories from West ORR ranged from 261 to 455‰; East ORR sites from 125 to 294‰ (Table 2; Fig. 4).

The time series for live (Fig. 4; Table 2) and dead roots (Table 2) showed that Δ14C values from both East ORR and West ORR were well above the atmospheric background during the entire 3-yr sampling period, demonstrating the substantial influence of the enrichment pulse. When significant differences were observed in Δ14C between 2001 and 2003, the trend was always towards decreasing Δ14C, with the largest decrease in the first year (2001–02). The decrease in Δ14C values over the entire study period ranged from 27 to 160‰ (West ORR) and from 4 to 133‰ (East ORR), depending on size class, depth, and live-or-dead status (Table 2).

Root diameter size class and changes in Δ14C values over time Live roots < 0.5 mm diameter exhibited statistically significant decreases in Δ14C value over the 2-yr period in four out of six comparisons (two locations × three depths), and 0.5–2-mm root Δ14C values decreased significantly in two out of six comparisons (Fig. 4). Total declines in Δ14C values towards background (the value of new photosynthate as determined from tree cellulose) over 2 yr were larger for < 0.5-mm live roots (45 and 97‰ for East and West ORR, respectively, based on averaging the three depth intervals; Table 2) than for 0.5–2-mm roots (37 and 36‰ for East and West ORR, respectively). Dead root Δ14C values exhibited a similar pattern (Table 2).

The FRTT indices for the 2001–03 period and each category of live roots are shown in Table 3. Averaging across all sites and size classes, regardless of statistical significance of decreases in Δ14C value, live roots < 0.5 mm in diameter had
a smaller FRTT index (4.1 ± 1.1 yr) than roots 0.5–2 mm in diameter (9.6 ± 2.7 yr). These calculations assume that all roots at a given soil depth within a given site (East or West ORR) behave as one homogeneous pool with a unimodal distribution of root ages within the population.

Relationship between soil depth and changes in $\Delta^{14}C$ values over time The decrease in live root $\Delta^{14}C$ values between 2001 and 2003 was largest in the O horizon, and declined with depth. Averaging the two size classes, total declines in $\Delta^{14}C$ values from 2001 values were 81, 24 and 17.5‰ for East ORR O horizon, 0–15- and 30–60-cm intervals, respectively, and 79, 59 and 53‰ for West ORR O horizon, 0–15- and 30–60-cm intervals, respectively (Fig. 4; Table 2). The three shortest FRTT indices were for O-horizon roots, and in all cases except West ORR 0.5–2-mm roots, FRTT indices increased with depth (Table 3). Averaging across all sites and size classes, regardless of statistical significance of decreases in $\Delta^{14}C$ value, gave average FRTT indices of 3.8 ± 2.4, 7.5 ± 2.8 and 9.2 ± 3.6 yr for O-horizon, 0–15-mm and 30–60-mm roots, respectively.

### Discussion

Fine-root mass and its distribution by live or dead category, size class, and depth

The mean total fine-root mass (< 2 mm diameter, live plus dead) for the four sites across 3 yr (788 g m$^{-2}$) was similar to earlier estimates from the upland-oak TDE site (750 g m$^{-2}$) located near the WB site (Joslin & Wolfe, 1999) and an upland oak site on an Ultisol comparable to soils at WB and TVA (828 g m$^{-2}$; Joslin & Henderson, 1987). Fine-root mass for other temperate deciduous forests has been reported in the same range (320–950 g m$^{-2}$; Hendrick & Pregitzer, 1993; 780 g m$^{-2}$; Jackson et al., 1997).

### Table 2

<table>
<thead>
<tr>
<th>Year sampled*</th>
<th>Depth</th>
<th>Side of reservation</th>
<th>Live &gt; 0.5–2 mm</th>
<th>Live &lt; 0.5 mm</th>
<th>Dead &gt; 0.5–2 mm</th>
<th>Dead &lt; 0.5 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>O horizon</td>
<td>East</td>
<td>202 (11)</td>
<td>240 (11)</td>
<td>223 (13)</td>
<td>298 (27)</td>
</tr>
<tr>
<td>2002</td>
<td>O horizon</td>
<td>East</td>
<td>130 (11)</td>
<td>169 (11)</td>
<td>153 (3)</td>
<td>160 (31)</td>
</tr>
<tr>
<td>2003</td>
<td>O horizon</td>
<td>East</td>
<td>126 (3)</td>
<td>154 (11)</td>
<td>143 (3)</td>
<td>163 (13)</td>
</tr>
<tr>
<td>Change 2001–02</td>
<td>O horizon</td>
<td>East</td>
<td>72</td>
<td>71</td>
<td>70</td>
<td>139</td>
</tr>
<tr>
<td>Change 2002–03</td>
<td>O horizon</td>
<td>East</td>
<td>426 (33)</td>
<td>366 (36)</td>
<td>459 (13)</td>
<td>376 (47)</td>
</tr>
<tr>
<td>2001</td>
<td>O horizon</td>
<td>West</td>
<td>330 (6)</td>
<td>306 (14)</td>
<td>330 (15)</td>
<td>263 (33)</td>
</tr>
<tr>
<td>2002</td>
<td>O horizon</td>
<td>West</td>
<td>297 (12)</td>
<td>337 (28)</td>
<td>296 (13)</td>
<td>294 (47)</td>
</tr>
<tr>
<td>Change 2001–02</td>
<td>O horizon</td>
<td>West</td>
<td>96</td>
<td>60</td>
<td>129</td>
<td>113</td>
</tr>
<tr>
<td>Change 2002–03</td>
<td>O horizon</td>
<td>West</td>
<td>33</td>
<td>–31</td>
<td>34</td>
<td>–31</td>
</tr>
<tr>
<td>2001</td>
<td>0–15 cm</td>
<td>East</td>
<td>192 (4)</td>
<td>255 (15)</td>
<td>230 (13)</td>
<td>270 (10)</td>
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<tr>
<td>2002</td>
<td>0–15 cm</td>
<td>East</td>
<td>182 (3)</td>
<td>248 (11)</td>
<td>203 (5)</td>
<td>246 (11)</td>
</tr>
<tr>
<td>2003</td>
<td>0–15 cm</td>
<td>East</td>
<td>163 (5)</td>
<td>236 (12)</td>
<td>211 (19)</td>
<td>253 (11)</td>
</tr>
<tr>
<td>Change 2001–02</td>
<td>0–15 cm</td>
<td>East</td>
<td>10</td>
<td>7</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>Change 2002–03</td>
<td>0–15 cm</td>
<td>East</td>
<td>19</td>
<td>12</td>
<td>–8</td>
<td>–7</td>
</tr>
<tr>
<td>2001</td>
<td>0–15 cm</td>
<td>West</td>
<td>372 (14)</td>
<td>321 (9)</td>
<td>424 (15)</td>
<td>392 (22)</td>
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<tr>
<td>2002</td>
<td>0–15 cm</td>
<td>West</td>
<td>332 (8)</td>
<td>312 (13)</td>
<td>330 (10)</td>
<td>310 (10)</td>
</tr>
<tr>
<td>2003</td>
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<td>West</td>
<td>279 (15)</td>
<td>294 (8)</td>
<td>302 (6)</td>
<td>297 (6)</td>
</tr>
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<td>West</td>
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<td>7</td>
<td>91</td>
<td>89</td>
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<tr>
<td>Change 2002–03</td>
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<td>53</td>
<td>18</td>
<td>28</td>
<td>14</td>
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<tr>
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<td>30–60 cm</td>
<td>East</td>
<td>236 (6)</td>
<td>235 (13)</td>
<td>240 (7)</td>
<td>229 (21)</td>
</tr>
<tr>
<td>2002</td>
<td>30–60 cm</td>
<td>East</td>
<td>232 (10)</td>
<td>258 (12)</td>
<td>227 (9)</td>
<td>258 (19)</td>
</tr>
<tr>
<td>2003</td>
<td>30–60 cm</td>
<td>East</td>
<td>207 (8)</td>
<td>229 (19)</td>
<td>204 (9)</td>
<td>225 (11)</td>
</tr>
<tr>
<td>Change 2001–02</td>
<td>30–60 cm</td>
<td>East</td>
<td>4</td>
<td>–23</td>
<td>13</td>
<td>–29</td>
</tr>
<tr>
<td>Change 2002–03</td>
<td>30–60 cm</td>
<td>East</td>
<td>4</td>
<td>29</td>
<td>23</td>
<td>33</td>
</tr>
<tr>
<td>2001</td>
<td>30–60 cm</td>
<td>West</td>
<td>365 (18)</td>
<td>375 (28)</td>
<td>417 (28)</td>
<td>399 (29)</td>
</tr>
<tr>
<td>2002</td>
<td>30–60 cm</td>
<td>West</td>
<td>325 (11)</td>
<td>329 (24)</td>
<td>324 (12)</td>
<td>314 (22)</td>
</tr>
<tr>
<td>2003</td>
<td>30–60 cm</td>
<td>West</td>
<td>314 (14)</td>
<td>315 (11)</td>
<td>288 (5)</td>
<td>289 (5)</td>
</tr>
<tr>
<td>Change 2001–02</td>
<td>30–60 cm</td>
<td>West</td>
<td>40</td>
<td>46</td>
<td>93</td>
<td>85</td>
</tr>
<tr>
<td>Change 2002–03</td>
<td>30–60 cm</td>
<td>West</td>
<td>11</td>
<td>9</td>
<td>36</td>
<td>25</td>
</tr>
</tbody>
</table>

$n = 8$; values in parenthesis represent standard errors.

*Cores sampled in January or February of year indicated. Any new growth was from previous year’s growing season.
Both live and dead root masses remained relatively constant across three annual sampling events at the four sites, suggesting that both live and dead fine-root mass were near steady state. The mass of live fine roots was approximately equal to the mass of dead fine roots in the mineral soil to 90 cm. Jackson et al. (1997) similarly found that the live fine-root fraction averaged 56% in temperate deciduous forests. By contrast, in the O horizon live root mass was two to three times larger than dead root mass. This could mean either (1) more rapid decomposition of dead roots in the O horizon relative to mineral horizons; or (2) longer turnover times for live fine roots in the O horizon. More rapid decomposition was corroborated by two results. First, the $\Delta^{14}C$ trends and FRTT indices indicate shorter turnover times for live roots in the O horizon than in mineral horizons. Second, the $\Delta^{14}C$ values of dead roots in the O horizon decreased more rapidly than those of dead roots from the mineral horizons (Table 2).

Fine live-root turnover time as assessed by dilution of the $^{14}C$ label

All pools sampled at all sites retained a significant amount of the enrichment through January–February 2003, > 2.5 yr after the initial enrichment (Fig. 4). Therefore the mean single-pool turnover time of all live fine roots < 2 mm sampled in this study must be on the order of years and not months. Live fine-root $\Delta^{14}C$ values ranged, across all depths, sites and size classes in 2003, from 125 to 235‰ (East ORR) and from 277 to 335‰ (West ORR). These values were considerably higher than that of new root growth taken from ingrowth screens in the 2002 growing season (112 and 260‰ for East and West ORR, respectively). These results confirm that a major portion of the live root mass that we sampled had average turnover times much longer than one growing season.

While the live fine roots had an average turnover time of the order of years, the dead root $\Delta^{14}C$ data indicate that there must be a component of fast-cycling live fine roots with turnover times of the order of months. Within 18 months on the enrichment pulse, the $\Delta^{14}C$ values of the dead-root pools on

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**Table 3** Fine-root turnover time index (yr) by depth, site, and diameter size class

<table>
<thead>
<tr>
<th>Parameter</th>
<th>East ORR</th>
<th>West ORR</th>
<th>East ORR</th>
<th>West ORR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O horizon</td>
<td>&lt; 0.5 mm</td>
<td>1.4*</td>
<td>2.3*</td>
<td>1.5*</td>
</tr>
<tr>
<td>0–15 cm</td>
<td>4.9</td>
<td>3.2*</td>
<td>14.4</td>
<td>7.6</td>
</tr>
<tr>
<td>30–60 cm</td>
<td>8.1</td>
<td>4.9*</td>
<td>18.3</td>
<td>5.5*</td>
</tr>
</tbody>
</table>

* Decreases in $\Delta^{14}C$ values over the 2 yr of the study were calculated from statistically significant decreases ($P < 0.05$).
the East ORR (Table 2) were 44–119% higher than that of new roots grown in the previous growing season on the East ORR (179%). Such rapid and pronounced elevations of dead root $\Delta^{14}C$ values suggest significant inputs from recent mortality of highly enriched live root material. Minirhizotron and root-screen studies in other forested ecosystems report that approx. 50% of roots do not survive > 1 yr (Fahey & Hughes, 1994; Burton et al., 2004). Our results indicate that our live root samples, collected annually in January–February, were not appropriately representing the population of ‘fast-turnover roots’. Minirhizotron and root-screen studies, combined with our observation of rapid enrichment of dead fine-root pools with $^{14}C$, strongly suggest that live fine-root populations must contain both short-lived and long-lived roots.

Recent work in other deciduous temperate forests has shown that fine roots have mean turnover times of 1.2–18 yr (Matamala et al., 2003; Gaudinski et al., 2001; Tierney & Fahey, 2002). These turnover time estimates were all made assuming a single pool of roots with a normal distribution of FRTT. However, our data and results of recent work (Tierney & Fahey, 2002; Luo, 2003; Trumbore & Gaudinski, 2003) indicate that such a unimodal approach is not an accurate representation of below-ground dynamics. The use of a unimodal distribution for turnover time is contradicted by the observation that one component of the fine-root system has a turnover time on the order of several years, while another component has a turnover time on the order of months.

The mounting evidence for complex, potentially bimodal patterns in root mortality in temperate forests (Gaudinski et al., 2001; Tierney & Fahey, 2002; Matamala et al., 2003; Trumbore & Gaudinski, 2003; Majdi et al., 2005) probably also has relevance for grass and shrubland ecosystems as well. The mean FRTTs (which assume unimodal distribution in turnover time for roots sampled) for forest, grass and shrubland, presented in a review of 190 published studies by Gill & Jackson (2000), were 1.7, 1.8 and 2.9 yr, respectively, yet study-specific turnover times within these three global ecosystem types (including temperate, tropical and boreal) vary from 0.5 to 12, 0.6 to 20 and 0.8 to 8.1 yr, respectively. The wide range for a given ecosystem type is probably partly caused by species and methodological differences. However, we hypothesize that grassland and shrubland FRTTs have ranges as wide as forests, partly because fine-root populations in these ecosystems have non-normal and possibly bimodal age distributions that are similar to trees. Indeed, minirhizotron studies in grasslands have shown bimodal distributions in fine-root survivorship (Fitter et al., 1998).

Live-root turnover time and diameter size class

The FRTT indices presented in Table 3 are an oversimplification of fine-root ecology as the $\Delta^{14}C$ results discussed above can be explained only with a minimum of two populations of roots, cycling at very different time intervals. However, these indices did allow for comparison of $\Delta^{14}C$ declines across size class and depth categories. Statistically significant decreases in $\Delta^{14}C$ values over the measurement period occurred in six out of 12 categories (Table 3). However, lack of statistical significance does not suggest that estimates of $\Delta^{14}C$ decline were any less real, only that the rate of root turnover was too slow to produce a statistically significant change during the 2-yr measurement period. As described in Materials and Methods, FRTT indices were calculated using all values, regardless of whether decreases in $\Delta^{14}C$ values were statistically significant.

Support for a relationship between fine-root diameter and turnover time has been seen in recent work using both minirhizotrons and isotopic approaches. Minirhizotron approaches have shown statistically significant correlations between turnover time and diameter for live roots < 1 mm diameter when diameter was measured in 0.1-mm increments (Wells & Eissenstat, 2001; Tierney & Fahey, 2002; Baddeley & Watson, 2005). For example, Tierney & Fahey (2002) found the likelihood of root survival within a cohort increased dramatically with diameter (43% for each 0.1 mm for roots < 0.5 mm; $P < 0.001$). Isotopic studies have shown that FRTT increases 1–3 yr with diameter size class, between < 1, 1–2 and 2–5 mm in one study (Matamala et al., 2003), and increases similarly between < 2– or = 2-mm diameter size classes in another (Gaudinski et al., 2001). These studies, along with the data presented here (Table 2; Fig. 4), provide evidence for a relationship between live FRTT and fine-root diameter.

In contrast, a relationship between diameter and the FRTT index was not corroborated by our exploratory $\Delta^{14}C$ analysis of a small subset of roots separated into much finer diameter size classes (< 0.3, 0.3–0.5, 0.5–0.8, 0.8–1.1 and 1.1–2 mm). In this analysis there was little difference in $\Delta^{14}C$ among live-root diameter size classes in 2001 fine roots. The small sample size, combined with the mixed species sampling, may have been a factor in the lack of relationship between diameter and the FRTT index because species can vary widely in mean root diameter (Pregitzer et al., 2002).

The trends in FRTT index show a large degree of variation between East and West ORR for a given diameter size class, despite generally similar forest types. It is likely that other parameters are also related to FRTT index and could influence the strength of its relationship to diameter if not accounted for. Root-branching structure is a key characteristic of root systems and governs both root form and function (Fitter, 2002). First- and second-order fine-root branches (the most distal portions of the fine-root system) have been shown to have greater N content and specific root length in 10 North American tree species (Pregitzer et al., 2002; Guo et al., 2004). Guo et al. (2004) also found that cellulose content increased dramatically from first- to fifth-order roots, and suggest that cellulose content may be related to branch order and root age. Wells et al. (2002) provided direct minirhizotron evidence...
that ‘apparent first-order’ roots in peach have median life spans of 3–4 months, whereas ‘higher-order roots’ have median life spans of 7–8 months. Tissue density, mycorrhizal colonization and secondary phenolic compounds may also affect FRTT (Eissenstat et al., 2000). Further exploration of the impact of these factors on FRTT and their relationship with diameter will probably allow us to estimate FRTT with greater accuracy than sorting roots by diameter alone.

Root turnover time and soil depth

Our data suggest that the FRTT index was smaller in the organic horizon than in the mineral soil, and that within the mineral soil horizons, FRTT indices also tended to increase with depth.

Other studies of temperate tree species have also shown an increase in FRTT with depth, with the most rapid fine-root turnover occurring in the upper 20 cm (Hendrick & Pregitzer, 1996; Gaudinski et al., 2001; Wells et al., 2002; Baddeley & Watson, 2005). Soil bulk density, clay content, moisture, nutrient content, temperature and organisms generally change with depth in the soil profile, and FRTT is probably affected by these characteristics.

Future research directions

Acknowledgements that fine-root populations contain short-lived and long-lived pools is a significant leap in our conceptual approach to below-ground research. Unfortunately, accurately quantifying below-ground C fluxes and a better understanding of fine-root ecology are still elusive because we do not know the proportions of short-lived vs long-lived root pools or the distribution of turnover times within these pools. We must explore new ways of sampling and modeling these below-ground dynamics. While separating fine roots into diameter size classes appears to produce a meaningful division, other factors such as position on the root branch system, chemical composition and mycorrhizal association (Pregitzer et al., 1997; Bidartondo et al., 2001; Wells & Eissenstat, 2001; King et al., 2002; Pregitzer et al., 2002) may also need to be incorporated in order to obtain the most accurate and consistent estimates of FRTT. Inclusion of such strategies has largely been avoided because of the difficulties of sampling roots in soil. (But cf. Guo et al., 2004 for a novel way to characterize biomass by branch order.) It is likely that a combination of minirhizotron, isotopic and coring techniques, used in concert with new root-sorting approaches, will be required to further improve our understanding of fine-root dynamics.

Modeling of fine roots has undergone little development since early attempts in the 1970s, largely because of the lack of solid data on the behavior of fine roots (Luo, 2003; Majdi et al., 2005). Despite a range of methodological approaches, the fine-root modeling in the literature is remarkably uniform in its use of a one-pool approach with a normal distribution of turnover times. To account for the complexities of below-ground fine-root dynamics, these models should recognize the high probability of non-normal age distributions for live and dead fine-root populations, multiple live and dead root carbon pools, or both (Trumbore & Gaudinski, 2003; Majdi et al., 2005). Translocation of plant-derived C below ground can be constrained by measurements of soil respiration and leaf litter fall (Giardina & Ryan, 2002). Additionally, measurement of isotope fluxes in soil gas as well as in root tissues should be used to constrain fine-root turnover (Trumbore et al., 2006). Validation of new sampling and modeling approaches will probably require working at sites where all below-ground C fluxes are actively measured.

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References


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