New Phytologist

Research

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

J. D. Joslin[1], J. B. Gaudinski[2], M. S. Torn[3], W. J. Riley[3] and P. J. Hanson[4]

[1]Below-ground Forest Research, Apartado 104-5655, Monteverde, Puntarenas, Costa Rica; [2]Department of Environmental Studies, University of California, Santa Cruz, CA, USA; [3]Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA; [4]Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA

Author for correspondence:
J. D. Joslin
Tel: +1 506 645 7043
Fax: +1 506 645 5702
Email: devjoslin@hotmail.com

Received: *9 May 2006*
Accepted: *15 June 2006*

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.

New Phytologist (2006) **172**: 523–535

doi: 10.1111/j.1469-8137.2006.01847.x

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 & Fahey, 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 & Eissenstat, 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 minirhizotron 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 & Camp (1974) and Trumbore *et al.* (2002), we have used the rate at which ^{14}C enrichment (the $^{14}C : {}^{12}C$ 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 Hapludults mixed with some typic 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 $\Delta^{14}C$ 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 $\Delta^{14}C$ 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 $\Delta^{14}CO_2$ in 2001 and 2002 was 81 ± 2 and 75 ± 2‰, respectively (Levin & Kromer, 2004). EBIS tree-ring cellulose $\Delta^{14}C$ values are

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

Site	Site location	Relative ^{14}C enrichment	Soil subgroups and series	Basal area (m^2 ha^{-1})*	Dominant overstory vegetation
Tennessee Valley Authority (TVA)	West ORR	High	Typic Paleudults: Fullerton series, Bodine series	31.3	Chestnut oak, scarlet oak, white oak, southern red oak
Pine Ridge (PR)	West ORR	High	Inceptic Hapludult: Armuchee Typic Dystrudept: Lehew series	30.8	Chestnut oak, scarlet oak, red maple, white oak, yellow poplar
Walker Branch (WB)	East ORR	Low	Typic Paleudult: Fullerton series	33.3	Chestnut oak, white oak, scarlet oak, southern red oak, red maple
Haw Ridge (HR)	East ORR	Low	Inceptic Hapludult: Armuchee Typic Dystrudepts: Lehew & Muskingum series	32.4	Chestnut oak, white oak, scarlet oak, southern red oak

*Basal area measurements taken in 2003.

slightly higher than atmospheric background in the northern hemisphere, probably because of local low-level atmospheric contamination, use of re-respired $^{14}CO_2$ 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 m^2 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 ^{14}C 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 ^{14}C is significant. However, the effect of respiration and exudation on the depletion of root ^{14}C has been minimized in this study by collecting roots at least 12 months after the atmospheric ^{14}C pulse. Allowing an entire growing season to elapse before sampling ensured that practically all the pulse-derived ^{14}C in labile root C pools was respired, and the remaining pulse-derived ^{14}C 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 ^{14}C 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 ^{14}C 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 $^{14}CO_2$ from recent photosynthate. The $\Delta^{14}C$ content of a portion of these roots would initially approach that of the atmospheric maximum. Subsequently, as atmospheric levels return to near backround, the $\Delta^{14}C$ content of this population would decline rapidly as the roots with high ^{14}C content were replaced by roots formed from plant photosynthate that is less enriched in atmospheric $^{14}CO_2$. By contrast, long average turnover times (years) would result in peak root $\Delta^{14}C$ values substantially below the atmospheric maximum, and a longer time to return to background levels.

This study measured the ^{14}C content of bulk live and dead fine-root tissues. Structural root components (primarily cellulose) do not exchange C after creation, thus the ^{14}C 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 $\Delta^{14}C$ values largely represent the structural C components, for several reasons.

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 (Wurth *et al.*, 2005).

Second, our time-series sampling began over a year after the enrichment pulse (in January–February 2001), so virtually all the ^{14}C-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 ^{14}C label was used in the construction of new roots in years following the initial ^{14}C pulse. To quantify such use, we sampled the Δ^{14}C values of newly grown roots using ingrowth screens. These new root Δ^{14}C 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 ^{14}C (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 Δ^{14}C 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 Δ^{14}C 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 Δ^{14}C 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 ^{14}C-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

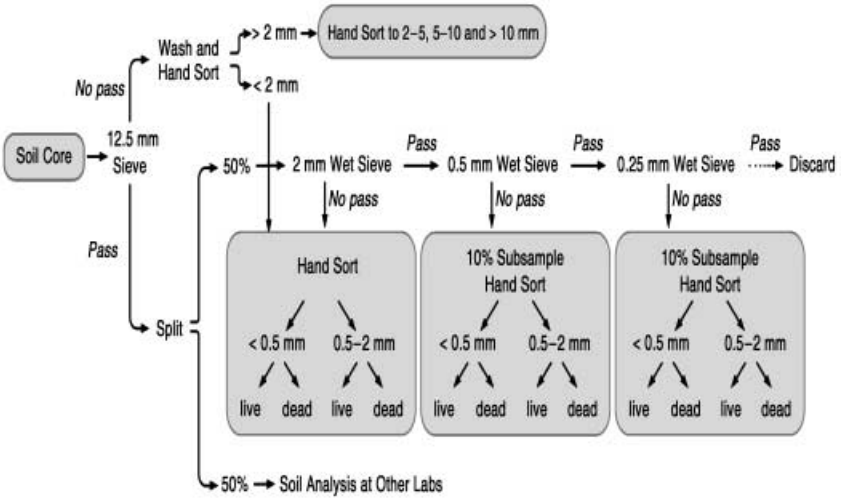

Fig. 1 Methods for sorting each mineral soil core. At each of four sites there were three replicate core holes from each of four plots taken at the following mineral soil depths: 0–15-, 15–30-, 30–60- and 60–90-cm depth intervals. Three replicate cores from each plot were composited by depth before sorting. Roots from the O horizon (sampled separately) and all mineral soil depths were sorted into < 0.5- or 0.5–2-mm size classes. Roots from the O horizon, 0–15 cm and 30–60 cm were also sorted into live vs dead categories. Live–dead fractions were interpolated for 15–30 cm; for the 60–90-cm depth interval they were assumed to be equal to the 30–60-cm. For each root category, eight live and eight dead samples were composited to four live and four dead samples for $\Delta^{14}C$ analysis (see text for details).

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 × eight plots × two size classes × two states (live and dead roots) × 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 (SPEX 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 $\delta^{13}C$ 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 ^{12}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:

$$d\Delta_r/dt = \Delta_p/T - \Delta_r/T - \lambda\Delta_r \qquad \text{Eqn 1}$$

Here Δ_r is the $\Delta^{14}C$ value (‰) of the root pool at time t (yr) and Δ_p is the $\Delta^{14}C$ value (‰) of new photosynthate. Δ_p 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 (λ) is negligible compared with the other terms, and is ignored. Solving equation 1 for Δ_r as a function of time by integration, and rearranging, gives:

$$T = -\Delta_t/\ln[(\Delta_r - \Delta_p)/(\Delta_{r0} - \Delta_p)] \qquad \text{Eqn 2}$$

where Δ_t is 2 yr and Δ_{r0} is the $\Delta^{14}C$ 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

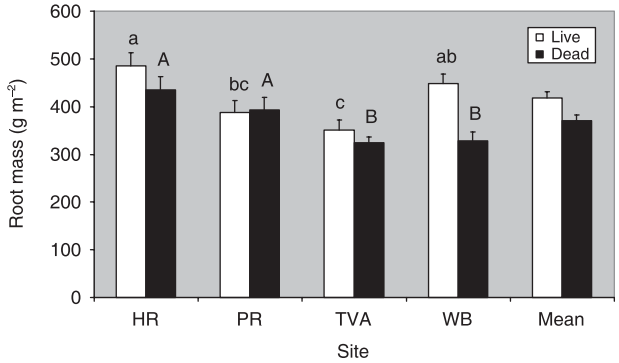

Fig. 2 Comparison across sites of mean live fine-root (< 2 mm) mass and mean dead fine-root mass, averaged over 3 yr. Error bars, SEM. Mean root mass values for sites with the same letter (lower case for live roots, upper case for dead) were not significantly different at $\alpha = 0.05$. Sites: HR, Haw Ridge; PR, Pine Ridge; TVA, Tennessee Valley Authority; WB, Walker Branch.

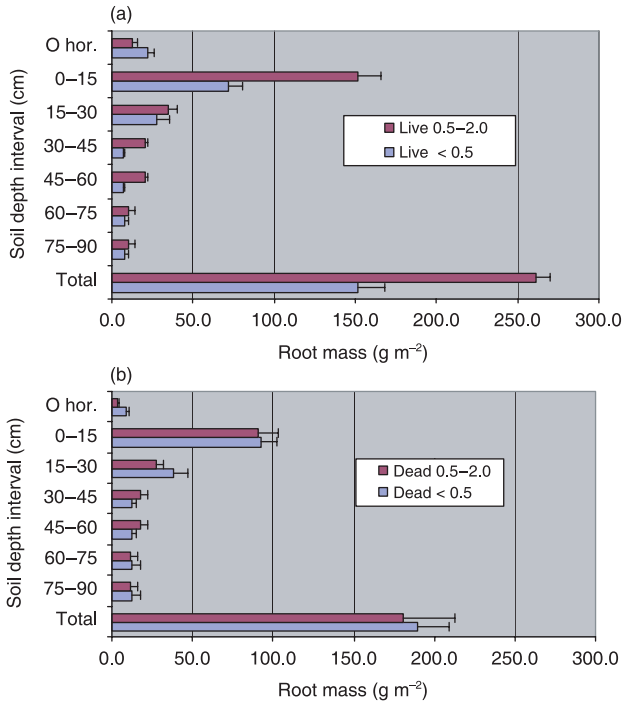

Fig. 3 Depth distribution of fine-root mass by size class and live (a) and dead (b) characterization. Mean values for all four sites. To maintain visual comparability across 15-cm mineral soil intervals in this graphic depiction, estimates for each 30-cm depth interval below 30 cm has been distributed into two 15-cm intervals, using the assumption of equal root density for each of the two adjacent 15-cm intervals. As described in Materials and Methods, for the depth interval 15–30 cm, live and dead percentages were extrapolated from measurements taken from depth intervals immediately above and below, while total fine-root mass was directly measured. Similarly, for the 60–90-cm depth intervals, live and dead percentages were assumed equal to those measured for the 30–60-cm interval. Error bars, SEM. In the cases of 30-cm intervals subdivided into 15-cm intervals, error bars shown are for 30-cm intervals.

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 $\Delta^{14}C$ values over time

General patterns There were no statistically significant differences in $\Delta^{14}C$ values between the two West ORR sites (PR and TVA). Similarly, there were no statistically significant differences in $\Delta^{14}C$ 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 $\Delta^{14}C$ 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 $\Delta^{14}C$ 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 $\Delta^{14}C$ between 2001 and 2003, the trend was always towards decreasing $\Delta^{14}C$, with the largest decrease in the first year (2001–02). The decrease in $\Delta^{14}C$ 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 $\Delta^{14}C$ values over time Live roots < 0.5 mm diameter exhibited statistically significant decreases in $\Delta^{14}C$ value over the 2-yr period in four out of six comparisons (two locations × three depths), and 0.5–2-mm root $\Delta^{14}C$ values decreased significantly in two out of six comparisons (Fig. 4). Total declines in $\Delta^{14}C$ 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 $\Delta^{14}C$ 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 $\Delta^{14}C$ value, live roots < 0.5 mm in diameter had

Table 2 $\Delta^{14}C$ values for time series of live and dead fine-root samples for roots < 0.5 and 0.5–2 mm diameter

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

$n = 8$; 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.

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).

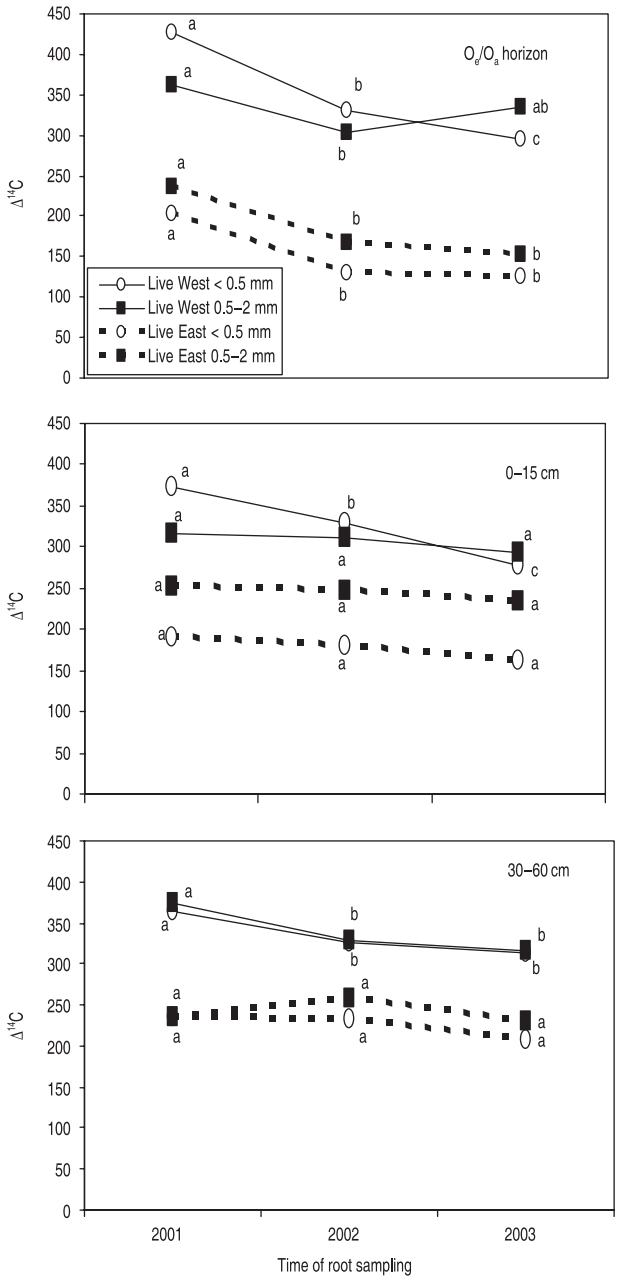

Fig. 4 Temporal pattern of the $\Delta^{14}C$ signature of live roots segregated by size class and by West Oak Ridge Reservation (ORR) vs East ORR sites. Results depicted separately for three soil depth intervals: O_e/O_a horizon, 0–15 cm and 30–60 cm mineral soil depth. Data points within the same size class, end of the ORR, and soil depth with the same letter were not significantly different at $\alpha = 0.05$. For reference, background atmospheric $\Delta^{14}CO_2$ for East ORR in 2001 and 2002 was 107 ± 3 and 95 ± 4‰ for West ORR; 213 ± 28 and 187 ± 4‰, respectively (see Materials and Methods for further details).

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).

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

Parameter	East ORR	West ORR	East ORR	West ORR
Depth	< 0.5 mm	< 0.5 mm	0.5–2 mm	0.5–2 mm
O horizon	1.4*	2.3*	1.5*	10.1
0–15 cm	4.9	3.2*	14.4	7.6
30–60 cm	8.1	4.9*	18.3	5.5*

Fine-root turnover time (FRTT) indices were calculated from changes in $\Delta^{14}C$ values of live roots over the period 2001–03 as described in Materials and Methods, assuming a donor-controlled system with log-normal behavior and a symmetric root-age distribution. Our data suggest at least a two-pool system, so that the FRTT indices given here should not be construed as estimates of FRTT for this population and should only be used for comparison.
*, Decreases in $\Delta^{14}C$ values over the 2 yr of the study were calculated from statistically significant decreases ($P < 0.05$).

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

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.

Acknowledgements

Funding for the EBIS project was provided by the US Department of Energy, Office of Science, Biological and Environmental Research as a part of the Terrestrial Carbon Processes Program, and by the Office of Science, US Department of Energy under Contract DE-AC02-05CH11231. EBIS project participants appreciate access to and use of Tennessee Valley Authority (TVA) land under Contract 105906 between TVA and the Oak Ridge National Laboratory. Many thanks to Don Todd for field and laboratory assistance, Jessica Westbrook and Deb Williard for sample preparation, Becky Ostertag and Whendee Silver for advice with root washing and sorting, Weixin Cheng and Todd Dawson for use of laboratory facilities and access to student assistants, and Chris Swanston for stewarding and processing samples at the Lawrence Livermore National Laboratory Center for Accelerator Mass Spectrometry. Numerous people assisted in the washing, separating and sorting of roots, but special recognition is due to Carrie Brandt, Sarah Carnes, Josh Moats, Casey Mullen, Corey Mullen, Sean Robertson and Kim Welch.

References

Baddeley JA, Watson CA. 2005. Influences of root diameter, tree age, soil depth and season on fine root survivorship in *Prunus avium*. *Plant and Soil* **276**: 15–22.

Barbaroux C, Breda N, Dufrene E. 2003. Distribution of above-ground and below-ground carbohydrate reserves in adult trees of two contrasting broad-leaved species (*Quercus petraea* and *Fagus sylvatica*). *New Phytologist* **157**: 605–615.

Barbour MM, Roden JS, Farquhar GD, Ehleringer JR. 2004. Expressing leaf water and cellulose oxygen isotope ratios as enrichment above source reveals evidence of a Péclet effect. *Oecologia* **138**: 426–435.

Bidartondo MI, Ek H, Wallander H, Soderstrom B. 2001. Do nutrient additions alter carbon sink strength of ectomycorrhizal fungi? *New Phytologist* **151**: 543–550.

Burton AJ, Pregitzer KS, Crawford JN, Zogg GP, Zak DR. 2004. Simulated chronic NO_3^- deposition reduces soil respiration in northern hardwood forests. *Global Change Biology* **10**: 1080–1091.

Caldwell MM, Camp LB. 1974. Belowground productivity of two cool desert communities. *Oecologia* **17**: 123–130.

Eissenstat DM, Wells CE, Yanai RD, Whitbeck JL. 2000. Building roots in a changing environment: implications for root longevity. *New Phytologist* **147**: 33–42.

Fahey TJ, Hughes JW. 1994. Fine root dynamics in a northern hardwood forest ecosystem, Hubbard Brook Experimental Forest, NH. *Journal of Ecology* **82**: 533–548.

Farquhar GD, Barbour MM, Henry BK. 1998. Interpretation of oxygen isotope composition of leaf material. In: Griffiths H, ed. *Stable isotopes: integration of biology, ecological and geochemical processes.* Oxford, UK: Bios Scientific, 27–62.

Fitter AH. 2002. Characteristics and functions of root systems. In: Waisel Y, Eshel E, Kafkafi U, eds. *Plant roots, the hidden half.* New York: Dekker, 15–32.

Fitter AH, Graves JD, Self GK, Brown TK, Bogie DS, Taylor K. 1998. Root production, turnover and respiration under two grassland types along an altitudinal gradient: influence of temperature and solar radiation. *Oecologia* **114**: 20–30.

Gaudinski JB, Trumbore SE. 2003. Chapter 11. Soil carbon turnover. In: Hanson PJ, Wullschleger SD, eds. *North American temperate deciduous forest responses to changing precipitation regimes.* New York: Springer, 190–209.

Gaudinski JB, Trumbore SE, Davidson EA, Cook AC, Markewitz D, Richter DD. 2001. The age of fine-root carbon in three forests of the eastern United States measured by radiocarbon. *Oecologia* **129**: 420–429.

Giardina CP, Ryan MG. 2002. Total belowground carbon allocation in a fast-growing *Eucalyptus* plantation estimated using a carbon balance approach. *Ecosystems* **5**: 487–499.

Gill RA, Jackson RB. 2000. Global patterns of root turnover for terrestrial ecosystems. *New Phytologist* **147**: 13–31.

Gower ST, Pongracic S, Landsberg JJ. 1996. A global trend in belowground carbon allocation – can we use the relationship at smaller scales? *Ecology* **77**: 1750–1755.

Guo DL, Mitchell RJ, Hendricks JJ. 2004. Fine root branch orders respond differentially to carbon source–sink manipulations in a longleaf pine forest. *Oecologia* **140**: 450–457.

Hanson PJ, Edwards NT, Tschaplinski TJ, Wullschleger SD, Joslin JD. 2003. Chapter 22. Estimating the net primary and net ecosystem production of a southeastern upland *Quercus* forest from an 8-year biometric record. In: Hanson PJ, Wullschleger SD, eds. *North American temperate deciduous forest responses to changing precipitation regimes.* New York: Springer, 378–395.

Hanson PJ, Swanston CW, Garten CT Jr, Todd DE, Trumbore SE. 2005. Reconciling change in Oi-horizon ^{14}C with mass loss for an oak forest. *Soil Science Society of America Journal* **69**: 1492–1502.

Hendrick RL, Pregitzer KS. 1993. The dynamics of fine root length, biomass, and nitrogen content in two northern hardwood ecosystems. *Canadian Journal of Forest Research* **23**: 2507–2520.

Hendrick RL, Pregitzer KS. 1996. Temporal and depth-related patterns of fine root dynamics in northern hardwood forests. *Journal of Ecology* **84**: 167–176.

Hendricks JJ, Hendrick RL, Wilson CA, Mitchell RJ, Pecot SD, Guo D. 2006. Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. *Journal of Ecology* **94**: 40–57.

Horwath WR, Pregitzer KS, Paul EA. 1994. C-14 allocation in tree soil systems. *Tree Physiology* **14**: 1163–1176.

Hua QM, Barbetti M, Worbes M, Hea J, Levchenko VA. 1999. Review of radiocarbon data from atmospheric and tree ring samples for the period 1945–97 AD. *IAWA Journal* **20**: 261–283.

Jackson RB, Mooney HA, Schulze E-D. 1997. A global budget for fine root biomass, surface area, and nutrient contents. *Proceedings of the National Academy of Sciences, USA* **94**: 7362–7366.

Johnson DW, Van Hook RI. 1989. *Analysis of biogeochemical cycling processes in Walker Branch Watershed.* New York: Springer.

Joslin JD. 1983. The quantification of fine root turnover in a white oak stand. PhD Dissertation. Columbia, MO, USA: University of Missouri.

Joslin JD, Henderson GS. 1982. A test of the budget method: a revised approach to the measurement of fine root turnover. In: Muller RN, ed. *Central Hardwood Forest Conference: Proceedings of the Fourth Meeting, 1982.* Lexington, KY, USA: University of Kentucky, 220–228.

Joslin JD, Henderson GS. 1987. Organic matter and nutrients associated with fine root turnover in a white oak stand. *Forest Science* **33**: 330–346.

Joslin JD, Wolfe MH. 1999. Effects of minirhizotron installation disturbance upon root observation data. *Soil Science Society of America Journal* **63**: 218–221.

Joslin JD, Wolfe MH. 2003. Chapter 16. Fine-root growth response. In: Hanson PJ, Wullschleger SD, eds. *North American temperate deciduous forest responses to changing precipitation regimes.* New York: Springer, 274–302.

Joslin JD, Wolfe MH, Hanson PJ. 2001. Factors controlling the timing of root elongation intensity in a mature upland oak stand. *Plant and Soil* **228**: 201–212.

King JS, Albaugh TJ, Allen HL, Buford M, Strain BR, Dougherty P. 2002. Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. *New Phytologist* **154**: 389–398.

Levin I, Kromer B. 2004. The tropospheric $^{14}CO_2$ level in mid-latitudes of the northern hemisphere (1959–2003). *Radiocarbon* **46**: 1261–1272.

Luo YQ. 2003. Uncertainties in interpretation of isotope signals for estimation of fine root longevity: theoretical considerations. *Global Change Biology* **9**: 1118–1129.

Majdi H, Pregitzer K, Moren AS, Nylund JE, Agren GI. 2005. Measuring fine root turnover in forest ecosystems. *Plant and Soil* **276**: 1–8.

Matamala R, Gonzalez-Meler MA, Jastrow JD, Norby RJ, Schlesinger WH. 2003. Impacts of fine root turnover on forest NPP and soil C sequestration potential. *Science* **302**: 1385–1387.

McClaugherty CA, Aber JD, Melillo JM. 1982. The role of fine roots in the organic matter and nitrogen budgets of two forested ecosystems. *Ecology* **63**: 1481–1490.

McLaughlin SB, McConathy RK, Barnes RL, Edwards NT. 1980. Seasonal changes in energy allocation by white oak (*Quercus alba*). *Canadian Journal of Forest Research* **10**: 379–388.

Milchunas DG, Lauenroth WK. 1992. Carbon dynamics and estimates of primary production by harvest, C-14 dilution, and C-14 turnover. *Ecology* **73**: 593–607.

Milchunas DG, Lauenroth WK, Singh JS, Cole CV, Hunt HW. 1985. Root turnover and production by C-14 dilution – implications of carbon partitioning in plants. *Plant and Soil* **88**: 353–365.

Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL. 2002. Fine root architecture of nine North American trees. *Ecological Monographs* **V72**: 293–309.

Pregitzer KS, Kubiske ME, Yu CK, Hendrick RL. 1997. Relationship among root branch order, carbon, and nitrogen in four temperate species. *Oecologia* **111**: 302–308.

Pregitzer KS, Laskowski MJ, Burton AJ, Lessard VC, Zak DR. 1998. Variation in sugar maple root respiration with root diameter and soil depth. *Tree Physiology* **18**: 665–670.

Santantonio D, Grace JC. 1987. Estimating fine root production and turnover from biomass and decomposition data: a compartment flow model. *Canadian Journal of Forest Research* **17**: 900–908.

SAS Institute. 1989. *SAS/STAT1 Users' guide, Version 6*, 4th edn, *EDZ, Vols 1 & 2.* Cary, NC, USA: SAS Institute.

Stuiver M, Polach H. 1977. Reporting of ^{14}C data. *Radiocarbon* **19**: 355–363.

Tierney GL, Fahey TJ. 2002. Fine root turnover in a northern hardwood forest: a direct comparison of the radiocarbon and minirhizotron methods. *Canadian Journal of Forest Research* **32**: 1692–1697.

Trumbore SE, Gaudinski JB. 2003. The secret lives of roots. *Science* **302**: 1344–1345.

Trumbore SE, Gaudinski JB, Hanson PJ, Southon J. 2002. Quantifying ecosystem-atmosphere carbon exchange with a ^{14}C label. *Eos, Transactions, American Geophysical Union* **84**: 265–268.

Trumbore S, DaCosta ES, Nepstad DC, DeCamargo PB, Martinelli L,

Ray D, Restom T, Silver W. 2006. Dynamics of fine root carbon in amazonian tropical ecosystems and the contribution of roots to soil respiration. *Global Change Biology* **12**: 217–229.

Vogel JS, Nelson DE, Southon JR. 1987. ^{14}C background levels in an accelerator mass spectrometry system. *Radiocarbon* **29**: 323–333.

Vogt KA, Persson H. 1991. Measuring growth and development of roots. In: Lassoie JP, Hinckley TM, eds. *Techniques and approaches in forest tree ecophysiology.* Boca Raton, FL, USA: CRC Press.

Vogt KA, Vogt DJ, Palmiotto PA, Boon P, Ohara J, Asbjornsen H. 1996. Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. *Plant and Soil* **187**: 159–219.

Wells CE, Eissenstat DM. 2001. Marked differences in survivorship among apple roots of different diameters. *Ecology* **82**: 882–892.

Wells CE, Glen DM, Eissenstat DM. 2002. Changes in the risk of fine root mortality with age: a case study in peach (*Prunus persica*). *American Journal of Botany* **89**: 79–87.

Wurth MKR, Pelaez-Riedl S, Wright SJ, Korner C. 2005. Non-structural carbohydrate pools in a tropical forest. *Oecologia* **143**: 11–24.