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The age of fine-root carbon in three forests of the eastern United States measured by radiocarbon

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Abstract Using a new approach involving one-time measurements of radiocarbon (^{14}C) in fine (<2 mm diameter) root tissues we have directly measured the mean age of fine-root carbon. We find that the carbon making up the standing stock of fine roots in deciduous and coniferous forests of the eastern United States has a mean age of 3–18 years for live fine roots, 10–18 years for dead fine roots, and 3–18 years for mixed live+dead fine roots. These ^{14}C-derived mean ages represent the time C was stored in the plant before being allocated for root growth, plus the average lifespan (for live roots), plus the average time for the root to decompose (for dead roots and mixtures). Comparison of the ^{14}C content of roots known to have grown within 1 year with the ^{14}C of atmospheric CO_2 for the same period shows that root tissues are derived from recently fixed carbon, and the storage time prior to allocation is <2 years and likely <1 year. Fine-root mean ages tend to increase with depth in the soil. Live roots in the organic horizons are made of C fixed 3–8 years ago compared with 11–18 years in the mineral B horizons. The mean age of C in roots increases with root diameter and also is related to branching order. Our results differ dramatically from previous estimates of fine-root mean ages made using mass balance approaches and root-viewing cameras, which generally report life spans (mean ages for live roots) of a few months to 1–2 years. Each method for estimating fine-root dynamics, including this new radiocarbon method, has biases. Root-viewing approaches tend to emphasize more rapidly cycling roots, while radiocarbon ages tend to reflect those components that persist longest in the soil. Our ^{14}C-derived estimates of long mean ages can be reconciled with faster estimates only if fine-root populations have varying rates of root mortality and decomposition. Our results indicate that a standard definition of fine roots, as those with diameters of <2 mm, is inadequate to determine the most dynamic portion of the root population. Recognition of the variability in fine-root dynamics is necessary to obtain better estimates of belowground C inputs.

Keywords Radiocarbon · Fine root dynamics · Ecosystem carbon balance · Belowground carbon allocation · Soil organic matter

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Introduction

The residence time of carbon in terrestrial ecosystems depends on how carbon is allocated by plants and the rate of decomposition of plant tissues and their alteration products in soils. Methods for estimating C allocation to aboveground biomass and net primary productivity (ANPP) are well developed (Schlesinger 1997). However, our ability to estimate C allocation, storage, and fate belowground is still poor (Gower et al. 1996; Vogt et al. 1996). Fine roots (<2 mm diameter) make up ~2.5% of total terrestrial plant biomass and have been estimated to receive one-third of global net primary productivity (NPP) (or 20 gigatons of C per year) assuming roots grow and die in 1 year (Jackson et al. 1997). The accuracy of this estimate depends critically on the assumption of fine-root turnover time.

The most commonly used method for estimating the mean age of root carbon is to assume that the mean age

equals the turnover time, defined as the standing stock of live roots divided by the estimated annual production of new roots or annual loss through death and decomposition (Vogt et al. 1996). The underlying assumption is that the fine-root population is at steady state and is homogeneous, i.e., the probability of root death or decomposition is the same for every root in the population (Rodhe 1992). Direct methods for estimating rates of root production and loss include sequential coring, in-growth root cores, root screens, minirhizotrons or rhizotrons, and litter bags (Fahey and Hughes 1994; Vogt et al. 1998). Sequential coring techniques have large uncertainties due to large spatial and temporal variability in root distributions (McClaugherty and Aber 1982; Fogel 1990), whereas other methods have significant disturbance effects (Vogt et al. 1998; Joslin and Wolfe 1999). In an effort to minimize labor-intensive and time-consuming direct methods, indirect methods such as the N budget (Aber et al. 1985; Nadelhoffer et al. 1985), or carbon flux approaches (Raich and Nadelhoffer 1989) have been developed.

Fine-root turnover times reported in the literature are generally 2 years or less (Gill and Jackson 2000), though some studies report turnover times of several years (Joslin and Henderson 1987; Milchunas and Lauenroth 1992; Ostertag 2001). Type of vegetation, soil nutrient status, and presence or lack of mychorrhizal association clearly affect root mean age. However, the method used to determine root turnover time also appears to influence the result. In hardwood forests of the northeastern United States, estimates of turnover times for roots based on root screens and minirhizotrons in the upper parts of the soil profile are on the order of months (Hendrick and Pregitzer 1992, 1993; Fahey and Hughes 1994; Eissenstat and Yanai 1997; Johnson et al. 2000). In the same kind of forest, sequential coring and in-growth cores typically yield turnover times of less than 2 years (Vogt et al. 1986; Fahey and Hughes 1994; Powell and Day 1991). The longest turnover time estimates, which can range from <1 to 3 years (Aber et al. 1985; Nadelhoffer et al. 1985), are associated with nutrient budget techniques. Despite a lack of agreement between field methods, mean ages for fine roots incorporated into ecosystem models reflect a general consensus that the majority of fine roots grow and die within 1 year (Hoffmann 1995; Rasse et al. 2001).

We present a new method based on radiocarbon (^{14}C) that allows direct estimation of the mean age of carbon in fine roots. The radiocarbon approach takes advantage of the elevated levels of ^{14}C in atmospheric CO_2 that resulted from thermonuclear weapons testing in the early 1960s (Fig. 1). This global ^{14}C isotope "spike" can be used to trace the time elapsed since C in plant tissues was fixed from the atmosphere by photosynthesis, and to estimate C cycling rates in an ecosystem. Following the nuclear test ban treaty in 1963, the amount of ^{14}C in atmospheric CO_2 has decreased due to exchange with the ocean and terrestrial biosphere, and dilution by burning of ^{14}C-free fossil fuels. The rate of decline has slowed

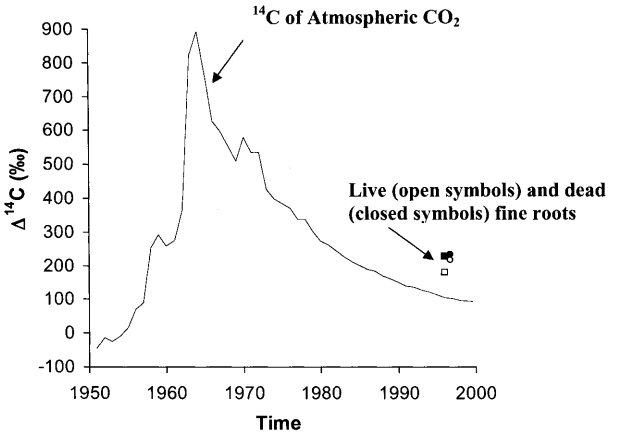


Fig. 1 The time record of $\Delta^{14}C$ in atmospheric CO_2 (Northern Hemisphere) and the mean fine-root $\Delta^{14}C$ values for live and dead fine roots sampled from a mixed deciduous stand in Harvard Forest, MA in 1996 (from Gaudinski et al. 2000). All measurements have been corrected to remove mass-dependent fractionation effects. *Open square* represents live roots from the O horizon (178±10‰, *n*=10), *closed square* represents dead roots from O horizon (226±12‰, *n*=7), *open circle* represents live roots from the A horizon (217±22‰, *n*=3) and the *closed circle* represents dead roots from the A horizon (233±12‰, *n*=2). Note that there is a significant difference between live and dead root values only in the O horizon samples. The samples represented by *discrete points* were taken at the same time (1996), but have been separated on the graph to make differences visible

with time (Fig. 1), with annual change of $\Delta^{14}C$ averaging ~–8‰ $year^{-1}$ in the 1980s and early 1990s. Between 1996 and 1999, the period of this study, rates of decrease slowed to ~–4‰ $year^{-1}$ (Levin and Hesshaimer 2000). The accuracy of the ^{14}C measurement by accelerator mass spectrometry (AMS) with graphite prepared in our laboratory is ±6‰, which allows us to resolve the time elapsed since organic matter was fixed from the atmosphere to within 1–2 years over the past five decades.

The radiocarbon content of fine roots (both live and dead) has been shown to be significantly higher than that of contemporary atmospheric $^{14}CO_2$ at a mixed deciduous forest in central Massachusetts, United States (Gaudinski et al. 2000) (Fig. 1). If, as is generally thought, fine roots grow and die within 1 year, and if they grow from carbon fixed from the atmosphere within the last year, then the ^{14}C content of their tissues should equal that of atmospheric CO_2 for that year. However, the data of Gaudinski et al. (2000) show that $\Delta^{14}C$ values in fine roots are significantly greater than atmospheric $\Delta^{14}CO_2$, implying either that the fine roots sampled are living significantly longer than 1–2 years or that they are made from carbon reserves stored in the plant for several years prior to translocation to fine roots.

In this paper, we expand our original observations of ^{14}C in fine roots to two other temperate forest sites, in Howland, Maine and the Calhoun Experimental Forest, South Carolina. We also add archived live roots from Harvard Forest sampled in 1979. We test the two hypotheses explaining the origin of the high ^{14}C values in fine

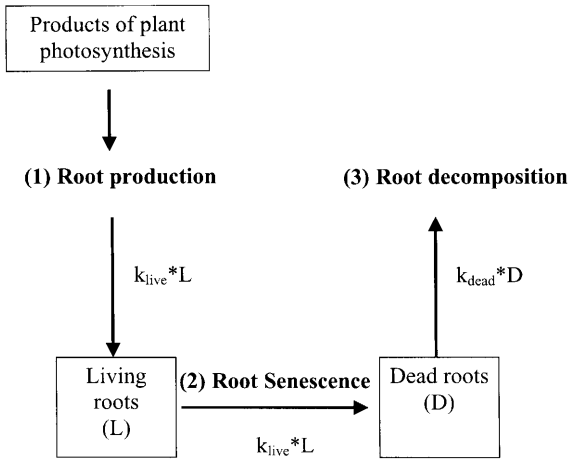


Fig. 2 The conceptual approach to understanding fine-root dynamics used in this paper. Carbon fixed from the atmosphere is used to produce new roots (*1*). Living roots eventually senesce or are killed by herbivory and become dead roots (available for decomposition). Note that the definition of a dead root includes only those roots that are dead and decomposing. The rate of transfer of material from the live to dead root pool (*2*) is assumed to follow first-order kinetics, with k_{live} reflecting the probability of fine-root senescence. Dead roots are assumed to decompose to produce CO_2 with first-order kinetics [(*3*); k_{dead} is the decay constant]. *L* and *D* represent the stock of C in live and dead fine-root pools respectively

roots (stored photosynthate versus long mean ages) by measuring the ^{14}C of roots known to have grown within a 1-year time period. We use two different models to estimate the mean age of C in fine roots by comparing their ^{14}C signatures to the time history of ^{14}C in atmospheric CO_2.

Radiocarbon provides a measure of the time elapsed since C was fixed from the atmosphere by photosynthesis. Hence, the measure integrates over: (1) the time C was stored in the plant prior to root formation; (2) the amount of time spent as a live root (its life span); and (3) any time elapsed after root death and prior to decomposition and loss as CO_2. If the residence time of C in the plant prior to allocation to root growth is short, ^{14}C measurements of live roots will represent a measure of their life span, while ^{14}C measurements of dead roots represent the sum of their life span and time spent in the soil after death and prior to decomposition. For live and dead root mixtures, the estimated mean age will exceed the true mean age by an amount that depends on the relative mass of live and dead roots.

Most studies differentiate live and dead roots when sorting based on arbitrary and qualitative measures such as resiliency or color of the root tissue. Such criteria do not necessarily correlate with active nutrient uptake and growth, criteria that can also be used to define a live root. Roots that are dormant and not growing or conducting nutrients and are also not decomposing might realistically be sorted as either live or dead. Here, we define dead roots as those available for decomposition. Figure 2 shows our conceptual model for carbon flow through live and dead fine-root pools. However we recognize that roots sorted by physical characteristics are not likely to be just those actively growing or conducting nutrients (for live roots) and just those available to decompose (for dead roots).

Another issue addressed in this work is the definition of a "fine" root. At the ecosystem level, a fine root has often been defined based on root diameter (i.e., roots <2 mm in diameter). Using this definition many studies have shown that roots <2 mm diameter are a dynamic component of forest ecosystems (Pregitzer et al., in press). However, over 75% of roots actively absorbing nutrients in most North American trees are less than 0.5 mm in diameter (Hendrick and Pregitzer 1993; Pregitzer et al. 1997) and recent work suggests that such a definition (<2 mm in diameter) lumps together roots that may cycle carbon at different rates (Wells and Eissenstat 2001; Gill and Jackson 2000). In this work we analyze ^{14}C in roots of different size classes to assess possible differences in dynamics among them.

Materials and methods

Sites

We sampled fine roots from three temperate forests in the eastern United States: a coniferous forest in Howland, Maine (Howland), a mixed hardwood forest in Massachusetts (Harvard Forest), and a loblolly pine plantation in South Carolina (Calhoun Experimental Forest).

The Howland site (45°12′N, 68°44′E) is dominated by red spruce and eastern hemlock. The soils are developed on glacial till and are classified as Typic Haplorthods. The forest was selectively logged around 1900 (Hollinger et al. 1999).

The Harvard Forest site (43°32′N, 72°11′E) is dominated by red oak and red maple with some eastern hemlock (Goulden et al. 1996). The soils are also developed on glacial till and are classified as Typic Distrochrepts. The area of our study site was cleared in the mid-1800s, plowed and used primarily for pasture. The pasture was abandoned between 1860 and 1880 (Foster et al. 1992). The regrowing forest was largely leveled by a hurricane in 1938 but has been growing undisturbed since.

The Calhoun Experimental Forest site (34°5′N, 81°4′W) is a loblolly pine plantation planted in 1957. The soils are developed on granitic-gneiss and are classified as Typic Kanhapludults (Markewitz et al. 1998, Richter and Markewitz 2001). The Calhoun soils were cultivated for cotton, dating back to nearly 1800 (Richter and Markewitz 2001).

Sample collection

We originally sampled fine roots at the Harvard forest in 1996, and used qualitative indices (resiliency and color) to separate live and dead roots. We observed that the ^{14}C contents of both cohorts were higher than atmospheric $^{14}CO_2$ for that year (Gaudinski et al. 2000). Dead roots had on average slightly higher ^{14}C values than live roots in that study (Fig. 1). Samples collected for this new study were not sorted into live and dead roots. Therefore, the ^{14}C values for bulk root samples reported here represent a measure of the time elapsed between C fixation and decomposition. The two exceptions to this are roots from the Calhoun Experimental Forest and archived roots from Harvard Forest sampled in 1979 where only live root samples were analyzed.

In spring 1999 at the Howland and Harvard Forest sites, we collected roots known to be less than 1 year in age by placing

screens horizontally at the base of the organic horizons where fine roots are abundant, following the experimental design of Fahey and Hughes (1994). Roots that grew through the screens were harvested in late August 1999 and were therefore less than 1 year in age. For comparison we collected samples of mixed live+dead fine roots for discrete depth intervals from soil pits dug in late July 1999. Roots from both screens and pits were separated from soil and divided into two size classes (<0.5 mm, 0.5–1 mm).

The roots from the Calhoun Experimental Forest were originally sampled as part of a separate study and thus sampling protocols and treatment varied from the two northern sites. Roots at the Calhoun Experimental Forest were collected from 6 cm diameter soil cores taken at three depth intervals (organic horizon, 0–15 cm, and 15–30 cm) in late July 1998. Live roots were separated from dead based on qualitative characteristics and sorted into two size classes (<2 mm and >2 mm). Only live roots were analyzed for this study.

All samples were either frozen or refrigerated, and then oven-dried at 60°C for at least 24 h. Roots were processed using an acid/alkali/acid procedure to remove easily hydrolyzable carbon, such as carbohydrates, that may post-date root formation. This procedure leaves behind primarily structural carbon components, such as cellulose and lignin. Root samples were soaked in 1M HCl in 20-ml glass vials for 15 min, repeatedly soaked in 1M NaOH until the liquid remained clear, and soaked for 15 min in 1M HCl. Finally, samples were soaked three times for 15 min in deionized water to remove residual salts. During all stages of pre-treatment the glass sample vials were placed in a hot water bath (approximately 80°C) and sonicated. The samples were then dried at 60°C, and converted to graphite as described below.

We were also able to obtain archived live fine roots (<0.5 mm and 0.5–3 mm) sampled in a similar mixed hardwood forest at Harvard Forest in 1979 by Charles McClaugherty (McClaugherty and Aber 1982). The samples were originally sorted by hand into size classes and then into live and dead categories. Again, live and dead separation was by qualitative characteristics. The samples were dried, ground, and stored in glass vials. We report radiocarbon results for the live samples only, because the ground dead root samples contained significant amounts of soil organic matter. Acid/alkalai/acid hydrolysis was performed on these ground samples after placing them in a polyester, heat sealed bag (ANKOM Technology, F57 filter bags) to prevent sample loss.

Atmospheric record of $\Delta^{14}C$ in CO_2

We interpret the $\Delta^{14}C$ in root tissues as the mean age of C, using two time-dependent models that simulate incorporation of bomb-^{14}C into the fine-root pool. A necessary input to both models is the history of ^{14}C in atmospheric CO_2 incorporated by plants. We use the $\Delta^{14}C$ record of atmospheric CO_2 (Northern Hemisphere) based on grapes grown in Russia for 1950–1977 (Burchuladze et al. 1989), and direct atmospheric measurements for 1977–1996 that represent summer means (May through August) taken at Schauinsland Black Forest, Germany, at an elevation of 1205 m above sea level (Levin and Kromer 1997). The Schauinsland site has the best available $^{14}CO_2$ record for a polluted continental setting similar to our sites on the east coast of the United States. After 1996 we assume a continued decrease of 4±2‰ year^{-1} (Levin and Hesshaimer 2000), which yields values of 100.3‰, 96.3‰, and 92.3‰ for 1997, 1998, and 1999 respectively. The 1997 and 1998 values are consistent with preliminary growing season means for Schauinsland for 1997 and 1998 (Ingeborg Levin, personal communication).

Radiocarbon (^{14}C) analysis

We converted all root samples to graphite by sealed-tube zinc reduction (Vogel 1992) and measured the ^{14}C content at the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory. We express radiocarbon data as $\Delta^{14}C$, the difference in parts per thousand (per mil or ‰) between the $^{14}C/^{12}C$ ratio in the sample compared to that of a universal standard (oxalic acid I, decay-corrected to 1950). All samples are corrected to a common $\delta^{13}C$ value of –25‰ to correct for the effects of mass-dependent isotope fractionation on measured ^{14}C values. This accounts for plant-based photosynthetic discrimination of atmospheric ^{14}C of CO_2 (^{14}C is assumed to fractionate twice as much as ^{13}C).

The $\delta^{13}C$ values used to correct $\Delta^{14}C$ for roots from Harvard and Howland Forests ranged from –23.09 to –29.65‰, though most were between –26 and –29‰. $\delta^{13}C$ values for roots from Calhoun Experimental Forest were not measured and were assumed to be –27‰. The error in the $\Delta^{14}C$ value derived from a 2‰ error in the $\delta^{13}C$ value assumed for ^{14}C correction is 2‰, less than the measurement precision.

Modeling fine-root age

We used two methods to estimate the mean age for fine roots from radiocarbon data. This average age represents the total time expired since the C used to form the root tissue was fixed from the atmosphere. For live and dead root mixtures, the mean age will exceed the average root life span by an amount that depends on the relative mass of live and dead roots. With either method, the time lag between C fixation from the atmosphere and growth of a new root is included in the overall age estimate.

The first method assumes that all structural carbon in the root grew in a single year, and that no new C has been added to the root since that time. In this case, the average age of the root is determined by comparing the $\Delta^{14}C$ of structural C directly to the record of $\Delta^{14}C$ of CO_2 in the atmosphere. This method is most appropriate for estimating the age of a single root as opposed to a composite sample that represents a population of roots.

For composite samples a time-dependent, steady-state model (method 2) is more appropriate because it integrates the $\Delta^{14}C$ concentration of atmospheric CO_2 over the past n years (where n equals the average age of the population). This model assumes that variation in radiocarbon values of a population of similar root samples, and hence any variation in ages of that population of roots, is normally distributed around the mean. The equation used in method 2 is:

$$C_{(t)}\times R_{\mathrm{root}(t)} = I\times R_{\mathrm{atm}(t)} + C_{(t\text{-}1)}\times R_{\mathrm{root}(t\text{-}1)}\times(1\text{-}k\text{-}\lambda)$$

where C is equal to the stock of fine-root carbon in g C m^{-2}, I equals the inputs of C by new production of fine roots in g C m^{-2} year^{-1}, k equals the (turnover time^{-1}) for roots in year^{-1}; k may be k_{live} for live roots or k_{dead} for dead roots. For mixed live+dead roots the k value will be that which best represents the mean turnover rate for the mixture. $R_{root} = \left(\frac{\Delta^{14}C_{root}}{1000}\right) - 1$. $R_{atm(t)} = \left(\frac{\Delta^{14}C_{atm(t)}}{1000}\right) - 1$. R_{atm} varies with year between 1950 and 2000 according to Fig. 1. λ is equal to the radioactive decay constant for ^{14}C (1/8267 years) and t equals the time (year) for which the calculation is being performed.

The two methods for using $\Delta^{14}C$ values to estimate a mean age are illustrated in Fig. 3. The $\Delta^{14}C$ measured for fine roots sampled in 1999 in this example is 145‰. Using method 1 we estimate mean age of 10±1 years for the roots because the atmosphere last had $\Delta^{14}CO_2$ of 145‰ in 1990 (1999-1990=10 years including the 1999 growing season). Using method 2, we predict a mean age of 8 years. The difference between the two models is not significant for samples that have $\Delta^{14}C$ values less than 110‰ (<5 years mean age), and is only 1–2 years for values between 110 and 185‰ (up to 14 years mean age). For roots with $\Delta^{14}C$ values greater than 185‰, the difference in the two methods becomes quite large. In fact, in 1999, $\Delta^{14}C$ values between 174–200‰ do not have a unique solution when applying method 2, and values greater than 200‰ cannot be explained with method 2. In such cases, we used method 1 to estimate mean age.

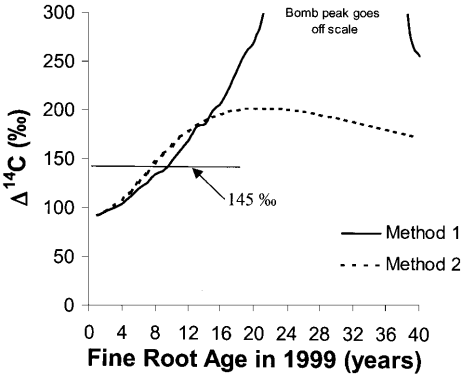


Fig. 3 Results from two modeling approaches for calculating fine-root age as a function of $\Delta^{14}C$ content. *Method 1* uses the atmospheric $\Delta^{14}C$ of CO_2 as a proxy for calculating fine-root age. *Method 2* is a steady-state time-dependent model also based on the atmospheric $\Delta^{14}C$ record of CO_2

Results

$\Delta^{14}C$ values for roots sampled from soil pits are consistently higher than atmospheric $^{14}CO_2$ in the year of sampling (Fig. 4). The corresponding mean ages of C in roots show a large range, between 3 and 30 years (Fig. 4).

Comparison of root screens with bulk roots at Harvard and Howland forests

Fine roots known to have grown in 1999, and to be less than 1 year old, have $\Delta^{14}C$ values consistent with the 1999 $\Delta^{14}C$ of atmospheric CO_2 (92±2‰; Fig. 5). Of 13 measurements from root screens, all but two are within 2 SDs (12‰) of the atmospheric $\Delta^{14}C$ of CO_2 for 1999 (92±2‰), and all but four are within 1 SD (6‰) of this value (Fig. 5). Given the slowed rate of decline of ^{14}C in atmospheric CO_2 in the late 1990s, the estimated mean ages of C in newly grown roots are less than 2 years, and likely less than 1 year.

Mixed live+dead roots sampled in 1999 from the soil pits at Howland and Harvard Forest had consistently higher $\Delta^{14}C$ values than those sampled from nearby root screens (Fig. 5; Table 1), and also had a larger range of $\Delta^{14}C$ values. The estimated mean ages for C in these roots range from 3–18 years (Fig. 4).

Mean age of C in live roots

Live roots were picked from total soil roots at two of our sites, Harvard forest (sampled in 1979 and 1996), and Calhoun forest (in 1998) and are elevated in ^{14}C relative to atmospheric CO_2 in the year they were sampled. At Harvard Forest live roots sampled in 1996 had $\Delta^{14}C$ values that exceeded atmospheric $\Delta^{14}CO_2$ values by 25–206‰ (Fig. 4) which correspond to mean ages of

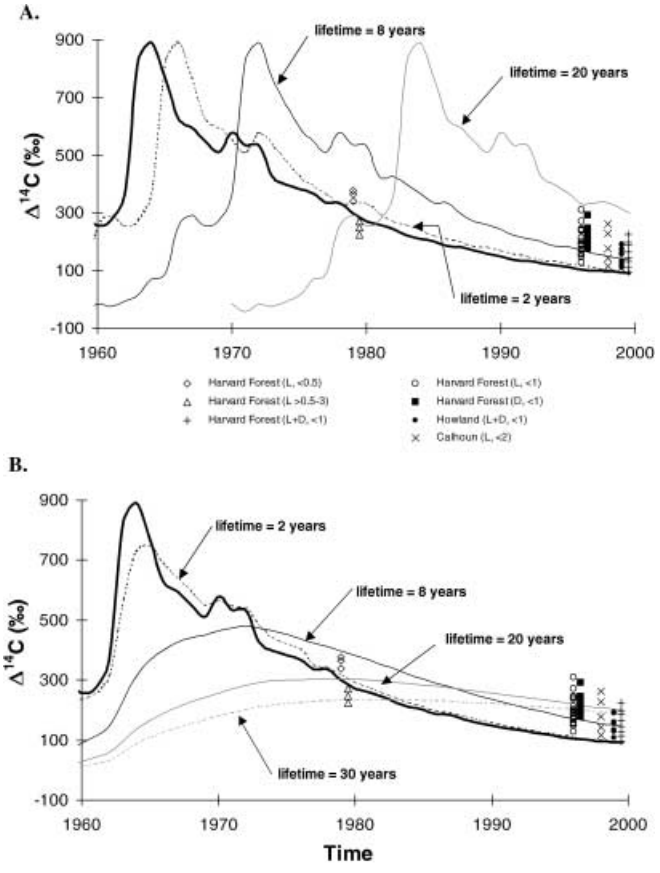


Fig. 4 The radiocarbon values of fine roots for all depth intervals measured at Howland, ME (1999), Harvard Forest, MA (1979, 1996, and 1999) and The Calhoun Experimental Forest, SC (1998) using the atmospheric $\Delta^{14}C$ of CO_2 as a proxy for calculating fine-root age, i.e., method 1 (Fig. 4A) and a steady-state time-dependent model also based on the atmospheric $\Delta^{14}C$ record of CO_2, i.e., method 2 (Fig. 4B) (*L* live roots, *D* dead roots, *numbers* indicate diameter range of fine roots sampled). Precision with accelerator mass spectrometry (AMS) for modern samples is ±6‰ and thus error bars are smaller than the *symbols* shown

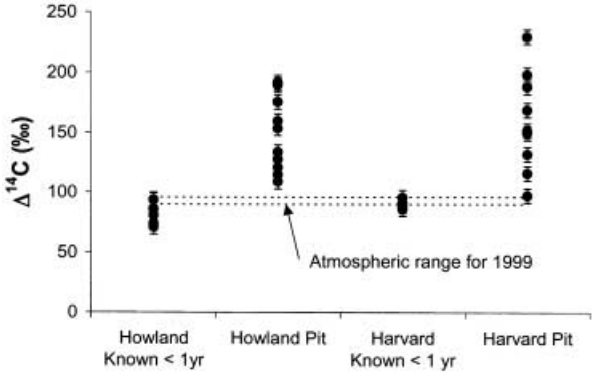


Fig. 5 Radiocarbon values for fine roots (<0.5 mm and 0.5–1mm in diameter) from the Howland and Harvard Forest sites sampled in 1999. Roots *known <1 yr* were sampled from root screens placed in the organic horizon in spring of 1999 and harvested in fall 1999 and are less than 1 year old. *Pit* samples are roots sampled from soil pits dug in July 1999. The atmospheric range in $\Delta^{14}C$ of CO_2 in 1999 is 92±2‰ (Levin and Kromer 1997; Levin and Hesshaimer 2000)

3–18 years. The $\Delta^{14}C$ values of dead roots were higher than those for live roots from the same horizon, though the difference is only significant in the O horizon (Fig. 1). The average age of C in O horizon roots (1996) is 8 years for live roots and 14 years for dead roots (calculated by taking the average of all the live or dead mean ages respectively; data not shown).

Table 1 $\Delta^{14}C$ of fine roots sampled from soil pits at all three sites. Values in *parentheses* are 1 SD of the accelerator mass spectrometry (AMS) radiocarbon measurement

SITE	$\Delta^{14}C$ (‰)	$\Delta^{14}C$ (‰)
Howland-1999[a] live+dead (mixed)		
Depth (cm)	<0.5 mm in diameter	0.5–1 mm in diameter
O horizon (+10–4)	128 (6)	192 (6)
O horizon (+4–0)	160 (6)	154 (6)
0–7	134 (6)	121 (5)
7–13	109 (6)	115 (6)
13–30	190 (6)	175 (6)
Harvard Forest-1999[a] live+dead (mixed)		
Depth (cm)	<0.5 mm in diameter	0.5–1 mm in diameter
O Horizon (+5–0)	97 (6)	132 (6)
0–2	197 (6)	229 (6)
2–6	116 (6)	149 (9)
6–30	188 (7)	168 (7)
Harvard Forest-1979[b] live only		
Depth (cm)	<0.5 mm in diameter	0.5–3 mm in diameter
0–15	340 (6)	249 (6)
15–30	377 (8)	273 (6)
30–45	366 (7)	225 (6)
Calhoun Experimental Forest-1998[c] live only		
Depth (cm)	<2 mm in diameter	>2 mm in diameter
O horizon (+2–0)	118 (5)	
0–15	146 (5)	180 (6)
15–30	230 (6)	264 (6)

[a] Atmospheric $\Delta^{14}C$ in 1999 was 92±2‰ (Levin and Kromer 1997; Levin and Hesshaimer 2000)
[b] Atmospheric $\Delta^{14}C$ in 1979 was 299±9‰ (Levin and Kromer 1997)
[c] Atmospheric $\Delta^{14}C$ in 1998 was 96±2‰ (Levin and Kromer 1997; Levin and Hesshaimer 2000)

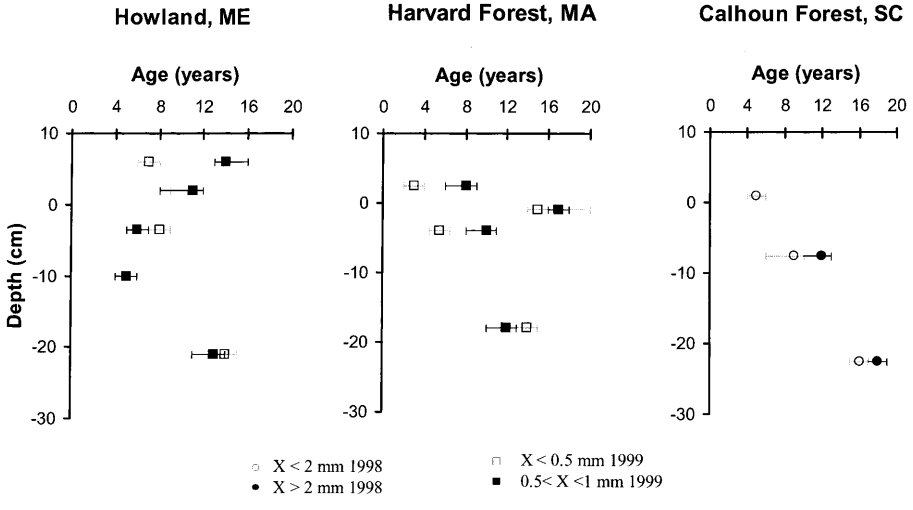
The $\Delta^{14}C$ values for archived live roots collected in 1979 at Harvard Forest (Table 1) are higher than those sampled in 1996 because they grew closer to the peak ^{14}C concentrations in 1964. The $\Delta^{14}C$ of atmospheric CO_2 in 1979 was 299‰. The values for live roots <0.5 mm in diameter are 340–377‰, which correspond to mean ages of 3–5 years. $\Delta^{14}C$ values for roots between 0.5 and 3 mm in diameter (225–273‰) were lower than atmospheric $\Delta^{14}CO_2$ in 1979. This can only be explained if the larger diameter roots contain a significant fraction of carbon fixed prior to the peak in atmospheric ^{14}C of CO_2 in 1964 as well as carbon fixed post-1964. The mean ages of C in live roots 0.5–3 mm in diameter calculated using method 2 were 22–32 years (Fig. 4).

Live roots sampled in 1998 at the Calhoun forest similarly had $\Delta^{14}C$ values that ranged from 22 to 168‰ higher than 1998 atmospheric $^{14}CO_2$ values (Table 1). Mean ages of C in these roots inferred from $\Delta^{14}C$ range from 5 to 18 years (Fig. 4).

Variations in root age with depth and size class

Much of the variation in the mean ages of C in roots reflect systematic changes in the $\Delta^{14}C$ of roots with depth or size class (Fig. 6). Error bars on the figure indicate the range of mean ages based on the two different modeling

Fig. 6 The estimated age of fine roots sampled at Harvard Forest and Howland (1999) and The Calhoun Experimental Forest (1998) using the atmospheric $\Delta^{14}C$ of CO_2 as a proxy for calculating fine-root age (method 1). The *error bars* indicate the range in mean age resulting from the SE in the $\Delta^{14}C$ measurement, plus differences between ages interpreted by method 1 versus a steady-state time-dependent model also based on the atmospheric $\Delta^{14}C$ record of CO_2 (method 2). For example, a sample with a $\Delta^{14}C$ value of 160±6‰ sampled in 1999 has an estimated age of 11±1 years using method 1, but 10±1 years using method 2. The mean age we plot here is thus 11+1 and –2 years. For a number of samples with $\Delta^{14}C$ values >200‰ method 2 cannot yield an estimate of the mean age of root C and in such cases, we used only results from method 1 to estimate root age. The *depths* shown indicate the midpoint of the sampling horizon or depth interval. *Depths greater than zero* represent the midpoint of the organic (O) horizon



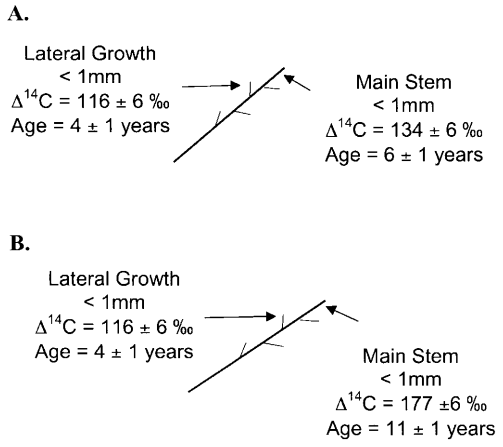


Fig. 7 $\Delta^{14}C$ values for portions of two different individual live fine-root systems (arbitrarily labeled *A* and *B*) growing in the organic horizon at Harvard Forest in 1997. The *ages* shown are calculated using the atmospheric $\Delta^{14}C$ of CO_2 as a proxy for calculating fine-root age only (method 1). The $\Delta^{14}C$ of the atmosphere in 1997 is 100±2‰ (Levin and Kromer 1997; Levin and Hesshaimer 2000)

techniques (see Fig. 6 caption for more detail). The profiles in Fig. 6 show trends in the mean age of fine-root C with depth in the soil profile and fine-root diameter. At the Calhoun Experimental Forest, a monospecific loblolly pine plantation, the age of live root C increases from 4–6 years near the surface to 15–18 years at 22 cm depth. Roots >2 mm in diameter are 2–3 years older than roots <2 mm in diameter at all depths.

Roots were not separated by species or into live and dead classes at Howland or Harvard Forest in 1999. At Harvard forest, differences in age between root size classes (here <0.5 mm and 0.5–1 mm) are clearly apparent, again with larger diameter roots in general made of older C. The highest $\Delta^{14}C$ values for both size classes were observed in the first mineral (A) horizon; however below this horizon root C age increases with depth. In the well developed Spodosols at Howland forest, the age of fine-root C is related to soil horizon, with the youngest roots at ~8–10 cm depth where humic materials accumulate beneath an E horizon (a zone of nutrient leaching). Roots <0.5 mm in diameter in the O horizon are 7 years younger than roots 0.5–1 mm in diameter although the size age relationship is not consistent throughout.

Age of root C and root architecture

Radiocarbon contents of intact fine roots vary significantly as a function of branching order. Portions of two individual fine-root systems were collected in late August 1997 at Harvard Forest and analyzed for the $\Delta^{14}C$ of structural C in the main stem and lateral growth off that stem (Fig. 7). Each $\Delta^{14}C$ value shown is from a single root system, not a composite from several root systems. The $\Delta^{14}C$ values for main stem structural carbon are elevated relative to ^{14}C of atmospheric CO_2 by 34 and 77‰ (6 and 11 years mean age of C) for Fig. 7A, B, respectively. Structural C from lateral growth off of the main stems are elevated relative to atmospheric ^{14}C of CO_2 by 16‰ (4 years mean age of C).

Discussion

Fine root ages

The radiocarbon measurement of bulk roots picked from soils reflects the total average time elapsed since C making up the roots was fixed from the atmosphere. Our measurements of ^{14}C in newly formed root tissues clearly demonstrate that the C used to grow new root structural tissue was fixed within the previous 1–2 years (Fig. 5). Thus, higher-than-atmospheric $\Delta^{14}C$ values in fine roots represent old C that resides in structural root tissues for several years to decades before it returns to the atmosphere through decomposition.

One alternative explanation for the presence of old C in fine roots is that roots may actively take up C via mycorrhizal associations from surrounding soil organic matter with higher $\Delta^{14}C$ values. This is unlikely, however, because fine-root $\Delta^{14}C$ content is greater than that of soil organic matter at many depths in the soil profile. For example, the $\Delta^{14}C$ values of soil organic matter below 15 cm depth are <0‰ at all three sites studied here (Richter et al. 1999; Gaudinski et al. 2000; Gaudinski 2001), yet the roots found there are enriched in bomb-^{14}C (with values from 108–260‰). For the uptake hypothesis to be correct, between 20 and 100% of the structural carbon would have to come from soil organic carbon for nearly all of the roots we sampled from the soil pits. While it is possible some exchange is taking place, it is unlikely to contribute such large amounts to root structural carbon pools.

The elevated $\Delta^{14}C$ values of live roots picked from the soil at the Calhoun Experimental and Harvard Forests relative to atmospheric CO_2 for the same year indicate the mean age of C in living roots is 5–18 years (for roots <2 mm diameter). If we assume a 0- to 2-year lag between C fixation and root growth, the root mean ages we infer are 3–16 years, much longer than have been reported in the literature. Identification of living root tissue by hand-picking is often problematic, and it could be argued that our samples of "live" roots include a significant mass of "dead" or dormant root tissues. If this is true, much of the residence time for C in "live" root tissues inferred from radiocarbon might be due to these dead roots which have high ^{14}C values due to very slow rates of decomposition. However, root decomposition rates measured using litter bags at the Harvard Forest (McClaugherty and Aber 1982) showed loss of ~20% of the initial root mass in the 1st year. The mean time for roots to decompose is thus ~5 years, too short to explain some of the very long C residence times we observe in live roots. Clearly, the radiocarbon measurements imply

longer residence times for C in live, fine (<2 mm diameter) roots than have been previously reported.

Classifying the dynamic component of fine roots

Our ^{14}C data indicate systematic variations in the mean age of fine-root C with root diameter, soil depth, and branching order. Ages for roots <0.5 mm in diameter average 2–3 years less than roots 0.5–1 mm in diameter at Howland and Harvard Forest (Fig. 6). Similarly roots <2 mm in diameter at the Calhoun Experimental Forest were 2–3 years younger than roots >2 mm in diameter. This trend is in agreement with work done by Wells and Eissenstat (2001) who found significant differences in life span for apple roots that differed in diameter by only a few tenths of millimeters. Using minirhizotrons to observe individual roots they found the risk of root death decreased with increasing diameter. Roots <0.3 mm had a median life span of <2 months, roots whose diameters were 0.3–0.5 mm had median life spans of 3–5 months while roots 0.5–1.1 mm in diameter had median life spans of 7 months or more. In a literature review of 190 published studies, Gill and Jackson (2000) also found that fine-root mean ages increased as the size classes that were sorted increased with roots 0–1 mm and 0–2 mm in diameter having mean ages of 0.6 and 1.2 years respectively. The work of Wells and Eissenstat (2001) and the review by Gill and Jackson (2000) show variation on the order of months while we show variation on the order of years. However, all three studies clearly show the commonly used definition of fine roots as those with <2 mm diameter is problematic because it lumps together populations of roots that cycle carbon at significantly different rates.

Fine-root ages also generally increase with depth at the Harvard and Calhoun Experimental Forests. At Harvard Forest and the Calhoun Experimental Forest the age ranges for live roots are 3–17 and 5–18 years for the whole profiles respectively, but only 3–8 years and 5 years in the respective organic horizons (Fig. 6). The profile at Howland shows decreasing age with depth through the spodic (Bh) horizon (a zone of nutrient accumulation), implying nutrient status (and not only depth) may have a significant impact on fine-root life spans (Fig. 6). Individual fine roots also show multi-year age differences between root tips and the stem from which they grow. Age differences of 2 and 7 years between different parts of the same fine root (Fig. 7) indicate that branching order may be an important factor in determining the age of fine-root C. Roots grow in complex networks through soil, and growing roots tips can function differently than the stems from which they grow (which may only serve to transport nutrients and water taken up at more active sites). Radiocarbon measurements can thus aid in understanding the patterns of root growth in soils.

Reconciling radiocarbon estimates with other measures of fine-root dynamics

Minirhizotrons and screen counting techniques are biased towards roots with short life spans because they selectively look for changes in root length, or root appearance and disappearance at the root tips. Radiocarbon measurements of root structural tissues are biased toward longer C residence times because the roots most likely to be hand-picked from the soil are often larger and more persistent. The dynamics of any small, yet fast-cycling, components will consequently be underestimated by radiocarbon (Gaudinski et al. 2000; Trumbore 2000). In other words if a large fraction of the fine-root mass has high radiocarbon content (i.e., older mean age of C) and a relatively small fraction of the fine-root mass has lower radiocarbon content (i.e., younger mean age), then the mass-weighted average radiocarbon content will indicate a relatively long age of C in fine roots. This problem may be exacerbated for fine roots because smaller diameter roots have been shown to have a higher specific root length on a length per gram basis (Pregitzer et al. 1997). Thus, the smallest diameter roots will represent even less of the mass of a sample than they would if all roots had a constant specific root length.

Our finding of relatively long fine-root mean ages based on radiocarbon can be reconciled with the much shorter turnover times estimated from minirhizotrons and screen counting techniques or steady-state mass balance methods (dividing stock by production or loss rate) if fine-root populations have varying rates of root mortality and decomposition. For example, a simple steady-state model based on minirhizotron observations that 50% of fine roots die within ~0.6 years would estimate a mean age of live fine roots of 0.86 years [0.6/ln(2)], much less than our ^{14}C-derived estimates. This example assumes that all roots have an equal probability of dying (and thus mean age equals turnover time; Rodhe 1992). However, if the mortality rate declined for roots that survive the first 0.6 years, the mean age of roots would exceed the turnover time. Thus our results are not at odds with observations of fine roots that live and die within 1 year if some fraction of the fine-root population survive as long as several years to decades. In fact, roots observed in long-running minirhizotron experiments (4 years) have been shown to live the duration of the experiment (Johnson et al. 2000). Differences of fine-root mean ages as a function of depth and size class (Fig. 6) and branching order (Fig. 7) further underscore the heterogeneity of fine-root populations

Future research directions

Collaborative work using this radiocarbon technique in conjunction with other methods will aid in understanding both the different results obtained by the different methods and further our understanding of fine-root ecology. Careful emphasis on quantitative sorting by diameter

size class, depth and live and dead fine roots will allow ^{14}C-derived mean ages to be used to calculate both rates of fine-root turnover and decomposition (in g C m^{-2} $year^{-1}$) for different cohorts of roots. As discussed above, the ^{14}C-technique is biased towards the largest mass and therefore the longer-lived and more slowly decomposing roots. To minimize this effect, future sampling efforts should be carefully timed to occur at the seasonal maximum in new root growth. Destructively sampling root systems from young trees of known age or root ingrowth cores sampled over many years would also help in mapping out the belowground C allocation patterns in ecosystems.

Belowground C cycling

Globally, fine roots have been estimated to comprise 33% of NPP (Jackson et al. 1997), and about 50% of NPP in forest ecosystems (Vogt et al. 1998) assuming fine roots live and die in 1 year. Our finding that fine roots vary significantly in mean age, and can indeed live and persist in the soil environment for a decade or longer, emphasizes the uncertainty in our current understanding of belowground C allocation. Whether this implies that present estimates of a large NPP allocation to fine roots are inaccurate is uncertain. Possibly, the smallest diameter roots or root exudates are dynamic enough to account for a large NPP flux. However, in order to satisfy both the need for substantial annual NPP allocation belowground *and* a fine-root stock that has mean ages from 3–20 years, fine roots (<2 mm diameter) must have a large variation in mean ages that range from months to decades. This is possible if fine roots have varying rates of root mortality and decomposition as a function of diameter, depth, branching order and nutrient status. Improved understanding of ecosystem carbon balance will require combined measurement approaches that further explore the ecology of fine roots.

Conclusions

Radiocarbon measurements of root tissues provide a new tool for studying the dynamics of fine roots in soils. Our measurements show that fine-root carbon resides in soils for on average several years up to two decades. The long mean ages derived from radiocarbon do not reflect C storage in plants prior to root growth, because the ^{14}C values of recently grown root tissues are consistent with that of recent photosynthate. Slow decomposition rates alone can not be responsible, both because we find high ^{14}C values in live roots as well as live+dead mixtures, and because decomposition rates reported in the literature are too fast to explain more than ~5 years of the total mean age. We observe systematic variations of root age with diameter class, soil horizon, and branching order that should prove useful in guiding further studies of fine-root dynamics. Our mean ages derived from radiocarbon exceed turnover times estimated using mass balance approaches and observed lifespans using minirhizotrons, implying that fine-root populations vary considerably in rates of mortality and decomposition.

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