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Use of stored carbon reserves in growth of temperate tree roots and leaf buds: analyses using radiocarbon measurements and modeling

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Abstract

Characterizing the use of carbon (C) reserves in trees is important for understanding regional and global C cycles, stress responses, asynchrony between photosynthetic activity and growth demand, and isotopic exchanges in studies of tree physiology and ecosystem C cycling. Using an inadvertent, whole-ecosystem radiocarbon (^{14}C) release in a temperate deciduous oak forest and numerical modeling, we estimated that the mean age of stored C used to grow both leaf buds and new roots is 0.7 years and about 55% of new-root growth annually comes from stored C. Therefore, the calculated mean age of C used to grow new-root tissue is $\sim$ 0.4 years. In short, new roots contain a lot of stored C but it is young in age. Additionally, the type of structure used to model stored C input is important. Model structures that did not include storage, or that assumed stored and new C mixed well (within root or shoot tissues) before being used for root growth, did not fit the data nearly as well as when a distinct storage pool was used. Consistent with these whole-ecosystem labeling results, the mean age of C in new-root tissues determined using 'bomb-^{14}C' in three additional forest sites in North America and Europe (one deciduous, two coniferous) was less than 1–2 years. The effect of stored reserves on estimated ages of fine roots is unlikely to be large in most natural abundance isotope studies. However, models of root C dynamics should take stored reserves into account, particularly for pulse-labeling studies and fast-cycling roots ($<$1 years).

Keywords: ^{14}C, carbon cycling, carbon isotope, carbon reserves, fine-root turnover time, mean age of carbon, radiocarbon, stored carbon

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Introduction

Carbon (C) reserves in plants play several roles in ecosystem C cycling. They are an important resource for mature trees under stressful conditions, such as after fire or pest outbreaks, and they are also used on a routine basis, over a range of time scales (Chapin *et al*., 1990), to moderate the effect of variations in C production. Photosynthate produced during the day may be used at night after stomates have closed. Seasonally, reserves are used to fuel initial bud break in the spring in both deciduous and coniferous forests (Hoch *et al*., 2003) and early wood growth in some oak species (Kramer & Kozlowski, 1979; Barbaroux *et al*., 2003). New-root growth is thought to be fueled primarily from recent photosynthate (Horwath *et al*., 1994; Pregitzer & Friend, 1996). However, in deciduous species, root growth in winter and spring-root growth can occur before canopy leaf-out (Joslin *et al*., 2001) and thus must be fueled by reserves.

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Little is known about the age of C in storage reserve pools of mature trees (Trumbore *et al*., 2002). Many tree physiology studies have observed a buildup of C reserves in the fall, and that these reserves are used for root and shoot growth in the spring (McLaughlin *et al*., 1980). However, neither the age of C in reserve pools nor the extent of its use in the growth of new leaf, wood, and root tissues has been well quantified for most mature tree species (Trumbore *et al*., 2002). Determining the mean age and amount of stored C used in tissue growth has become increasingly important in the last decade, because of new methods that rely on changing isotopic (^{13}C and ^{14}C) concentrations of atmospheric CO_2 to determine belowground C-cycling rates (Gaudinski *et al*., 2001; Tierney & Fahey, 2002; Trumbore *et al*., 2002; Matamala *et al*., 2003; Keel *et al*., 2006). Radiocarbon studies using the changing concentration of $^{14}CO_2$ in the atmosphere following thermonuclear weapons testing in the early 1960s ('bomb-^{14}C' technique; Trumbore, 1993) have generally assumed that inputs to soil organic matter from roots and leaves have the isotopic signature of the atmosphere at the time of their growth (Trumbore *et al*., 1995; Gaudinski *et al*., 2000; but for exceptions, see De Camargo *et al*., 1999; Torn *et al*., 2005). However, if storage C is used, then a mixture of recently fixed and previously stored C determines the isotopic composition of new tissues. Therefore, to accurately determine the turnover time of plant tissues and C exchanges with soil organic matter, the amount and mean age of stored C used in growth of new tissues may need to be taken into account.

The turnover time of a C pool (defined here as the ratio of annual average pool mass to cumulative annual flux leaving the pool) is equivalent to its mean age for pools that are well mixed and have a first-order (exponential) loss process. This equivalence results because the C age and transit time distribution curves are equivalent (Rodhe, 1992). The models used in this paper assume that the storage pool is well mixed and has a first-order loss process, thereby insuring that the mean age of C leaving the storage pool is equivalent to the storage pool turnover time. We note that this equivalence does *not* occur for C in the roots, because root populations have an age distribution curve different from their transit time distribution curve because younger roots tend to leave the fine root population more rapidly than older roots (Wells & Eissenstat, 2001; Tierney & Fahey, 2002; Trumbore & Gaudinski, 2003).

Assessment of stored C inputs to new growth is particularly important for isotopic methods that estimate fine-root turnover time (Gaudinski, 2001). Studies using isotopic methods have found that fine roots (i.e., roots <2 mm in diameter) have turnover times ranging from 1 to more than 20 years (Gaudinski *et al*., 2001; Tierney & Fahey, 2002; Matamala *et al*., 2003; Joslin *et al*., 2006; Keel *et al*., 2006; Trumbore *et al*., 2006) and are challenging the assumption that fine roots, as a cohort, live for about 1 year (Jackson *et al*., 1997). Gaudinski *et al*. (2001) showed that in three temperate, deciduous forests of the United States, the mean age of C making up new (<1 year old) root growth was less than the resolution of the studies' measurements using the bomb-^{14}C approach (1–2 years). Thus, storage C, if present, did not affect the ^{14}C-derived fine-root mean age of 3–18 years. These results, however, could not rule out the possibility of a significant use of C fixed in the previous one or two growing seasons. In fact, $\sim 33\%$ of the new-root tissue of scrub oak in central Florida has been shown to be from stored C (Langley *et al*., 2002), and stored C was a significant component of autotrophic root respiration in boreal forests (Czimczik *et al*., 2006; Schuur & Trumbore, 2006; Carbone *et al*., 2007), implying that stored C is allocated to roots for various functions. If stored reserves are used in new-root growth, not accounting for them could cause large overestimation of root turnover time (e.g., as much as 31% and 293% if storage reserves have a mean age of 6 months or 2 years, respectively; Luo, 2003).

Some plant tissues, such as leaf buds, and roots that develop in winter in deciduous species, grow entirely from stored C. Other tissues, such as wood, some leaf buds, and most fine roots, may grow from a combination of stored and recent photosynthate. The main goal of our research was to quantify the role of stored C in the new-root and leaf-bud growth of mature, temperate forest trees. For a mature, deciduous forest in Oak Ridge, Tennessee, we addressed the following questions: (1) What is the mean age of stored C reserves contributing to new leaf-bud and fine-root growth? (2) On an annual basis, how much new-root growth comes from stored C reserves? To answer these questions, we took advantage of a whole-forest pulse label at the Oak Ridge Reservation (ORR), Tennessee, resulting from an unplanned ^{14}C release in 1999 (Trumbore *et al*., 2002). While the release was unplanned and not discovered until several months afterwards, we obtained time-series ^{14}C measurements in new roots, leaf buds, and parasitic plants growing between 1999 and 2002. We used the leaf-bud data with a one-pool model to determine the mean age of stored C used to grow leaf buds. Data from parasitic plants that grow only on root-derived C were used as an additional proxy for the age of stored C supplying root growth. Data from new roots, in combination with a recently developed numerical root model (Radix; Riley *et al*., submitted), were used to quantify the amount and mean age of storage C used to grow new roots. In addition, the results from

both modeling approaches were used to estimate the mean age of C used to grow new-root tissue from the mixture of stored and recently fixed C.

We also investigated whether growth from stored C could affect previous results for three temperate forests, based on bomb-^{14}C, that fine roots have long lifetimes (Gaudinski *et al*., 2001). To do this, we used the bomb-^{14}C approach of Gaudinski *et al*. (2001) to estimate the mean age of C in new fine-root tissues in coniferous forests in Sweden and on the west coast of the United States, as well as a mixed deciduous forest at Harvard Forest, Massachusetts, previously studied in Gaudinski *et al*. (2001).

Site descriptions

Locally enriched ^{14}C sites

This study was conducted at three sites on the ORR in Tennessee, the site of Oak Ridge National Laboratory (36°N, 84°W; Fig. 1). The local enrichment in atmospheric $^{14}CO_2$ at ORR was presumably from a nearby hazardous waste incinerator, and was discovered by $^{14}CO_2$ measurements in air and soil respiration made in 1999. Subsequent measurements of tree-ring cellulose from white oak trees (Trumbore *et al*., 2002) showed that East ORR received a large $^{14}CO_2$ pulse in 1999 (Appendix A, Trumbore *et al*., 2002 and http://ebis.ornl.gov/pretreat.html). The three sites studied here, Walker Branch (WB), Haw Ridge (HR), and the Throughfall Displacement Experiment site (TDE; adjacent to WB), are all located on East ORR (Fig. 1). Although the ^{14}C pulse also affected the West ORR, only data from the eastern sites are used in the current analysis because root growth collection screens were in place at TDE during the 1999 pulse, enabling collections of new roots that grew during and shortly after the labeling event. This work represents one component of a large multi-institution study of forest C cycling using radiocarbon labeling, called the Enriched Background Isotopic Study (EBIS; Trumbore *et al*., 2002; Hanson *et al*., 2005; Swanston *et al*., 2005; Cisneros-Dozal *et al*., 2006; Joslin *et al*., 2006; Treseder *et al*., 2006).

The sites are located in the upland oak forest type on ridge and upper slope positions. Vegetation is chiefly white oak (*Quercus alba* L.), chestnut oak (*Q. prinus* L.), and red maple (*Acer rubrum* L.), with scattered pine (*Pinus echinata* Mill. and *P. virginiana* Mill.), yellow

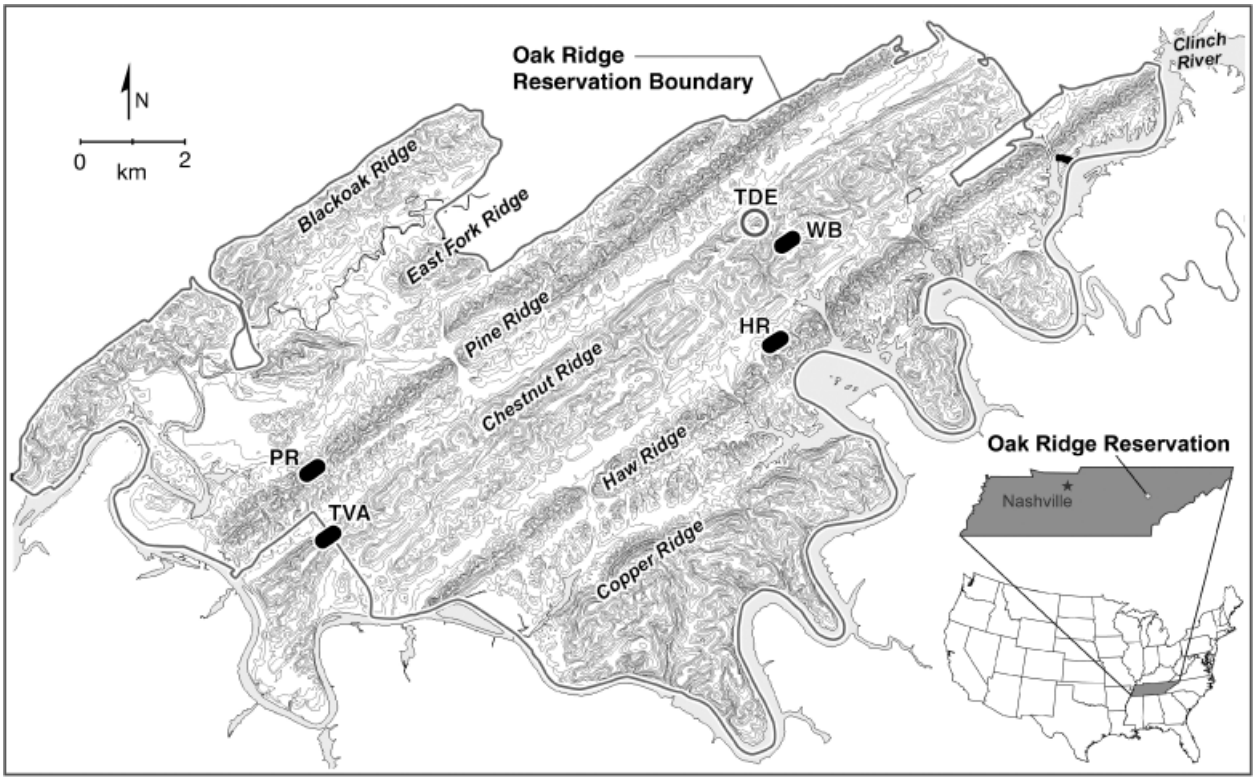

Fig. 1 Location map for the Oak Ridge Reservation. Shown are all four sites that are part of the Enriched Background Isotopic Study project [Walker Branch (WB), Haw Ridge (HR), Tennessee Valley Authority (TVA), and Pine Ridge (PR)] as well as the Throughfall Displacement Experiment site (TDE) which was originally developed for a separate experimental program (see the text for more details).

poplar (*Liriodendron tulipifera* L.), black gum (*Nyssa sylvatica* Marsh.), and sourwood (*Oxydendron arboreum* L.). The age of overstory trees in all the three sites is variable and ranges from 40 to 150 years (Hanson *et al*., 2005). Mean annual precipitation is 1358 mm and mean annual temperature is 14.1 °C (Johnson, 1989). The soils at WB and TDE are Ultisols, highly weathered Typic Paleudults developed over cherty dolomitic limestone parent material. At HR, the soils are mostly Ultisols, Inceptic Hapludults, mixed with some Typic Dystrudepts developed over shale and/or sandstone parent material.

Bomb -^{14}C sites

Harvard Forest is a mixed deciduous forest located near the town of Petersham in central Massachusetts (42.54°N, 72.18°W). The study area, located on the Prospect Hill Tract, was cleared in the mid-1800s and then plowed and used primarily for pasture until 1860–1880 (Foster, 1992). The regrowing forest has been undisturbed since being leveled by a hurricane in 1938. The dominant tree species in the study area are northern red oak (*Quercus rubra* L.) and red maple (*A. rubrum*) with some hemlock (*Tsuga canadensis* Carr.) and white pine (*Pinus strobes* L.). Mean annual air temperature is 8.5 °C and mean annual precipitation is 1050 mm. The soils developed on predominantly granitic glacial tills and are classified as Entisols.

The Blodgett Experimental Forest (Blodgett Forest) is a mixed coniferous forest located near the town of Georgetown in the Sierra Nevada, California (38.53°N, 128.30°W). The data presented here are from forest management unit 630, comprising trees that are about 90 years old. The dominant species is ponderosa pine (*Pinus ponderosa* Laws), with intermixed white fir (*Abies concolor* Lind. & Gord.), douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco], and incense cedar [*Calocedrus decurrens* (Torr.) Florin]. Mean annual air temperature was 8.5 °C in 2002 and 2003 (J. Bird, unpublished results) and mean annual precipitation is 1774 mm. The soils developed on granitic parent material and are classified as Alfisols.

Knottåsen is a coniferous forest located near the town of Jädraås in central Sweden. The study area (61.00°N, 16.13°E) was clear-cut in 1963. Before that time, the forest comprised norway spruce that were 120 + years old. Present stands were planted in 1965, primarily with 2-year-old norway spruce [*Picea abies* (L.) Kars.] seedlings, with some scots pine (*Pinus sylvestris* L.) mixed in. Mean annual air temperature is 3 °C and mean annual precipitation is 613 mm. The soils developed mainly on sandy glacial tills atop bedrock composed of granitic and volcanic sediments and are classified as Spodosols.

Methods

Site-specific atmospheric $^{14}CO_2$ histories

This paper uses two types of atmospheric $^{14}CO_2$ enrichment to trace the time elapsed since C in plant tissues was fixed from the atmosphere by photosynthesis (i.e., its mean age). The first utilizes a local enrichment of $^{14}CO_2$ at ORR (Fig. 2), while the second uses the global enrichment in background atmospheric $^{14}CO_2$ caused by thermonuclear weapons testing (bomb-^{14}C) in the late 1950s and early 1960s (Fig. 2). Both approaches require an accurate record of atmospheric $^{14}CO_2$ at the sites studied.

In the absence of direct atmospheric samples, tree rings can be used as a proxy for atmospheric $^{14}CO_2$ content (Hua *et al*., 1999). At each site, we assembled a local tree-ring record of atmospheric ^{14}C; additional proxy indicators that we used are discussed below. All tree cores were taken with a 4.35 mm Haglof increment borer (Haglöf Lángsele, Sweden) and stored in a dry location until processed. Each core was separated into annual rings, and each ring was pretreated using the Jayme–Wise soxhlet extraction process and bleached to a final product that is operationally defined as holocellulose (Gaudinski *et al*., 2005).

Locally enriched ^{14}C sites at ORR. Because the local, 1999 ^{14}C release at ORR was unplanned, direct measurements of local atmospheric $^{14}CO_2$ content during and immediately after the release do not exist. Therefore, we relied on plant-based proxy records to estimate the atmospheric $^{14}CO_2$ inputs to the ORR ecosystem. Certain plant tissues or their respiration can be used as proxies because photosynthate, the organic substrate used in both tissue growth and autotrophic respiration, has the same $\Delta^{14}C$ value as the CO_2 from which it is derived (isotopic fractionation during photosynthesis does not alter the Δ unit; Stuiver & Polach, 1977).

Two issues arise with this approach. First, these plant-based proxies are more representative of photosynthetic $^{14}CO_2$ uptake integrated over weekly to monthly time intervals, rather than actual atmospheric values at the time of the release, which probably varied dramatically on time scales of minutes to hours due to changing wind speed and direction. Therefore, we call our estimation of ^{14}C inputs to ORR the EBIS atmospheric radiocarbon proxy curve (ARPC). It should not be construed as an accurate reconstruction of atmospheric $^{14}CO_2$ over time.

Second, proxy measurements may not actually represent the atmospheric $^{14}CO_2$ concentration over the specified time interval due to use of stored C. For example, tissue that grew in 2000, but with stored C

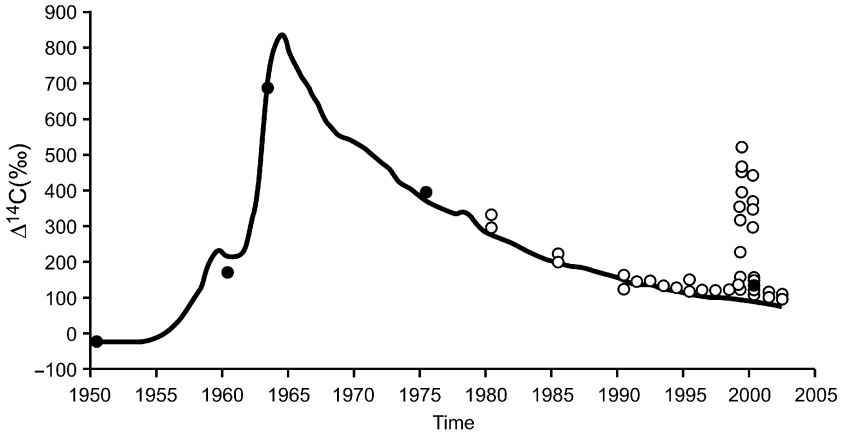

Fig. 2 $\Delta^{14}C$ values for background northern hemispheric air and local East Oak Ridge Reservation (ORR) tree rings ($n = 1$ in each case). Northern hemispheric air values (straight line) come from Levin & Hesshaimer (2000) for 1950–1976 and Levin & Kromer (2004) for 1977–2003. The tree-ring cellulose data from 1950 to 1975 (filled circles) come from one white oak on Throughfall Displacement Experiment site; data from 1976 to 2002 (open circles) come from four different trees (three white oaks, one chestnut oak) from Walker Branch and Haw Ridge. They are separated into annual increments except during 1999 and 2000, where they were separated into three increments per year. The elevated tree-ring values in 1999 and 2000 show clear influence of a strong local ^{14}C pulse at ORR. See Fig. A1 for smaller scale presentation of post-1998 tree-ring data.

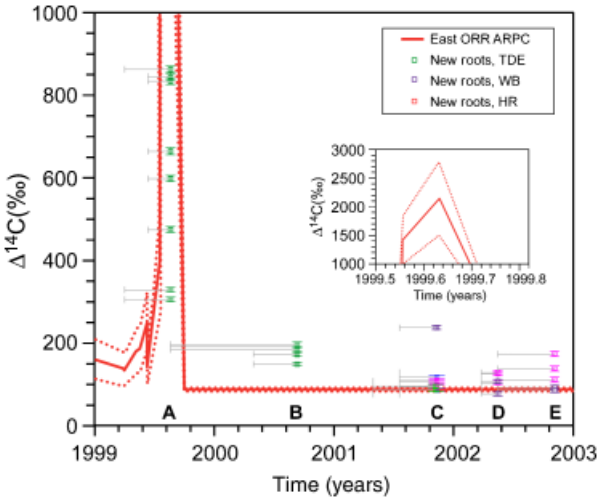

Fig. 3 Atmospheric radiocarbon proxy curve (ARPC) for East Oak Ridge Reservation and $\Delta^{14}C$ values of new roots over time. Dashed lines represent one standard deviation of the ARPC curve. Letters indicate the 'cohort' to which roots belong and key them to information in Table 1. For the new-root values, the horizontal line and the placement of each data point constrain the time interval during which the root tissues could have grown.

fixed after the 1999 pulse (entirely or in part), or newly fixed CO_2 but from a re-respired 1999 source, would be more enriched in ^{14}C than if it grew from C fixed only in 2000. In fact, it appears that the wood cellulose samples did indeed utilize stored C (see Appendix A). To address this issue, we use proxy measurements *only* to estimate local ^{14}C inputs before and during the 1999 pulse. After the 1999 pulse, atmospheric ^{14}C is assumed to return to a locally measured 'background' and remain at that level through 2002. Details on the ARPC and the data utilized to create it are in Appendix A. The final values used for the ARPC are shown in Fig. 3.

Bomb-^{14}C sites away from ORR. Atmospheric $^{14}CO_2$ content has been decreasing since the peak of the ^{14}C-bomb spike in the mid-1960s (Fig. 2), because of dilution of ^{14}C through exchange with C in the oceans and terrestrial biosphere. The rate of decrease was rapid at first, but has slowed with time. In the northern hemisphere, the annual change in atmospheric $^{14}CO_2$ was roughly $-8‰\ yr^{-1}$ in the 1980s and early 1990s, and roughly $-6‰\ yr^{-1}$in the late 1990s and early 2000s (Levin & Kromer, 2004). The decrease will continue due to ^{14}C dilution by use of ^{14}C-free fossil fuels (a.k.a. the Suess effect). The analytical error in ^{14}C measured with accelerator mass spectroscopy (AMS) reported here is 4–6‰, which is similar to the annual rate of $^{14}CO_2$ decrease over the time period of this study.

In the natural background sites, hereafter referred to as 'bomb-^{14}C sites,' use of stored C reserves will make the ^{14}C value of new-root tissues higher than that of the current atmosphere because of the decreasing trend of atmospheric ^{14}C content. A significant, positive difference between $\Delta^{14}C$ values in new-root growth and atmospheric CO_2 allows us to calculate the influence of stored C reserves (Gaudinski *et al.*, 2001).

Negative differences indicate fossil fuel CO_2 incorporation into photosynthate. Positive differences, however, do not rule out the possibility of local atmospheric depressions in isotopic values due to fossil fuel contributions.

For the three bomb-^{14}C sites, annual atmospheric measurements or proxies are considered equivalent to plant ^{14}C uptake, because the annual rate of change in atmospheric $^{14}CO_2$ for the past two decades is small ($<8‰\,yr^{-1}$) and known (Fig. 2). Past studies have used hemispheric ^{14}C atmospheric records (i.e., Burchuladze *et al*., 1989; Levin & Kromer, 1997, 2004; Levin & Hesshaimer, 2000) as estimates of local atmospheric $^{14}CO_2$ (Gaudinski *et al*., 2001; Tierney & Fahey, 2002; Trumbore *et al*., 2006). However, hemispheric $^{14}CO_2$ records by themselves do not account for local influences of fossil fuel and re-respired CO_2 within the forest canopy. Therefore, at each of our three sites, we compared three types of data that can be used to represent the atmospheric ^{14}C content. First, we used the ^{14}C signature of global background atmospheric CO_2 at Schauinsland, Germany, which is considered the most representative published record for both continental Europe and North America (Levin & Kromer, 2004). Second, we sampled the ^{14}C content of local tree-ring cellulose at Blodgett Forest (1976–2004), Harvard Forest (1951–2003), and Knottåsen (1972–2003). Tree rings were analyzed from one tree core at each site and were measured at multi-year intervals before 1998 and annually after 1998. Tree species sampled were ponderosa pine, northern red oak, and norway spruce at Blodgett Forest, Harvard Forest, and Knottåsen, respectively. Finally, direct measurements of local air $^{14}CO_2$ were obtained at Harvard Forest in 2001, Knottåsen in 2001 and 2002, and Blodgett Forest in 2002 and 2003. Except at Harvard Forest, air samples were taken in evacuated 6 L stainless steel canisters (Scientific Instrumentation Specialists, Moscow, ID, USA). Air was passed through an inlet to restrict the fill rate and dry the air (using magnesium perchlorate). It took approximately 45 min to fill the canister. At Harvard Forest, air samples were collected through a molecular sieve trap as described in Gaudinski *et al*. (2000). Air samples were taken in an open location, at least 1 m off the ground, to obtain well-mixed air. The number of air sampling dates per year used in the calculation of the annual $\Delta^{14}CO_2$ value varied from 1 to 3, and the number of air samples taken on each date varied from 1 to 3 (see the Supporting Information).

Sampling of new tissues

Roots. To investigate the magnitude of stored C reserves in new-root tissues, we collected new-root growth by slicing into the soil with a shovel and placing a nylon screen (1 mm mesh) stretched across a circular embroidery hoop (15 cm diameter) vertically into the soil, so that the top of the hoop was 1–5 cm below the surface of the organic horizon. After placement, soil or organic horizon (as appropriate) was carefully packed around the entire screen. At ORR, roots that grew through the screen mesh were harvested and cleared multiple times between 1999 and 2002 to track the input of elevated ^{14}C to new-root growth over time (Table 1). During 1999–2001, samples were collected at TDE, because screens to collect new-root growth were already in place (for another study) before the original 1999 release. In 2001, we collected new-root growth at all three sites (TDE, WB, and HR), and in 2002, we sampled only WB and HR.

At the bomb-^{14}C sites, we collected new-root growth during only one time interval (Table 1), because the annual decrease in $\Delta^{14}C$ was small and fairly constant. After collection, at both EBIS and bomb-^{14}C sites, roots were transported back to the laboratory and refrigerated or frozen until processed. EBIS roots were dried (50 °C for a minimum of 48 h) and ground in a Spex Certprep 8000 M Mixer Mill (Spex CertiPrep, Metuchen, NJ, USA) to a fine homogeneous powder (5–10 min). Roots from bomb-^{14}C sites were handled identically except that they underwent soxhlet extraction followed by bleaching (Gaudinski et al., 2005). At the Blodgett site, we also collected new roots from soil cores (from the O horizon and 0–10 cm) by selecting only the very fleshy white or very light colored unsuberized roots that were clearly the current year's growth. We used a stainless steel corer 5.35 cm in diameter. Once separated from the soil and other roots, the new roots were handled and processed as described above for bomb-^{14}C sites.

The nonstructural C in new roots is derived mostly from recent photosynthate and is likely only days to weeks old (Horwath *et al*., 1994). In contrast, the structural component, such as cellulose, is not replaced over the root lifespan (Sternberg *et al*., 1986; Farquhar *et al*., 1998; Barbour *et al*., 2004). For roots from bomb-^{14}C sites, we pretreated tissue to isolate the cellulose by a soxhlet extraction, followed by bleaching (Gaudinski *et al*., 2005), thereby ensuring measurement of the oldest C making up the root. Root samples from ORR were not pretreated, because we wanted to compare the ^{14}C signature of bulk (i.e., not pretreated) new-root tissue with the ^{14}C content of leaf buds, expanding leaves, and parasitic plants which had already been measured for ^{14}C as bulk tissues. We also wanted to compare ^{14}C in new roots with that in mixed-age populations of roots sampled from cores (as bulk nonpretreated roots) for other aspects of the EBIS project. Given that the modeling approaches we applied to EBIS samples in this study are based on rates of decline in ^{14}C over time, and that the difference

Table 1 Sampling and processing information for new-root samples

EBIS sites‖	Screens emplaced	Screens harvested	Number of screens with roots*	Number of ^{14}C samples measured†	Pretreatment method‡	$\delta^{13}C$ range§ (‰)	Graphing cohort¶
TDE	04/02/99	08/20/99	3/3	3	None	−28.1	A
	06/12/99	08/20/99	6/6	5	None	−28.1	A
	08/20/99	09/10/00	10/10	3	None	−28.1	B
	04/30/00	09/10/00	3/3	2	None	−28.1	B
	05/01/01	11/07/01	12/12	3	None	−28.1	C
Walker Branch	07/20/01	11/11/01	9/12	3	None	−28.1	C
	03/26/02	05/15/02	9/12	2	None	−28.1	D
	05/15/02	11/06/02	8/12	3	None	−28.1	E
Haw Ridge	07/20/01	11/11/01	10/12	3	None	−28.1	C
	03/26/02	05/15/02	10/12	3	None	−28.1	D
	05/15/02	11/06/02	8/12	3	None	−28.1	E
Bomb-^{14}C sites							
Harvard Forest	04/08/01	11/08/01	12/12	6	Sox/bleach	−26.8 to −27.8	None
Blodgett	04/09/02	06/07/02	4/12	2	Sox/bleach	−20.0 to −27.1	None
Knottåsen	09/03/01	08/12/02	8	4	Sox/bleach	−23.7 to −24.5	None

*Top number represents the amount of screens put in the field. Bottom number represents the number of screens that had roots growing through them.
†Samples represent roots from one screen or a composite of roots from two screens.
‡Pretreatment was via a soxhlet/bleaching (sox/bleach) method (Gaudinski *et al*. 2005).
§Each sample measured individually on bomb-^{14}C samples; for EBIS sites, a value of −28.1‰ was assumed (see the text for details).
¶Roots were grouped and graphed in five different cohorts (A–E) depending on when they grew.
‖The large ^{14}C release at ORR occurred sometime between June 12 and July 22, 1999.
EBIS, Enriched Background Isotopic Study; TDE, Throughfall Displacement Experiment site.

in age between structural and nonstructural C is small for new roots because they are so young, the use of exclusively bulk tissues should not affect our results.

Leaf buds and expanding leaves. We sampled a time series of new leaf buds and expanding leaves from white and chestnut oak and red maple trees in East ORR between 2000 and 2003. New buds were collected in mid-march of 2000 and 2001 and expanding leaf tips in early April of 2000 and 2001 (see Appendix A for sampling dates). Samples were kept cool and transported to the laboratory, where they were dried (50 °C) and ground. Leaf buds and expanding leaves were assumed to grow entirely from stored C reserves (Kramer & Kozlowski, 1979).

Parasitic plants. As an additional indicator of the isotopic signature of stored C fueling growth of new tissues, we collected samples of nonphotosynthesizing perennial parasitic plants, which grow only on C originating from the root exudates of their tree hosts. Samples of the cone-shaped inflorescence of Squaw root [*Conopholis americana* (L.) Wallr] and Indian pipe (*Monotropa uniflora* L.) were collected in East ORR during spring 2000, 2001, 2002, and 2003. Samples were kept cool and transported to the laboratory, where they were dried (50 °C for a minimum of 48 h) and ground. Squaw root is associated only with trees of the genus *Quercus* (Baird & Riopel, 1986).

Radiocarbon analysis

To determine ^{14}C content, we converted all samples (solids and atmospheric gas) to graphite by either sealed-tube zinc reduction (Vogel, 1992) at Lawrence Berkeley National Laboratory or hydrogen reduction (Vogel *et al*., 1987) at the Center for Accelerator Mass Spectrometry (CAMS) at Lawrence Livermore National Laboratory. The graphite was measured for ^{14}C content at CAMS for all samples except tree rings from the bomb-^{14}C sites which were analyzed at the W.M. Keck-CCAMS facility at University of California, Irvine. Radiocarbon results are expressed as $\Delta^{14}C$ (‰) according to Stuiver & Polach (1977). The $\Delta^{14}C$ unit is normalized to a $\delta^{13}C$ value of –25‰, which removes the effects of mass-dependent isotopic fractionation, such as the discrimination against atmospheric ^{14}C during photosynthesis. The $\delta^{13}C$ values used to normalize $\Delta^{14}C$ for new roots, at the bomb-^{14}C sites, were measured in each sample after pretreatment. For EBIS samples, we used

−25‰ for leaf buds, expanding leaves, and parasitic plants (−25.0 ± 0.4‰ represents the mean of five measured parasitic plant samples); −28.1‰ for new-root growth (−28.1 ± 0.06‰ represents the mean of 113 fine-root samples from EBIS root cores; Joslin *et al*., 2006); or sample-specific $\delta^{13}C$ values (see the Supporting information).

Mean age of stored C in leaf buds, expanding leaves, and parasitic plants at East ORR (one-pool model)

Leaf buds and expanding leaves are made completely from C reserves stored in previous years (Kramer & Kozlowski, 1979). We estimated the mean age of storage reserves used for leaf buds and expanding leaves at East ORR with a one-pool, donor-controlled model analogous to the approach described by Trumbore *et al*. (2002), except that we explicitly take into account the $\Delta^{14}C$ value of background atmospheric CO_2 (Joslin *et al*., 2006). In this model (hereafter referred to as the 'one-pool model'), the C flux out of the pool is linearly proportional to pool size, inversely related to turnover time, and yields an exponential relationship for ^{14}C content with time:

$$N_i = (N_0 - A_b)e^{-t/\tau} + A_b, \quad (1)$$

where N is the measured $\Delta^{14}C$ value (‰) of the leaf buds, expanding leaves and, parasitic plants (and represents the isotopic signature of the storage pool). The subscript '0' indicates the initial year of measurement, '*i*' indicates successive years of measurement, A_b is the background $\Delta^{14}C$ value of photosynthate fixed from atmospheric CO_2 before or after the pulse, t is the time (years), and τ is the C pool turnover time (years). We took A_b to be constant [88‰ ± 13(SD)‰; see Appendix A] over the period after the pulse in 1999 through 2002, because we do not have accurate measurements of the local A_b during this time, and the standard deviation of the mean (for measurements taken in 1999 and spring/early summer 2000) encompasses the global background measurements for 1999 through 2002 [90‰, 87‰, 81‰, and 75‰, respectively: Levin & Kromer (2004)]. We estimated τ by exponential regression of the data [using Eqn (1)], and the mean for A_b (88‰). We also calculated the range in τ given by using the mean $A_b \pm$ SD and the different plant tissue types. We assumed that the drop in $\Delta^{14}C$ value of N over time after the local 1999 pulse was caused by dilution of the storage pool by new photosynthetic inputs with a $\Delta^{14}C$ value of A_b. Radioactive decay of ^{14}C is not included in the model because, with a half-life of 5700 years, it is too slow to have an effect over the 3-year time scale of this study.

Amount of stored C used to grow new roots (one-pool model)

The amount of stored C contributing to new-root growth (f_s) can be calculated using isotope mass balance, including the radiocarbon value of C supplying new-tissue growth, which was estimated as the *y*-intercept of exponential curve fits to the ^{14}C data, of (1) leaf buds, expanding leaves, and parasitic plants (i.e., storage C growth), and (2) new-root growth. The mass balance equation is:

$$\Delta_{ng} = \Delta_{nc} f_{nc} + \Delta_s f_s, \quad (2)$$

where Δ_{ng} and Δ_s are the radiocarbon values at time zero of the C supplying new-root growth and storage C growth, respectively. Δ_{nc} is the $\Delta^{14}C$ value for C fixed from new photosynthate (which is 0‰, because we subtract A_b from the initial data to perform the regressions). f_{nc} and f_s represent the fraction of new-root growth coming from newly fixed C or storage C, respectively.

Mean age and amount of stored C used in fine-root growth annually at East ORR (Radix)

To calculate the mean age and amount of stored C supplying new-root growth, we used the EBIS root-screen time-series data and a new model of fine-root dynamics (Radix1.0; Riley *et al*. submitted). Similar to the model described above, Radix simulates donor-controlled fluxes. However, Radix has the following added components for simulating realistic physiological complexities: (1) short-lived (months) and long-lived (years to decades) roots, each with right-skewed age populations; (2) stored C inputs to new fine-root growth and fine-root respiration; (3) seasonal variation in fine-root respiration and growth rates; and (4) uncertainty in forcing variables and model parameters.

A simplified version of Radix was used for this paper which simulates only new-root growth (Fig. 4). In the model, fine roots grow using stored and/or newly photosynthesized C and are represented by one pool (new roots; L_1). L_1 loses C via respiration (R_1). Mortality turnover of L_1 was not simulated because the roots were sampled (harvested) after less than 1 year so that mortality turnover was assumed to have small influence on the results. A fraction of new photosynthate (f_s) is allocated to the storage pool; the remainder ($1-f_s$) goes directly into L_1 (see Fig. 4). Losses from the storage pool result from transfer to L_1. The transfer is controlled by the storage pool turnover time (τ_s) and size. The goal of the Radix modeling was to estimate values of f_s and τ_s that best fit the time series of new-root ^{14}C measurements. The best fit estimates were achieved with a chi-

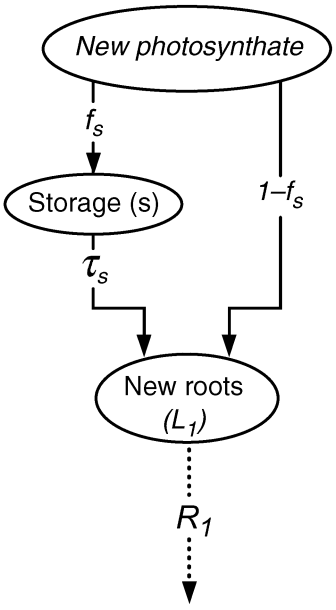

Fig. 4 Modified Radix model [see Riley *et al.* (submitted) for full model description]. In Radix, new photosynthate (f_s) is represented by belowground primary productivity, τ_s represents the steady-state turnover time of the storage pool, and R_1 is the respiration from the live root pool (L_1). For this study, root death is assumed to occur only at root harvest (see Table 1 for root harvest dates).

square (χ^2) analysis which minimizes the squared difference between model predictions and measured $\Delta^{14}C$ values, weighted by measurement uncertainty (Press *et al.*, 1989). The relative χ^2 values indicate how well predictions using a particular combination of f_s and τ_s fit the ^{14}C data. The smaller the χ^2 value, the better the fit.

Model inputs and constraints. Between November and April, when tree leaves in the ORR deciduous forest have senesced or dropped, there is no gross primary productivity. There can, however, be new-root growth and respiration from stored C reserves. At WB, leaf expansion occurs on average between 10 April (5% completion) and 11 May (95% completion; Joslin *et al.*, 2001; Hanson *et al.*, 2003). Previous radiocarbon labeling of mature white oaks at WB showed that in April, leaves are accumulating C (much of it from stored reserves) and not translocating C out of the leaf (Edwards *et al.*, 1989). However, during May through October, leaves translocate a large part of the newly photosynthesized C (Edwards *et al.*, 1989). Thus, we assume all new photosynthate produced in April is used for aboveground leaf growth (and not used belowground).

In the model, the periods of May–July and August–October receive 72% and 28%, respectively, of annual belowground primary productivity (BGPP). This partitioning is estimated from minirhizotron observations at TDE of growth in the length of fine roots (<2 mm diameter), in November–March (5%), April (10%), May–July (65%), and August–October (20%; Joslin *et al.*, 2001; J.D. Joslin, unpublished results). Because none of the C used between November and April (when 15% of root growth occurs) is recently fixed, we divide the remaining 15% BGPP evenly between the 6 months of May–October (i.e., 2.5% of the annual new-root growth occurs each month, May–October).

We assumed a lower bound for the fraction of BGPP allocated to the storage pool (f_s) of 0.15, because 15% of new fine-root growth occurs between November and April, when no new photosynthate is being generated. To impose seasonality in use of storage reserves, we set the C fluxes out of the storage pool explicitly, such that 10% occurs in April, and 1% occurs in each of the 5 months between November and March. These partitions are based on data from Joslin *et al.* (2001) and J.D. Joslin (unpublished results). Any growth from storage C above 15% of BGPP (i.e., $f_s > 0.15$) is partitioned equally among the remaining 6 growing season months.

The model prescribes a steady annual oscillation in storage pool size (i.e., the annual variation in storage pool size is identical between years). The turnover time of the storage pool (τ_s) reported here is the annual flux-weighted value, calculated as the ratio of the annual mean storage pool size, S_m ($g\,C\,m^{-2}$), and the cumulative C flux out of the storage pool. S_m is an important determinant of τ_s but was not constrained by available data. Instead, S_m is imposed (as an initial condition), and we varied S_m (instead of τ_s directly) to generate a range of τ_s values for the χ^2 simulations. We used 20 years as an upper bound on τ_s (Gaudinski *et al.*, 2001).

Values for root respiration (R_1; maintenance + growth) were set at 0.020, 0.033, 0.055, and $0.064\,\mu g\,C\,g^{-1}\,s^{-1}$ for the four seasonal periods (November–March, April, May–July, and August–October, respectively). Harvest of L_1 roots was prescribed based on actual harvest dates for the root screens, and grouped into five root cohorts labeled A–E (see Table 1, Fig. 3).

Once we determined the best-fit values for f_s and τ_s, we defined this to be our 'nominal' case and investigated the sensitivity of model predictions to several different parameterizations (Table 2). First, we investigated the impact of our parameterization of seasonality in use of storage C by running the model

Table 2 Simulations run as part of a sensitivity analysis for fraction of new photosynthate allocated to the storage pool (f_s) and the turnover time of the storage pool (τ_s) in Radix

Run number	Run description	f_s	τ_s* (years)	Seasonality	χ^2
1	Nominal	0.55	0.7	Yes	1.3
2	No seasonality	0.55	0.7	No	1.5
3	Min f_s	0.15	0.7	Yes	30
4	5-year τ_s	0.55	5	Yes	15
5	10-year τ_s	0.55	10	Yes	21
6	Inclusive storage	1	0.35	Yes	9
7	No storage	0	0	Yes	154

*Annually flux-weighted value.

with no seasonality in storage use (i.e., an equal proportion of the total annual growth from storage is used in each of the 12 months; Run 2). Second, we investigated the sensitivity of χ^2 values to f_s and τ_s by running Radix with the minimum observed value of f_s (0.15, Run 3) and values of τ_s that were much longer than the nominal τ_s (5 years, Run 4 and 10 years, Run 5). Finally, we investigated the sensitivity to use of stored C by assuming (1) that all new photosynthate and stored C mix together in one pool, rather than being stored and moved separately ($f_s = 1$; Run 6) or (2) that there is no use of stored reserves ($f_s = 0$; Run 7). For Run 6, we determined the best-fit τ_s that went with $f_s = 1$.

Mean age of C used to grow new-root tissue

Root tissues grow using C from storage and/or newly fixed photosynthate. Using the best-fit values of f_s and τ_s derived from the Radix and one-pool models, we estimated the mean age of C used to grow new roots (C_r) for both modeling approaches. Using Eqn (3), we average the mean ages of the two sources of C supplying root growth:

$$C_r = \tau_s f_s + \tau_{rf}(1 - f_s), \quad (3)$$

where τ_{rf} is the turnover time of new photosynthate (assumed to be 1 week). We note that C_r represents the mean age of tissues in 'new roots' as well as 'new tissue' growing on existing roots that may be several years in age.

Results

Mean age of stored C used to grow leaf buds, expanding leaves, and parasitic plants

Leaf-bud, new-leaf, and parasitic-plant ^{14}C content at East ORR. Isotopic signatures of leaf buds and expanding leaves at East ORR showed a significant degree of ^{14}C incorporation, from the 1999 release, in 2000, followed by rapid return to background atmospheric ^{14}C levels in 2001 (Fig. 5). Compared with leaf buds and expanding leaves of maple, leaf buds and expanding leaves of oak had a higher peak ^{14}C value and more rapid return to background levels, indicating a faster turnover time of the storage pool in oak. In March and April 2000, oak buds and expanding leaves ranged between 317‰ and 527‰, whereas maple buds ranged from 350‰ to 393‰. In March 2001, ^{14}C levels had dropped to 127–143‰ for oak, but only to 188‰ for maple [note that there was only one maple leaf sample in 2001 (Fig. 5)]. Parasitic plants sampled in summer 2000 had values between 406‰ and 516‰ in June and between 320‰ and 419‰ in August (Fig. 5 and Appendix A). These values dropped to less than 100‰ by late April 2003.

The mean ages of C in leaf-bud growth, as estimated from an exponential fit to the data [Eqn (1)], are 0.5 and 1.0 years for oak and maple, respectively (Table 3, Fig. 5). The shorter mean age for oak derives from the faster decline in $\Delta^{14}C$ values and implies that its reserve pool cycles more quickly relative to maple. The mean age of C fueling leaf bud and leaf growth, combining data for both species, was 0.6 years (Table 3). The mean age of C in parasitic plants was 0.7 years based on samples from early spring only, when we are sure that their growth represents only stored C, as well as when using data from all measurement periods. This concurrence may be due to the fact that the curve fit is strongly influenced by the three measurements in April 2003 (Fig. 5). The mean age of C used in new growth as calculated from all leaf-bud and parasitic-plant data was also 0.7 years. We note that the sample sizes of the different datasets (oak, maple, and parasitic plants) are not equal, and the curve fitting is strongly influenced by a few points at the tail end of the datasets. Therefore, interpretations of interspecies differences should be used with caution. Additionally, the parasitic plants are perennials with a subterranean plant body (Baird & Riopel, 1986). Therefore, it is possible that stored C from the subterranean plant body, fixed before the 1999 pulse, was used in the growth of the flowering stalk. However, given the high $\Delta^{14}C$ values measured in spring 2000, and their rapid decline, it is unlikely that a large amount of 'prepulse' stored C was used.

Using the range in A_b of $\pm$ 13‰ changed the mean age of any given tissue by $\pm$ 0.1 to 0.3 years, yielding a range in mean C storage age of 0.4–1.10 years (Table 3). Our estimates differ from the 4–6 years reported by Trumbore *et al.* (2002), because there was an error in their calculation of τ (S.E. Trumbore, personal communication).

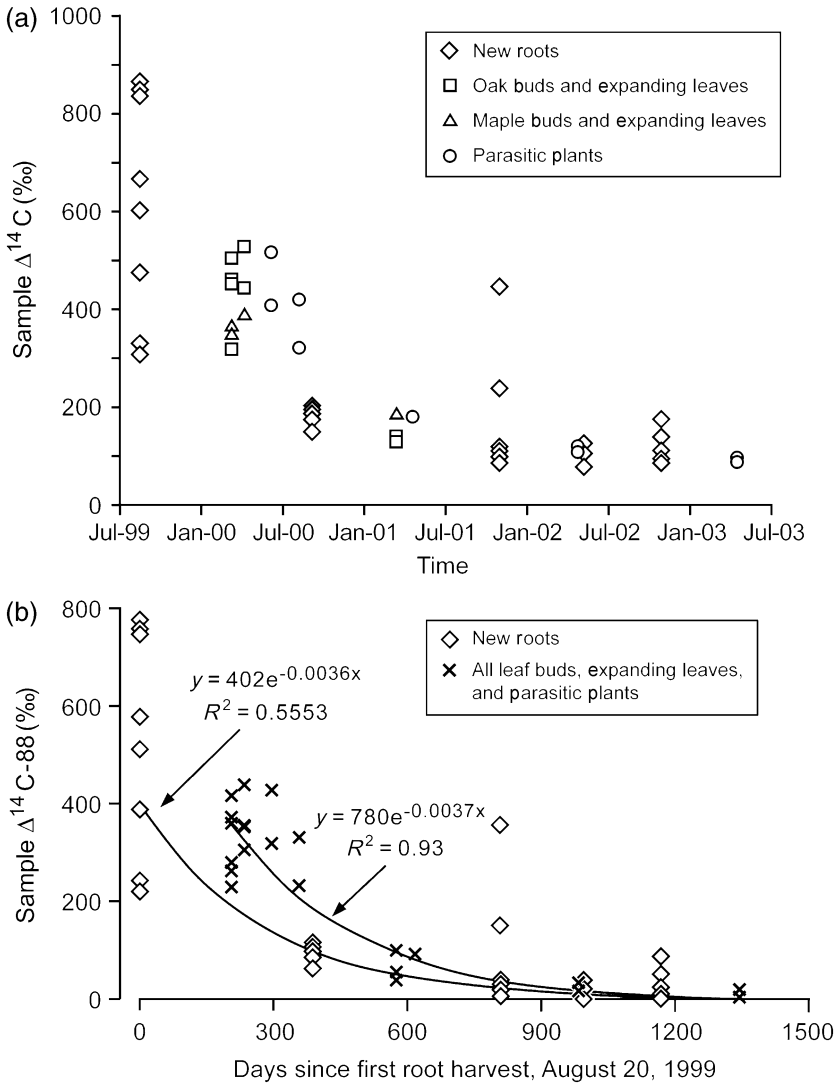

Fig. 5 (a) Local Enriched Background Isotopic Study pulse of ^{14}C was released between June 12 and July 22, 1999. The *x*-axis is the date of tissue harvest. (b) The same data as panel a, except that all values have had the $\Delta^{14}C$ value of newly fixed photosynthate (i.e., A_b or background; 88‰) subtracted.

Mean age and amount of stored C reserves used in root growth

Root ^{14}C content at East ORR. The isotopic content of new roots that grew into root screens at ORR between April 2 and August 20, 1999, showed a large degree of label incorporation (Fig. 3), with values ranging from 307‰ to 864‰. The large variability was likely due to different periods of root growth relative to the $^{14}CO_2$ atmospheric pulse as well as to species differences in C allocation to various pools. Because roots could have grown into the screen at any time after emplacement, we do not know the precise time of growth. Samples with higher ^{14}C values may have accumulated a larger fraction of their mass closer to the ^{14}C release.

Roots that grew in fall 1999 or the 2000 growing season (August 20, 1999, through September 10, 2000) had $\Delta^{14}C$ values between 150‰ and 198‰. Roots that grew during the 2001 growing season at TDE showed declines in isotopic signatures, with an average of 93 ± 5‰ in 2001 (Fig. 3). Roots that grew in 2001 at HR and WB (data pooled to calculate one mean; $n = 5$ or 6 in all cases) had $\Delta^{14}C$ values similar to TDE, but slightly higher means and standard errors (126 ± 23‰). Subsequent, minor ^{14}C releases or plumes from fossil fuel sources could explain such differences. Variability was greatest at WB, with two particularly high values in 2001. In 2002, roots were measured only at HR and WB, and showed no significant decline in ^{14}C relative to the 2001 growing

Table 3 Exponential curve-fitting parameters for one-pool model

Tissues	Equation*	τ (years)†	Time interval‡ (years)	r^2	n
Oak buds/expanding leaves	$1265e^{-0.0057x}$	0.5 (0.4–0.6)	1	0.9287	10
Maple buds/expanding leaves	$511e^{-0.0028x}$	1.0 (0.9–1.1)	1	0.9668	5
All leaf buds/expanding leaves§	$925e^{-0.0047x}$	0.6 (0.5–0.7)	1	0.8585	15
Parasitic plants¶	$1190e^{-0.0040x}$	0.7 (0.5–1.0)	3	0.9467	10
All leaf buds, expanding leaves, and parasitic plants	$780e^{-0.0037x}$	0.7 (0.5–1.0)	3	0.9300	25
New roots	$402e^{-0.0036x}$	0.8 (0.7–1.1)	3	0.5553	32

*The equation is $y = N_i - A_b = (N_o - A_b)e^{(-x/\tau)}$, where the background value (A_b) has been subtracted from the input data and is thus equivalent to 0‰; x is equivalent to time and τ is 1 divided by the exponent. See the text for further explanation of symbols.
†Values in parentheses represent range of results based on running the curve fit for background (88‰ ± 13‰; i.e., 101‰ or 75‰). The smallest value of τ is associated with A_b of 101‰ and the highest value of τ with A_b of 75‰.
‡Represents the period of time from the first tissue sampling to the last tissue sampling.
§Includes data for leaf buds and expanding leaves for both oak and maple trees.
¶Data included are for all sampling dates (see the text for details). The same A_b was also applied to parasitic plant measurements taken in April 2003 because the tissues grew from stored C fixed in the previous growing seasons over which A_b applies (i.e., 1999–2002).

season, whether they grew in the early half of the growing season (109 ± 9‰; March 26 to May 15, 2002) or the latter half of the growing season (116 ± 14‰; May 15 to November 6, 2002).

Storage C use in roots estimated using one-pool model. New-root $\Delta^{14}C$ values were lower than those of leaf bud, new leaf, and parasitic plants (Fig. 5a). This pattern is consistent with new-root growth being a mixture of stored C and newly fixed photosynthate, where the ^{14}C signature of the latter is the same as the background atmosphere (Fig. 5b). Using Eqn (2), and the y-intercept values for new-root growth (402‰; Table 3), and storage C (780‰; Table 3 equation for all leaf buds, expanding leaves, and parasitic plants), we estimate that 52% of the C in new roots comes from storage with a range of 35–64% given ± 13‰ in the value of background atmospheric $^{14}CO_2$. The range was 32–79% if the minimum (maple) and maximum (oak) y-intercept values were used.

We investigated the affect of our assumption of a constant A_b on our results for the one-pool model by forcing A_b to decrease 5‰ per year (Levin & Kromer, 2004; Supporting information for tree-ring cellulose from Blodgett Forest, Harvard Forest, and Knottåsen sites) beginning in 2001. The $\Delta^{14}CO_2$ values for 1999 and 2000 came from observations (see Appendix A) and the $\Delta^{14}CO_2$ values we used for 1999, 2000, 2001, 2002, and 2003 were 90‰, 86‰, 81‰, 76‰, and 71‰, respectively. The resulting τ-values were all within the range of those predicted using a constant A_b. However, they were all at the highest end of the range. Given the lack of data for 2001–2003, the enriched local $^{14}CO_2$ history, and the potential for use of re-respired $^{14}CO_2$, we contend the use of a higher constant A_b with the large error (± 13‰; resulting from the standard deviation of the 1999–2000 data) is the better approach.

Radix predictions. We estimated the fraction of new photosynthate (f_s) allocated to the storage pool and its turnover time (τ_s) by determining the values that gave the best fit (lowest χ^2) to the time series of measurements of $\Delta^{14}C$ in new roots. If we define the range of best-fit values as the region in Fig. 6 with χ^2 values <2.0, then the range of best-fit values for f_s is $0.5-0.6$, and for τ_s is $0.5-1.2$ years. The best χ^2 value (1.3) occurs for $f_s = 0.55$ and $\tau_s = 0.7$ years (Table 2).

The sensitivity of Radix predictions to seasonality of C inputs was minimal. Using the previously calculated best-fit values of f_s and τ_s, simulations with and without seasonality showed a very similar fit to the data and χ^2 values that were almost identical (Table 2 and Fig. 7, compare Runs 1 and 2). Based on these comparisons, we conclude that the model is not sensitive to the prescribed seasonal parameterization in the region where the best fit occurs.

Radix is sensitive to the values of f_s and τ_s. The sensitivity occurs primarily with fitting the last four data points. All values of f_s and τ_s predict highly enriched root $\Delta^{14}C$ values in 1999 (Fig. 7). Beginning 1 May, new photosynthate began to be generated and

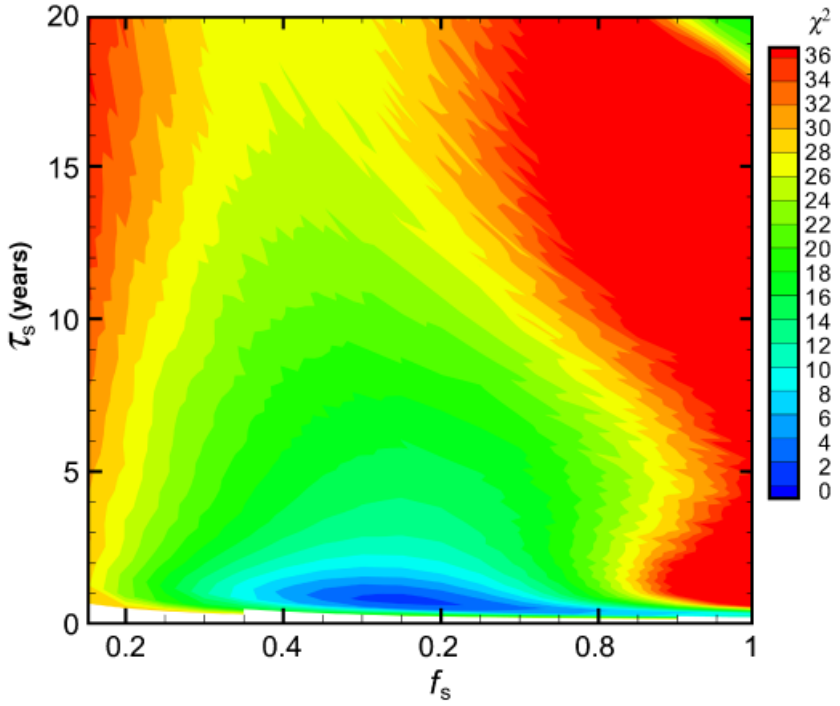

Fig. 6 Contour plot of χ^2 values, using East Oak Ridge Reservation atmospheric radiocarbon proxy curve over the space of f_s and τ_s showing the entire range evaluated. The lower the χ^2 value, the better a particular f_s, τ_s combination fits the time-series new-root ^{14}C data. These simulations were performed with seasonal use of storage reserves. White areas indicate that the storage pool size is unable to supply the required C throughout the year and thus are not valid combinations of f_s and τ_s.

most of the growth was from this newly produced and highly enriched C source. Thus, the $\Delta^{14}C$ value of the storage pool and the amount of it used had little impact on root $\Delta^{14}C$ values in early 1999. Post-1999, the low values for f_s (0.15, Run 3 and 0.0, Run 7) produced the poorest fit to the data, because the storage pool did not supply enough ^{14}C to sustain the enrichment at the measured levels. For simulations where $f_s = 0.55$, the effect of varying τ_s was not large (compare Runs 1, 4, and 5), although the longer τ_s values produced a poorer fit. Run 6 ($f_s = 1$), which simulated the mixing of stored and recently fixed C in one pool, matched the first and second data points well, but could not reproduce the higher $\Delta^{14}C$ values measured in 2001 and 2002.

Mean age of new-root tissues at East ORR. We calculated the mean age of C making up new-root tissues (C_r) using Eqn (3) and f_s and τ_s calculated from both the one-pool and Radix models. For the former, mean values for f_s and τ_s were 52% and 0.7 years, respectively, and C_r was 0.4 years (with a range of 0.2–0.8 years due to range in A_b and all nonroot values of τ_s in Table 3). For Radix, mean values for f_s and τ_s were 55% and 0.7 years, respectively, and C_r was 0.4 years (with a range of 0.3–0.7 years if the range in best-fit f_s and τ_s were used). In the one-pool analysis, we assumed that the age of storage C used for aboveground growth (i.e. leaf buds) was the same as that fueling new-root growth. We did not use the storage pool τ calculated from new roots (i.e., $\tau = 0.8$ years; Table 3), because new roots may contain a mixture of stored and recently fixed C (unlike leaf buds and expanding leaves which are grown entirely from stored C). The calculation was not sensitive to τ_{rf} (turnover time of new photosynthate); doubling τ_{rf} from 1 to 2 weeks increased C_r by only 0.01 years in each case.

Mean age of new roots using the bomb-^{14}C technique. At the three bomb-^{14}C sites, mean $\Delta^{14}C$ values of new roots differed from $\Delta^{14}C$ values of (1) local air by 2–7‰ and (2) tree-ring cellulose by 0–4‰ (Table 4). There were no significant differences between ^{14}C content of new roots and of either air or tree-ring cellulose samples ($\alpha = 0.05$; see Table 4) in any case. Therefore, storage inputs to new fine-root growth were undetectable using the bomb-^{14}C approach. The age of C used in new fine-root growth must be less than 1–2 years, which is the limit of the technique, given our AMS analytical error of 4–6‰, a decline in northern hemisphere atmospheric $\Delta^{14}CO_2$ between 1998 and 2003 of $\sim 6‰\,yr^{-1}$ (Levin & Kromer, 2004), and a decline of $\sim 5‰\,yr^{-1}$ in tree-ring $\Delta^{14}C$ values for 1998–2003 (based on measured tree-ring

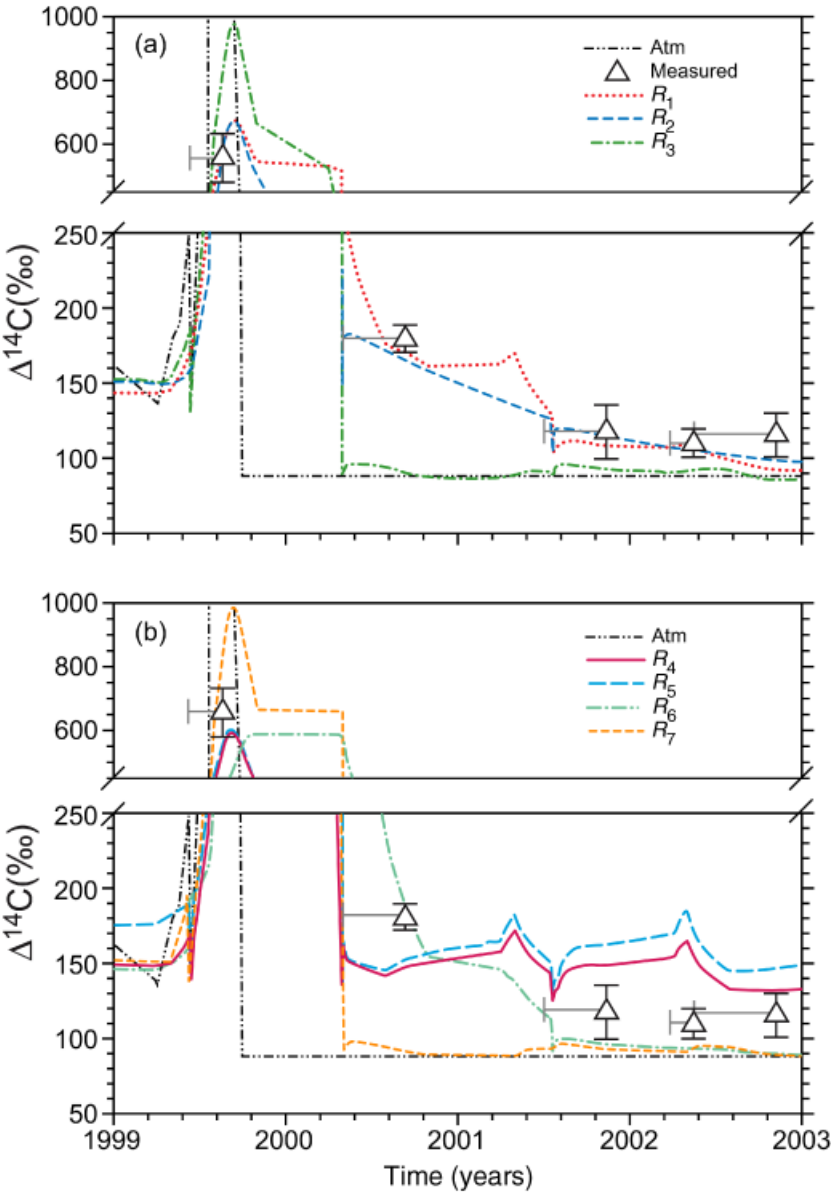

Fig. 7 Predicted $\Delta^{14}C$ values for new roots grown in root screens for seven scenarios (Runs 1–7; see Table 2) using East Oak Ridge Reservation atmospheric radiocarbon proxy curve. For the new-root values, the horizontal line and the placement of each data point constrain the time interval during which the measured root tissues could have grown. The root mass at the beginning of any growth interval is zero. Sharp changes in $\Delta^{14}C$ values indicate either root harvest or the turning on or off of new photosynthate as a source for new-root growth, or both.

values at each site; Supporting information). More samples with higher precision AMS measurements of ^{14}C would be required for a more accurate determination of the mean age of new-root tissues using the bomb-^{14}C signal. *In situ* ^{13}C or low-level ^{14}C labeling experiments (Carbone *et al.*, 2007) may be a more cost-effective approach for more precise quantification of C storage, if labeling could be accomplished on mature trees.

Summary of storage contributions

Analysis of $\Delta^{14}C$ values for leaf buds and expanding leaves using the one-pool model showed that aboveground tree tissues were grown using stored reserves with mean age that ranged between 0.5 and 1.0 years (Table 3).

We estimated that the mean age of C reserves contributing to new fine-root growth (τ_s) was 0.7 years by

Table 4 $\Delta^{14}C$ values for new roots, local air, local tree-ring cellulose, and global background air

Site	Blodgett Forest		Harvard Forest		Knottåsen		Harvard Forest	
Year	2002	*n*	2001	*n*	2002	*n*	1999	*n*
New roots	63 (5)	9	77 (1)	5	81 (3)	4	90 (2)*	5
Local air	69 (2)	9	75 (1)†	2	74 (2)	3	93 (3)‡	3
Local tree-ring cellulose	62 (2)	1	80 (2)	1	77 (2)	1	90 (2)	1
Global background air§	75 (2)		81 (2)		75 (2)		90 (2)	
New roots minus local air	−6		2		7		−3	
New roots minus tree-ring cellulose	1		−3		4		0	
P-value new roots/local air¶	0.1468		0.1675		0.0546		Not done	
P-value new roots/local tree-ring cellulose‖	0.8640		0.1070		0.3070		Not done	

Units are $\Delta^{14}C$‰ and values in parentheses represent standard error.
*Gaudinski *et al*. (2001).
†Air samples collected in 2002, we added 4‰ to average value to estimate 2001.
‡J.B. Gaudinski & S.E. Trumbore (unpublished results).
§Levin & Kromer (2004).
¶*P*-value obtained from Student's *t*-test (two samples assuming unequal variance).
‖*P*-value obtained from Student's *t*-test (one sample assuming known value, i.e., wood value).

two approaches. First, Radix had best-fit τ_s of 0.7 years with a range of 0.5–1.2 years. Second, the one-pool model, using the ^{14}C content of parasitic plants growing on root exudates before full canopy leaf-out, showed a mean age of stored C of 0.7 years with a range 0.5–1.0 years (Table 3).

The fraction of new fine-root C coming from stored reserves annually was calculated to be 55% with a range of 50–60% using Radix, and 52% with a range of 32–79% using τ estimated from the one-pool model.

We calculated the annually averaged mean age of C in new-root tissues (after stored C and newly fixed C mix), C_R, at ORR to be 0.4 years (range 0.3–0.7 years) using Radix and 0.4 years (range 0.2–0.8 years) using the one-pool model. At the three bomb-^{14}C sites, the mean age of C in new-root tissues was less than 1–2 years.

Discussion

Despite uncertainties in ^{14}C inputs to the ORR ecosystem, we are confident in our estimates of both the mean age of C reserves used to grow leaf buds and new roots, and the amount of new-root growth coming from stored C reserves annually. This is because our two independent model approaches, the one-pool model (for which we did not need to quantify ^{14}C inputs) and Radix (for which we estimated ^{14}C inputs via East ORR ARPC), gave very similar results.

The use of storage C may be different in other ecosystem types such as boreal forests which tend not to be water limited or Mediterranean ecosystems where there is typically no summer rain. For example, three studies in boreal black spruce forests in Alaska and Canada found the mean age of stored C pools supplying root respiration, and therefore presumably root growth, to be >3–5 years (Czimczik *et al*., 2006; Schuur & Trumbore, 2006; Carbone *et al*., 2007). Additionally, in a seasonally dry scrub oak system of central Florida, one-third of the C in new-root tissues came from photosynthate fixed before the year of the study. However, a mean age for this C could not be estimated (Langley *et al*., 2002). Further work is needed, particularly in nontemperate ecosystems, to understand the amount and seasonal timing of stored C use in mature forests.

Isotopic studies of fine-root dynamics and patterns of C allocation, particularly ^{13}C or low-level ^{14}C pulse-chase studies (e.g., Carbone *et al*., 2007), could be used to investigate the influence of stored carbohydrate on time scales less than 1–2 years. In past isotopic studies, in-growth cores (used to sample new-root growth) have typically been sampled at annual intervals or longer (Langley *et al*., 2002; Matamala *et al*., 2003) or not placed in the soil until several months after labeling began (Matamala *et al*., 2003). As such, they miss information on seasonal use of stored carbohydrate. In this study, for example, the influence of storage is most clear in the isotopic measurements of roots sampled in the year immediately after the ^{14}C release. For our study, time-series measurements of root ^{14}C content in April, May–July, and August–September of 2000, as well as in April 2001, would have helped constrain seasonal patterns of storage use and improved confidence in model esti-

mates of f_s and τ_s. Labeling studies are also needed to investigate the amount and age of stored reserves used under varying types and degrees of plant stress.

In the deciduous forest at ORR, the storage C used to grow new above- and belowground plant tissue was fixed 0.7 years ago, and the mean age of C in new-root tissues is 0.4 years. At the three other forests studied here (one deciduous, two coniferous), and in two broadleaf tropical forests in the Eastern Amazon of Brazil (Trumbore *et al*., 2006), the mean age of storage C used to grow new roots was <1–2 years. Taken together, the seven sites span a broad range of forest and ecosystem types and suggest that in several different forest types, storage reserves generally turn over quickly (are used quickly relative to stock). This implies that storage C may provide limited capacity to buffer tissue growth from stress or periods of low productivity that last more than 1 year.

It is possible, however, that trees have other storage pools with longer turnover times that were not accessed during these studies. The results reported here represent storage C use during years without anomalous environmental stress. None of the sites studied here experienced acute climatic stress, pest outbreaks, or fire during our study. The amount and age of C reserves used might vary under different degrees of stress such as mild vs. intense fires or droughts. Under extreme conditions, plants might access different reserve pools that could be older. Once photosynthate is converted to storage materials (primarily starch), it is commonly deposited into parenchyma cells where it stays until remobilized and transported outward radially toward the cambium. The starch is available along deep-to-shallow radial axes from the inside of the tree, outwards, and therefore the sink organs very likely use the nearest (and therefore youngest) carbohydrate available (Kagawa *et al*., 2006).

Implications for fine-root modeling

Isotopic methods for estimating mean ages of roots compare measured isotopic values of roots with a known and changing record of isotope values in the atmosphere (Gaudinski *et al*., 2001; Tierney & Fahey, 2002; Trumbore *et al*., 2002; Matamala *et al*., 2003; Keel *et al*., 2006). These previous studies assumed that plant tissues grow entirely from new photosynthesis and have the same isotopic signature as the atmosphere at the time of their growth (Trumbore *et al*., 1995; Gaudinski *et al*., 2000), and thus have a mean age of 0 years. Our work shows that this assumption is not entirely correct (for roots or leaves). For roots specifically, given that we estimated an age of the C in new-root tissues of 0.4 years, this error will make up a large proportion of the estimated mean age only for fast ($\leq$ 1 years) cycling roots. The error associated with this assumption decreases as the turnover time of roots increases.

To test the impact of our findings, on published estimates of fine-root lifetimes, we modified the model published in Gaudinski *et al*. (2001) to include reserve use of 55%, with a mean age of storage C of 1 year. The resulting root mean ages decreased by 0–2 years in all cases, with a new range of 1–17 years. Although this change is within the error of their method, it is a systematic bias and future applications of the technique should account for storage C use whenever possible.

Root populations comprise roots that cycle on time scales ranging from months to several years (Gaudinski *et al*., 2001; Tierney & Fahey, 2002; Trumbore & Gaudinski, 2003; Majdi & Andersson, 2005). Therefore, models of fine-root dynamics (with or without an isotopic component) need to be able to handle this complexity by using, for example, multiple pools and/or specified age distributions within a pool (Majdi & Andersson, 2005; Joslin *et al*., 2006; Trumbore *et al*., 2006). This study shows that inclusion of a storage pool is also important for models of fine-root dynamics. Additionally, because the amount of storage C used to grow new roots is significant ($\sim$ 55%), it should be accounted for in ecosystem C budgets. For example, including stored C would be important in reconciling above- and belowground net primary productivity in periods when primary production is not constant across years.

Models of root dynamics vary in their assumptions about how C is stored and utilized. Radix assumes that storage exists as a distinct pool and that C used for root growth or maintenance comes either from this storage pool, or from the new photosynthate pool, or both (Fig. 4). However, there are other possible model constructs. Luo (2003) and Luo *et al*. (2004) assumed that stored nonstructural C and new nonstructural C always mix well and are contained in the same pool before being used to drive root growth (Fig. 4; analogous to our simulation with $f_s = 1$). Matamala *et al*. (2003, 2004) assumed that all new growth is supported by recent C assimilation with no storage input (Fig. 4; analogous to our simulation with $f_s = 0$ and $\tau_s = 0$).

We used the ORR new-root time-series data and Radix to test which of these three constructs – distinct as done with Radix; inclusive as in Luo (2003) and Luo *et al*. (2004); or no-storage as in Matamala *et al*. (2003, 2004) – best fit the ORR data (Table 2, Fig. 7). In the *distinct* and *inclusive* cases, we used the value for τ_s that gave the smallest χ^2 in each case (0.70 and 0.35, respectively). The *distinct* construct produced the best fit to the data with a χ^2 of 1 (Run 1). The simulations with the *inclusive* storage pool (in which

all new and old C mix well before use for growth) had a best-fit χ^2 of 9 (Run 6). The inclusive simulation underpredicted $\Delta^{14}C$ values in summer 1999 (Fig. 7) and appears to be less responsive to the isotopic pulse relative to the *distinct* construct. The no-storage-pool χ^2 best-fit value was 154 (Run 7). The large difference between the *no-storage-pool* construct and both of the other two constructs supports the interpretation that storage is significant for new-root growth in the ORR forest. We conclude that the best framework to model C storage use has a distinct storage C pool and allows C for root growth or respiration to be drawn from both storage and new photosynthate.

Conclusion

In a deciduous forest in Tennessee, in years when trees are not under acute stress, the age of storage reserves contributing to both above- and belowground tissue growth is young (0.7 years), and the mean age of C used to grow new-root tissue is 0.4 years. In three additional temperate forests (two coniferous, one deciduous), the mean age of C in new roots was less than 1–2 years. Thus, C reserves appear to cycle quickly and their impact on calculation of fine-root lifetimes is small particularly for roots that live for many years. However, a significant percentage of stored C is used to grow root tissues ($\sim 55\%$) and this should be considered in future modeling efforts (both isotopic and nonisotopic). Research is needed on the seasonal and interannual variability in stored C use, as well as to evaluate the use of stored C as a function of ecosystem stress. These would allow us to learn more about impacts of asynchrony between photosynthetic activity and growth demand on seasonal and annual time scales.

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Appendix A

This appendix describes the creation of the Atmospheric Radiocarbon Proxy Curves (ARPCs) for the East and West sides of the Oak Ridge Reservation (ORR) used as forcing for Radix in Gaudinski *et al*., this issue; Riley *et al*., submitted). The West ORR Enriched Background Isotopic Study (EBIS) sites are named Pine Ridge (PR) and Tennessee Valley Authority (TVA). The East ORR EBIS sites are Walker Branch (WB) and Haw Ridge (HR). Data taken from the Throughfall Displacement Experiment (TDE) site and the P5 site (used for C inventory studies), both within 2 km of the East ORR WB site, are also used. See Gaudinski *et al*. (this issue) for a map and detailed site descriptions.

Characterization of atmospheric $^{14}CO_2$ inputs at ORR

The ORR has a locally enriched atmospheric $^{14}CO_2$ history and thus requires its own record of atmospheric $^{14}CO_2$. In 1999, release of $^{14}CO_2$ some time between June 12 and July 22, 1999, caused very large changes in atmospheric $^{14}CO_2$ levels over hour to daily time scales. Because this local ^{14}C release was unplanned, direct measurements of ORR atmospheric $^{14}CO_2$ content during and immediately after the 1999 enrichment do not exist. Therefore, we rely on plant-based records as proxies to estimate atmospheric $^{14}CO_2$ inputs to ORR photosynthesis. Plant tissues or their autotrophic respiration can be used as proxies for atmospheric values because photosynthate, the organic substrate used in both tissue growth and autotrophic respiration, has the same $\Delta^{14}C$ value as the CO_2 from which it is derived (the $\Delta^{14}C$ unit corrects for isotopic fractionation during photosynthesis; Stuiver & Polach, 1977).

Two issues arise with this approach. First, these plant-based proxies are representative of photosynthetic $^{14}CO_2$ uptake integrated over weekly to monthly time intervals, rather than atmospheric values at the time of the release, which probably varied dramatically on time scales of minutes to hours due to changing wind speed and direction. Second, proxy measurements may not accurately represent the atmospheric $^{14}CO_2$ content over the specified time interval due to use of stored C in current growth and respiration. Nevertheless, these proxies are the best available data for reconstructing local $^{14}CO_2$ inputs to the ORR ecosystem before and during the 1999 pulse. After the 1999 pulse, we assume that atmospheric ^{14}C returned to a locally measured 'background' and remained at that level through 2002. This assumption of a return to background is based on multiple sample records, as described below.

Atmospheric $^{14}CO_2$ background

We estimated a pre- and postenrichment 'background' of 88 ± 13 (SD)‰ based on point atmospheric samples taken at 10 cm above the soil surface on three dates between April 1, 1999, and June 12, 1999 (before the enrichment), and four dates between August 19, 1999, and June 10, 2000 (after the enrichment). The sampling date of July 22, 1999, was excluded because it clearly includes some of the 1999 enrichment pulse (Fig. A1). Samples were taken through molecular sieve traps as described in Gaudinski *et al*. (2000). Over the 4-year time period of this study, we utilize a constant mean with the standard deviation ($\pm$ 13‰) instead of the standard error ($\pm$ 5‰), because 88 ± 13‰ encompasses the global background measurements for 1999, 2000, 2001, and 2002 (90‰, 87‰, 81‰, and 75‰, respectively; Levin & Kromer, 2004). Additionally, given the lack of data on background atmospheric $\Delta^{14}C$ content for 2001–2002, the enrichment history, and the possible use of re-respired $^{14}CO_2$, we felt this rather conservative approach more appropriate than using an annually changing correction based on published values for the northern hemisphere (Levin & Kromer, 2004) or our data from other, bomb-^{14}C, sites.

Were there additional ^{14}C perturbations to the ORR atmosphere?

Some plant and soil CO_2 samples show elevated ^{14}C levels in 2000. This is likely due to use of 1999 C, and it is unlikely that a large pulse affected East ORR in early 2000 due to two lines of evidence. First, point air samples taken on April 23 and June 10, 2000, on the East ORR are both below 90‰. Second, new root tissues that grew between August 1999 and September 2000 do not show signs of additional enrichment [see Gaudinski *et al*. (this issue) and the Supporting information]. We do not have data for West ORR for this time period; however, it is not likely that there was another large pulse because the East ORR would probably have been affected.

We used air sampling to rule out the possibility of additional pulses after September 2000 to the end of this study. Air samples (collected 1–1.5 m above the ground) on both East and West ORR were collected by drawing air into a 32 L evacuated canister, through a capillary tube restrictor chosen so that the canister would take 2 weeks to fill (hereinafter referred to as '2-week air').

We did not use the canister samples to estimate the local $\Delta^{14}C$ value of free troposphere $^{14}CO_2$ for two reasons. First, the strength of the vacuum decreased over time as the canister filled. Thus, the amount of air sampled daily also decreased over time and each day is not represented in equal proportion. Second, because the canisters sampled 24 h a day, the night-time air was overrepresented relative to daytime air, because night-time air has higher CO_2 concentrations due to stable nocturnal conditions that trap re-respired CO_2 from plants and soil, which may have contained older, release-derived ^{14}C.

The measured $\Delta^{14}C$ values of 2-week air varied widely and include both positive and negative $\Delta^{14}C$ values (Fig. A1). Negative $\Delta^{14}C$ values clearly indicate a significant contribution of fossil fuel emissions to ambient CO_2. The record suggests, however, that there were no other significant ^{14}C releases or ^{14}C depletion of photosynthate during the growing season from September 2000 through 2002. Based on observations, we define the growing season as May through September. At WB, leaf expansion typically occurs between 10 April (5% completion) and 11 May (95% completion; Joslin *et al*., 2001; Hanson *et al*., 2003a). Previous radiocarbon labeling of mature white oaks at WB showed that in April, leaves are accumulating C (much of it from stored reserves) and not translocating C out of the leaf (Edwards *et al*., 1989). During May through October, leaves translocate a large proportion of the newly photosynthesized C (Edwards *et al*., 1989). Photosynthetic assimilation rates drop dramatically beginning in mid to late September (Wilson & Hanson, 2003), and therefore we do not include October in our definition of growing season.

There are a few 2-week air samples with high (>200‰) or low (<0‰) values. These data indicate that two moderately large ^{14}C releases affected the ORR and that fossil fuel contamination also occurred. However, all these events were in early April, late September, or October, and not during the growing season. Thus, we assume they did not significantly affect $\Delta^{14}C$ content of ORR photosynthate. Data from new roots in 2001 and 2002 support this assumption. The values for new-root $\Delta^{14}C$ were either less than those in the previous year (as in 2001) or not significantly different from those in the previous year (as in 2002). Additionally, the East ORR 2-week air values of 366‰ in late September 2000 and 184‰ in late July 2002 (Fig. A1) likely represent a large amount of re-respired CO_2 and not a new release of ^{14}C. We conclude this because the contemporaneous West ORR canisters, located much closer to the presumed source, do not also show a large degree of enrichment (133‰ and 68‰, respectively; see the Supporting Information).

Creation of ORR ARPCs

The East and West ORR ARPCs were based on published atmospheric records, local measurements of tree-ring cellulose, tree leaves, soil respiration, and local atmospheric measurements. For the time period 1880–1980, the East and West ARPC curves were estimated based on published background northern hemispheric air values from Levin & Kromer (1997), Levin & Hesshaimer (2000), and Levin & Kromer (2004) for the time periods 1880–1950, 1950–1976, and 1977–1980, respectively. For the time period 1980–1998, average annual $\Delta^{14}C$ values were based on cellulose from tree rings measured at the two East and West ORR EBIS sites (HR, WB and PR, TVA, respectively) for the years 1980 and 1985, and annual increments for 1990–1998 ($n = 1$–2 tree cores in all measured years).

For the years 1999 and 2000, we divided each year's tree ring into three increments that represent growth in early spring, spring/summer, and summer to characterize inputs of atmospheric ^{14}C. However, it is clear that the tree-ring increments did not capture the peak ^{14}C uptake by plants, because the $\Delta^{14}C$ values for 1999 East ORR tree-ring cellulose (113–520‰; Fig. A1) are much lower than (1) East ORR new roots in 1999 [306–861‰; see Gaudinski *et al*. (this issue) and the Supporting Information] and (2) maximum measured East ORR soil respiration $\Delta^{14}C$ values (1577‰ and 2009‰, respectively; Supporting Information). Therefore, tree-ring samples, even when split into seasonal increments, did not reflect peak plant ^{14}C uptake. Hence, we used a time series of several available plant-based proxy records. We had to use different proxy records for East and West ORR because the data available to construct each record are different.

East ORR ARPC

For 1999, we synthesized the East ORR ARPC using the 1999 tree-ring and soil respiration data from WB, HR, TDE, and P5. For soil respiration, we used measured $\Delta^{14}CO_2$ values of respiration coming from the soil surface and from within the soil profile (see the Supporting information). In order to use the soil respiration data to estimate part of the ARPC curves, we had to partition autotrophic and heterotrophic sources, because only the autotrophic $\Delta^{14}C$ values were appropriate as proxies for atmospheric values.

Respiration partitioning

Autotrophic respiration (Δ_a) was assumed to be from recently fixed C and representative of atmospheric ^{14}C inputs (Δ_{ARPC}), whereas heterotrophic respiration (Δ_h) was assumed to come from microbial respiration of soil organic matter of mixed ages. Measurements of the soil respiration CO_2 flux (F_m) and its ^{14}C signature (Δ_m) were used to estimate Δ_a (equivalent to Δ_{ARPC}). F_m is a composite of heterotrophic (F_h) and autotrophic (F_a) fluxes:

$$F_m = F_h + F_a. \quad \text{(A1)}$$

The ^{14}C composition (Δ, with subscripts as above) of the soil surface flux is an analogous composite:

$$(F_h + F_a)\Delta_m = \Delta_h F_h + \Delta_a F_a. \quad \text{(A2)}$$

Combining Eqns (A1) and (A2), and denoting the ratio of heterotrophic to autotrophic respiration as r, gives

$$\Delta_a = (1 + r)\Delta_m - r\Delta_h. \quad \text{(A3)}$$

For these calculations, we assumed $r = 1$ (50% heterotrophic and 50% autotrophic) and $\Delta_h = 100$‰ (corresponding to the expected average value of microbial respiration during the

period of record; Cisneros-Dozal *et al*., 2006). The sensitivity of Δ_a to Δ_h is small, particularly for large Δ_a.

We used a Monte Carlo approach to impose uncertainty in Δ_{ARPC}. In this approach, a geometric mean (GM; taken to be the Δ_{ARPC} record as described above) and geometric standard deviation (GSD) were imposed, and many simulations were run, each sampling from this distribution. Results are given as mean and standard deviations for the ensemble simulation. For the East ORR, a GSD of 1.3 was used. The resulting Δ_{ARPC} is shown in Fig. A2.

West ORR ARPC

We have no soil respiration measurements for West ORR, so for 1999, we imposed the timing of the pulse to be the same as that of the pulse on the East ORR. In other words, we assumed that enrichment at both sites resulted from the same release. The peak value in 1999 was set at the average value of leaves measured in 2000 on the West ORR [2111 ± 1811 (SD)‰; see http://ebis.ornl.gov/pretreat.html] and Δ_{ARPC} at the end of 1999 is assumed to be the same as for the East ORR (i.e. it had returned to background). Again, a Monte Carlo approach was used, but for the West ORR, a larger GSD of 1.7 was imposed to reflect the larger uncertainty in the West ORR Δ_{ARPC} (Fig. A2).

Uncertainty in East and West ORR ARPCs

Several factors create large uncertainty in the East and West ORR ARPC curves. The timing, duration, and magnitude of the local ^{14}C release are unknown, and are estimated with plant proxy data that integrate over varying time scales and can be influenced by stored C reserves. The inputs of ^{14}C after the initial 1999 pulse are also uncertain both with respect to an average background value and our knowledge of whether there were additional small releases. We know there were at least two large releases that affected the whole ORR (late November 2000 and early April 2002). However, we assume because they did not occur during the growing season that the plants did not assimilate significant quantities of these secondary enrichments. It also appears there were a few fossil fuel contamination events (particularly at the West ORR) that may have caused depression of ^{14}C values. Finally, potential further ^{14}C enrichment via photosynthesis of respired 1999-enrichment-derived ^{14}C, particularly in the lower canopy, is unknown and not taken into account. We ascribed large GSDs to each atmospheric ^{14}C curve (1.3 and 1.7 for East and West ORR curves, respectively) to propagate these uncertainties through our model analyses. Limitations in interpretation of EBIS data, based on the large uncertainty in the ARPC, are discussed in Riley *et al*. (submitted).

Stored C use in plant proxy data

Our data show that the tree-ring cellulose samples included stored C (Fig. A1). First, $\Delta^{14}C$ values for leaf bud and new expanding leaves (which are made entirely from storage) measured in spring 2000 are similar to tree-ring cellulose values in spring 2000. Second, tree-ring cellulose values from late-season growth in 1999 are similar in value to the early-season growth in 2000. This implies that initial growth used a high percentage of stored C relative to later in the season. Such an interpretation is consistent with empirical estimates that roughly 25% of white oak wood growth occurs before full canopy leaf development (Hanson *et al*., 2003b).

The elevated tree-ring cellulose values in 2000 on both sides of ORR could also be explained by an additional pulse in early 2000. However, as discussed above, we are confident this did not occur on the East ORR, and while we cannot completely rule it out, it most likely did not occur on the West ORR (Fig. A1).

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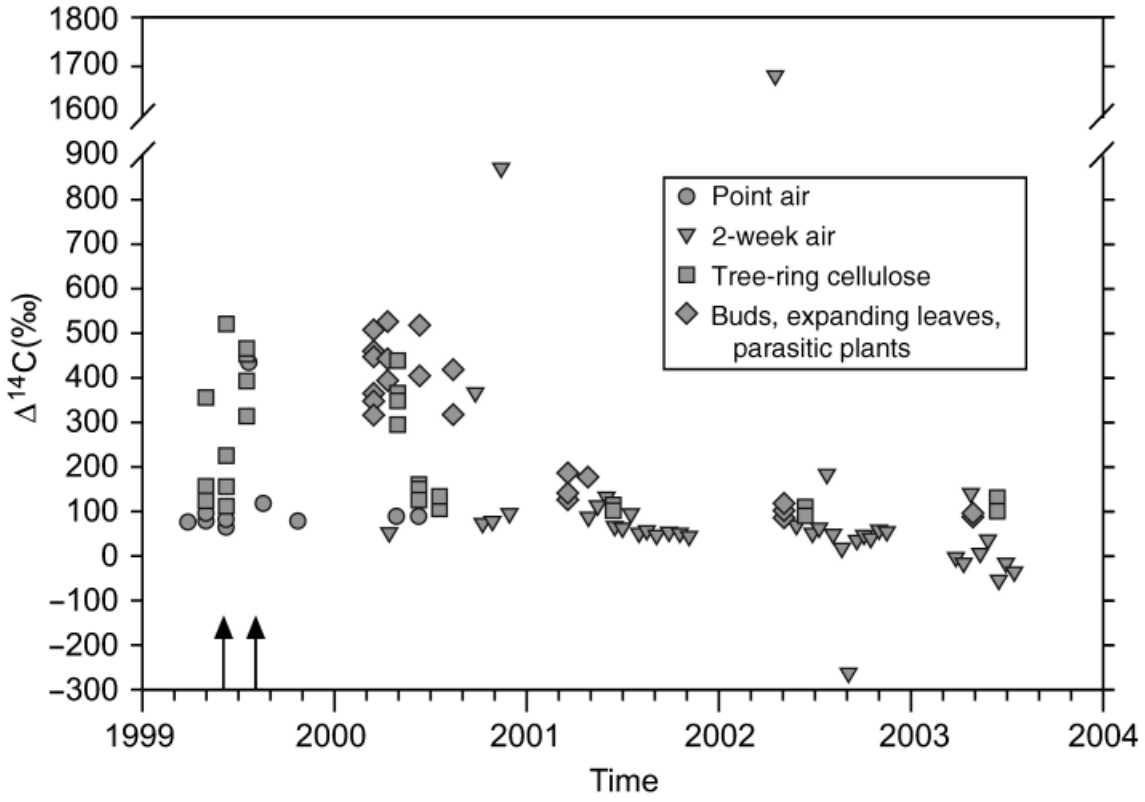

Fig. A1 $\Delta^{14}C$ values of air and plant proxy indicators on East ORR. The enrichment pulse occured sometime between the two arrows.

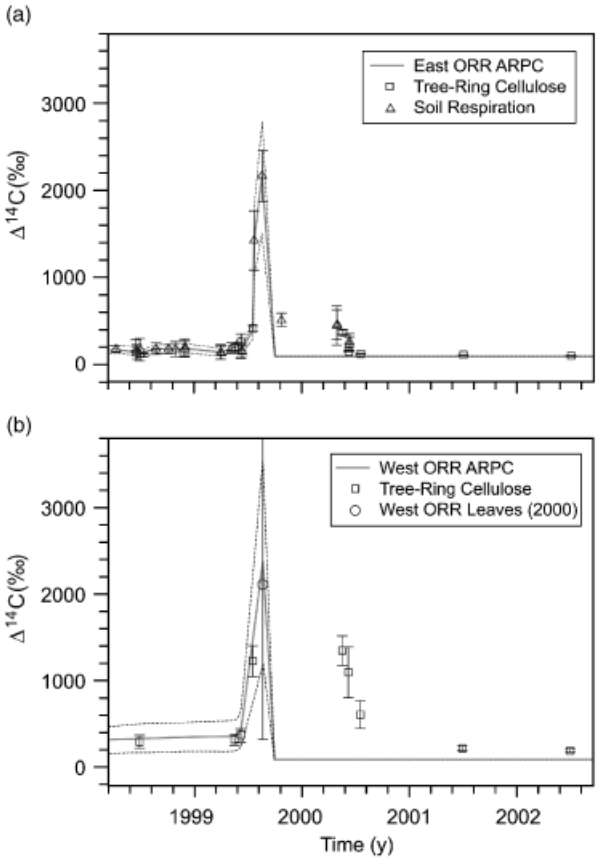

Fig. A2 Atmospheric radiocarbon proxy curves for East ORR (a) and West ORR (b). Dashed lines represent one standard deviation of the ARPC curves. Error bars represent standard error for tree-ring Cellulose and soil respiration data and standard deviation for West ORR leaves.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. EBIS sites: Point air samples taken in molecular sieve traps.
Table S2. EBIS sites: Two week integrated air samples taken in 32 L cannisters.
Table S3. EBIS sites: Tree ring samples.
Table S4. EBIS sites: New root samples taken from root screens.
Table S5. EBIS sites: Soil gas samples taken from within soil profile.
Table S6. EBIS sites: Surface flux soil gas samples.
Table S7. EBIS sites: Bud, new leaf and parasitic plant samples (no pretreatment of tissues).
Table S8. EBIS sites: Root core samples.
Table S9. Bomb-^{14}C sites: Point air samples.
Table S10. Bomb-^{14}C sites: Tree ring samples.
Table S11. Bomb-^{14}C sites: New root samples.

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