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REGULAR ARTICLE

Partitioning of belowground C in young sugar maple forest

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Abstract
Background and aims Trees allocate a high proportion of assimilated carbon belowground, but the partitioning of that C among ecosystem components is poorly understood thereby limiting our ability to predict responses of forest C dynamics to global change drivers.
Methods We labeled sugar maple saplings in natural forest with a pulse of photosynthetic ^{13}C in late summer and traced the pulse over the following 3 years. We quantified the fate of belowground carbon by measuring ^{13}C enrichment of roots, rhizosphere soil, soil respiration, soil aggregates and microbial biomass.
Results The pulse of ^{13}C contributed strongly to root and rhizosphere respiration for over a year, and respiration comprised about 75 % of total belowground C allocation (TBCA) in the first year. We estimate that rhizosphere carbon flux (RCF) during the dormant season comprises at least 6 % of TBCA. After 3 years, 3.8 % of the C allocated belowground was recovered in soil organic matter, mostly in water-stable aggregates.
Conclusions A pulse of carbon allocated belowground in temperate forest supplies root respiration, root growth and RCF throughout the following year and a small proportion becomes stabilized in soil aggregates.

Keywords Rhizosphere · Rhizodeposition · Root respiration · Soil respiration · Aggregates · Carbon budget

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Introduction

Global changes in atmospheric CO_2 concentration and climate have stimulated increased interest in understanding the carbon dynamics of ecosystems. Forests play a particularly prominent role in the global C cycle acting as a significant C sink (Houghton 2005) and responding to changing atmospheric CO_2 concentration (Korner et al. 2005) and climate change (Boisvenue and Running 2006). Uncertainty about the short-and long-term fate of photosynthetically-fixed C in forests, and especially soils, constrains our ability to predict the interactions and feedbacks among forests, atmospheric CO_2 and climate. In particular, predictions of the response of ecosystem respiration and soil C sequestration

to environmental changes are limited by incomplete understanding of how plant carbon is allocated to and utilized by different ecosystem components (Carbone et al. 2007). Photosynthetic C is allocated within trees to supply cellular growth and respiration, and a large proportion of the assimilated C is allocated belowground where it enters a variety of pools with varying turnover rates including, fine and coarse roots, microbial biomass, soil organic matter (Keel et al. 2006). Although the magnitude of total belowground carbon allocation (TBCA) is well known from ecosystem C flux measurements (Raich and Nadelhoffer 1989, Davidson et al. 2002), the partitioning of TBCA among soil components and fluxes is not well understood. Because the magnitude and timing of belowground C allocation and its subsequent transformations are likely to be sensitive to changes in environment and disturbance regimes, better understanding of the partitioning of belowground C in forests is needed.

Total soil respiration (TSR) consists of respiration by roots and mycorrhizae and by soil heterotrophs and typically comprises over half of total ecosystem respiration in temperate and boreal forests (Granier et al. 2000, Hogberg et al. 2001). Soil heterotrophic respiration is fueled by root turnover and aboveground litterfall as well as rhizosphere carbon flux (RCF). Recent studies indicate that TSR in temperate forests is supplied in roughly equal proportions by root/mycorrhizal and heterotrophic respiration (Hanson et al. 2000, Hogberg et al. 2002). Although the rapid link between root and rhizosphere respiration and photosynthetic C assimilation has been demonstrated (Ekblad and Hogberg 2001, Steinmann et al. 2004), a significant proportion of root processes is supplied by carbon reserves temporarily stored in the trees (Gaudinski et al. 2009). Distinct seasonal allocation patterns in temperate zone trees allow the use of excess assimilates to fuel maintenance respiration in the dormant season, new growth in the spring and subsequent growth flushes in summer (Dickson 1991). This role of tree C reserves may be particularly sensitive to stressful conditions associated with environmental change and natural disturbances (Gaudinski et al. 2009).

The transport of photo-assimilated C to roots and the long-term fate of TBCA in forests has received limited study (Endrulat et al. 2010) in part because of practical problems of measurement. Recent syntheses (Jones et al. 2009) have argued that isotope pulse-labeling approaches are most effective for this purpose. The advantage of using ^{13}C for this study is that the pulse of ^{13}C could be detected even in the large plant and soil pools with high precision for a relatively long time. An analogous approach based on an inadvertant release of radioactive ^{14}C provided parallel results but with attendant environmental hazards (Gaudinski et al. 2009). Based upon the observations from tree girdling experiments of the rapid link between photosynthetic C fixation and soil respiration (Hogberg et al. 2001), we hypothesized that the ^{13}C label would be detected in a large pulse of root and rhizosphere respiration immediately after labeling in early fall. Based on principles of tree assimilate distribution and storage (Dickson 1991), we hypothesized that root growth and respiration would be supplied throughout the following winter and summer from stored labeled C. We also hypothesized that the labeled, plant labile pools would be largely depleted by the end of the first growing season (Gaudinski et al. 2009), resulting in constant, low enrichment of TSR supplied mostly by heterotrophic activity. We also expected RCF (Grayston et al. 1997) to result in C accumulation in the rhizosphere soil, and together with turnover of roots and microbes, RCF would supply relatively stable soil carbon stored in soil aggregates (Six et al. 2000).

Materials and methods

Site and ^{13}C pulse labeling Sugar maple trees growing in a natural forest stand in the Arnot Forest in central New York were labeled with ^{13}C. The Arnot Forest is a hardwood forest on the northern Allegheny Plateau (Fain et al. 1994). Annual precipitation averages 90 cm and average June and December temperatures are 22° and −4 °C, respectively. Soils are acidic Dystrochrepts with pH 4.5–5.0. The site chosen for study was in a ca. 90-yr-old stand that had been selectively harvested in 2000, leaving 50 overstory trees per ha; this thinning released a dense understory of young sugar maple trees. At the time of labeling in September 2006, this sapling layer averaged 2.5 m in height.

The labeling procedure has been described in detail by Horowitz et al. (2009). Four aluminum-frame chambers (2.5 m tall x 3 m diameter) were positioned over pure sugar maple sapling groves (about 10 stems/

m^2) in June 2006. The maximum distance between the chambers was 50 m, and all were on moderately-well drained soils on a gentle NE-facing slope. Although sugar maple saplings were ubiquitous across the stand, these groves were more uniformly pure sugar maple than average. The soil and root systems in each chamber were isolated by trenching around the chambers to 0.5 m depth, lining the trenches with 6 mil polyethylene and backfilling with soil. After enclosing the chambers with 6 mil polyethylene, the saplings were labeled with ^{13}C by injecting 40 atom % enriched $^{13}CO_2$ into each chamber on 13 sunny days between 1 and 20 September 2006 (hereafter, "year 0"); this procedure was designed to maximize the incorporation of the label into plant tissues. The procedure involved sealing the tops of the chambers at dawn on each day, scrubbing ambient CO_2 in chamber atmosphere to ca. 60 ppm and then adding $^{13}CO_2$ until total CO_2 concentration reached about 500 ppm. Chamber tops were removed each day after about 0.5 to 1 h when chamber CO_2 was reduced by photosynthesis to nearly constant concentration.

Sample collection and processing All freshly-fallen leaf litter was removed from the chambers in fall of year 0 (2006) and year 1 (2007) for use in a separate experiment (Fahey et al. 2011). The total dry mass of leaf litter in each year was measured and subsamples were obtained for isotope analysis. Unlabeled sugar maple litter from an adjacent, 90-yr-old sugar maple stand was added in each year to replace the removals.

Roots were collected from the 0–5 cm soil depth on three dates after leaf senescence in fall of year 0 (10 October, 24 October, 10 November), on two dates prior to leaf out in spring (30 April, 15 May) and in late summer (15 August) of year 1. These samples were obtained by loosening soil and excavating small root fans with attached rhizosphere soil. The aim was to obtain roots and rhizosphere soil while minimizing soil disruption which was a recurring challenge within the chambers of limited size. Adhering rhizosphere soil was manually removed (Phillips and Fahey 2006), roots were sorted visually into fine (<1 mm) and coarse (1–5 mm) classes, and samples were dried (55 °C) and stored for isotope analysis. Roots and soil were also sampled on 1 June and 15 October of year 2 (2008) and 15 October of year 3 (2009). For these samples four soil cores (5 cm diameter) were collected to 10 cm depth, roots were manually separated from soil and sorted by size class, and roots and soil were dried, pooled within chambers and stored for isotope analysis. Roots and soil for natural abundance measurements were collected in fall of year 0 in adjacent areas outside the chambers. Four cores were collected by the same method and processed as above.

Root in-growth cores were used to obtain newly-grown fine roots for isotope analysis over four intervals: 15 April to 13 July and 13 July to 2 October of year 1, and 15 November to 15 May and 1 June to 15 October of year 2. On each date two soil cores (5 cm diameter) were excavated to 10 cm depth in each chamber, soil was sieved to remove roots, the core hole was lined with coarse mesh and sieved soil was backfilled. Root in-growth samples were sieved from soil, washed with d H_2O, dried and stored for isotope analysis. Reference samples were also obtained from four ingrowth cores in adjacent areas outside the chambers.

We collected CO_2 emitted from soil in the chambers and from adjacent reference sites using a NaOH trap method (Phillips and Fahey 2005). Sampling was conducted on two dates in fall of year 0 (26 September, 7 November), seven dates from April to October of year 1 and seven dates from June to November of year 2. The CO_2 emitted from the soil was trapped in 10 mL of 0.1 mol/L NaOH inside two sealed 5-cm diameter cylinders in each chamber over an 8 h daytime incubation period. Samples were pooled within chambers, sealed in vials and stored for isotope analysis. Following the addition of 1 M $BaCl_2$, the base trap solution was titrated with 0.1 M HCl. To collect the $BaCO_3$ precipitate for ^{13}C analysis, the precipitate was washed with degassed dH_2O, centrifuged at 2,200 rpm for 5 min., and the supernatant decanted. This washing procedure was repeated three times and any remaining solution was evaporated at 65 °C for 24 h. ^{13}C was measured on a Finnegan isotope ratio mass spectrometer (IRMS) at the University of Georgia. Vanadium pentoxide was added as a catalyst to ensure complete recovery of ^{13}C during combustion of $BaCO_3$ precipitate (Harris et al. 1997).

Soil CO_2 emission was measured using a LI-COR soil respiration chamber (model LI-6000-09; LI-COR BioSciences, Lincoln, Nebraska) and LI-6200 infrared gas analyzer (Norman et al. 1992). Four soil collars were placed in each chamber and measurements were conducted on seven dates from early June to early November of year 2 following the procedure detailed by Fahey et al. (2005a).

Microbial biomass C and ^{13}C were measured using the chloroform-fumigation method (Jenkinson and Powlson 1976) on 0–5 cm soil cores collected from each chamber in October of year 2. Soils were fumigated to kill and lyse microbial cells and fresh soil was used to inoculate samples with microbes that used lysed cells as substrate. Flushes of CO_2 over a 10 day incubation at field moisture content were assumed to be proportional to C in microbial biomass after applying a standard (0.45) proportionality constant. Gas chromatography was used to measure CO_2 and a Europa Integra IRMS to measure ^{13}C at the University of California-Davis.

After drying to constant mass at 70° C, tissue and soil samples were finely ground and homogenized for isotope analysis. Leaf litter was ground with a mortar and pestle; fine roots and soil with a ball mill. The isotopic composition (^{13}C) of these samples was measured on a Finnegan IRMS at the Cornell Stable Isotope Laboratory. At each of the IRMS laboratories appropriate NIST standards were used for normalization correction (CBT, HCRN), instrument linearity (methionine) and precision purposes (NIST calibrated rice, poplar, BCBG). Samples from the October year 2 soil collections were processed for analysis of isotope enrichment of soil aggregate fractions following a procedure modified from Fonte et al. (2007). Subsamples of air-dried soil were wet sieved to generate three aggregate size classes: macroaggregates (>250 μm), microaggregates (53–250 μm) and silt plus clay (<53 μm) as well as low-density organic matter that floated in water. The macroaggregate fraction was further separated by wet sieving following the procedure of Six et al. (2000) to yield three size classes: coarse particulate organic matter (>250 um), microaggregates, and silt plus clay. Subsamples of each of these fractions were finely ground and analyzed for total C, and C isotope ratios by IRMS at Cornell, as described above.

Data analysis Recovery of added ^{13}C in soil pools and fluxes was calculated after correcting for atom % ^{13}C concentration of reference pools and fluxes measured in areas adjacent to the chambers (termed excess ^{13}C). The excess ^{13}C pool in soil was estimated for 0–10 cm depth based on bulk density measured in similar soils at Arnot Forest (0.56 g/cm^3; Fahey et al. 2011). For the three dates when rhizosphere soil $\delta^{13}C$ was measured (see above), we assumed no enrichment in bulk soil (Keel et al. 2006) and that rhizosphere represented 26 % of soil mass, the figured obtained by Phillips and Fahey (2006) using the same method for nearby sugar maple stands on the same soil type. To account for roots to 30 cm depth, we assumed that roots collected from 0 to 10 cm soil had the same isotope signature as those from 10 to 30 cm. We estimated excess $^{13}CO_2$ flux from soil respiration on the basis of short-term measurements of $\delta^{13}CO_2$ of soil emissions and LI-COR chamber measurements of soil CO_2 flux. In general, these calculations were performed using linear interpolations to obtain estimates during the intervals between measurements. For the winter period (December-March) we assumed soil CO_2 emission was the same as estimated for nearby sugar maple stands on the same soil type in the Arnot Forest by Fisk et al. (2004), i.e. 16 g C m^{-2} month^{-1}, and we applied the average of $\delta^{13}CO_2$ values from the last autumn and first spring measurement in each year (Fig. 1). Also, spring (April-May) soil CO_2 emissions were assumed to mirror those in fall (October-November), as previously indicated for Arnot Forest (Fisk et al. 2004). All the assumptions above result in uncertainty for our budgetary calculations, as detailed in the Discussion.

Statistical significance of differences across dates within years in $\delta^{13}C$ of TSR was evaluated using ANOVA. In our ANOVA, year (0, 1, 2, 3) was fixed, month of sampling was random and nested within season (spring, summer, or fall) with chambers as replicates for the dependent variable, $\delta^{13}CO_2$. This model was used because of large between-year differences and because the day of the year for sampling differed between years. Statistical differences in $\delta^{13}C$ and in the excess ^{13}C pool (relative to natural abundance) in fine roots and rhizosphere soil were evaluated with one-way ANOVA; values of the dependent variables ($\delta^{13}C$ and excess ^{13}C) were log transformed to fit normal distribution. Post-hoc comparisons for these ANOVAs were made using Tukey's test. Differences in $\delta^{13}C$ across soil aggregate fractions were evaluated using repeated measures ANOVA as all the values of each replicate were obtained from the same soil sample.

Results

We were able to detect the partitioning of a large pulse of photo-assimilated ^{13}C through the tree-soil system across 3 years. During leaf senescence

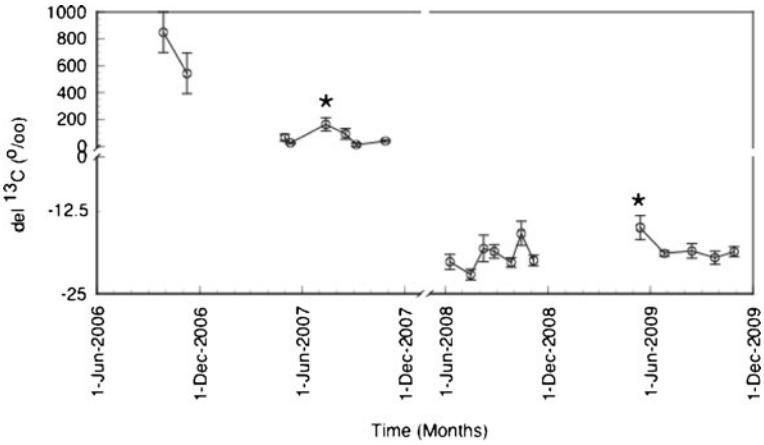

Fig. 1 Per mil enrichment of ^{13}C (δ ^{13}C, ‰) of total soil respiration collected from chambers in which sugar maple sapling stands were labeled with $^{13}CO_2$ in early September 2006 (labeled leaf litter removed). Error bars indicate standard errors (n=4 chambers). Within-years significant differences are indicated by asterisks ($p<0.05$)

soon after labeling, ^{13}C in fine and coarse roots rapidly increased (see Horowitz et al. 2009) reaching peak values by November of year 0, but remaining highly enriched in year 1 and in spring of year 2, before declining significantly thereafter (Table 1; ANOVA for fine roots: $F_{6,21}=11.34$, $p<0.001$; coarse roots: $F_{6,21}=.90$; $p<0.05$). By comparison, enrichment of $^{13}CO_2$ in soil emissions (TSR) declined rapidly from a peak value of 848±151 ‰ 6 days after completion of labeling to −20±1 ‰ by spring of year 2 (Fig. 1). Moreover, during year 1 significant seasonal fluctuations in $\delta^{13}C$ of TSR were observed (month * year; $F_{9,71}=1.95$, $p<0.05$), with a seasonal minimum in mid-May at the time of leaf emergence, followed by a pronounced peak in early summer. The large rhizosphere soil pool showed the slowest enrichment of ^{13}C; only slight enrichment was observed in fall of year 0 (−23±1 ‰), but it increased markedly over winter (−13±5 ‰) and reached a peak value in late summer of year 1 (−2±6 ‰). During the growing season of year 2 excess ^{13}C in soil declined significantly by over half ($F_{5,18}=7.62$, $p<0.001$), with a smaller, non-significant decline in year 3. Two years after labeling the highest $\delta^{13}C$ enrichment among soil fractions was in low density particulate organic matter (presumably mostly dead fine roots), and significantly higher enrichment was observed in both macroaggregates and micro-aggregates than in adsorbed pools on silt plus clay (Fig. 2).

We estimated the fluxes and pool sizes of excess ^{13}C in the plant-soil system through time after labeling correcting for natural abundance in roots, soil and TSR (Table 2). The daily excess ^{13}C flux from soil was calculated as the product of TSR and excess ^{13}C in

Table 1 Enrichment of ^{13}C ($\delta^{13}C$, ‰) in fine roots (<1 mm) and coarse roots (1–5 mm) collected following ^{13}C labeling of photosynthesis of sugar maple saplings in September 2006 (year 0). In columns values with different letters are significant different ($p<0.05$)

Date	$\delta^{13}C$ (‰±SE)	
	Fine roots	Coarse Roots
Year 0—November	89 (25) [a]	97 (28)[a]
Year 1—April	101 (21) [a]	72 (25)[ab]
Year 1—May	88 (49) [a]	32 (24)[b]
Year 1—August	60 (21) [a]	28 (16)[b]
Year 2—June	79 (30) [a]	73 (33)[ab]
Year 2—October	10 (21) [b]	34 (29)[b]
Year 3—October	−23 (3) [c]	−2 (19)[c]

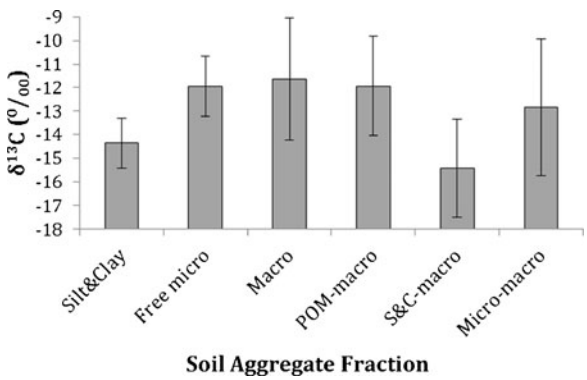

Fig. 2 Per mil enrichment of ^{13}C (δ ^{13}C, ‰) of six soil aggregate fractions in 0–5 cm soil collected from labeling chambers in October 2008 (year 2). Free micro=microaggregates, Macro=macroaggregate, POM-macro=particulate organic matter in macroaggregates, S&C-macro=silt and clay in macroaggregates, and Micro–macro=microaggregates held within macroaggregates. Error bars indicate standard errors (n=4)

Table 2 Excess ^{13}C pool in fine roots (<1 mm diameter) and rhizosphere soil and excess ^{13}C flux in total soil respiration for sugar maple saplings labeled with $^{13}CO_2$ in September 2006. Soil respiration fluxes are cumulative for the interval ending on the date noted

Date	Excess ^{13}C (mg/m^2±SE)		
	Rhizosphere soil	Fine roots	Soil respiration
Year 0—October	47 (10)	372 (110)	–
Year 1—May	242 (57)	314 (114)	1,614 (148)
Year 1—August	531 (111)	243 (92)	726 (103)
Year 2—June	530 (227)	336 (104)	369 (23)
Year 2—October	197 (21)	180 (100)	163 (3)
Year 3—October	125 (37)	41 (27)	249 (4)

TSR using the interpolations described in Methods. The largest ^{13}C fluxes in TSR occurred in year 0 immediately after labeling, but high fluxes also were observed in year 1. These fluxes can be compared with the soil and root (<5 mm diameter) pool sizes and temporal changes in their magnitude (Table 2). For example, excess ^{13}C in TSR during year 0 and the growing season of year 1 greatly exceeded accumulation in roots and rhizosphere soil; thereafter the significant decline of excess ^{13}C in roots ($F_{5,18}$=4.44, $p<0.01$) and soil was comparable to TSR.

A whole-ecosystem budget of the ^{13}C pulse enrichment also was estimated. Much higher ^{13}C enrichment was observed for year 1 than year 0 leaf litter and the total flux of excess ^{13}C for the 2 years (885 mg ^{13}C/m^2) was larger than the maximum pool size in small roots (336±104 mg ^{13}C/m^2) or rhizosphere soil (531±111 mg ^{13}C/m^2) but smaller than TSR flux during the first 2 years after labeling (2,872±207 mg/m^2; Table 1). By comparison the pool of excess ^{13}C in microbial biomass in 0–10 cm soil in fall of year 2 was very small (3.9 mg ^{13}C/m^2), although it was highly enriched in ^{13}C (δ^{13} C−1±3 ‰). An estimate of TBCA of the ^{13}C pulse is the sum of TSR in year 0/1 (2,340 mg ^{13}C/m^2; Table 1) and the maximum pool size in roots and soil (867±215 mg ^{13}C/m^2), i.e. 3,207 mg ^{13}C/m^2. The immediate allocation of this TBCA to root and rhizosphere respiration is about 40 % based on fall/winter TSR. Allocation to RCF through the dormant season was about 7.5 % of TBCA and allocation to new roots a minimum of 9 % (i.e., based on root pool size in June of year 2 and assuming no turnover of new roots). An additional 9 % of TBCA supplied some of RCF during the following growing season.

Newly-grown fine roots collected using in-growth cores exhibited much lower ^{13}C enrichment than observed for *in situ* roots. In-growth cores roots collected in July and October of year 1 averaged $\delta^{13}C$ −17.8±1.1 ‰ and −16.9±0.9 ‰, respectively. In-growth roots collected in mid-May of year 2 had slightly but not significantly higher $\delta^{13}C$ (−13.6±4.6 ‰), but in-growth roots produced during the growing season of year 2 were not enriched compared with natural abundance values (−27.8±0.2 ‰ *vs.* −27.6±0.2 ‰).

Discussion

We followed a large pulse of photo-assimilated ^{13}C into the belowground components of a young sugar maple forest and quantified the partitioning of this pulse among soil pools and fluxes over 3 years. In particular, we evaluated the temporal sequence of C partitioning to total soil respiration (TSR), root growth, rhizosphere carbon flux (RCF) and relatively stable soil organic matter (SOM). Our observations demonstrated that the pulse of late-season belowground C was allocated to immediate root and rhizosphere respiration during the dormant season (about 40 % of TBCA), but that a large proportion of the pulse was stored in roots and supplied subsequent root respiration, root growth and RCF for an entire growing season. Thereafter, the pulse apparently was utilized in heterotrophic respiration and after 3 years about 3 % of the pulse was stabilized in SOM, mostly in aggregates and particulate organic matter derived from root turnover. This is one of the first reports of the mid-term partitioning of belowground C among forest ecosystem components and will help inform understanding of the likely responses of belowground C dynamics to changes in CO_2, climate and disturbance regimes.

Our first hypothesis, based on observations from a boreal evergreen forest (Ekblad and Hogberg 2001), was that photo-assimilated ^{13}C would be rapidly transported belowground to fuel root and rhizosphere respiration. This hypothesis was supported by very high ^{13}C enrichment observed on our first measurement date for TSR 6 days after the last labeling event (20 September; Fig. 1). Although abiotic soil $^{13}CO_2$ efflux

might contribute slightly to this enrichment, observations of Subke et al. (2009) indicated that abiotic efflux from soil pores could be detected for only 2 days under labeling conditions similar to ours.

Enrichment of ^{13}C in TSR remained high during the fall ($\delta^{13}C$=684 ‰ on 7 November; Fig. 1), illustrating that translocation of photosynthate to root systems occurred both during and after leaf senescence and resulted in a gradual increase of $\delta^{13}C$ in fine and coarse roots during the fall (Horowitz et al. 2009). The high metabolic demand of roots contributes to the sink strength driving carbohydrate transport to roots during autumn senescence (Farrar and Jones 2000), but much of this C probably goes into temporary storage pools because minimal root growth of sugar maple occurs after leaf senescence (Tierney et al. 2003). We also observed relatively large accumulation of ^{13}C in rhizosphere soil between October and May (Table 2), indicating that substantial RCF occurs during the dormant season. Cold soil temperatures through most of this interval would suppress heterotrophic utilization of this RCF, promoting its accumulation.

Our second hypothesis was that the fall pulse of TBCA would contribute significantly to root respiration, growth and RCF during some or all of the following growing season, conforming with general principles of tree assimilate distribution and storage (Dickson 1991). Our observations supported this hypothesis, as the ^{13}C enrichment of TSR, fine roots and rhizosphere soil remained very high throughout year 1 (Fig. 1, Table 1, 2). These results corroborate recent observations of Gaudinski et al. (2009) that over half of annual new root growth in a temperate deciduous oak forest was supplied by stored C (*vs.* fresh assimilate). Similarly, Endrulat et al. (2010) demonstrated the remobilization of starch from a late fall ^{13}C pulse into new fine roots the next spring in a temperate conifer forest. However, the seasonal patterns in ^{13}C enrichment of TSR that we observed during year 1 provided new evidence about the timing of partitioning of stored C in temperate deciduous forest trees. A pronounced (though non-significant) decline in $\delta^{13}CO_2$ of TSR during leaf out in May was followed by a large and significant ($p<0.05$) increase during early summer. The early decline coincided with a decline in coarse root $\delta^{13}C$ (Table 1) presumably reflecting the mobilization of stored C to the strong aboveground C sink (Cannell and Dewar 1994); perhaps the coincident decline in $\delta^{13}C$ of TSR reflected consequent diversion of stored C from roots. The early summer peak in $\delta^{13}C$ of TSR coincides with the peak of root growth in sugar maple and other temperate deciduous trees (Joslin et al. 2001, Tierney et al. 2003), strongly suggesting that new root growth and additional growth respiration (Veen 1981) are substantially dependent on stored carbon. Thus, any factors reducing the availability of stored C (*e.g.*, canopy disturbance, late-summer drought) might be likely to disrupt peak root production during the growing season, with possible consequences for soil resource acquisition. Conversely, extension of the growing season in fall owing to climate warming would be likely to increase stored C and its allocation to RCF and root production.

The sustained high ^{13}C enrichment of fine root biomass from year 0 through year 1 and spring of year 2 presumably reflects a complex balance between remobilization of root storage pools (Endrulat et al. 2010) and coincident growth and mortality of fine roots. Although our measurements cannot distinguish among these processes, it is notable that in fall of year 1, $\delta^{13}C$ of structural fiber (mostly lignocellulose) in very fine roots (root order 1–2; Fahey et al. 2012) was 93.32 ±11.18 ‰, suggesting that stored ^{13}C was allocated to the growth of these short-lived roots (Hendrick and Pregitzer 1993, Tierney and Fahey 2002), and supporting our earlier assertion that the early summer, year 1 peak in $\delta^{13}C$ of TSR was associated with stored ^{13}C allocated to root growth respiration.

The strikingly lower ^{13}C enrichment of newly-grown fine roots collected from in-growth cores (range=−13.56 to −17.80 ‰ during year 1 and spring of year 2) compared with *in situ* roots (59.62 to 88.13 ‰; Table 1) supports recent observations of the complexity and heterogeneity of C supply to new root growth (Guo et al. 2004, Joslin et al. 2006, Vargas et al. 2011). Clearly, roots in the in-growth cores were grown almost entirely with new photoassimilate. The ^{13}C in the *in situ* roots consisted of both stored ^{13}C (i.e. reflected in their high initial enrichment; Table 1), as well as growth of new daughter roots derived from this stored ^{13}C. One possible explanation of this striking difference is based on the observation of Eissenstat and Achor (1999) that most of the roots colonizing in-growth cores are "pioneer" roots derived from the cut ends of a few lateral roots. If these pioneer roots and their daughter laterals are supplied mostly by new photoassimilate whereas new fibrous,

lateral roots arising directly from other mother laterals receive lots of C stored in those laterals, then such a large contrast in ^{13}C enrichment could occur. This observation emphasizes the need to use caution when interpreting root growth and production measured with in-growth cores (Tierney and Fahey 2007).

Between fall of year 0 and spring of year 1 $\delta^{13}C$ of TSR declined markedly and remained relatively constant supporting our third hypothesis that nearly all of the labile ^{13}C storage in the trees would be exhausted during year 1. Leaf and fine root tissues produced during the growing season in year 2 were not significantly higher than natural abundance values; hence, ^{13}C enrichment of TSR in year 2 and 3 probably represented almost entirely heterotrophic metabolism of RCF and decaying dead roots. The pool of ^{13}C in rhizosphere soil more than doubled during the growing season of year 1, suggesting that RCF (including C allocation to extra-matrical mycorrhizal hyphae) greatly exceeded its utilization by soil heterotrophs. This observation clearly indicates that a substantial portion of RCF is derived from stored photosynthate from the previous growing season. Undoubtedly some turnover of labeled fine roots occurred during this interval, but based on the method we employed to collect rhizosphere soil (i.e., brushing adhering soil from roots), root turnover was probably not a major source of ^{13}C in soil samples from August of year 1. However, during years 2 and 3 bulk soil samples were used to quantify the soil ^{13}C pool; these samples would have included considerable root detritus. Continued heterotrophic processing of root-derived carbon was indicated by the parallel decline of ^{13}C enrichment of both soil and fine root pools during years 2 and 3, as well as the continued elevated $^{13}CO_2$ flux in TSR (Table 2). Notably, ^{13}C enrichment of microbial biomass (−0.967‰±2.705) was much higher than for TSR in fall of year 2.

Rhizosphere carbon flux is recognized as a major soil carbon flux in forest ecosystems (Grayston et al. 1997), but accurate measurement is notoriously difficult. Phillips and Fahey (2005) used a ^{13}C pulse-labeling method and estimated that RCF during the growing season comprised 7 % of net photosynthesis in sugar maple saplings. Our conservative estimate of RCF during fall and winter was about 7.5 % of TBCA. In addition, the observation that ^{13}C continued to accumulate in the rhizosphere during the growing season in year 1 (representing 9 % of TBCA) suggests that RCF is considerably greater than estimated by Phillips and Fahey (2005). For example, assuming that TBCA represents about 55 % of net photosynthesis in sugar maple dominated northern hardwood forest (Fahey et al. 2005b), we would estimate that total annual RCF is at least 16 % of net photosynthesis in young sugar maple forest. A substantial proportion of RCF enters non-labile pools including, for example, glycoproteins produced by arbuscular mycorrhizal trees like sugar maple (Wright and Upadhyaya 1996).

In support of our fourth hypothesis that a substantial proportion of the TBCA pulse would contribute to relatively stable soil C, 3 years after pulse labeling about 3.8 % of TBCA was recovered in SOM. Separation of SOM into aggregate fractions (Fig. 2) illustrated the processes resulting in this mid-term stabilization of belowground C. The highest ^{13}C enrichment was observed in low density particulate organic matter (presumably mostly dead roots) and in macroaggregates and free microaggregates. Lower enrichment was noted for silt plus clay fractions (Fig. 2). Both RCF and the rapid turnover of microbial biomass and mycorrhizal hyphae may contribute to the enrichment of aggregate fractions and the possible role of glycoproteins associated with the arbuscular mycorrhizae of sugar maple in formation and stabilization of aggregates is noteworthy (Rillig 2004).

We emphasize that our budgetary estimates are subject to several sources of uncertainty and error associated with the assumptions described under Methods. First, to estimate ^{13}C flux of TSR we used linear interpolations between measurement dates both for TSR and $\delta^{13}CO_2$ of TSR. Perhaps most important for flux estimates was the overwinter interpolation (November-April); although winter TSR is undoubtedly low in soils near 0° (Groffman et al. 2006), $\delta^{13}CO_2$ was high (Fig. 1). If the change in $\delta^{13}CO_2$ departed from the linear decline that we assumed, significant error in the TSR ^{13}C flux would result. Second, we assumed that rhizosphere soil comprised 26 % of soil mass (Phillips and Fahey 2006); departures from this assumption would result in proportional errors in RCF estimates. Moreover, our approach would overestimate RCF to the extent that root tissues may have been included in rhizosphere soil, and it would underestimate RCF because of heterotrophic utilization of rhizosphere C. The challenges of quantifying RCF are well known (Jones et al. 2009). Third, to calculate the root ^{13}C pool we assumed that 10–

30 cm roots had the same $\delta^{13}C$ as 0–10 cm roots and we ignored deeper roots. Because roots of different orders form differently (Guo et al. 2008), some error from this assumption is likely. However, if deeper roots had lower $\delta^{13}C$ this source of error would be counterbalanced by the additional mass of roots at depths >30 cm. Finally, variation in the $\delta^{13}C$ signature of both rhizosphere soil and roots was highly variable (Table 1, 2) both within chambers and across chambers, contributing to uncertainty in pool sizes and fluxes.

Our budgetary estimates can be compared to some literature values obtained with different methods to help evaluate confidence in pool sizes and fluxes. In a summary of TBCA for global forests based on measurements of TSR and aboveground litterfall, Davidson et al. (2002) reported a mean value of 3.8 (range=1.5–6.8) for the ratio of TBCA/aboveground litterfall C flux. Our estimate of this ratio for excess 13 flux is 3.6 (i.e., 3,207/885 mg $^{13}C/m^2$; Table 2). This value is somewhat higher than for a complete C budget for a sugar maple forest in NH, USA (2.23; Fahey et al. 2005a); our value may overestimate proportional TBCA because aboveground C demand at the time of labeling in late summer would be lower than the growing season average. Hogberg et al. (2002) estimated that about 75 % of TBCA goes to root and soil respiration in a boreal pine forest. Our comparable figure is 73 % based on year 0+1 TSR and maximum soil and root pool sizes (i.e., 2,340/3,207 mg $^{13}C/m^2$). Estimation of this proportion is complicated in our approach by coincident turnover occurring in the root and rhizosphere pools; that is, our peak ^{13}C pool size estimate of RCF and root production does not account for ^{13}C supply to these pools that counterbalances losses to root mortality or heterotrophic respiration. Nevertheless, these comparisons suggest that our C budget estimates are reasonably accurate.

In conclusion, ours is the first study to trace a large pulse of photo-assimilated ^{13}C into belowground pools and fluxes over the long term in a natural forest, thereby providing useful insights for predicting responses of forest C dynamics to environmental change. We show that stored C from late summer photosynthesis is utilized for root growth and RCF during the following growing season. Thus, these processes could be particularly sensitive either to disruption or enhancement of late-season photosynthetic activity associated with changing climate or canopy disturbance. Our results also indicate substantial RCF during the dormant season (over 6 % of TBCA). Thus, accounting for growing season RCF of stored TBCA plus immediate allocation of fresh photosynthate to RCF (Phillips and Fahey 2005), we suggest that total RCF in young sugar maple forest exceeds 16 % of net photosynthesis. Together with fine root turnover this RCF supplies C sequestered in stabilized SOM. After 3 years nearly 4 % of the photosynthetic ^{13}C pulse was retained in mineral soil mostly in microaggregates and macroaggregates. Longer term tracing of this pulse in SOM would be useful. Moreover, studies of how the partitioning of TBCA among soil ecosystem components varies across the growing season would provide a more complete picture of belowground carbon dynamics in temperate forests.

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