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Stored carbon partly fuels fine-root respiration but is not used for production of new fine roots

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Summary

- The relative use of new photosynthate compared to stored carbon (C) for the production and maintenance of fine roots, and the rate of C turnover in heterogeneous fine-root populations, are poorly understood.
- We followed the relaxation of a ^{13}C tracer in fine roots in a *Liquidambar styraciflua* plantation at the conclusion of a free-air CO_2 enrichment experiment. Goals included quantifying the relative fractions of new photosynthate vs stored C used in root growth and root respiration, as well as the turnover rate of fine-root C fixed during [CO_2] fumigation.
- New fine-root growth was largely from recent photosynthate, while nearly one-quarter of respired C was from a storage pool. Changes in the isotopic composition of the fine-root population over two full growing seasons indicated heterogeneous C pools; < 10% of root C had a residence time < 3 months, while a majority of root C had a residence time > 2 yr.
- Compared to a one-pool model, a two-pool model for C turnover in fine roots (with 5 and 0.37 yr^{-1} turnover times) doubles the fine-root contribution to forest NPP (9–13%) and supports the 50% root-to-soil transfer rate often used in models.

Introduction

Fine roots, typically defined as roots < 2 mm in diameter, are a significant component of net primary production in terrestrial ecosystems (Jackson *et al.*, 1997). The respiration of CO_2 from the growth and maintenance of fine roots is an important component of the terrestrial C cycle, and may account for as much as half of soil CO_2 efflux (Taneva *et al.*, 2006; Brüggemann *et al.*, 2011) and up to 40% of total ecosystem respiration (Davidson *et al.*, 2006).

Although we are beginning to understand environmental drivers for rates of root respiration at the ecosystem level (Tang *et al.*, 2005; Trueman & Gonzalez-Meler, 2005; Drake *et al.*, 2008; Taneva & Gonzalez-Meler, 2011), our understanding of the sources of C fueling root respiration (i.e. recent photosynthate compared with C stored in the plant) is less clear (Körner, 2003; Trumbore, 2006). New photosynthate was thought to be the main source of C for fine-root respiration (Högberg *et al.*, 2001; Trueman & Gonzalez-Meler, 2005), but studies using a radiocarbon tracer found a significant contribution of stored C to the root-respired CO_2 (Czimczik *et al.*, 2006; Schuur & Trumbore, 2006). The amount of stored C used in root respiration may differ between species or vary seasonally (Kuptz *et al.*, 2011; Hopkins *et al.*, 2013). While new evidence indicates that root-respired CO_2 may be a mixture of recent photosynthate and stored C, quantitative studies assessing the contribution from these pools to root-respired CO_2 are lacking.

The ability for plants to move C quickly to roots for respiration contrasts with the observations that at least some C in fine roots is multiple years old (Matamala *et al.*, 2003; Gaudinski *et al.*, 2010). The presence of older C in fine-root mass may indicate that stored C was used in the production of new roots. Stored C was not used in production of new fine roots in coniferous (Matamala *et al.*, 2003) and deciduous tree plantations (Trueman & Gonzalez-Meler, 2005) exposed to elevated concentrations of atmospheric CO_2. By contrast, as much as 55% of C used for the production of new roots was from a storage C pool in temperate and subtropical oak forests (Langley *et al.*, 2002; Gaudinski *et al.*, 2009). Furthermore, there appears to be differences in the contribution of stored C depending on root diameter. In *Pinus sylvestris*, roots < 0.5 mm diameter were produced from recent C, while roots 0.5–2 mm diameter were produced using C that was up to 10 yr old (Sah *et al.*, 2011). Significant amounts of stored C were also used for the growth of new fine roots in a diverse forest in Switzerland (Bader *et al.*, 2009).

The presence of older C in fine roots may also be due to multiple pools of C in the fine-root population that have different turnover rates (e.g. structural vs nonstructural C). While fine roots are an important source of C inputs to the soil (Rasse *et al.*, 2005), the rate at which fine-root C is transferred to the soil

system is not resolved. Theoretical modeling (Luo, 2003; Guo *et al.*, 2008) and isotopic approaches (Trueman & Gonzalez-Meler, 2005; Riley *et al.*, 2009; Gaudinski *et al.*, 2010; Keel *et al.*, 2012) have recently demonstrated heterogeneity in the turnover of C in fine roots in some cases (Matamala *et al.*, 2003, 2004), with some C turning over relatively quickly (on the order of months), and other C having a multiple-year lifespan. The relative magnitude of the C pool sizes, and the turnover rates for the 'fast' and 'slow' C pools is less clear. Gaudinski *et al.* (2010) placed 20% of fine root C into a fast (*c.* 1–3 yr) pool and 80% into a slow (decadal) pool. However, in a temperate *Pinus taeda* forest, use of one fine-root C pool provided similar production estimates for at least three methods deployed where isotopes (^{13}C and ^{14}C, Matamala *et al.*, 2003), minirhizotrons (Pritchard *et al.*, 2008) and sequential coring methods (Matamala & Schlesinger, 2000) converged into similar fine-root production estimates between 80 and 160 $g\,m^{-2}\,yr^{-1}$ (*c.* 5–10% of total NPP). Current models of the belowground C cycle often represent fine roots as a single pool with a fixed turnover rate (Parton *et al.*, 1987; Thornton *et al.*, 2007). However, the distribution of fine roots into 'slow' and 'fast' turnover pools can alter C cycling in ecosystems (Gaudinski *et al.*, 2010). Thus, if models are to accurately portray belowground productivity and the contribution of roots to soil C, characterization of heterogeneity in fine roots is a high research priority.

Here, we took advantage of a unique opportunity afforded by the conclusion of a long-term free-air CO_2 enrichment (FACE) experiment in a *Liquidambar styraciflua* (sweetgum) plantation at Oak Ridge National Laboratory, TN, USA, to track movements of C in the root system. The C in trees that was assimilated during 12 yr of CO_2 fumigation had a depleted isotopic C signal, and the relaxation of that signal as the trees assimilated less-depleted C after cessation of CO_2 fumigation provided a means of tracking C cycling processes (as in Trueman *et al.*, 2009). We measured changes in the isotopic composition of newly produced fine roots and root-respired CO_2, and monitored the dilution of the depleted isotope tracer in the fine-root pool over two growing seasons following the cessation of CO_2 fumigation. Our primary objectives were two-fold: quantify the relative use of new photosynthate and storage C for new root growth and root respiration; and determine the relative size and turnover rate for fine-root C pools.

Materials and Methods

Site description

This study was performed at the Oak Ridge National Laboratory (ORNL) free-air CO_2 enrichment (FACE) experiment, located in a sweetgum (*Liquidambar styraciflua* L.) plantation in eastern Tennessee, USA, described elsewhere (Norby *et al.*, 2001, 2002, 2004). Briefly, the experiment had four 25-m diameter FACE rings, two of which were fumigated with elevated $[CO_2]$ to *c.* 550 ppm for 12 yr, from 1998 to September 2009, with fumigation terminating after leaf drop. The other two rings were maintained at current, ambient $[CO_2]$, which ranged from 384 to 405 ppm during the course of the experiment. A fifth control ring without a FACE apparatus was not used in this experiment. The CO_2 used in the experiment had a consistent ^{13}C signature of *c.* −51‰; the carbon isotope composition of the atmosphere in the elevated $[CO_2]$ treatment was −21‰ during fumigation (Matamala *et al.*, 2003), compared to the ambient atmospheric value of *c.* −8‰. Leaf litter produced in elevated $[CO_2]$ remained consistent throughout the experiment, averaging -40.0 ± 0.4‰ compared to -29.4 ± 0.2‰ in ambient $[CO_2]$ (R. J. Norby, unpublished; Garten *et al.*, 2011).

In order to improve our understanding of ecosystem C cycling, particularly C residence time in ecosystem compartments (Epron *et al.*, 2012), it is important to take advantage of the few available long-term, ecosystem-scale, manipulations of ecosystem isotopic composition, such as FACE. However, a potential limitation of following the fate of ^{13}C during a FACE experiment (Keel *et al.*, 2006) is that the partitioning of the ^{13}C label can be confounded with the effects of elevated CO_2 on C allocation such that the results may not represent C cycling in current, ambient CO_2 (Epron *et al.*, 2012). We addressed this limitation in two ways. First, the analyses in the current study were carried out after the elevated $[CO_2]$ treatment had ended, removing the direct effect of elevated $[CO_2]$ on the processes of interest. Second, we examined C cycling in the final 2 yr of CO_2 enrichment to alleviate concerns regarding the possibility of residual effects of elevated $[CO_2]$ on C residence time in fine roots and found no difference in leaf-level photosynthesis, leaf area, stand net primary production (NPP) and, therefore, total C supply between the ambient and elevated $[CO_2]$ treatments (Norby *et al.*, 2010a). Furthermore, there was no evidence for differences in belowground allocation (fine-root production or soil respiration) between the historical ambient and elevated $[CO_2]$ treatments after fumigation ended (C. M. Iversen, unpublished). Hence, there is little evidence for important artifacts in our approach, especially in comparison to most other pulse-chase C isotope experiments (Epron *et al.*, 2012).

Fine-root sampling from in-growth cores

In order to determine the sources of C used for new root production, root in-growth bags were sequentially placed and extracted for a full growing season following the cessation of CO_2 fumigation in the elevated $[CO_2]$ plots. From October 2009 to October 2010, root in-growth bags (5-cm diameter × 10-cm depth), consisting of fiberglass 1 mm × 1 mm screen mesh filled with a sand and perlite mixture, were placed in pre-made holes in the elevated $[CO_2]$ treatment and retrieved after different incubation periods as described below. The two elevated $[CO_2]$ plots received 24 in-growth bags in October 2009, shortly after CO_2 fumigation was terminated. Eight of these bags were extracted from each plot before leaf-out (March 2010), eight bags were extracted just after leaf-out (late April 2010), and the remaining eight bags were extracted after a full year of incubation *in situ* (October 2010). Beginning in March 2010, 16 bags were inserted into the soil each month except in June as indicated above; eight of these bags were extracted after 4 wk and eight bags were extracted after 12 wk of placement. The differential in-growth bag incubation

times (4 or 12 wk) were to ensure that enough root material was retrieved for isotopic analysis, particularly for larger diameter roots during slower root-growth periods. A final set of eight root in-growth bags was placed in the soil in August 2010 and extracted in October 2010. In total, 96 root in-growth bags were placed in the soil; a timeline depicting in-growth bag placement and extraction is depicted in Fig. 1. The extracted in-growth bags were transported to the laboratory on blue ice, and frozen at −20°C before shipment to the University of Illinois at Chicago, where root retrieval and isotopic analyses were completed.

Fine-root sampling from intact cores

In order to monitor turnover of C in the fine-root pool and examine sources of C utilized for fine-root respiration, intact cores were extracted at regular intervals from the plots previously receiving elevated [CO_2] for two growing seasons following cessation of CO_2 fumigation. Intact soil cores (5-cm diameter) were taken to 10-cm depth from the elevated [CO_2] treatment at the time of extraction of in-growth cores in 2010 (see Fig. 1). Additional intact cores were extracted in June, August, September and October 2011, and a final set of cores was collected in February 2012. Intact soil cores were also extracted from the ambient [CO_2] treatment plots (i.e. plots never receiving [CO_2] fumigation) in May 2010, August 2011 and September 2011. During sampling periods in 2010 and early 2011, eight cores were taken in each plot. Starting in August 2011, the number of cores was three per plot as three replicates were sufficient for statistical purposes. The extracted intact soil cores were transported to the laboratory on ice, and frozen at −20°C before shipment to the University of Illinois at Chicago, where root retrieval and isotopic analyses were completed.

Fine-root separation and respiration C source measurements

For both in-growth cores and intact soil cores, samples were first thawed in a 4°C refrigerator for 4 h. Roots were separated from thawed soil and thoroughly rinsed with deionized water and ensured to be soil free by visual inspection and with a microscope (as in Matamala *et al.*, 2003). Live roots were separated using tensile strength (very few dead roots were found), and herbaceous roots, which were distinct from sweetgum roots in form, color and tensile strength were visually identified and removed. Roots were separated into roots < 1-mm diameter and roots > 1-mm but < 2-mm diameter. Few samples contained roots > 2-mm diameter, and they were not used for these analyses.

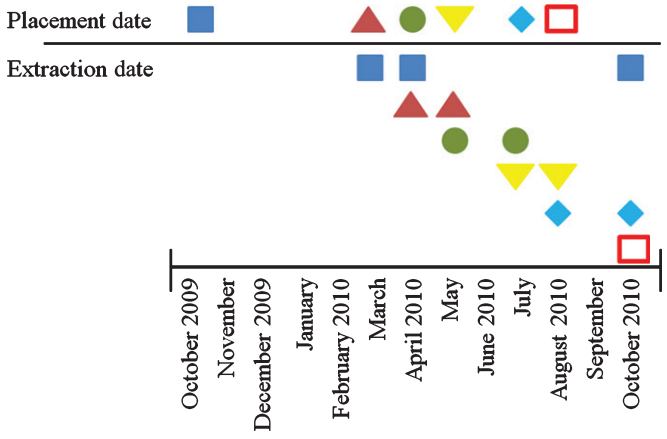

Fig. 1 A timeline of placement and extraction of in-growth cores used to analyze sources of carbon (C) for new fine-root growth in *Liquidambar styraciflua*. CO_2 fumigation ceased in September 2009.

Roots extracted from intact cores from several sampling periods were incubated to capture CO_2 respired from fine roots for isotopic analysis and C source determination (as in Gomez-Casanovas *et al.*, 2012), as the isotopic composition of root-respired CO_2 is preserved for several hours following root extraction (Millard *et al.*, 2008). From the plots previously receiving elevated [CO_2], roots from intact cores extracted in May and October 2010, and in May, August and September 2011, were incubated for root-respired CO_2. All roots extracted from ambient [CO_2] plots (May 2010, August 2011 and September 2011) were incubated for capture of root-respired CO_2. Roots < 1-mm diameter were incubated using a system similar to that described in Taneva & Gonzalez-Meler (2011) and Gomez-Casanovas *et al.* (2012). After extraction, roots were placed into a 140 cm^3 PVC chamber with a moist tissue to prevent drying. The chamber was then flushed with CO_2-free air and sealed for 1–2 h at 25°C. Following incubation, the CO_2 was collected in a gas flask and stored for < 1 wk before analysis for $^{13}CO_2$.

Roots collected from the in-growth and intact soil cores were oven-dried at 65°C for at least 48 h, and ground to a fine powder for ^{13}C analysis of the bulk root tissue (henceforth referred to as 'structural root tissue').

Stable C isotope analysis

All gas samples and structural root tissues were analysed for ^{13}C at the University of Illinois at Chicago (UIC) stable isotope laboratory. Gas samples were purified by cryogenic distillation, and pure CO_2 samples were analyzed for ^{13}C with a Gas Bench II (Thermo Finnigan, Bremen, Germany) coupled to a Finnigan Deltaplus XL isotope ratio mass spectrometer (IRMS, Thermo Finnigan). Structural root samples were run on a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA) coupled to the same IRMS. The $\delta^{13}C$ values are reported relative to the standard VPDB following the equation:

$$\delta^{13}C_{sample} = \left[\frac{\frac{^{13}C}{^{12}C}\text{sample}}{\frac{^{13}C}{^{12}C}\text{std}} - 1\right] \times 1000 \qquad \text{Eqn 1}$$

Partitioning of C sources

In order to differentiate between C fixed in the elevated [CO_2] treatment plots during fumigation (i.e. treatment C that was isotopically depleted) and C fixed after fumigation ceased (i.e. post-treatment C, normal air), we applied a two-end-member mixing model for both structural root C and root-respired CO_2

(Matamala *et al.*, 2003; Taneva *et al.*, 2006). For structural root C, the treatment C end-member was determined by roots sampled from intact cores in March 2010 before leaf-out (i.e. roots that were produced using only C produced during CO_2 fumigation). The post-treatment C end-member was determined by averaging the isotopic composition of all roots collected from intact cores from both growing seasons in the plots that never received [CO_2] fumigation (i.e. ambient [CO_2] plots). For root-respired CO_2, the end-member for treatment C was not directly measured as no roots were incubated until May 2010 (after some 'new' C had been incorporated). Instead, the end-member was calculated from the structural C end-member by adding an observed, and consistent, 4.5‰ enrichment in root-respired CO_2 with respect to structural root tissue seen by other studies (discussed below). The post-treatment C end-member for root-respired CO_2 was determined by averaging the isotopic composition of root-respired CO_2 from all roots collected from intact cores in the ambient [CO_2] plots that never received [CO_2] fumigation.

The amount of treatment C (i.e. isotopically depleted C fixed under elevated [CO_2]) in a sample (F_t) in % can be calculated from

$$F_t\,(\%) = \left[\frac{\delta^{13}C_{sample} - \delta^{13}C_{post-treatment}}{\delta^{13}C_{treatment} - \delta^{13}C_{post-treatment}}\right] \times 100 \qquad \text{Eqn 2}$$

($\delta^{13}C_{sample}$, $\delta^{13}C$ of the harvested roots (or the root-respired CO_2); $\delta^{13}C_{post\text{-}treatment}$, $\delta^{13}C$ of C incorporated after fumigation ceased; $\delta^{13}C_{treatment}$, $\delta^{13}C$ of C incorporated during fumigation with isotopically-depleted, elevated [CO_2].)

Estimation of C turnover

We determined whether there was heterogeneity in the turnover rate of C in the fine-root population through a linearization approach (as in Taneva *et al.*, 2006). We also fitted one-pool and two-pool exponential decay models to our data and determined the best model fit using several model parameters, including model R^2 (as in Keel *et al.*, 2012), the Akaike Information Criterion (AIC), a widely accepted metric for nonlinear model selection (Spiess & Neumeyer, 2010), and ANOVA tests between one-pool and two-pool models. In exponential decay models, $F(t) = a_1 e^{-k_1 t} + a_2 e^{-k2t}$, $F(t)$ is the percent of treatment C remaining, a is the initial amount of treatment C, and k is the decay rate of treatment C for each respective pool. In the one-pool model, a_2 and k_2 are equal to zero.

Statistical analysis

All statistical analyses were performed with R statistical analysis software, v2.15.1 (R Development Core Team, 2012). ANOVA models with sampling time as a replicate were utilized to analyze changes in sources of C for new root growth and for root respiration throughout the growing season with samples from the historical ambient [CO_2] plots combined and treated as one sampling period. An ANOVA model was also utilized to compare C isotopic composition between structural C and C in root-respired CO_2 in the historical ambient [CO_2] plots with sampling time as a replicate. Tukey's Honestly significant difference (HSD) tests were performed on ANOVA models to compare different sampling periods and treatment types. The rate of turnover and the pool size of C in fine roots were analyzed by the fitting of nonlinear models to our data (see previous section).

Results

C sources for new root growth

The first set of root in-growth bags extracted in March 2010, contained no sweetgum roots. For the population of roots < 1-mm diameter, all subsequent sets of in-growth cores contained enough root mass for isotopic analysis. Roots extracted in April 2010 had a $\delta^{13}C$ value of *c.* −35‰, which is 5.6‰ more depleted than that of roots grown under ambient CO_2 conditions (−29.4 ± 0.4‰). During the remainder of the growing season, roots were less depleted (averaging *c.* −30‰) and similar to never-fumigated roots (Fig. 2). The isotopic composition of the newly produced fine roots differed significantly between sampling periods, ($P < 0.001$, see Supporting Information Table S1 for ANOVA table), but only the cores extracted in April 2010, differed significantly isotopically from the post-treatment C end-member (Fig. 2, $P < 0.05$). After April 2010, very little isotopically depleted treatment C was found in newly-produced fine roots.

Despite the 12-wk incubation time for in-growth root bags, larger roots with a diameter between 1 and 2 mm were observed only in eight in-growth cores over five sampling periods (out of a total of 96 in-growth cores; Fig. S1). The isotopic composition of these few samples was consistent with the smaller diameter roots collected on the same date, indicating that new roots from both diameter classes were derived mostly with C fixed by the trees post-treatment (i.e. after fumigation with elevated CO_2 had ended).

C sources for root-respired CO_2

In roots sampled from the ambient [CO_2] treatment, the $\delta^{13}C$ of root-respired CO_2 was −24.9 ± 0.8‰ (mean ± SE, Fig. 3), which was 4.5‰ enriched compared to structural root tissue from the same plots ($P < 0.01$, Table S2), and this 4.5‰ enrichment was incorporated into our mixing model calculations for CO_2 respired from fine roots (Eqn 2). In the plots previously receiving elevated [CO_2], $\delta^{13}C$ of root-respired CO_2 ranged from −27.6 to −25.8‰ (Fig. 4). Sampling time had significant effects on the $\delta^{13}C$ of root-respired CO_2 ($P < 0.05$, Table S3). After application of a two end-member mixing model, *c.* 24% and 10% of C in root-respired CO_2 was derived from treatment C in 2010 and 2011, respectively (Fig. 5).

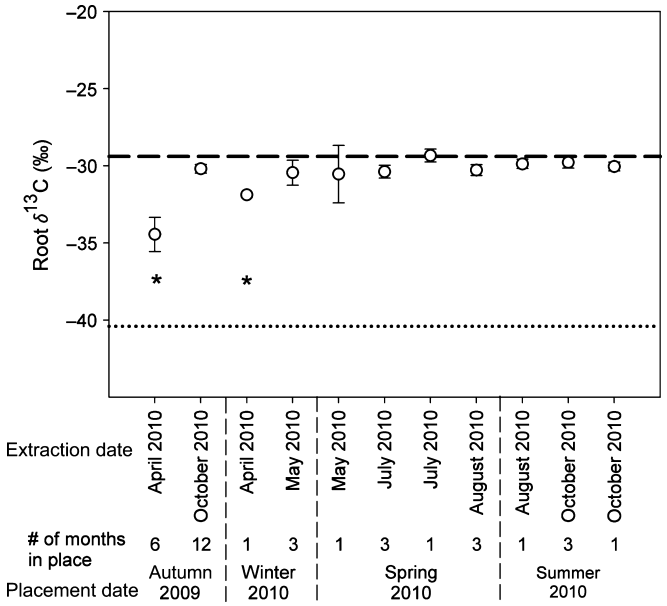

Fig. 2 Bulk root $\delta^{13}C$ for fine roots < 1 mm from in-growth cores in 2010. Data shown are means (± 1SE). The dotted line indicates the isotopic composition of carbon (C) incorporated into *Liquidambar styraciflua* biomass during fumigation (treatment C mixing model end-member) and the dashed line the isotopic composition of C incorporated into plant biomass after fumigation ceased (post-treatment C mixing model end-member). Significant difference from post-treatment end-member (dashed line) from Tukey's HSD tests on ANOVA model: *, $P < 0.01$.

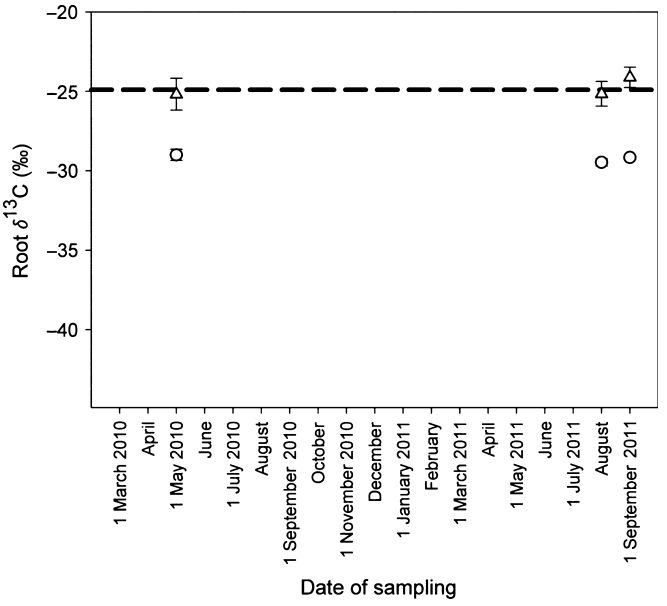

Fig. 3 Structural root $\delta^{13}C$ (circles) and root-respired CO_2 $\delta^{13}C$ (triangles) for *Liquidambar styraciflua* fine roots < 1 mm from intact cores in the ambient [CO_2] treatment. Data shown are means (± 1SE). Dashed line indicates the mean for all root-respired CO_2 $\delta^{13}C$ samples from the [CO_2] treatment. Tukey's HSD on an analysis of variance model indicates that bulk root $\delta^{13}C$ significantly differs from root-respired CO_2 $\delta^{13}C$ in all cases.

C turnover in fine roots

Roots sampled from intact soil cores, which represented the entire population of fine roots, including newly produced roots and older roots, had a more depleted isotopic signature than new roots alone. For roots < 1 mm diameter, the isotopic composition changed over the course of the study from −40.5‰ in March 2010 to −33.6‰ in February 2012 (Fig. 6). By February 2012, *c.* 40% of C in the population of roots < 1 mm diameter remained from the elevated CO_2 treatment that ended in September 2009 (Fig. 6). While visual examination of the $\delta^{13}C$ data shows an initial step change by April 2010 (Fig. 6), a linearity approach (Taneva *et al.*, 2006) did not detect multiple C turnover pools in the population of roots < 1 mm diameter, though we had less than half of the required 25 data points for this method (Friedlander *et al.*, 1981; Fig. S2). One-pool and two-pool exponential decay models were fitted to the data (see Table 1 for model parameters). In a one-pool vs two-pool exponential decay model comparison, R^2 was 0.92 and AIC was −18.0 for the one-pool and model R^2 was 0.93 and AIC was −25.3 for the two-pool model, both values indicating a better fit for the two-pool model. Additionally, an ANOVA test of the models resulted in significant difference between the models ($P < 0.05$). The two-pool model detected a 'fast' turnover root C pool comprising 9 ± 2.5% of total C, and a 'slow' turnover root C pool comprising 91 ± 2.4% of total C (Figs 7, S3). However, in the two-pool model, only the 'slow' pool parameters were statistically significant ($P < 0.001$). The fast root C pool detected by the two-pool model is consistent in magnitude (*c.* 9%) with the initial step change seen in the isotopic composition of roots after cessation of CO_2 fumigation (Fig. 6).

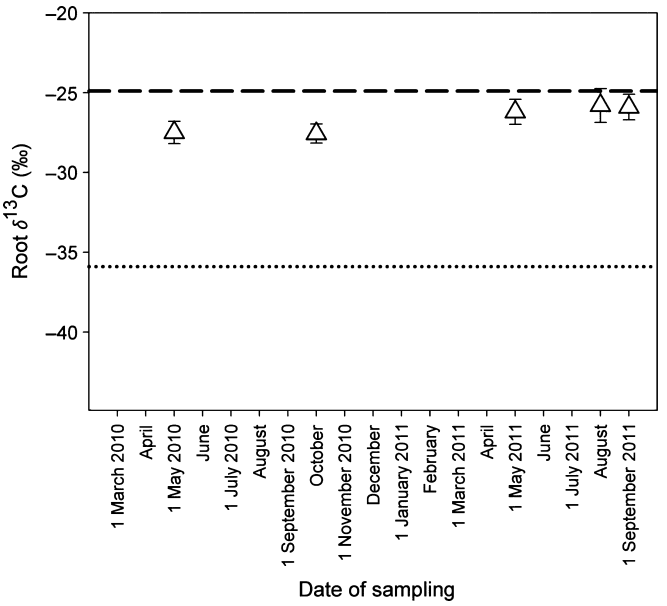

Fig. 4 Root-respired CO_2 $\delta^{13}C$ for fine roots < 1 mm from intact cores in the elevated [CO_2] treatment. Data shown are means (± 1SE). The dotted line indicates the isotopic composition of carbon (C) incorporated into plant biomass during fumigation (treatment C mixing model end-member) and the dashed line the isotopic composition of C incorporated into *Liquidambar styraciflua* biomass after fumigation ceased (post-treatment C mixing model end-member). Tukey's HSD on an analysis of variance model does not indicate a significant difference between measured root-respired CO_2 $\delta^{13}C$ and the post-treatment C $\delta^{13}C$ (dashed line).

The mean residence times (MRT = $-1/k$) of root C derived from the two-pool model were 0.2 and 2.7 yr for the 'fast' and 'slow' turnover pools, respectively (Table 1).

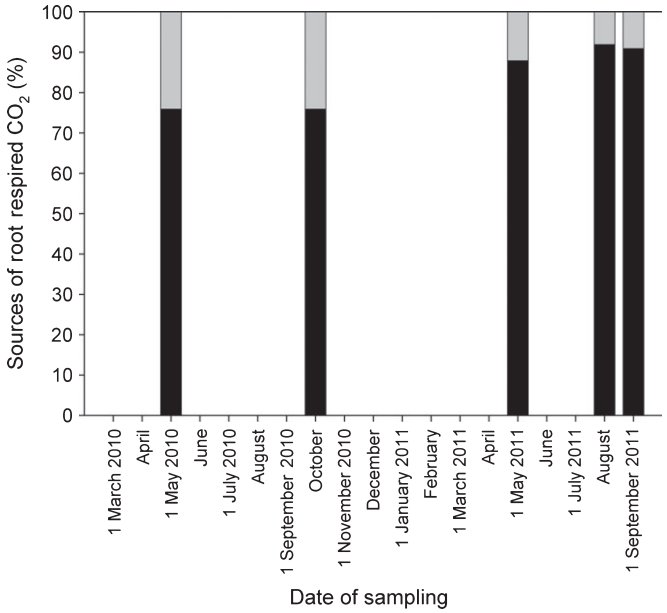

Fig. 5 Sources of carbon (C) for root-respired CO_2 for *Liquidambar styraciflua* fine roots < 1 mm from intact cores in the elevated [CO_2] treatment after applying a two end-member mixing model. Black bars, current year C; gray bars, storage C pool. Approx. 24% and 10% of C respired from fine roots is storage C in 2010 and 2011, respectively.

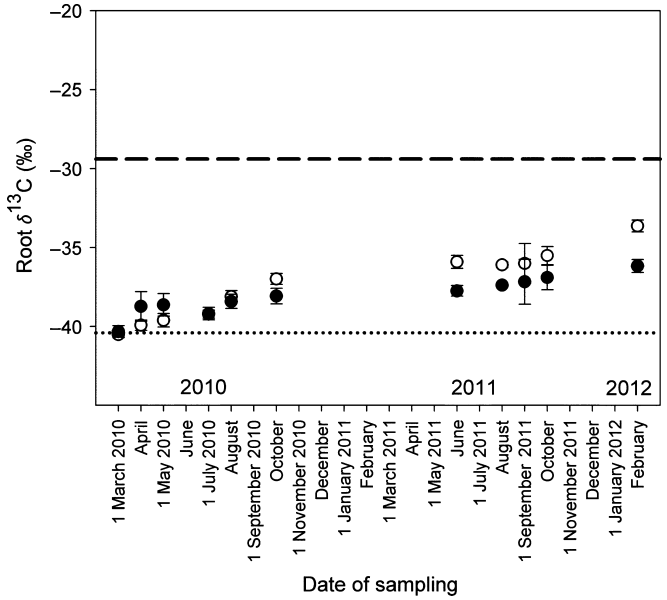

Fig. 6 Bulk root $\delta^{13}C$ for fine roots < 1 mm (open circles) and 1–2 mm (closed circles) from intact cores. Data shown are means (± 1SE). The dotted line indicates the isotopic composition of carbon (C) incorporated into *Liquidambar styraciflua* biomass during fumigation (treatment C mixing model end-member) and the dashed line the isotopic composition of C incorporated into plant biomass after fumigation ceased (post-treatment C mixing model end-member) for fine root < 1 mm. The post-treatment end-member for 1–2 mm roots is not shown, but is 0.2‰ depleted with respect to the < 1 mm roots.

For roots with a diameter between 1 and 2 mm, the isotopic composition changed over the course of our study from −40.3‰ in March 2010, to −36.2‰ in February 2012 (Fig. 6). By February 2012, nearly 60% of C in roots between 1 and 2 mm diameter remained from the CO_2 fumigation treatment that ended in September 2009 (Fig. 6). Similar to smaller diameter roots, an initial step change occurred in early spring 2010. A linearity approach did not detect multiple C turnover pools in our data (Fig. S2). In a one vs two-pool exponential decay model comparison, the model R^2 was 0.68, and AIC was −25.3 in the one-pool model, and model R^2 was 0.89 and AIC was −33.6 for the two-pool model; both values indicated a better fit for the two-pool model. Additionally, an ANOVA test of the models revealed a significant difference between the models ($P < 0.05$). The two-pool model indicated a 'fast' turnover pool comprising 9.5 ± 4.0% of total root C, and a 'slow' turnover pool comprising 87 ± 3.1% of total root C (Fig. 7). Like the smaller diameter roots, only the 'slow' pool parameters were statistically significant ($P < 0.001$). Mean residence time (MRT) of C derived from the two-pool model were 0.1 and 6.3 yr for the 'fast' and 'slow' pools, respectively.

Discussion

We utilized a unique opportunity afforded by the end of a long-term FACE experiment in a mature stand of *L. styraciflua*, where the isotopic composition of C fixed during [CO_2] fumigation was different from C fixed after fumigation ended in September 2009. In contrast to experimental designs using a one-time pulse labeling of an isotopic tracer, the dilution of labeled C incorporated into sweetgum biomass for 12 growing seasons by the newly fixed, unlabeled C (i.e. the 'relaxation' of the isotopically-depleted ^{13}C signature of C fixed during CO_2 fumigation) allowed us to quantify a significant (*c.* 24%) use of a storage C pool fueling root respiration and a lack of storage C for new root growth. Additionally, our results indicate a small (10% of total biomass) 'fast' (i.e. short MRT of fine-root C) turnover pool and a large (90% of total biomass) 'slow' (i.e. longer MRT of fine-root C) turnover pool in fine roots.

Post-carboxylation carbon isotope fractionation

Post-carboxylation isotope fractionation needs to be considered when quantifying C sources used for fine-root respiration (Werner *et al.*, 2011). Isotopic composition of root-respired CO_2 from plots never receiving CO_2 fumigation indicate a substantial and consistent 4.5‰ enrichment relative to the root biomass (Fig. 3). This enrichment is similar to that of other woody species including *Fagus sylvatica* (5‰, Formanek & Ambus, 2004) and somewhat larger than that seen in *Eucalyptus delegetenis* (0.7–3.1‰, Gessler *et al.*, 2007). Root-respired CO_2 in herbaceous plants, by contrast, has mostly been found to be depleted in ^{13}C with respect to root substrate (Bowling *et al.*, 2008; Werth & Kuzyakov, 2010; Zhu & Cheng, 2011).

The mechanisms creating isotopic depletion or enrichment in root-respired CO_2 with respect to root substrate in plants are not well understood (Bowling *et al.*, 2008), but may include the use of different biochemical pathways during primary C metabolism (Gessler *et al.*, 2009). Without considering post-carboxylation

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Table 1 Model parameters for exponential decay models explaining turnover of carbon (C) in *Liquidambar styraciflua* fine roots

		Slow pool (i.e. longer MRT)				Fast pool (i.e. shorter MRT, if applicable)				
Source	Model type	a_1	k_1	MRT (yr)	95% Turnover time (yr)	a_2	k_2	MRT (yr)	95% Turnover time (yr)	R^2
Roots < 1 mm diameter										
Current study	One pool	0.99 ± 0.022	−0.44 ± 0.027	2.0–2.6	7		N/A			0.92
Current study	Two pool	0.91 ± 0.024	−0.37 ± 0.026	2.4–3.2	8	0.09 ± 0.025	−4.15 ± 12.5	0.2	0.2	0.93
Matamala *et al.* (2003)	One pool	0.99	−0.8356	1.1–1.4	4		N/A			0.99
Roots 1–2 mm diameter										
Current study	One pool	0.99 ± 0.023	−0.26 ± 0.026	3.2–4.73	11.4		N/A			0.68
Current study	Two pool	0.87 ± 0.031	−0.16 ± 0.028	4.8–9	17.8	0.095 ± 0.04	−10 ± 13.7	0.1	0.1	0.89
Matamala *et al.* (2003)	One pool	1	−0.3333	2.7–3.3	9		N/A			0.98

MRT, mean residence time. N/A, not applicable.

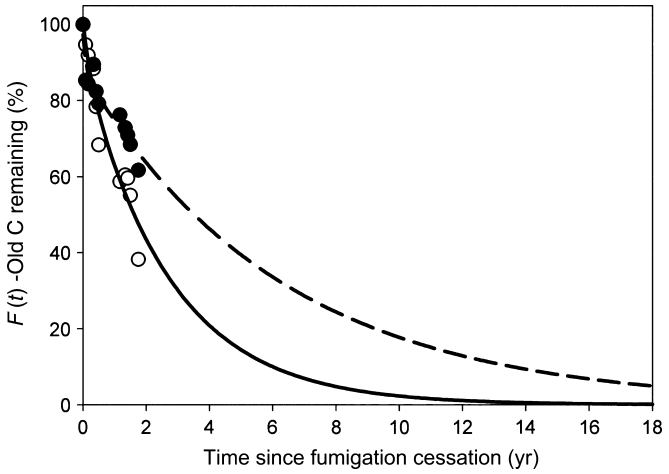

Fig. 7 Bulk root $\delta^{13}C$ for *Liquidambar styraciflua* fine roots < 1 mm (open circles) and 1–2 mm (closed circles) from intact cores presented as % treatment carbon (C) after applying a two end-member mixing model. Two-pool exponential decay models are fitted to the data: $F(t) = a_1e^{-k_1t} + a_2e^{-k2t}$, where $F(t)$ is % fumigation C remaining, a is initial amount of fumigation C and k is the decay rate of fumigation C for each respective pool. For < 1 mm roots, $F(t) = 0.91e^{-0.368t} + 0.09e^{-4.15t}$ and for 1–2 mm roots, $F(t) = 0.87e^{-0.16t} + 0.095e^{-10t}$. Mean residence time (MRT) = $-1/k$ and is 2.7 and 0.2 yr for the < 1 mm larger and smaller pools, respectively, and is 6.3 and 0.1 yr for the 1–2 mm larger and smaller pools, respectively.

fractionation, we would have wrongly concluded that no storage C is used for fine-root respiration, highlighting the importance of understanding plant processes that create post-carboxylation fractionation when performing isotope tracer studies. Isotope techniques are often employed to separate autotrophic and heterotrophic respiration components (Lin *et al.*, 1999). The values of the bulk substrate are sometimes used to estimate the isotopic composition of respiration components (Zhu & Cheng, 2011), while other authors have used the isotopic value of respired CO from different sources (Carbone & Trumbore, 2007; Taneva & Gonzalez-Meler, 2011; Gomez-Casanovas *et al.*, 2012). Accurate partitioning of components of ecosystem fluxes may require the incorporation of isotope effects during transport and metabolism (i.e. post-carboxylation fractionation), particularly if isotopic fractionation varies through time.

C sources fueling fine-root respiration

Despite the availability of C that is just a few days old for root-respired CO_2 (Högberg *et al.*, 2008; Gomez-Casanovas *et al.*, 2012; Hopkins *et al.*, 2013), roots may combine recent photosynthate with a storage C pool to fuel metabolic activity (Schuur & Trumbore, 2006). In our study, a significant portion of C utilized for fine-root respiration is derived from a storage pool (Fig. 4). In the first year following cessation of fumigation (2010), *c.* 24% of C was derived from storage in both spring and autumn (Fig. 5). In 2011 (2 yr after the end of treatment), *c.* 10% of C was derived from storage C fixed in 2009 or earlier. Results from the 2nd year following fumigation cessation (2011) are more difficult to interpret, as C incorporated during 2010 is isotopically indistinguishable to C incorporated during 2011, even though it is a 1-yr old storage pool. Thus, the 10% from a storage pool is C that is at minimum 2 yr old. Possibly, the difference in storage contribution to root-respired CO_2 (*c.* 14%) between 2010 and 2011 represents a storage contribution to root respiration that is *c.* 1 yr old, with the remainder (*c.* 10%) at least 2 yr old (though this assumes a constant use of 24% storage C as found in 2010). Regardless of age, our data are in agreement with other studies finding a significant use of storage C to fuel fine-root respiration (Czimczik *et al.*, 2006; Schuur & Trumbore, 2006). These results might represent a maximum value if enhanced carbohydrate availability due to legacy affects from the elevated [CO_2] treatment increased stand use of storage carbon.

C sources for new root growth

Uncertainties remain in understanding the C sources for fine-root production. In-growth cores placed during the dormant

season (October 2009–March 2010) contained no *L. styraciflua* roots, indicating no significant new fine-root production when no new photosynthate is available. In our study, new roots were produced using current-year photosynthate for the majority of the growing season (Fig. 2), although some stored C was used to produce new roots in April 2010 (Fig. 2). All subsequent fine-root production occurring after 50% leaf-out in mid-May (Norby *et al.*, 2003) was derived exclusively from current-year photosynthate. Cores in place for a full year (October 2009–2010) were also not isotopically different from current-year photosynthate, indicating that a majority of fine-root growth occurs during the growing season and comes from new photosynthate (i.e. the biomass of roots grown between October 2009, and April 2010, was small, and the isotopic signature of these roots was diluted by the large biomass of roots grown from April 2010 to October 2010, that had a strong signal of current-year photosynthate; Fig. 2). These results are consistent with studies showing little or no storage C used for production of new fine roots (Matamala *et al.*, 2003; Trueman & Gonzalez-Meler, 2005). However, other studies have reported up to 55% of new fine-root production comes from a C storage pool (Bader *et al.*, 2009; Gaudinski *et al.*, 2009). Few in-growth cores contained larger fine roots (> 1 mm diameter), but those that did are in agreement with new root growth utilizing mostly new C (Fig. S1). In contrast with our results, production of large-diameter fine roots in mature boreal forests utilized significant storage C (Sah *et al.*, 2011). There are several important implications for the lack of consistency between species or ecosystems in use of a storage C pool for fine-root production. First, when using isotopic tracers to determine C turnover rates in fine roots, evidence of use of storage C in fine-root production will complicate interpretation of results, making fine-root C turnover appear slower (Luo, 2003). Second, studies modeling belowground C allocation should consider differences between species, which may have large impacts on C cycling at ecosystem scales. Mechanisms that can account for differences in storage C for new fine-root production are not currently known and deserve further study.

Fine-root C turnover estimates

Both newly-fixed photosynthate and storage fuel root respiration, while new root growth is supported exclusively by recent photosynthate. The turnover rate of C used to produce fine roots was measured by tracking disappearance of the 12-yr treatment C isotopic tracer in the intact fine-root pool over two full growing seasons (Fig. 6). In contrast to the in-growth cores, roots collected from intact cores represented both old roots produced during CO_2 fumigation and new roots produced following fumigation cessation and therefore the rate of C replacement represents the C turnover of a given root pool (Table 1, Figs S3, S4; Matamala *et al.*, 2003). There was an initial step-change in the isotopic composition of fine roots (first few weeks; Fig. 6) suggesting the existence of a fast turnover fine-root C pool (i.e. short MRT in fine-root C) representing *c.* 9% of the total root biomass. Although data linearization (Taneva *et al.*, 2006) did not reveal a fast and a slow C pool, model R^2 values were higher and AIC scores lower for two-pool models compared to one-pool models for both diameter classes. Thus, our data indicate a small 'fast' pool with C turnover times of a few months (*c.* 10% of total fine-root C) and a larger 'slow' pool with C turnover times of multiple years (*c.* 90% of total C).

The mean residence time of C in fine roots estimated at the onset of CO_2 fumigation at this FACE site (Matamala *et al.*, 2003) was less than estimates from this study (Table 1). Part of the difference can be attributed to the identification of a fast root pool in this study, as MRT of root C is increased after removing the 'fast' C pool. Part of the difference between the two studies may also be due to an effect of elevated [CO_2] on root turnover, as root turnover appeared to be slower under elevated [CO_2] conditions at the site (Iversen *et al.*, 2008) Thus, our root C turnover estimates might be slower due to a larger standing root crop following elevated [CO_2] treatment. Despite the differences, C residence times in fine roots measured at the onset and cessation of fumigation with isotope tracers are in agreement with each other, and longer than minirhizotron estimates of root structure turnover at the same ORNL site, which was < 2 yr for fine roots < 2 mm diameter from 2001 through 2006 at elevated CO_2 and < 1 yr at ambient CO_2 (Iversen *et al.*, 2008).

Disparities between minirhizotron and isotope tracer studies on root (C) turnover and longevity may stem from several independent processes. While minirhizotron approaches are based on direct observations of production and senescence of individual root structures *in situ*, isotope tracers quantify residence time of C in root systems. Any re-absorption/mobilization of root C from senescing roots or root exudates (Jones *et al.*, 2009) will increase longevity of C compared to root structures. Minirhizotron installations also seem to promote turnover of roots due to soil disturbance, and it may take 3–5 yr for the system to stabilize (Iversen *et al.*, 2008; Pritchard *et al.*, 2008). Other potential reasons for differences between isotopic tracer experiments and minirhizotron studies are that soil coring and extraction can miss the smallest roots when separating from the soil matrix while minirhizotrons mostly observe the finest roots (Majdi *et al.*, 2005). Recent evidence suggests that fine-root production and mortality may occur in clusters of low-order roots that differ in their function and structure (Xia *et al.*, 2010), but only a fraction of the fine roots turnover rapidly (Guo *et al.*, 2008), as shown here (Fig. 6). It is clear from this and previous isotope tracer studies that some C persists in fine roots for multiple years. While a large portion of C in fine roots remains for multiple years, a small amount is turned over very quickly, in a few weeks or months. This 'fast' turnover pool may include 10% of total fine-root C, as found here (Fig. 6) or up to 20% as found elsewhere (Gaudinski *et al.*, 2010).

Implications for C cycle models

Studies quantifying C allocation for various plant processes and C turnover in plant organs (particularly belowground) are important for incorporation into terrestrial C cycle models at various scales (Iversen, 2010). Most ecosystem-scale or larger models currently incorporate a single C pool for root turnover (Fisher *et al.*,

2010; Gaudinski *et al.*, 2010). If we incorporate two C turnover pools in fine roots, as our best model fit indicates, then it is possible to calculate the contributions of roots to soil organic C (SOC) and soil organic N (SON) pools and validate those contributions with observed soil accruals at the site. For example, given fine root C and N contents of 80 g C m^{-2} and 2 g N m^{-2} at 10-cm soil depth (Iversen *et al.*, 2012), respectively, if 10% of the fine root mass has a 0.2-yr turnover (replacing the biomass five times in a year) and 90% of the fine root mass is replaced at a rate of 0.37 yr^{-1}, then the fast root turnover will produce *c.* 20 g C m^{-2} yr^{-1} and 1 g N m^{-2} yr^{-1}, and the slower turnover roots will produce *c.* 13 g C m^{-2} yr^{-1} and 0.7 g N m^{-2} yr^{-1}, if we assume that 50% of the root litter stays in the soil as soil organic matter (Parton *et al.*, 1987). The ORNL FACE showed increases in SOC (44 g C m^{-2} yr^{-1}) and SON (2.2 g N m^{-2} yr^{-1}) during the first 6 yr of CO fumigation (Jastrow *et al.*, 2005) that were sustained until the end of the CO_2 fumigation experiment at this depth (J. D. Jastrow, pers. comm.). Thus, fine-root C and N turnover represents *c.* 80% of the SOC accrual and *c.* 76% of the SON accrual observed in this experiment, with the remainder produced by coarser root turnover and leaf litter decomposition. Such an agreement shows that the belowground C cycle can be modeled properly with two heterogeneous pools for fine roots (Gaudinski *et al.*, 2010).

Uncertainties remain in quantifying the contribution of fine roots to total forest NPP, with estimates ranging from as high as 33% (Jackson *et al.*, 1997) to as low as 5–7% (Matamala & Schlesinger, 2000). Application of a two-pool model found a contribution of 9–30% of total NPP by fine roots, resulting in a reduction in overall C transfer from roots to soil by 20–80% (Gaudinski *et al.*, 2010). However, in that study, the turnover time of the 'fast' turnover C pool was on the order of 1 yr, and the turnover time of the 'slow' C pool had decadal times. If the smaller 'fast' C pool is replaced multiple times during a growing season (as our best model fit at ORNL FACE indicates), the smaller C pool could have a larger effect on NPP than suggested by its size. At ORNL FACE, NPP was *c.* 500–700 g C m^{-2} yr^{-1} in the final years of the experiment (Norby *et al.*, 2010b). Our one-pool model indicates 34.4 g C m^{-2} yr^{-1} from fine roots and our two-pool model indicates 66.6 g C m^{-2} yr^{-1}, resulting in fine roots contributing 5–7% and 9–13% of total NPP for the one-pool and two-pool models, respectively. Thus, the use of a two-pool model roughly doubled the contribution of fine roots to total NPP, and bring NPP estimates in close agreement with minirhizotron estimates of *c.* 9% (Norby *et al.*, 2010b). Thus, quantification of the size and rates of turnover of multiple C pools is important, as multiple replacements per year of even a small amount of fine-root biomass can have large consequences for fine-root NPP.

Conclusions

In this study, we monitored the relaxation of a C isotope tracer following the conclusion of a long-term FACE experiment. We found a substantial use of storage C fueling fine root respiration (*c.* 24% of total C), which appears to be consistent throughout the growing season. Additionally, a 4.5‰ post-carboxylation enrichment in root-respired CO_2 relative to the root substrate must be considered in interpretation of studies utilizing isotopic tracers to examine root respiration. In contrast to respiration, new fine-root substrate was produced exclusively from current-year photosynthate for a majority of the growing season. Our results confirmed relatively long turnover times for fine-root C (on the order of years) determined by previous isotope studies. Additionally, we have provided evidence for heterogeneity in C turnover in fine roots, as suggested by previous studies (Guo *et al.*, 2008; Gaudinski *et al.*, 2010). We found a small C pool with fast ($<<$ 1 yr) turnover and a large C pool with slow (multiple-year) turnover. When two pools of fine-root C were considered, disparities in different estimates of belowground NPP at the site were reconciled.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Bulk root $\delta^{13}C$ from fine roots 1–2 mm diameter from in-growth cores.

Fig. S2 A linearity approach to separating C pools with different turnover rates: log (% treatment C remaining) as a function of time after CO_2 fumigation cessation.

Fig. S3 A comparison of one-pool and two-pool exponential decay functions for fine roots < 1 mm diameter.

Fig. S4 A comparison of one-pool and two-pool exponential decay functions for fine roots 1–2 mm diameter.

Table S1 ANOVA results for bulk $\delta^{13}C$ of fine roots < 1 mm diameter from in-growth cores with sampling period as replicate

Table S2 ANOVA results for comparing bulk root $\delta^{13}C$ and fine-root respired CO_2 in ambient [CO_2] with sampling time and C source as replicates

Table S3 ANOVA results for comparing $\delta^{13}C$ of fine-root respired CO_2 in the elevated [CO_2] rings with sampling time as replicate

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