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Research paper

Timing and magnitude of C partitioning through a young loblolly pine (*Pinus taeda* L.) stand using ^{13}C labeling and shade treatments

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The dynamics of rapid changes in carbon (C) partitioning within forest ecosystems are not well understood, which limits improvement of mechanistic models of C cycling. Our objective was to inform model processes by describing relationships between C partitioning and accessible environmental or physiological measurements, with a special emphasis on short-term C flux through a forest ecosystem. We exposed eight 7-year-old loblolly pine (*Pinus taeda* L.) trees to air enriched with $^{13}CO_2$ and then implemented adjacent light shade (LS) and heavy shade (HS) treatments in order to manipulate C uptake and flux. The impacts of shading on photosynthesis, plant water potential, sap flow, basal area growth, root growth and soil CO_2 efflux rate (CER) were assessed for each tree over a 3-week period. The progression of the ^{13}C label was concurrently tracked from the atmosphere through foliage, phloem, roots and surface soil CO_2 efflux. The HS treatment significantly reduced C uptake, sap flow, stem growth and fine root standing crop, and resulted in greater residual soil water content to 1 m depth. Soil CER was strongly correlated with sap flow on the previous day, but not the current day, with no apparent treatment effect on the relationship. Although there were apparent reductions in new C flux belowground, the HS treatment did not noticeably reduce the magnitude of belowground autotrophic and heterotrophic respiration based on surface soil CER, which was overwhelmingly driven by soil temperature and moisture. The ^{13}C label was immediately detected in foliage on label day (half-life = 0.5 day), progressed through phloem by Day 2 (half-life = 4.7 days), roots by Days 2–4, and subsequently was evident as respiratory release from soil which peaked between Days 3 and 6. The $\delta^{13}C$ of soil CO_2 efflux was strongly correlated with phloem $\delta^{13}C$ on the previous day, or 2 days earlier. While the ^{13}C label was readily tracked through the ecosystem, the fate of root C through respiratory, mycorrhizal or exudative release pathways was not assessed. These data detail the timing and relative magnitude of C flux through various components of a young pine stand in relation to environmental conditions.

Keywords: carbon allocation, carbon partitioning, $^{13}CO_2$, loblolly pine, *Pinus taeda*, respiration, shade, soil CO_2 efflux.

Introduction

The relative distribution of new carbon (C) among different pools and processes within plant and soil systems (i.e., C partitioning, Litton et al. 2007) is a driving factor in ecosystem biogeochemical and hydrological cycling. It is especially important to understand the magnitude and timing of C fluxes through forest ecosystems because of the large role forests play in the global C cycle (Bonan 2008). However, in contrast to photosynthetic C assimilation, which is relatively well understood and robustly represented in ecosystem models (Farquhar et al. 2001), scientific understanding of C partitioning in forests continues to be relatively incomplete (Wardlaw 1990, Litton et al. 2007). This limits the capacity to model forest ecosystem metabolism and accurately predict the effects of global change on C cycling. Carbon cycling through the plant–soil–atmosphere is difficult to assess due to a lack of information on C transport rates within the plants, spatial heterogeneity of belowground structures and resources, and temporal variation in activity

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governing the underlying processes (Hanson et al. 2000, Subke et al. 2009, Kuzyakov and Gavrichkova 2010). Carbon partitioned to perennial biomass is readily measured and scaled through stand level allometry, yet dynamics of internal C partitioning (Wardlaw 1990), the large percentage of annual gross primary production (GPP) partitioned to ephemeral root systems (Nadelhoffer and Raich 1992) and the temporally variable fraction of that C released back to the atmosphere through root and heterotrophic respiration continues to demand investigation (Litton and Giardina 2008).

As a consequence, a major uncertainty regarding ecosystem C partitioning is focused on the incremental flux of C belowground, and the residence time of that C within the soil system (Litton and Giardina 2008, Chapin et al. 2009). Short- and long-term tracer studies using radio- and stable-C isotopes have been successfully used to examine C uptake, partitioning and residence time in forested ecosystems (e.g., Horwath et al. 1994, Hanson et al. 2000, Carbone et al. 2007, Högberg et al. 2008, Plain et al. 2009, Kuptz et al. 2011). Recently, high-resolution sampling of background levels of stable C isotopes (i.e., ^{12}C:^{13}C) has also been shown as a viable technique to link C uptake and respiratory release in ecosystems (e.g., Bahn et al. 2009, Wingate et al. 2010). At a global scale, it appears that increasing GPP increases the annual flux of C to all biomass components within forest ecosystems (i.e., 'a rising tide lifts all boats'; Litton et al. 2007). However, it is unclear whether the relationship between GPP and belowground C flux and partitioning holds in individual ecosystems on shorter timescales, or those subjected to rapid changes associated with climatic, environmental or biological changes (Chapin et al. 2009). Current photosynthate can play an important role in the amount of belowground C partitioning and soil CO_2 efflux (Liu et al. 2006, Högberg et al. 2008, Kuzyakov and Gavrichkova 2010), although this relationship depends on seasonal C sinks, tissue turnover rates and plant C storage pools (Carbone et al. 2007). The time lag between photosynthetic C uptake and release through soil CO_2 efflux ranges from 0 to 25 days in forest ecosystems, and is related to a number of different biological and environmental variables, including species, season, balance between C uptake and stored C, phloem transport rates, root distribution, soil moisture and temperature (Bahn et al. 2009, Kuzyakov and Gavrichkova 2010, Vargas et al. 2011). The quantity of current photosynthate rapidly lost through root or microbial respiration has been difficult to assess, and estimates range from 25 to 65% (Ekblad and Högberg 2001, Litton et al. 2007).

The representation of C allocation (flux, partitioning and biomass) in ecosystem models can have a large effect on estimates of global C flux and storage (Ise et al. 2010); however, the mechanistic basis is not well refined. This is particularly true for the percent of GPP partitioned to belowground structures and processes in current ecosystem and land surface models, which is often static and based on fixed coefficients (Thornton et al. 2002, Zobitz et al. 2008, Ostle et al. 2009). Experimental manipulations quantifying the dynamic partitioning of C within established forest ecosystems are needed to provide information to validate model C partitioning regimes, and potentially improve model structure.

Shading can be used to reduce GPP, impact the rate of internal plant C flux and shift C partitioning between sinks. A 65% shade treatment in loblolly pine reduced both photosynthesis and dark respiration within several weeks as foliage acclimated to lower radiation levels (Zhang et al. 1997). Such biochemical adjustments result in less C uptake, but concurrently lower foliar respiration rates which may thereby buffer shade-induced reduction in relative C available for export. Shading dampened and delayed propagation of a ^{13}C pulse-label through a grassland ecosystem (Bahn et al. 2009), presumably due to reduced C uptake, transport and respiratory demand. Eight-year-old *Pinus radiata* D. Don trees shifted C partitioning from bole to upper branch elongation following shading of the lower crown (Walcroft et al. 2002), illustrating the utility of shade treatments to modify internal C dynamics. We utilized shade treatments to manipulate GPP to assess timing and magnitude of C partitioning among leaves, phloem, fine roots and bulk soil pools in a short-term, continuously monitored, ^{13}C pulse-chase experiment in a young loblolly pine stand located in eastern Tennessee, USA. Our questions were twofold: (i) Is belowground C flux positively correlated with GPP? (ii) Do reductions in GPP delay flux of new photosynthate from foliage, through phloem, roots and soil? We hypothesized that shading would reduce total GPP, reduce C flux belowground and delay propagation of the ^{13}C signal through the plant–soil–atmosphere pathway.

Materials and methods

Site description and trenching manipulation

The research was conducted on individual trees within a small existing stand of planted *Pinus taeda* L. (loblolly pine) on the University of Tennessee Forest Resources Research and Education Center in Oak Ridge, Tennessee (36°00′N, 84°11′W). Improved, 1-year-old seedlings from the TN Department of Agriculture's forestry nursery were planted at 2.5×3 m spacing in 2003 over a ~20×100 m area. In August 2010, mean tree height was 7.2 ± 0.2 m and mean diameter (dbh) was 10.4 ± 0.5 cm. The soil was classified as clayey, mixed, thermic Ochreptic, and was an Armuchee silt loam with a 5–12% slope. The site was previously cultivated, then in the 1940s became dominated by invasive *Pueraria* sp. (kudzu) until mowing and herbicide control began in the 1980s. Non-pine vegetation including grasses and small shrubs were removed by clipping and herbicide prior to study initiation.

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Plots were established across two rows in the interior of the stand, with four adjacent sample trees selected from each row. In April 2010, a trench was excavated in-between the two rows of four trees (oriented east–west) using a small track machine with a 2.13 m wide bucket. Study trees 1–4 were on the south side of the trench, trees 5–8 were on the north side of the trench. During excavation the edge of the bucket cleanly cut most roots, although some large roots (> 1 cm diameter) were manually clipped to avoid potential damage to the trees or the trench wall. The resulting trench was ~ 1 m deep × 15 m long, with trench edges ~40 cm from the base of the study trees. Walls were lined with white landscaping fabric to minimize moisture loss, light penetration and heating of the pit face. A sump pump was installed to prevent buildup of water in the trench. Six 10 m aluminum poles with wire cable attached across the pole tops were installed surrounding the eight study trees. This rectangular frame was used to support the temporary plastic labeling chamber and the shade cloth treatments (described below).

Automated environmental and tree monitoring

Standard environmental monitoring included precipitation, relative humidity, photosynthetically active radiation (PAR), direct shortwave radiation, wind speed and air temperature at ~2 m height at a fully exposed location adjacent to the stand (~15 m from the study trees). Data were collected automatically every 30 min and stored on a logger (model CR10X, Campbell Scientific, Logan, UT, USA). Photosynthetically active radiation was also monitored just above the canopy for each of the two shade treatments.

Sap flow was monitored in each tree using thermal dissipation sensors (Dynamax Inc., Houston, TX, USA) installed at depths of 1.5 cm (E and W aspect) and 2.5 cm (SE aspect) at 0.75 m height. Sensors were insulated to reduce thermal errors. Data were sampled every 30 min and stored on a logger. Standard techniques were used to assess relative differences in sap flux density between treatments (Granier 1985, Warren et al. 2011). Sap flux density was scaled to whole-tree flux using radial patterns of sap flow and sapwood depth. Sapwood depth was based on bark thickness and assumed to extend to the center of the tree. Basal area growth was assessed for each tree every 30 min using automated band dendrometers (DR 26, EMS, Brno, Czech Republic), which recorded stem circumference at 1.3 m height every 30 min. Daily stem growth was calculated as the change in daily minimum cross-sectional area, which generally occurred shortly after sunset.

Soil CO_2 efflux rate (CER) was monitored ~0.5 m from each tree and 0.5 m from the trench, using automated gas exchange chambers (Li-8100, LI-COR Inc., Lincoln, NE, USA) that sampled every 20 min. The gas exchange system included integral soil moisture and temperature measurements (0–5 cm depth) at each location. Measurements taken during rain events or when soil was flooded were removed from the data set. Soil moisture was also measured vertically within the soil profile at each tree using multi-sensor frequency domain capacitance probes (EnviroSCAN, Sentek Pty Ltd., Adelaide, Australia). Sensors were deployed at 10, 20, 30, 40, 60, 80 cm depth for T1–T4 and 20, 30, 40, 50, 60, 100 cm depth for T5–T8 and automated measurements were collected every 30 min.

^{13}C labeling event

A ^{13}C label was applied to the study trees by enclosing all eight trees in a large translucent plastic chamber (~510 m^3) on the morning of 1 September 2010. To construct the chamber, bucket trucks and lifts were used to drape large sheets of 6 mil polyethylene greenhouse film over the entire aluminum pole—wire frame and down to the ground, with sides taped together and the bottom edges sealed with sandbags. The trench and soil surrounding each tree was covered with tarps to minimize direct diffusion of $^{13}CO_2$ into the soil system. The trees were labeled by adding 53 l of 99 atom % $^{13}CO_2$ (Cambridge Isotope Laboratories, Inc., Andover, MA, USA) to the enclosure over a 45-min period beginning at 08:31 h. The $^{13}CO_2$ was fed to two fans at a flow rate of ~0.66 l min^{-1}, and the fans were used to mix the $^{13}CO_2$ tracer within the enclosure during the labeling event. Based on the volume of the enclosure, we calculated an expected maximum $^{13}CO_2$ concentration of 21 atom %. Tree canopy temperature was monitored during the labeling event using a thermal imaging camera (Fluke Corp., Everett, WA, USA). The plastic enclosure was removed after 2 h at which point canopy temperature exceeded ~35 °C and the inside walls of the enclosure were covered in condensation—similar to impacts of labeling chambers in other studies (i.e., Carbone and Trumbore 2007). The [CO_2] and $\delta^{13}CO_2$ were monitored at six positions in the canopy (two upper, two middle and two lower) and at a location immediately outside of the enclosure during the exposure period using a Picarro G1101-i Isotopic CO_2 Analyzer (Picarro Inc., Sunnyvale, CA, USA). Each sampling position was measured for 10 min, but measurements from only the last 3 min of each data record were used for analysis (i.e., when the Picarro had reached steady state with respect to measurement of both CO_2 concentration and $\delta^{13}CO_2$). Per mil values from the Picarro (i.e., $\delta^{13}CO_2$) were converted to atom % $^{13}CO_2$.

The Picarro measures the $\delta^{13}CO_2$ in ambient air at a precision of 0.1‰. The Picarro factory calibration for [CO_2] was checked against a range of CO_2 standards (100–5000 ppm) (Scotty gas calibration standards from Sigma-Aldrich, St Louis, MO, USA). Measured CO_2 concentrations were within 6% of expected values. The Picarro factory calibration was the default configuration for $\delta^{13}CO_2$ and measurements at the top of the pine canopy were in agreement with expected values of $\delta^{13}CO_2$ in ambient air (~ −8‰). Analyses indicated that $\delta^{13}CO_2$ was

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not dependent on CO_2 concentration at values ≥300 ppm. Measured concentrations of $^{12}CO_2$ during the labeling experiment were ~450 ppm in the atmosphere, and ranged from 1200 to 19,000 ppm, on average, in soil CO_2 efflux. Concentrations of $^{13}CO_2$ were ~5 ppm in the atmosphere and ranged from 15 to 215 ppm, on average, in soil CO_2 efflux.

Shade treatments

After the labeling event, the greenhouse film was removed and replaced with black knitted shade cloth for the duration of the study. Two levels of shading were applied to the trees in order to reduce GPP and thereby manipulate C flux and partitioning within trees. A 30% shade cloth (light shade; LS) treatment was applied to the top and north, east and south sides of one group of four trees (T1, 2, 5, 6) as the control, and a 90% shade cloth (heavy shade; HS) treatment was applied to the top and north, west and south sides of the adjacent group of four trees (T3, 4, 7, 8). Actual daily PAR within the two treatments at the upper canopy surface averaged 11% (HS) or 68% (LS) of ambient.

Plant growth and physiology

Carbon flux and partitioning within the trees in response to treatments were assessed by monitoring both aboveground and belowground tree growth and physiological changes following imposition of treatments.

Light response curves, *A*–*Ci* curves (assimilation versus internal sub-stomatal CO_2 concentration) and A_{max} at 1500 μmol m^{-2} s^{-1} PAR were obtained for foliage in the upper and mid canopy of several trees prior to shade treatment using an infrared gas analyzer (model 6400, LI-COR Biosciences, Lincoln, NE USA). Following treatments, there were periodic measurements of assimilation at maximum treatment PAR conditions (i.e., 150–200 and 1000–1100 μmol m^{-2} s^{-1} for HS or LS treatments, respectively). Relative daily photosynthesis within each treatment was estimated based on diel patterns of PAR within each treatment.

Predawn leaf water potentials were obtained for the eight study trees and several additional trees in the surrounding plantation periodically during the study using a pressure chamber (PMS Instruments, Corvallis, OR, USA).

Root growth was assessed using minirhizotron tubes installed laterally into the pit face within the trench. Cellulose acetate butyrate minirhizotron tubes (5 cm diameter by 91 cm length) were installed in May 2010, at 5 and 30 cm depths (i.e., two tubes per tree), positioned 50 cm horizontally from the base of each tree These depths were chosen based upon initial measurements of *P. taeda* root standing crop distribution estimated from 45 cm deep soil cores collected in February 2010. Minirhizotron images (38 frames per tube, beginning ~5 cm into the soil profile from the pit face) were collected daily beginning on 27 August, and every 3 days from 11 September to 20 September 2010. Root length production and mortality were averaged over each tube, which was considered a statistical replicate. Roots were observed in only two of the eight deeper (30 cm depth) tubes; therefore, these analyses focus on the dynamics of the shallow root population (5 cm depth). Adjacent to the minirhizotron tubes, we installed rhizotron windows (50 cm × 50 cm) consisting of acetate (1.3 mm thickness) attached to a wooden frame. The windows were secured against the trench face (0–50 cm depth) at the base of each tree using long bolts. Sieved soil (2 mm) was packed into the windows from above to fill gaps such that acetate was stretched taut in its frame. The windows allowed access to newly formed root tissue for ^{13}C analysis.

Root standing crop beneath each of the soil gas exchange chambers was estimated following removal of shade treatments for the 0–5, 5–15 and 15–30 cm depth increments. A 5 cm diameter by 30 cm deep soil core was taken from the center of each soil gas exchange chamber. Roots were separated from the soil using a hydropneumatic elutriator with a 530 μm filter (Gillison Variety Fabrications, Benzonia, MI, USA), and subsequently sorted into pine and non-pine roots (primarily fibrous grass roots), and living and dead roots. Roots were oven-dried at 70 °C and weighed, and the biomass of living roots was used as a covariate in analyses of soil CO_2 efflux.

^{13}C in plant tissues—foliage, phloem, bulk roots, new roots

Plant foliar, phloem and root tissue samples were collected from individual trees prior to the labeling event (31 August) and five occasions after the labeling event (1, 2, 5, 9 and 21 September) to track the changes in $\delta^{13}C$ over time.

Foliar samples were collected throughout the canopy using a vertical lift. First and second flush needles from the current (2010) growing season and older needles were sampled. Needles were dried at 70 °C and ground to a fine powder for analysis.

Phloem tissue disks were collected from the east and west aspects of the lower bole 20 cm from the ground using a 10.5 mm diameter punch (cork borer). At each date, phloem sampling locations were progressively shifted over and down the bole 2–3 cm to avoid sampling wounded tissue. Phloem tissue was easily peeled and separated from the suberized outer bark layers, frozen with liquid N_2 and kept at −80 °C until extraction. For extraction, each phloem sample was cut into four quarters and 1/2 the sample retained for future analysis. Samples were pooled by tree by date. The tissue was then quickly rinsed twice with distilled water and incubated for 24 h at 4 °C in scintillation vials containing 2 ml distilled water (Gessler et al. 2004). After the incubation, the extracted phloem tissue was removed and dried. The supernatant was centrifuged, and 200 μl was added to a glass fiber filter in a tin cup and dried at 80 °C for ^{13}C isotopic analysis. We assumed

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the supernatant was primarily composed of extracted sieve tube sap, although cutting and the freeze/thaw process could allow for minor contamination (dilution) by other soluble cellular constituents from adjacent damaged cells. If so, the reported ^{13}C phloem concentrations would slightly underestimate the actual ^{13}C concentrations in phloem sieve tube sap.

Roots were collected from 5 cm diameter by 30 cm deep soil cores taken 20 cm horizontally from the base of each tree, separated by depth increment (0–5, 5–15 and 15–30 cm) and kept at −20 °C until processing. Thawed soil was passed through a 2 mm mesh sieve, and *P. taeda* roots (<2 mm) were removed with tweezers, quickly rinsed in distilled water, lyophilized and ground to a fine powder. Only the 0–5 cm depth increment was processed due to the fact that the minirhizotron observations did not observe root growth in deeper soil during the experimental manipulation. To further assess fate and longevity of the ^{13}C signal, new root tips growing against the face of the rhizotron windows were collected on 21 September 2010 and again in early May 2011. In addition, newly emerging buds, 2009 and 2010 foliage, and branch wood were sampled from each tree in early May 2011. Tissue was dried at 70 °C and ground for analysis.

Ground or extracted tissue samples were analyzed for ^{13}C using an Integra CN isotope ratio mass spectrometer (SerCon Ltd, Crewe, UK). Glucose ($\delta^{13}C = -10.2$‰) was used as the working standard for isotope analysis and was calibrated against reference material from the National Institute of Standards and Technology (NIST 8542, sucrose). The isotopic signature of C in plant tissues (leaves or roots) was expressed as $\delta^{13}C$ (‰) ($\delta^{13}C = [R_{SAMPLE}/R_{STANDARD} - 1] \times 1000$), where R is the $^{13}C/^{12}C$ ratio and the standard is carbonate from Pee Dee Belemnite.

^{13}C in soil CO_2 efflux

Following the labeling event, the appearance of ^{13}C in soil CO_2 efflux was monitored hourly using static chambers (volume = 5 l, surface area = 214 cm^2) made from white, translucent, high-density polyethylene. Each chamber contained a small vent (6 mm diameter) on the side to equalize interior and exterior atmospheric pressure. The top of the chamber contained a second hole that was connected to 6 mm diameter Tygon plastic tubing. The tubing was led to a Picarro G1101-i isotopic CO_2 analyzer for analysis of $\delta^{13}CO_2$ in the chamber. Chambers were placed near the base (within 1 m) of three trees in the control treatment (T2, 5, 6) and the HS treatment (T3, 7, 8). The analyzer sampled the gas in each chamber for 7–10 min, which included transit time for gas to reach the analyzer, several minutes for stabilization of transient responses and a final 3-min period of useable data. With a flow rate of 26 ml min^{-1}, each sampling event replaced ≤6% of the existing (soil) CO_2 in the chamber with new atmospheric CO_2 via the vent. Due to a strong concentration gradient from the interior to the exterior of the chamber and the small diameter of the vent, we assumed negligible diffusional influence of atmospheric CO_2 on the isotopic signature inside the chamber during periods when the chamber was not being sampled. Data were categorized as day or night (defined by sunrise and sunset). Atmospheric $\delta^{13}CO_2$ (near the ground) was also measured each hour at the site of the analyzer.

Vertical profiles of soil $^{13}CO_2$ gas were periodically collected (2, 4, 8 and 20 days post-label) at 5, 10, 20 and 30 cm depths. Stainless-steel tubes (2 mm diameter, 15 cm long) were inserted in the pit wall and 5–10 ml gas samples were extracted with a syringe inserted through a rubber stopper at the end of the tube and stored in evacuated vials. Additional samples were collected from soil beneath unlabeled trees. The samples were analyzed for [CO_2] and ^{13}C content at the University of California Davis Stable Isotope Facility using a PreCon-GasBench system interfaced to a Delta V Plus isotope ratio mass spectrometer (ThermoScientific, Bremen, DE, USA).

Data analysis

Treatment differences between mean values of environmental or physiological data were assessed using *t*-tests. Differences between regression slopes were assessed using analysis of variance techniques using statistical software (ver 9.1.3, SAS Institute, Cary, NC, USA). A repeated-measures mixed model was used to test for significant treatment differences in soil moisture content or net root turnover through time (contrasting treatment × date interactions). Differences were considered statistically significant at $P < 0.05$. Tracer half-life was determined by fitting an exponential decay function to each data set beginning with the peak ^{13}C values.

Results

Trenching

Excavation of the trench removed a significant portion of the root system (>20%), including long (>3 m) lateral roots from the upper 20 cm that radiated out from each tree. Despite root removal, there was no noticeable impact of the disturbance on study trees based on foliar retention or appearance in relation to other trees at the site. The trench revealed large roots vertically spanning the pit face to depths >1 m. Also evident was heterogeneity within the soil profile; specifically the depth to bands of shale fragments 1–5 cm across and covered with clay films varied due to both differential orientation of the shale layers and historical impacts of agriculture. Roots were evident growing laterally within the shale layers. Initially, some additional roots were exposed as soil collapsed from the trench walls under moist conditions, but this soil calving stabilized through time after installation of a sump pump to remove water from the pit.

Labeling event

Concentrations of CO_2 in the enclosure declined from 460 to 332 ppm over the 2-h labeling event (Figure 1). The maximum

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measured $^{13}CO_2$ concentration in the enclosure (15.3 atom %) was well above ambient (1.102 atom %), but ~25% less than the expected maximum $^{13}CO_2$ concentration. The peak in $^{13}CO_2$ in the enclosure coincided with the end of the tracer injection (Figure 1). Following the tracer release into the enclosure, the concentration of $^{13}CO_2$ declined over 1.25 h at a loss rate of ~0.13 atom % min^{-1}. Concentrations of $^{13}CO_2$ measured outside of the enclosure during label release were slightly elevated ($\delta^{13}C = 9‰$) relative to measurements made in the canopy prior to the labeling event (–12 to –8‰), indicating some leakage from the chamber.

Impact of shading on C uptake, growth and physiology

The LS treatment did not significantly reduce net C uptake by upper canopy foliage since the treatment level of PAR was above the light saturation point for these trees (Figure 2). In contrast, the HS treatment significantly reduced foliar C fixation, even during midday where reductions reached ~50% as compared with LS treatment. Maximum daily PAR rarely reached 200 μmol m^{-2} s^{-1} under HS treatments, which is a steep part of the light response curve, as opposed to LS treatments, which generally exceeded 1000 μmol m^{-2} s^{-1} (Figure 2). Peak daily PAR for the upper canopy during the study was 700–1270 μmol m^{-2} s^{-1} for LS trees and 100–260 μmol m^{-2} s^{-1} for HS trees. Consequently, cumulative reductions in modeled net C assimilation (A_{net}) for the upper canopy were minor for LS trees (<10%) but quite significant for HS trees (>65%) as compared with full sun conditions, based on modeled A_{net} using light response curves and 1/2 h measurements of PAR within each shade treatment. Over the study period, HS A_{net} was reduced by ~70% as compared with LS A_{net}. The reduction in C uptake by HS trees was less pronounced under low PAR conditions during wet or cloudy periods.

Prior to treatment initiation, mean whole-tree sap flow was ~20% greater for the four designated HS trees than for the four LS trees, with variation dependent on tree size and location (Figure 3a). After implementation of shade treatments, HS sap flow was equal to or lower than that in LS trees (Figure 3b). Sap flow generally declined for all trees initially, partly as a result of reduced soil moisture, because rates increased following precipitation (Figure 3b and c). Most stem growth during the 3 weeks following the labeling event occurred on 3 days following rain events; diameter growth on those days was 45% greater in LS compared with those in HS ($P = 0.07$) (Figure 3c). There was no correlation between the timing of stem growth and the timing of fine-root production. Fine-root production and mortality was evident in seven of eight trees during the study. There were no roots visible in minirhizotron tubes associated with one LS tree. Fine-root standing crop was reduced in the HS treatment relative to the LS treatment (Figure 3d) due to reduced root production in the HS treatment. The difference in root standing crop between treatments materialized concurrent with a strong rainfall event on Day 10 (Figure 3c and d).

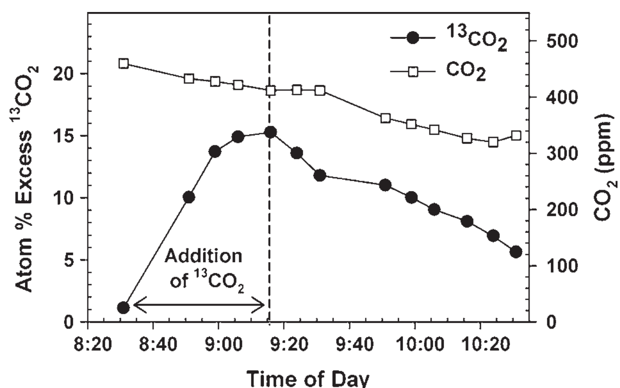

Figure 1. Change in atmospheric $^{13}CO_2$ and CO_2 within a translucent plastic chamber enclosing eight loblolly pines during a 2-h $^{13}CO_2$-labeling event in early September 2010.

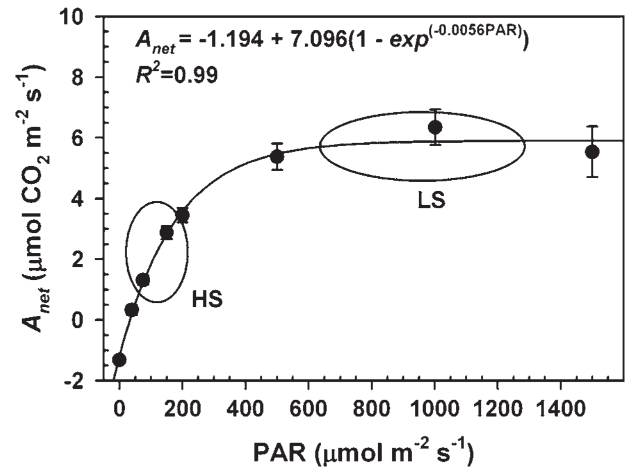

Figure 2. Mean light response curve for upper canopy first-flush 2010 loblolly pine foliage in early September 2010, depicting the response of net photosynthetic C assimilation (A_{net}) to increasing photosynthetically active radiation (PAR). Circled areas highlight the daily range of activity for the light shade (LS) and heavy shade (HS) treatments.

There was substantial variability of initial soil water content across the site and through the profile; interpolated soil moisture in the upper 1 m varied by up to 25% between trees. There were no treatment effects on mean soil water content over the course of the study (Figure 4a). However, there were significant treatment effects on the relative changes in soil water content at different depths through time, particularly for several days following rainfall events on Days 7, 10 and 15 (Figure 4b). By the end of the study, average residual water contents were slightly greater for the HS trees as compared with initial conditions at 20–40 cm ($t = 1.08$; $P = 0.16$) and 60 cm ($t = 1.67$; $P = 0.07$) and significantly greater at 80–100 cm ($t = 3.05$; $P = 0.01$) depths. Predawn leaf water potential was slightly increased (closer to zero) by HS treatments, but only under dry conditions. Four days after shade

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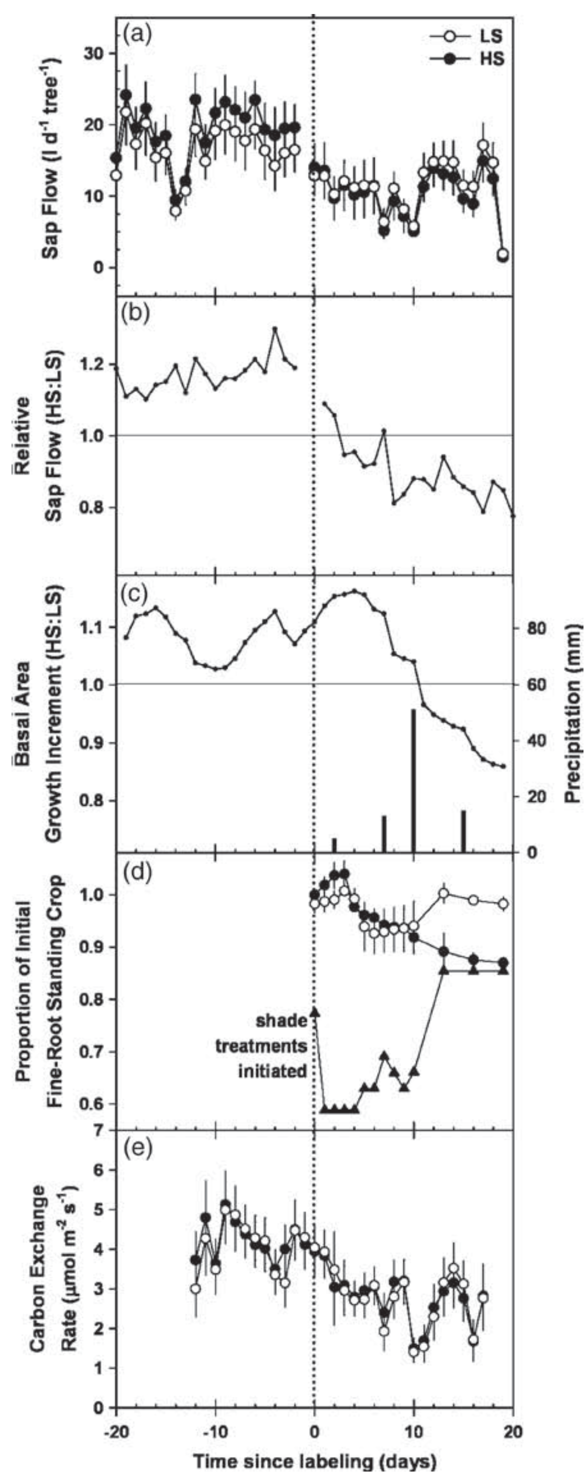

Figure 3. (a) Mean whole-tree sap flow for loblolly pines prior to and after exposure to light shade (LS) and heavy shade (HS) treatments ($n = 4 \pm$ SE). Impact of shading treatments on (b) relative sap flow rate, (c) relative growth rate ($n = 4 \pm$ SE) and (d) mean fine-root standing crop over the course of the experiment (circles, $n = 4 \pm$ SE); one HS tree outlier (filled triangles) experienced substantial root mortality during the first day of the study and was not included in analyses. Precipitation since labeling is depicted by bars in panel (c), which regulated the physiological responses of trees to shading. (e) Soil CER from soil adjacent to individual loblolly pine trees ($n = 4 \pm$ SE). Data were normalized to tree size (basal area × height).

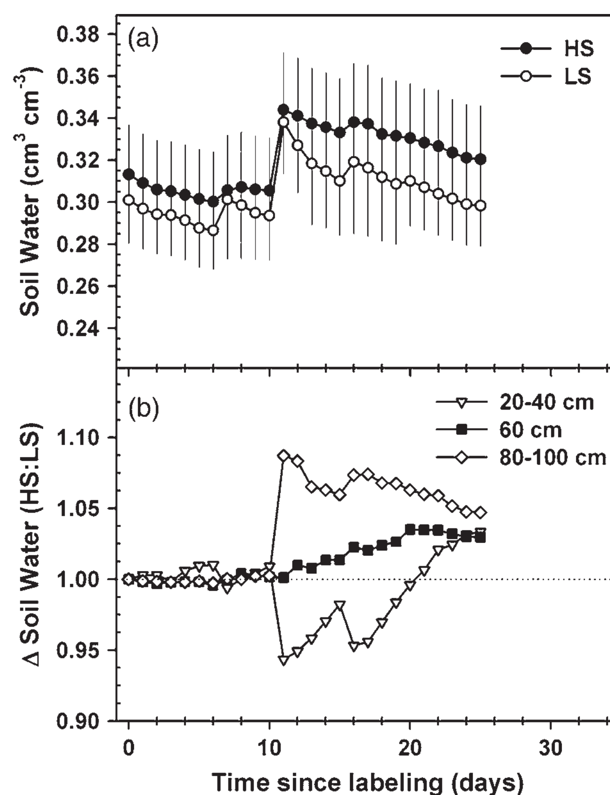

Figure 4. (a) Mean soil water content for the upper 1 m of soil (5–105 cm) after initiation of light shade (LS) or heavy shade (HS) treatments of loblolly pine trees ($n = 4 \pm$ SE). (b) Relative change in soil water content at different depth increments through time. Data from each sensor were normalized to 1 at Day 0. Values above 1 indicate greater water removal from the LS soil as compared with the HS soil.

treatments were applied, $\psi = -0.50 \pm 0.04$ MPa (HS) or -0.59 ± 0.03 MPa (LS) ($t = 2.16$; $P < 0.05$).

Total belowground autotrophic and heterotrophic respiration

Prior to application of the shade treatments, respiratory release of CO_2 as soil CER was ~30% greater beneath trees that were designated for HS compared with those designated for the LS treatment. Carbon dioxide efflux rate normalized to mean tree size [(basal area × height)/mean (basal area × height)] was similar between treatments (Figure 3e). There was no impact of shading treatments on CER during the experiment. Soil CER beneath seven of the trees was well correlated with root mass in the top 5 cm of soil ($R^2 = 0.88$) but not with root mass in the upper 30 cm, such that the observations do not allow separation of CER into autotrophic and heterotrophic respiration.

Carbon dioxide efflux rate was linearly related to the mean sap flow of trees adjacent to the chambers over the previous day for all eight trees (Figure 5). Eight of the 16 previous day regressions for pre- and post-treatment samples were significant at $P < 0.05$ (10 of 16 at $P < 0.10$). In contrast, the relationship between current-day sap flow and CER was not significant, with only 1 of

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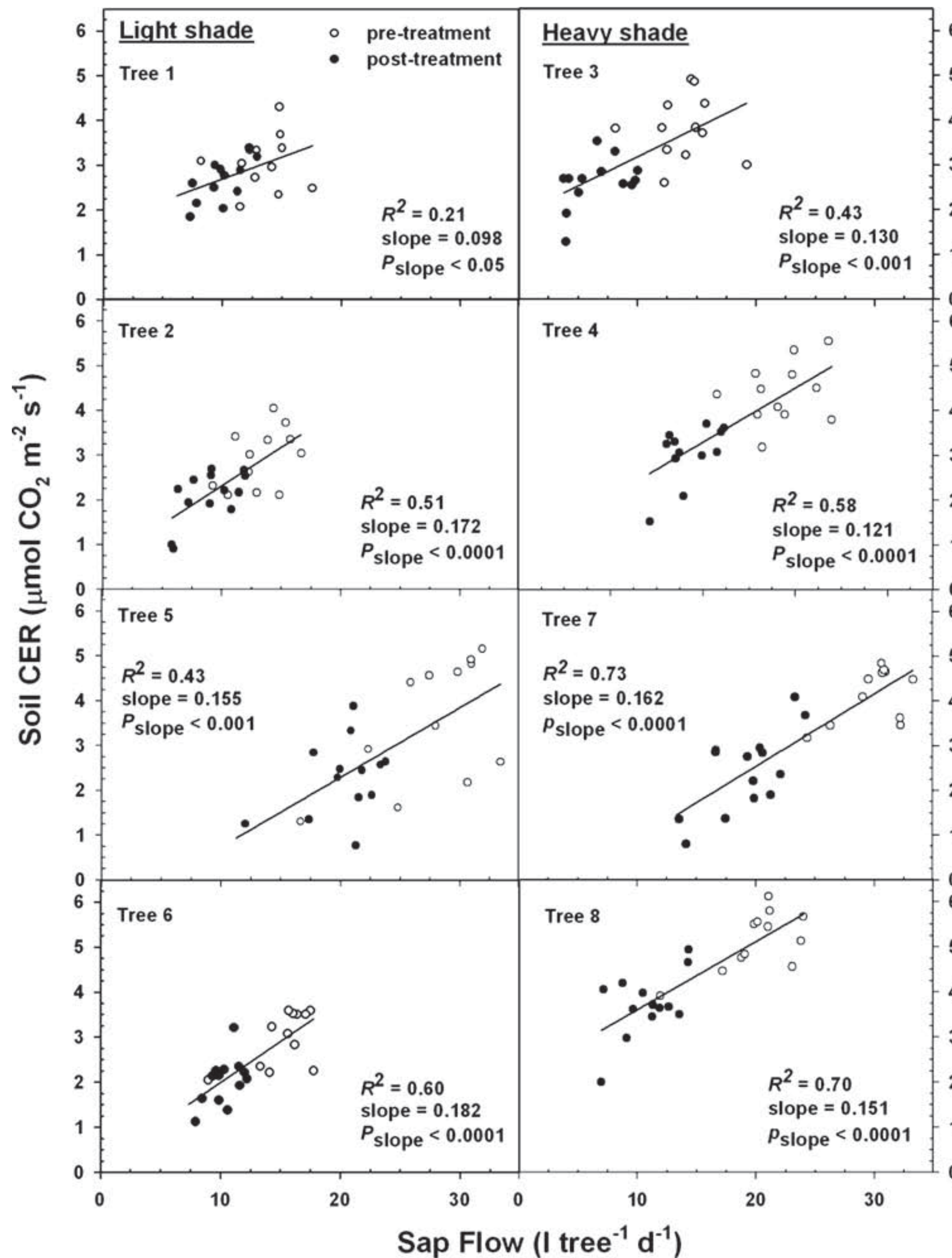

Figure 5. Soil CO_2 efflux (CER) rate in response to the magnitude of total daily sap flow during the previous day of paired loblolly pine trees that were exposed to LS or HS treatments. Data include 2 weeks prior to shade treatments (pre-treatment) and 3 weeks after initiation of shade treatments (post-treatment), excluding precipitation events. Linear regressions are shown for combined pre- and post-treatment data.

16 regressions at significance level $P < 0.10$. There were no significant treatment differences in the relationship between CER and sap flow, nor were there differences in the slope of the relationships through time (i.e., pre- or post-treatment).

Detection of ^{13}C in plant tissues and soil efflux

Immediately after the labeling event on 1 September 2010 (Day 0), foliar δ ^{13}C values were substantially elevated above pretreatment background levels (Figure 6a). There was a rapid loss of ^{13}C from the canopy of labeled trees within 24 h of the labeling event. There was no shading treatment difference in $δ^{13}C$ initial uptake or rate of decline through time. At the end of the sampling period (Day 20), 3 weeks after the labeling event, foliar $δ^{13}C$ values in trees under both HS and LS treatments remained significantly elevated by ~4‰, above pretreatment levels (–28.0‰). The half-life of ^{13}C in the foliage was ~0.5 day. There was no shade treatment effect on retention of the label within foliage.

Carbon-13 concentrations in phloem peaked by Day 2 for both shade treatments (Figure 6b); however, the enrichment

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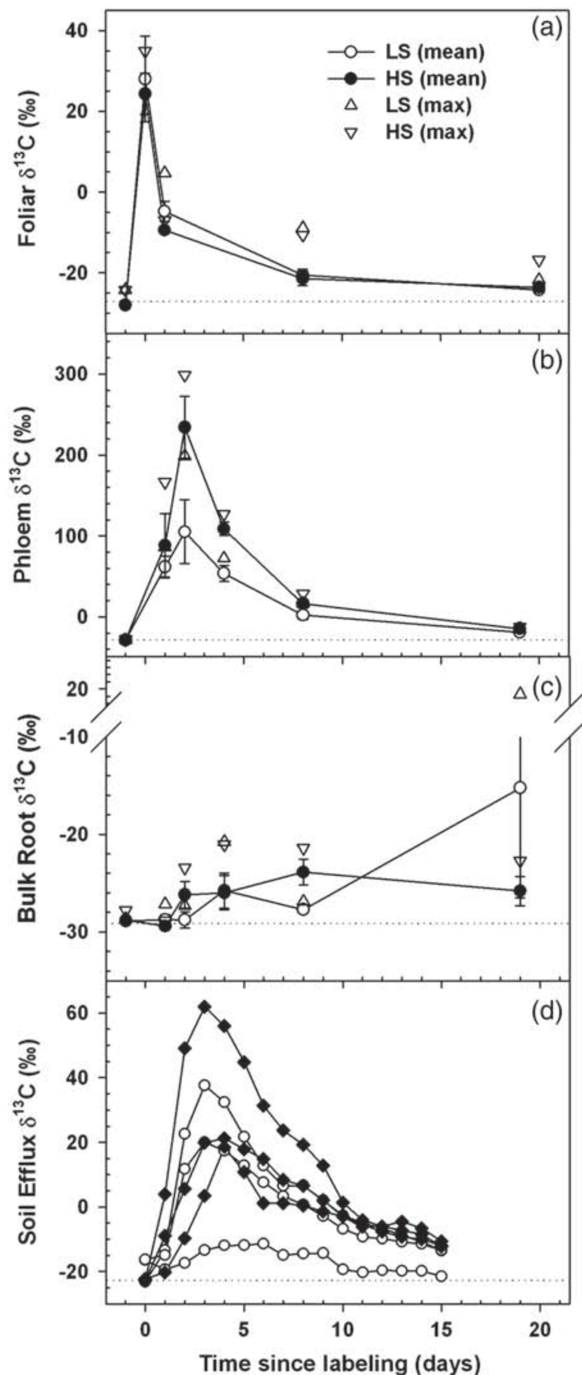

Figure 6. Change over time in $\delta^{13}C$ of loblolly pine (a) foliar, (b) phloem and (c) root tissue, as well as (d) soil surface efflux $\delta^{13}C$ under light shade (LS) or heavy shade (HS) treatments following labeling with air enriched in $^{13}CO_2$ on 1 September 2010 (Day 0). Data in panels a, b and c are means of four samples (±SE) by treatment and maximum values for each date. Data in panel d represent individual locations directly beneath treatment trees. The dotted horizontal lines depict background baseline values of $\delta^{13}C$.

was greater for HS treatments (t = 2.36; $P < 0.05$), driven by relatively low peak enrichment for two LS trees (T1, 5). There was no shade treatment effect on ^{13}C half-life (4.7 ± 0.4 days) in phloem tissue.

The label was detected in a single bulk root sample by Day 2, and in most root samples by Day 4 (Figure 6c). Carbon-13 in bulk root samples from LS treatments declined by Day 8, while HS treatments remained steady. Most root samples from both treatments were approaching background ^{13}C levels by Day 19, although one sample (from an LS root) was highly enriched, which increased the mean LS ^{13}C value for that date (Figure 6c). The high ^{13}C signal detected in the LS bulk root sample on Day 19 was indicative of ^{13}C incorporation into new root biomass, and not of soluble root C concentrations as storage or phloem sugars. In fact, roots collected from behind the rhizotron windows on Day 20 (new roots that had grown during the treatment period) were also highly enriched above background levels, with evidence of the label in three of eight sampled roots from each shade treatment (values from –1‰ to +143‰). The label was still detectable 8 months later in three of eight pooled HS samples (e.g., 5–10 roots per tree) collected in spring 2011. One of the enriched HS samples was from new root tips that had initiated after the treatments ended, while the other two were from roots that had grown during the treatment period. There was no residual ^{13}C enrichment detected in any branch, foliar or wood sample collected in spring 2011 (range –26 to –30‰).

$^{13}CO_2$ efflux from soil was immediately enriched above background values following cessation of the labeling event and removal of tarps covering the soil and trench, reaching values ranging from –8 to +150‰ (compared with background values of –28.0 ± 0.1‰; n = 32). This efflux quickly dissipated and approached background levels within 7 h as the mean $\delta^{13}CO_2$ in soil efflux declined from 50 to –15‰. This short-term enrichment had a half-life of only 1.7 h and reflected diffusion and return to the atmosphere of tracer $^{13}CO_2$ that had entered the surface soil during the labeling event. Longer-term dynamics indicated increasing $\delta^{13}CO_2$ in soil CO_2 efflux with peak values occurring between Days 3 and 6 following tree labeling (Figure 6d). Locations with greater peak $\delta^{13}CO_2$ values occurred earlier than locations with lesser peak $\delta^{13}CO_2$ values. Following peak $\delta^{13}CO_2$ in soil CO_2 efflux, $\delta^{13}CO_2$ declined over ~2 weeks to levels measured in air near the surface of the ground prior to the labeling event (i.e., –14 to –11‰). Mean (±SE) daytime and night-time CO_2 concentrations in unchambered air sampled near the surface of the ground were 420 ± 2 and 477 ± 7 ppm, respectively, over a 15-day measurement period. Carbon dioxide concentration within chambers varied widely (1200–19,000 ppm) and was strongly associated with daily precipitation, peaking on Days 2, 7, 10 and 15 when the study site received approximately 5, 13, 51 and 15 mm of precipitation, respectively.

Carbon-13 efflux from soil adjacent to Tree 5 was much lower than all other samples, reaching only –10‰ (Figure 6d). The signal dropped to near background levels after only 11 days, more quickly than it did at the other locations. There was no shade treatment effect on the half-life of ^{13}C efflux from soil (8.4 ± 0.4 days). Across shade treatments, soil ^{13}C efflux was

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significantly related to phloem sampled on the previous day ($r^2 = 0.48$ or 0.42, for LS and HS, respectively) or 2 days prior (Figure 7; $r^2 = 0.47$ or 0.53, for LS and HS, respectively), but not related to ^{13}C in phloem sampled on the same day ($r^2 = 0.16$).

Roots and soil extracted from 20 cm soil cores beneath the six $^{13}CO_2$ sampling chambers were assessed for ^{13}C at the end of the study. With the exception of a single root sample, there was no ^{13}C enrichment of roots or soils. Background fine-root $\delta^{13}C$ was −28‰, background coarse root $\delta^{13}C$ was −26 to −30‰ and soil $\delta^{13}C$ was ~ −26‰.

Detection of ^{13}C in soil air

Air extracted from multiple soil depths 2 days post-labeling was enriched in $^{13}CO_2$ beneath five of the eight trees. The enrichment increased by Day 4, when the soil air drawn from beneath six of eight trees (including all HS trees) was enriched in ^{13}C (Figure 8). Of the 32 samples (8 trees × 4 depths), 22 had $\delta^{13}C$ values averaging −25.5 ± 0.1‰, indicating no influence of the labeling event. The remaining 10 samples ranged from −19.8 to 22.3‰, with no distinguishable depth-dependent pattern, although label was not detected at the deepest soil depth (30 cm). The $\delta^{13}C$ of soil air was correlated with CO_2 concentration of the soil gas, which ranged from 1013 to 2072 ppm ($R = 0.66$). These results are consistent with the assumption that the label entered the soil as root or microbial respiration, but the influence of roots on the soil environment was too heterogeneous for quantitative analysis.

Discussion

The labeling event successfully introduced a pulse of ^{13}C into new photosynthate throughout the pine canopies. As

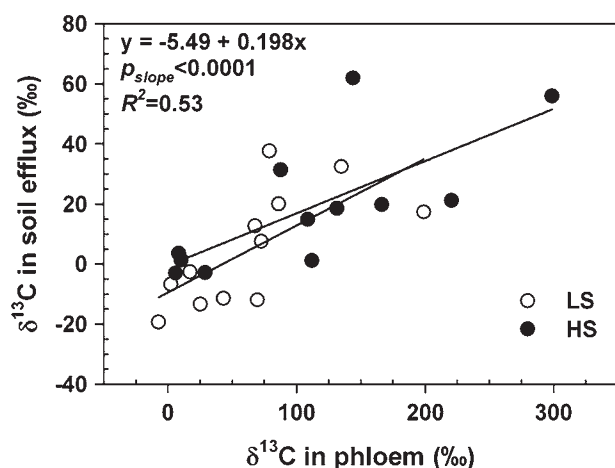

Figure 7. Soil ^{13}C efflux in relation to adjacent loblolly pine tree phloem ^{13}C sampled 2 days earlier following $^{13}CO_2$ labeling of the canopy. Data are from 1, 2, 4 and 8 days post-labeling for light shade (LS) or heavy shade (HS) treatments (n = 3 tree trees per treatment). Linear regression lines shown for the two treatments did not differ, thus the regression equation was derived from all six trees.

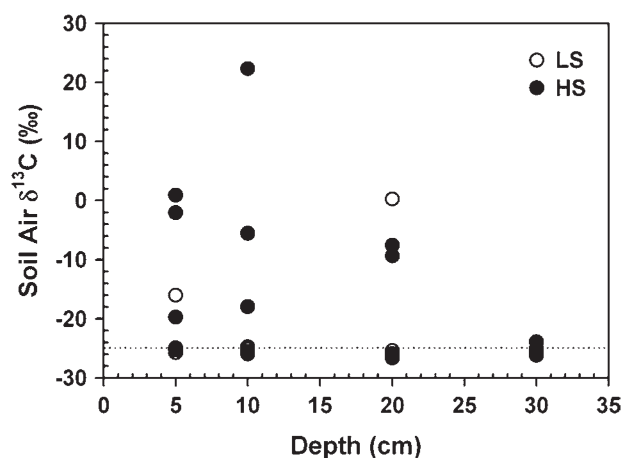

Figure 8. $^{13}CO_2$ enrichment of soil air extracted from various depths beneath light shaded (LS) and heavy shaded (HS) loblolly pine trees 4 days after tree canopies were labeled with $^{13}CO_2$ (n = 4 trees per treatment per depth). The dotted horizontal line depicts background baseline values of $\delta^{13}C$.

expected, the HS treatment subsequently reduced foliar C uptake, and thereby reduced C available for partitioning to both aboveground and belowground sinks, as evidenced by reduced stem growth and fine-root biomass. The progression of the ^{13}C label from the atmosphere through plant tissue and into the soil occurred rapidly for both shade treatments; however, contrary to our hypothesis, HS did not delay the propagation of the ^{13}C signal through the plant–soil–atmosphere pathway. In addition, although there were apparent reductions in new C flux belowground, the HS treatment did not noticeably reduce the magnitude of belowground autotrophic and heterotrophic respiration based on surface soil CER. This suggests that for this *P. taeda* stand, the rapid cycling of new photosynthate through the ecosystem was a minor component of total daily CER during autumn.

Labeling event

Three processes potentially contributed to the decline in $^{13}CO_2$ following release of the tracer into the enclosure: (i) uptake by the canopy, (ii) absorption of CO_2 by water vapor that had condensed on the inside of the plastic enclosure, and (iii) leakage of $^{13}CO_2$ from the enclosure to the soil or the atmosphere. Although some leakage of $^{13}CO_2$ was indicated by measurements made on the outside of the enclosure, uptake by the canopy or absorption of $^{13}CO_2$ by water vapor on the inside of the enclosure were considered to be the most likely mechanisms causing $^{13}CO_2$ (and total CO_2) decline over the 2-h exposure period. Measurements of $\delta^{13}C$ in soil CO_2 efflux in the static soil chambers indicated both short-term dynamics of ^{13}C-tracer release to the atmosphere following diffusion of tracer into the soil, and longer-term dynamics of ^{13}C transport from foliage to roots.

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Measurements of $\delta^{13}CO_2$ in soil CER prior to the labeling event (−28‰) were consistent with previously published data (Cerling et al. 1991). The depletion of $^{13}CO_2$ following diffusion into the soil was rapid, and decreasing variability in $\delta^{13}CO_2$ measurements over time indicated there were some places where tracer $^{13}CO_2$ seeped beneath the ground tarp in the enclosure and created ^{13}C 'hot spots' in the surface soil. Soil moisture was a primary driver of longer-term dynamics of CER based on CO_2 concentrations in the static chambers, but had no detectable effect on $\delta^{13}CO_2$ efflux. Carbon-13 labeled exudates in the rhizosphere may have created a readily available substrate for heterotrophic soil microorganism that, when combined with $^{13}CO_2$ from root respiration, resulted in greater buffering of variability in $\delta^{13}CO_2$ than chamber CO_2 concentrations. A large precipitation event on Day 10 post-labeling was associated with the highest chamber CO_2 concentrations and indicated that static soil chamber measurements were tracking relative changes in CER as measured by the automated soil gas-exchange system.

In another $^{13}CO_2$ labeling experiment, using 5 l of 95 atom % enriched $^{13}CO_2$ in a 200 m^3 chamber, Högberg et al. (2008) achieved an average enrichment of 4.7 atom % ^{13}C in CO_2 and increased the $\delta^{13}C$ value of Scots pine needles by 18‰. In our larger ~500 m^3 chamber, with approximately a 10-fold increase in the amount of $^{13}CO_2$ injected, we increased the $\delta^{13}C$ value of loblolly pine needles by 54‰. The calculated loss rate of $^{13}CO_2$ during the labeling event from both experiments was similar; ~0.4% of the $^{13}CO_2$ in the enclosures was lost during each minute of exposure. Plain et al. (2009) succeeded in obtaining much greater enrichments of foliar $\delta^{13}C$ (>1000‰) in beech trees by using an enclosure with a smaller volume (19.5 m^3), longer exposure times and 25 l of 99 atom % enriched $^{13}CO_2$. Time of day, low soil moisture and canopy temperatures potentially created high stomatal resistance in our experiment and would explain, in part, the relatively low ^{13}C-enrichment in pine needles immediately after the labeling event.

Is belowground C flux positively correlated with GPP?

The HS treatment reduced PAR to below the light saturation point and thereby reduced foliar C uptake and canopy water use. The strong reduction in photosynthate within leaves led to reduced available C for partitioning to growth or respiration throughout the tree. As a result, there was decline in stem growth and fine-root standing crop under HS. However, HS did not concurrently reduce soil CER. Despite a linear relationship between sap flow and soil CER, the large variation in soil CER among trees limited detection of significant treatment effects. There was also difficulty in linking CER to root respiration (based on total root biomass). The poor correlation between total root biomass and CER illustrates confounding factors of incomplete sampling, root turnover, xylem CO_2 flux (e.g., Aubrey and Teskey 2009), soil CO_2 diffusion (Subke et al. 2009) and substantial heterotrophic contributions (Maier and Kress 2000, Hanson et al. 2000, Vargas et al. 2011). In the future, complete sampling of root distribution beneath the CER chambers to depth will facilitate separation of respiration between autotrophic and heterotrophic sources. Our single soil core from the center of the CER chamber could easily have missed a large root that would have a disproportionate contribution to CER, and thus reduce potential correlation between root biomass and CER.

In addition to spatial heterogeneity in CER, drought and late season reductions in GPP limited new photosynthate available for partitioning belowground. The CER in September was 75% less than the CER in early August. Multiple other factors increased the heterogeneity of belowground responses, particularly the lack of distinct spatial or physical separation of treatments, soil properties, pit and equipment installation and sampling artifacts. Since the treatment trees were not separated from one another, there was overlap of roots from different treatments. Long lateral roots emanated from the trees, such that at a location 0.5 m from the base of a tree (e.g., at the CER chamber) there could exist roots from several other labeled or unlabeled trees that would dilute the influence of individual treatment trees on their adjacent soils. Also, there was substantial variation in soil texture and bulk density caused by the shale layers, and consequentially large spatial variation in soil moisture that could affect root proliferation and respiratory activity. Heterogeneity of root water extraction, infiltration of water following precipitation events, and lateral and vertical redistribution of water within soils further diluted evidence of responses to shade treatments. Even so, based on relative changes in soil water content and sap flow there was a treatment effect on soil water extraction by individual trees. Root production was also impacted by treatments despite fairly recent installation of the minirhizotron tubes. Tubes were installed just 3 months prior to the experimental manipulation, which is likely shorter than necessary for full colonization of the tubes by the root population. While not conducive to annual production dynamics, the tubes were nonetheless able to provide insight into short-term dynamics of a representative root population to shading. Lateral tube installation from the pit face (as opposed to angled installation from the surface) enhanced observation of roots where root density was greatest, and increased the ability to distinguish root responses to shade treatments.

Sequential soil coring, ingrowth cores and minirhizotrons have been used to estimate C contributions to net fine root production in *P. taeda*, which vary substantially both seasonally and due to resource availability (e.g., Nadelhoffer and Raich 1992, Albaugh et al. 1998, King et al. 2002, Pritchard et al. 2008). In our late-season study there was significant fine root mortality across shade treatments as the soil moisture declined. Following the Day 10 rainfall event, root production increased

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in the LS treatments, but not the HS treatments, suggesting a lack of new C available for root growth. Similar to our study, seasonal mortality of fine, ephemeral roots in 10-year-old *P. taeda* occurred concurrent to leaf fall in autumn, followed by a surge in new root production (King et al. 2002). The next year, both soil CER and root biomass declined during drought in late summer, then increased following rainfall (Maier and Kress 2000), and it appeared that heterotrophic respiration was more sensitive than autotrophic respiration to the changing environmental conditions. Similarly in a young *Fagus sylvatica* L. forest, late season declines in CER were related to low soil moisture and declining temperature, although CER remained responsive to precipitation inputs (Epron et al. 2004). Across studies of *P. taeda* stands in the southeastern USA, seasonal CER remained fairly stable and was primarily driven by soil environmental conditions (Maier and Kress 2000, King et al. 2004, Wiseman and Seiler 2004, Gough et al. 2005), which suggests that short-term C flux associated with net root production was a minor component of CER. While seasonality of soil CER has been related to sinks and root growth (Wardlaw 1990, Högberg et al. 2001, Wingate et al. 2010, Epron et al. 2011), late season soil CER may not necessarily be strongly influenced by or directly linked to GPP, as changes in GPP are corollary to and confounded by the actual physical driving factors affecting CER, specifically temperature and soil moisture (Stoy et al. 2007).

Do reductions in GPP delay flux of new photosynthate from foliage through phloem, roots and soil?

Despite much lower GPP, there was no apparent delay in the transport of new C through the plant–soil–atmosphere pathway based on the dynamics of the ^{13}C signal. Since the ^{13}C label and shade treatments were applied the same day, we should expect that the reduction in GPP and subsequent decline in phloem loading resulted in a decrease in a pressure wave through the phloem (cf. Mencuccini and Hölttä 2010*a*) and decreased C flux through roots. Yet CER was not affected by the shade treatments, nor was the appearance of ^{13}C label in the phloem or as CER. The rapid loss of ^{13}C from the canopy after the labeling event was due to a combination of autotrophic respiration of $^{13}CO_2$, and translocation of ^{13}C-labeled photosynthate to wood and roots. There are no data to evaluate the relative importance of the two processes, but the rapid appearance of ^{13}C in phloem sap on the day following the labeling event confirms foliar export of ^{13}C-labeled compounds commenced soon after the assimilation of $^{13}CO_2$ by the canopy. Similarly, in *P. sylvestris* $\delta^{13}C$ in phloem sap peaked the day following labeling (Högberg et al. 2008). In labeled *P. pinaster*, $^{13}CO_2$ in continuously measured stem respiratory efflux peaked 1–4 days post-labeling, and corresponded to $\delta^{13}C$ in sampled phloem tissue extracts (Dannoura et al. 2011). Our results were consistent with prior studies showing rapid and substantial transfer of newly assimilated C from tree canopies, through phloem to the rhizosphere, resulting in a close association between canopy and rhizosphere C dynamics (Horwath et al. 1994, Högberg et al. 2001, 2008, Steinmann et al. 2004, Mencuccini and Hölttä 2010*a*, Epron et al. 2011). Measurements of ^{13}C in soil CO_2 efflux indicated that the transfer of newly assimilated C to the rhizosphere occurred within a few days following the labeling event, and that the translocated ^{13}C-labeled photosynthates were quickly metabolized and respired by tree roots. We observed peak $^{13}CO_2$ concentrations in soil respiration at 3–4 days after tree labeling, which was consistent with the timing of ^{13}C transfer to the rhizosphere in ^{13}C-labeled scots pine (Högberg et al. 2008), ^{13}C-labeled maritime pine (Epron et al. 2011) and ^{14}C-labeled hybrid poplar trees (Horwath et al. 1994). Moreover, similar to previous studies (Horwath et al. 1994, Epron et al. 2011), the C tracer in soil respiration beneath our ^{13}C-labeled loblolly pines declined quickly, and in an approximately linear fashion, over a period of ~2 weeks. Any ^{13}C remaining in the pines after this period was probably associated with biomass or long-term pools of C storage.

Root and heterotrophic respiration are dependent on GPP, but the primary drivers of daily CER in our study were the environmental conditions. Carbon dioxide efflux rate generally declined through the study as mean temperature declined, yet remained highly responsive to rainfall events. Patterns of soil CER at our site were consistent with results from other studies in young *P. taeda* stands that found that most of the variation in CER was dependent on soil temperature, but under dry conditions soil moisture was also an important predictor of CER (Maier and Kress 2000, King et al. 2004, Wiseman and Seiler 2004, Palmroth et al. 2005, Stoy et al. 2007). The linear correlation between sap flow and next day CER does not necessarily indicate cause and effect. Rather, the underlying processes controlling sap flow and CER, i.e., stomatal conductance or respiration respectively, are similarly regulated by environmental conditions.

Since net canopy photosynthesis is proportional to stomatal conductance (Farquhar and Sharkey 1982), GPP is directly linked to transpiration and therefore sap flow. Higher sap flow thus indicates increased C uptake and phloem loading, resulting in greater C available for belowground flux and ultimate release. This study indicates that production of new photosynthate, as indicated by sap flow, was rapidly transported through stem phloem into roots where it was released as respiration beginning the next day. The strong relationship between sap flow and next day CER was not dependent on mutual correlation with temperature (Liu et al. 2006) since there was no relationship between sap flow and CER on the same day. The correlations between $\delta^{13}C$ of phloem tissue and $\delta^{13}C$ of soil CO_2 efflux on the next day, and second day (but not the same day) provide further evidence that current photosynthate is rapidly moving through the system.

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Similar relationships have been seen in other forest species (Ekblad and Högberg 2001, Högberg et al. 2001; Bhupinderpal-Singh et al. 2003, reviewed by Kuzyakov and Gavrichkova 2010). Following exposure of Scots pine to enriched $^{13}CO_2$, Högberg et al. (2008) observed that δ^{13}C peaked in phloem samples after 1 day and in soil respiration after 2–4 days. Recent analyses and mechanistic modeling efforts that address confounding variables (e.g., temperature, soil diffusion) have successfully characterized timing and strength of the coupling of photosynthesis and soil respiratory activity (Sampson et al. 2007, Stoy et al. 2007, Vargas et al. 2011).

Even though the soil CO_2 was enriched in ^{13}C, there was little indication of ^{13}C enrichment in other belowground components. There was no ^{13}C enrichment of bulk soil samples, and bulk root samples which primarily consisted of old roots were only slightly enriched. However, there was substantial enrichment of some of the new root tips that developed during the experiment, and the label was still evident in some of the roots grown during the experiment the following spring. This would suggest that $^{13}CO_2$ assimilated over the time frame of the experiment was not stored in existing roots, but rather was incorporated into new root biomass or quickly depleted through respiration or release to the rhizosphere. The heterogeneity of new root tip enrichment is likely a condition of both differential foliar uptake of the $^{13}CO_2$ label resulting in patchy translocation through the root system, and differential existing soluble C supply and demand within the root system.

Modeling links

The type of controlled experiments as conducted in this study can provide qualitative and quantitative information for developing and testing dynamic tree and stand-level C partitioning models that rely on environmental conditions, physiology and allometry to determine C allocation patterns at variable spatial and temporal resolution (e.g., Siqueira et al. 2006, Ogle and Pacala 2009). A promising addition to these models would be inclusion of a mechanistic partitioning approach based on the Münch pressure-flow theory that allows for rapid feedback and model adjustment to changing external conditions. The theory assumes mass flow of solutes within phloem based on source (foliar) loading and sink (stem or root) unloading, which creates differential osmotic and pressure gradients that regulate C transport and partitioning within the system. This theory has been validated in the laboratory and has been accepted by most plant physiologists (Salisbury and Ross 1992), although in situ timing, magnitude and impact of pressure gradients between source and sink within forest ecosystems have not been resolved (Kayler et al. 2010, Kuzyakov and Gavrichkova 2010, Mencuccini and Hölttä 2010*a*, 2010*b*). Directly testing the model would involve comparing the predicted carbohydrate concentrations in sources and sinks and the fluxes between them against measurements. However, our destructive sampling methodology did not allow for the high-frequency measurements necessary to examine diel patterns in phloem loading, transport and unloading. The recent application of tuneable diode laser absorption spectroscopy to track ^{13}C through an ecosystem (e.g., Plain et al. 2009, Wingate et al. 2010, Dannoura et al. 2011, Epron et al. 2011) is a novel and non-destructive approach that might be successfully applied to test the pressure-flow theory.

Conclusions

We were successful in manipulating C availability and incorporating a ^{13}C label into the trees. There was adequate label to track the rapid flux of C from the canopy to the phloem in the lower bole, and the subsequent appearance of ^{13}C in roots and soil CO_2 efflux. Although some roots clearly accumulated the ^{13}C label, others did not, which illustrates the sampling challenges posed by differential C flux through old roots, dilution with existing soluble carbohydrate pools and partitioning into new root biomass. Likewise, soil gas contained the ^{13}C label at some locations but not at others. The extreme heterogeneity in belowground $^{13}CO_2$ distribution precluded analysis of C partitioning by depth or between soil and root respiration, without knowledge of proximity and activity of roots to each sampling location. This late-season study found relatively low rates of CER, which were driven by soil temperature and moisture, despite significant changes in ephemeral net root production. The experiment was valuable for providing information about the timing of C transport through the plant–soil system.

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