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ECOSPHERE

Seasonality and partitioning of root allocation to rhizosphere soils in a midlatitude forest

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Citation: Abramoff, R. Z., and A. C. Finzi. 2016. Seasonality and partitioning of root allocation to rhizosphere soils in a midlatitude forest. Ecosphere 7(11):e01547. 10.1002/ecs2.1547

Abstract. Root growth, respiration, and exudation are important components of biogeochemical cycles, yet data on the timing and partitioning of C to these processes are rare. As a result, it is unclear how the seasonal timing, or phenology, of root C allocation is affected by the phenology of its component processes: growth of root tissue, respiration, mycorrhizal allocation, and exudation of labile C. The objective of this study was to estimate the phenology and partitioning of C belowground across the growing season in a midlatitude forest located in central Massachusetts. Fine and coarse root production, respiration, and exudation were summed to estimate a monthly total belowground C flux (TBCF) in two hardwood stands dominated by *Quercus rubra* and *Fraxinus americana*, respectively, and one conifer stand dominated by *Tsuga canadensis*. We observed significant stand-level differences in belowground C flux and the partitioning of C to root growth, mycorrhizal fungi, exudation, and respiration. The deciduous hardwood stands allocated C belowground earlier in the season compared to the conifer-dominated stand. The deciduous stands also allocated a greater proportion of TBCF to root growth compared to the conifer-dominated hemlock (*T. canadensis*) stand. Of the three stands, red oak partitioned the greatest proportion of TBCF (~50%) to root growth, and hemlock the least. Low root growth rates in hemlock may be related to the arrival and spread of the invasive pest, hemlock wooly adelgid (*Adelges tsugae*), during the study period. Ongoing research in the eastern hemlock stand may yet determine how whole tree allocation and partitioning change as a result of this infestation.

Key words: belowground allocation; C budget; *Fraxinus americana;* Harvard Forest; midlatitude forest; minirhizotron; phenology; *Quercus rubra;* rhizosphere; root growth; *Tsuga canadensis*.

Received 1 May 2016; revised 7 July 2016; accepted 12 July 2016. Corresponding Editor: Jesse B. Nippert.

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Introduction

Carbon (C) allocation belowground via the root system can account for over half of net primary production (NPP; Keyes and Grier 1981, Helmisaari et al. 2002) and, along with litterfall, is the main conduit by which atmospheric C is incorporated into soil organic matter (SOM; Cotrufo et al. 2013). Roots convert some proportion of the C allocated belowground to CO_2 via maintenance respiration, but also produce new roots and store carbohydrates for future use. Through turnover, exudation into the rhizosphere, and allocation to mycorrhizal fungi, root tissues affect microbial activity as well as the content and the composition of soil organic C pools (Finzi et al. 2015). As such, the components of belowground C allocation—tissue growth, respiration, and exudation—are important to biogeochemical cycles in terrestrial ecosystems.

To date, few studies have measured seasonal variations in total belowground C allocation or

its various components. This makes it difficult to understand how the belowground C flux is partitioned among root growth, respiration, mycorrhizal allocation, and exudation and the relative contributions of each component to the total flux across the growing season (Drake et al. 2011). However, a number of studies point to notable variations in the timing of root growth that relate to respiration and exudate fluxes. For example, Cardon et al. (2002) report multiple peaks in soil respiration, suggesting periodic root production and/or exudation, in oak species alternating with flushes of aboveground growth. Others also observe asynchronicity between above- and belowground growth across multiple biomes, finding that deciduous trees have more synchronous phenology than evergreen trees (Steinaker et al. 2009, Abramoff and Finzi 2015). Interspecific differences in the timing of root growth have also been observed in a common garden experiment, where the peak in growth differed up to 49 d between plant species in the same location, indicating that belowground phenology relies in part on internal cues in addition to temperature (McCormack et al. 2014).

Up to 70% of belowground C allocation is respired by roots during maintenance and growth metabolism (Fahey et al. 2005, Litton et al. 2007). While the temperature dependence of root tissue respiration is well established (Atkin et al. 2000), there are still notable variations in tissue-specific root respiration rate between colocated plants of different species (Reich et al. 1998). These differences may be the result of variation in the extent to which roots acclimate to seasonal temperatures, or variation in C allocated to root metabolism (Burton and Pregitzer 2003, Loveys et al. 2003). The reserve pool of nonstructural carbohydrates (NSC) in roots may serve as a temporary sink for C allocated belowground, the size of which may vary across the season and between stands (Gough et al. 2009, Dietze et al. 2014). Direct, seasonal measurements of both NSC concentration and root respiration on intact or severed roots are rare, with notable exceptions (Drake et al. 2008, Gough et al. 2009).

In addition to respiration, root activity stimulates the release of soil C to the atmosphere via priming effects. Plants exude organic C and nitrogen (N) substrates from growing root tips that are often consumed by the root-associated microbial community (Hirsch et al. 2013). This exudation stimulates microbial biomass growth and extracellular enzyme activity, thereby increasing the decomposition rate of SOM and liberating plant available nitrogen (Kuzyakov 2010, Phillips et al. 2011, Finzi et al. 2015). There are few measurements that estimate the amount of labile C exuded annually to microbes (Phillips et al. 2008, Pritchard et al. 2008), and little is known about the seasonality of exudation.

The objective of this study was to assess the phenology and partitioning of C allocated belowground across the growing season in three midlatitude stands at the Harvard Forest. The hardwood tree species northern red oak (*Quercus rubra*) and white ash (*Fraxinus americana*) dominate the overstory each of two stands. The conifer tree species eastern hemlock (*Tsuga canadensis*) is monodominant in the third stand. We were particularly interested in red oak because of its emergence as the canopy dominant throughout the Harvard Forest over the last two decades (Urbanski et al. 2007, Keenan et al. 2012) and in hemlock because it is a late-successional dominant species that is on the verge of local extirpation as a result of the invasive insect, hemlock woolly adelgid (Orwig 2002, Finzi et al. 2014). Based on the observed high NPP in deciduous stands at Harvard Forest, and a high ratio of total belowground carbon flux-to-aboveground net primary production in needleleaf evergreen relative to deciduous forests (Litton et al. 2007), we hypothesize that (1) deciduous stands initiate root growth earlier in the growing season than the conifer stand, and (2) deciduous stands allocate more C belowground, but (3) the conifer stand partitions a larger fraction of GPP belowground than the deciduous stands.

Materials and Methods

Study site

This study was conducted in the Prospect Hill tract of the Harvard Forest Long Term Ecological Research site, a 120+-year-old secondary-growth forest located in Petersham, Massachusetts, USA (42′ N, 72′ W, elevation 340 m; Wofsy et al. 1993, Goulden et al. 1996). The dominant species are northern red oak and red maple (*Acer rubrum*), with smaller populations of eastern hemlock, white ash, white pine (*Pinus strobus*), and red pine (*Pinus resinosa*). The site is located on former

Table 1. Stand-level soil characteristics (±SE).

Stand	Total C content (g C/m^2)	Total N content (g N/m^2)	O horizon mass (g/m^2)	pH	Soil C:N ratio	Heterotrophic respiration (g C·m^{-2}·gs^{-1})	Root biomass (g C/m^2)	Microbial biomass (g C/m^2)
White ash	5989^b ± 330	431^a ± 24	–	5.1	13.9^b ± 0.17	460^a ± 40	190^c ± 9	240^a ± 27
Red oak	7709^a ± 438	354^b ± 23	131^a ± 8	5.2	21.8^a ± 0.72^a	491^a ± 32	305^a ± 15	14^c ± 1
Eastern hemlock	6922^a ± 246	290^b ± 13	105^b ± 7	3.7	23.9^a ± 0.70	427^a ± 45	241^b ± 11	69^b ± 5

Notes: Heterotrophic respiration was estimated as the difference between soil CO_2 efflux and root respiration (Table 4). Total C content refers to organic and mineral horizon soil C down to 15 cm.

Letters indicate a statistically significant difference in means using analysis of variance at the $P < 0.05$ level.

agricultural land that was abandoned in the mid-1800s allowing forest regrowth beginning late in the 19th century (Foster et al. 2003). Forest uptake of C has increased since 1990 from ~2 Mg C·ha^{-1}·yr^{-1} to ~5 Mg C·ha^{-1}·yr^{-1} (Urbanski et al. 2007, Keenan et al. 2012). Soils are Typic Dystrochrepts derived from glacial deposits of granite, schist, and gneiss.

Plots were established in three monodominant stands: white ash, red oak, and eastern hemlock. Each stand occupies an area of 3.4, 8.3, and 10.9 ha, respectively. We established six biometry plots per stand ($N = 18$) and 10 minirhizotron tube plots per stand ($N = 30$). The basal area in each 8 m radius biometry plot is composed of 80% dominant tree species, with the inner 5-m area containing only the dominant species. The three stands differ in soil chemistry and biogeochemistry (Brzostek and Finzi 2012). The white ash stand has a lower ratio of C-to-N content in the soil than the oak and hemlock stand (Table 1).

Root production and biomass

Root production and turnover were measured April–December 2012, March–November 2013, and April–November 2014 using a BTC-100× high-magnification minirhizotron camera system (Bartz Technology Company, Carpinteria, California, USA). Measurements were made biweekly during the growing season in 2012 and monthly in 2013, in 2014, and during the snow-free dormant season of each of the three years. There was no sampling from December 2012 to March 2013 and November 2013 to April 2014.

The camera system was inserted into cellulose acetate butyrate tubes installed at a 45° angle to a vertical soil depth of 40 cm. Thirteen tubes were installed in the center of each minirhizotron tube plot at Harvard Forest 10+ years ago ($n = 4$ in red oak, $n = 9$ in eastern hemlock). Seventeen tubes were installed in November 2012 ($n = 6$ in red oak, $n = 1$ in eastern hemlock, and $n = 10$ in white ash) for a sample size of $n = 10$ for each stand in 2013 and 2014. Minirhizotron tubes installed in November 2012 likely severed existing roots during placement and may have increased root growth rates in the following seasons. To test for this effect, we conducted an analysis of variance using growth or mortality as the dependent variable and sample date and whether or not the sample came from a minirhizotron tube installed in 2012 as fixed effects for 2013 and 2014.

The minirhizotron camera captures 39 sequential images that are 13.5 × 17 mm in size along the upper axis of each tube at each sampling interval. The resulting images were processed using the open source imaging software Rootfly (Rootfly Development Team, Version 2.0.2, GNU General Public License, Clemson, South Carolina, USA). In each image, every root or root segment's length and diameter were annotated. Length (mm) and diameter (mm) were scaled to mass (g) using a site-specific relationship based on $n = 20$ fine root (<2 mm) segments per species, excavated from the top 15 cm of soil in each stand. For each root sample, we recorded length and diameter and dried it to constant mass at 60°C for 4 d. The polynomial fit to mass as a function of length and diameter was of the form:

$$\text{Mass} = \text{length} \times (a \times \text{diameter}^2 - b \times \text{diameter}^2) \quad (1)$$

where the coefficients [a, b] were [3×10^{-4}, 1×10^{-5}], [6×10^{-4}, 5×10^{-5}], and [4×10^{-4}, 9×10^{-5}], for white ash, red oak, and eastern hemlock, respectively. The R^2 value for this relationship was greater than 0.94 for each species. Root biomass was estimated from the images assuming

that the viewing depth for a minirhizotron image is 0.7848 mm (Taylor et al. 2014). Given this assumption of viewing depth, we calculated the diameter of the imaged root using the method of Taylor et al. (2014). Assuming that roots are cylindrical, the relationship between the true diameter (D) and diameter perceived (p) at depth (f) is:

$$D = \frac{\left(f^2 + \frac{1}{4}p^2\right)}{f} \quad (2)$$

Daily fine root growth and mortality (g root/d) in each minirhizotron image for each sampling interval were calculated as:

$$\text{Growth (g root/d)} = \frac{m_{t_2} - m_{t_1}}{t_2 - t_1} \quad \text{when } m_{t_2} - m_{t_1} > 0 \quad (3)$$

$$\text{Mortality (g root/d)} = \frac{m_{t_2} - m_{t_1}}{t_2 - t_1} \quad \text{when } m_{t_2} - m_{t_1} < 0 \quad (4)$$

where m is the total mass of roots traced on day of year (DOY) t_1 or t_2. Root production refers to total fine root growth summed over an interval of time. Root biomass increment is the sum of growth and mortality for that interval. We consider root biomass increment as defined here to be an estimate of the change in standing crop over the growing season. Root production and biomass increment measurements were scaled to g $C \cdot m^{-2} \cdot d^{-1}$ using the assumption that each minirhizotron image is representative of a 0.173 cm^3 (13 × 17 × 0.7848 mm) volume of soil (Taylor et al. 2014). The depth of each image was calculated using:

$$\text{Depth (cm)} = \text{dist (cm)} \times \sin\theta \quad (5)$$

where dist is the distance of the window from the top of the tube and θ is the angle of the tube relative to vertical (45°).

The standing biomass of roots was estimated from field samples in 2012. We collected three 10 × 10 cm samples of the organic horizon and three 5 cm diameter mineral soil samples to a depth of 15 cm monthly in each plot. Roots were removed and sorted into fine (<2 mm), coarse (>2 mm), live and dead pools. Live vs. dead status was determined using tensile strength and color (Vogt and Persson 1991, Matamala and Schlesinger 2000). Brittle roots with dark vascular tissue were classified as dead. We recognize that there is a wide range of structural and functional heterogeneity within the pool of roots that are <2 mm (McCormack et al. 2015). However, the purpose of this study is to estimate bulk movements of C through root tissues rather than partition root process rates on the basis of root architecture, so we employed a diameter size cutoff for simplicity and consistency. Sorted roots were dried and weighed to obtain standing biomass for live fine roots (g/m^2). Subsamples of roots from monthly soil coring were assayed for carbon content (%C) using an elemental analyzer (model NC2500; CE Instruments, Milan, Italy). Fine root standing biomass for the organic horizon down to a depth of 15 cm in the mineral soil was scaled up to g C/m^2 by adjusting for the horizontal area of the soil core, the carbon content of roots in each stand, and rock content.

Root respiration

CO_2 efflux was measured directly on recently severed fine roots using an infrared gas analyzer (LI6400; LiCor Biosciences, Lincoln, Nebraska, USA). Measurements were made monthly from March to October 2013 on three samples per stand. Respiration rates have been measured successfully using severed roots in previous studies (Burton and Pregitzer 2003, Burton et al. 2012). Furthermore, we compared respiration measurements of an attached and severed root system for each stand on three separate days and confirmed that respiration rates were similar between the two types of roots (correlation coefficient = 0.78) and stable up to approximately 7 h (Data S1).

Two measurements per sample were made on each sample date within 7 h of collection, one in the field at ambient temperature and one in the laboratory at a constant temperature. Field measurements of CO_2 efflux were fit to the Arrhenius equation,

$$R_S = A \times e^{(-E_a/RT)} \quad (6)$$

where R_S is the respiration rate (μmol $CO_2 \cdot s^{-1} \cdot g^{-1}$), E_a is the activation energy (kJ/mol), A is a pre-exponential factor (μmol $CO_2 \cdot s^{-1} \cdot g^{-1}$), T is temperature (Kelvin), and R is the gas constant (kJ·$Kelvin^{-1} \cdot mol^{-1}$). The parameters E_a and A were estimated using nonlinear curve fitting in

SigmaPlot (version 10.0; Systat Software, San Jose, California, USA). We then estimated growing season rates of R_S using daily soil temperature measured at 10 cm depth from HOBO data loggers installed in March 2013 in each stand. Mass-specific R_S (μmol CO_2·g root^{-1}·s^{-1}) was scaled to g C·m^{-2}·s^{-1} using the mass of root per square meter ground surface area and converting from μmol to μg.

Nonstructural carbohydrates

The pool of NSC was estimated as the sum of the concentration of sugars (assumed to be glucose:fructose:galactose in 1:1:1 ratio) and starch using the method of Chow and Landhäusser (2004). We collected three ~1- to 2-g fine root samples from each biometry plot monthly from May to November 2011 and four times from March to November 2012. Roots were excavated, washed, and frozen in liquid nitrogen until analysis. Sugars were extracted from dried and finely ground root tissue using a 12:5:3 methanol:chloroform:water solution before being developed with 2% phenol and concentrated sulfuric acid. Absorbance was measured at 490 nm using a digital spectrophotometer (Spectronic 20D+; Thermo Scientific, Waltham, Massachusetts, USA). Starch was extracted using a 0.005 N sulfuric acid solution at 95°C and developed as described above.

Root exudation

Root exudates were collected from six fine root systems per stand in June and August 2012, and April, May, July, and October 2013 following the method of Phillips et al. (2008, 2011). In brief, roots were excavated 48 h prior to collection, washed, and incubated in a moist soil–sand mixture. Roots were placed into cuvettes with glass beads and a C-free nutrient solution 24 h prior to collection. At the time of collection, exudate-containing nutrient solution was extracted with two additional flushes of C-free nutrient solution to ensure that exudates adhering to glass beads were flushed into solution. Samples were transported back to the laboratory on ice and analyzed for nonpurgeable organic carbon content using an elemental analyzer (Shimadzu TOC-VCSH analyzer, New Haven, Connecticut, USA). Exudation rate (g C·g root^{-1}·d^{-1}) was scaled to g C·m^{-2}·d^{-1} using fine root biomass (g root/m^2) from soil cores.

Total belowground C flux

To estimate the quantity of C allocated belowground during the growing season (herein abbreviated as "gs"), we define a simplified belowground C flux budget (sensu Litton et al. 2007, Drake et al. 2011) using two different approaches. First, a top-down estimate of total belowground C flux ($TBCF_{top}$, g C·m^{-2}·gs^{-1}) is defined as:

$$TBCF_{top} = F_{efflux} + F_{leaching} - F_{litter} + \Delta(C_{roots} + C_{soil}) \quad (7)$$

where F_{efflux} is the growing season rate of soil respiration, $F_{leaching}$ is the flux of dissolved organic C into streamwater, F_{litter} is litterfall, and $\Delta(C_{roots} + C_{soil})$ is the growing season change in the C pool associated with fine roots and soil. F_{efflux} for oak and hemlock was estimated from a 22-year synthesis of soil CO_2 efflux data using a range of methods across multiple stand types (Giasson et al. 2013). For the ash stand, F_{efflux} was estimated by summing root and heterotrophic respiration. We assumed that heterotrophic respiration was the result of microbial decomposition of litter, exudates, and soil organic matter. We used soil temperature measurements to model soil organic matter decomposition using an empirical relationship with temperature derived from soil incubations (Drake et al. 2013*b*). F_{litter} was measured using litter baskets (Frey and Ollinger 1999, Munger and Wofsy 1999, Hadley 2009, Barker Plotkin 2010, Brzostek 2012, Lemos 2013). We estimated ΔC_{roots} using net root production from this paper. We set $\Delta C_{soil} = 0$, assuming that it is small relative to the timescale of this study (Gaudinski et al. 2000).

Second, a bottom-up estimate ($TBCF_{bottom}$, g C·m^{-2}·gs^{-1}) is defined as:

$$TBCF_{bottom} = F_{roots} + F_{resp} + F_{exudates} + F_{fungi} \quad (8)$$

where F_{roots} is fine root production, F_{resp} is fine and coarse root respiration, $F_{exudates}$ is root exudation, and F_{fungi} is mycorrhizal production and respiration. F_{roots}, $F_{exudates}$, and fine root respiration are estimated using measurements from this study. Annual estimates of ΔC_{roots} and F_{roots} were averaged across the three years of the study for the oak and hemlock stands and two years for the ash stand. We also averaged the two annual estimates of $F_{exudates}$. Coarse root (>2 mm)

respiration was estimated using the observation that mass-specific rates of coarse root respiration are approximately 70% lower than fine root respiration rates in the same stand (Pregitzer et al. 1998, Desrochers et al. 2002, Fahey et al. 2005). Coarse root respiration (μmol CO_2·g $root^{-1}$·s^{-1}) was scaled to g C·m^{-2}·gs^{-1} using coarse root biomass to 15 cm from soil pits in evergreen and hardwood stands at Harvard Forest and the Harvard Conservation Trust (Harvard, Massachusetts, USA, 42°31′ N, 71°32′ W), respectively (Lemos 2013). Mycorrhizal production was estimated using a relationship between temperature and mass-specific fungal growth developed by Averill et al. (2015). We scaled mass-specific growth to g C·m^{-2}·gs^{-1} using measurements of microbial biomass in each stand. We determined the fungal proportion of microbial biomass using an empirical relationship between fungal-to-bacterial (F:B) ratio and soil C:N (Waring et al. 2013). We estimated the mycorrhizal proportion of fungal biomass using relative abundance of ectomycorrhizal fungi in the hemlock stand, making the additional assumption that this relative abundance is generalizable to the oak and ash stands at the Harvard Forest (Averill et al. 2015). We openly acknowledge the uncertainty in this assumption, particularly in the ash stands that are largely dominated by arbuscular mycorrhizal fungi. Soil microbial biomass in the hemlock stand was measured in June, July, August, and September of 2012 (Averill et al. 2015). Soil microbial biomass in the ash stand was measured in July of 2011 (C. Averill, *unpublished data*). Microbial biomass data in oak-dominated hardwoods stands were taken from Drake et al. (2013*a*) and Sorensen et al. (2016). We estimated the contribution of mycorrhizae to heterotrophic respiration in proportion to their contribution to total microbial biomass. Given that mycorrhizal production is estimated from parameters from multiple sources, we consider this estimate of production and respiration to be more uncertain than the other pools and fluxes presented here and stress that this estimate is based primarily on a theoretical partitioning of the microbial biomass measured in these stands.

Belowground C pools were derived from the Harvard Forest Data Archive and this study. Soil C content data were taken from published sources (Nadelhoffer et al. 1999, Bowden et al. 2009, Orwig and Foster 2009, Brzostek 2012, Lemos 2013, Frey et al. 2014, Sorensen et al. 2016) and one unpublished source (J. Drake, *unpublished data*). Standing biomass of roots was estimated using data from this study as well as a previous study conducted on the same plots (Lemos 2013). We did not include transpiration of dissolved inorganic C or throughfall leaching in the calculation of C outputs or inputs as these terms are generally <5% of the C budget in other midlatitude forest stands (Fahey et al. 2005, Drake et al. 2011). Coarse root production may account for 5–10% of total C inputs (Fahey et al. 2005, Drake et al. 2011), but we had no data on coarse root production at Harvard Forest and therefore did not include it. Thus, our belowground C flux estimate is conservative.

Monthly values of gross primary production (GPP) were estimated using partitioned NEE measured in 2012 at the eddy-covariance towers located in a mixed hardwood stand and a hemlock stand at Harvard Forest (ORNL-DAAC 2013). Data were not available to estimate GPP in the ash stand. For the purpose of this budget, we assumed ash GPP was equivalent to oak-dominated hardwoods. This assumption makes the belowground partitioning of GPP in the ash stand less certain. We used PhenoCam data (http://phenocam.sr.unh.edu/) to determine maximum canopy greenness in red oak and eastern hemlock stands.

Data analysis

All statistical analyses were performed in R Statistical Software (R Core Team 2013). Stand-specific and seasonal-to-interannual variations in fine root growth, mortality, respiration, NSC concentration, and exudation were modeled using a mixed-effects model with stand, sample date, and year as fixed effects and plot as a random effect. A Tukey's HSD post hoc test was used to test for differences between stands. Analysis of variance (ANOVA) was used to test for the effect of newly installed tubes on root growth and mortality, to test for the effect of stand on root tissue (N), to test for the effect of stand and soil horizon on root biomass, and to test for the effect of stand type and estimation method (top-down or bottom-up) on TBCF. Estimates of TBCF were generated using data sets of varying sample size, and standard errors

were propagated by quadrature. To test for an effect of stand type and estimation method, we assumed a conservative effective sample size of 6 (the number of plots per stand) for each TBCF estimate and resampled 1000 times from a normal distribution using the mean and standard error of each TBCF estimate. *P*-values from each of 1000 ANOVA models were averaged, and $P < 0.05$ was used as the threshold for significance. We also used ANOVA to test for differences in C content, N content, and organic horizon mass between stands, averaging across subsamples. All mixed-effects and ANOVA models were constructed using the *aov* function in base R.

Linear regression was used to correlate root growth with root mortality, and to correlate root growth with soil temperature for each stand separately. Multiple linear regression was used to model mass-specific respiration as a function of soil temperature, precipitation, and stand. We used a linear mixed-effects model to model root mortality as a function of temperature, precipitation, stand, year, and day of year with tube as a random effect. Precipitation and day of year were not significant effects and were dropped from the final model. We also used a linear mixed-effects model to model root growth as a function of temperature, precipitation, stand, year, and day of year with tube as a random effect using the *lmer* function in the *lme4* package in R (Bates et al. 2014). In the mixed-effects, ANOVA, and regression models, fine root growth and mortality were log-transformed to meet assumptions of normality.

Results

Root production and biomass

There was a measurable but transient effect of tube installation on root production in red oak. In 2013, mean red oak root growth was 0.34 g $C \cdot m^{-2} \cdot d^{-1}$ higher in newly installed tubes compared to tubes established a decade earlier ($F_{2,79} = 2.7$, $P = 0.07$), but this difference diminished in 2014 to 0.09 g $C \cdot m^{-2} \cdot d^{-1}$ ($P = 0.12$). There was no detectable installation effect on red oak root mortality or on the single hemlock tube installed in 2012. All white ash tubes were newly installed in November 2012, so it was not possible to establish an effect of installation on white ash root production.

Root growth was positively correlated with soil temperature in each stand (ash: $\beta = 0.08$, $P < 0.001$, $R^2_{adj} = 0.26$; oak: $\beta = 0.11$, $P < 0.001$, $R^2_{adj} = 0.30$; hemlock: $\beta = 0.08$, $P < 0.001$, $R^2_{adj} = 0.15$), but was not correlated with precipitation ($P > 0.1$). As a result, root growth was concentrated in midsummer, but with distinct stand-level differences (Fig. 1a–f). Red oak root growth occurred in one to three flushes over the growing season, with highest mortality in mid-to-late summer (Fig. 1a–c). Root mortality was not correlated with either soil temperature ($P = 0.13$) or precipitation ($P = 0.76$). Hemlock root growth was low throughout the growing season with small production peaks occurring in the fall in 2013 and 2014. White ash root growth peaked in midsummer with high mortality in late summer. In red oak and white ash stands, the peak in maximum canopy greenness occurred ~20 d earlier than in the eastern hemlock stand, but the peak in root growth occurred ~50 d earlier. As a result, the deciduous stands had a smaller offset between maximum canopy greenness and peak root growth than did the hemlock stand. Similar to the phenology of fine root production, biomass increment increased in early summer to midsummer in the deciduous stands. In 2012, hemlock fine root biomass increment was positive, but in 2013 and 2014, there was no net production of fine roots.

Root biomass in soil cores was significantly higher in the red oak ($P < 0.001$) and eastern hemlock ($P < 0.1$) stand compared to ash, largely because of a surface organic horizon ($F_{1,111} = 47.9$, $P < 0.001$). We did not detect a significant difference in root biomass between sampling dates. Root biomass estimated from minirhizotron images was also significantly ($P < 0.05$) higher in the red oak compared to the ash stand, with 40% of red oak roots concentrated in the organic horizon and top 5 cm of mineral soil (Table 2). White ash stands at Harvard Forest lack an organic horizon, possibly the result of bioturbation by exotic earthworms that are not present in the red oak or eastern hemlock stands. In this stand, surface litter is incorporated directly into the mineral soil horizon in <1 yr and there is relatively little variation in the root depth profile (Table 2). The concentration of N in root tissue was significantly higher in the ash stand (1.12%) compared to the red oak (0.72%) and eastern hemlock (0.68%) stands ($F_{2,68} = 90.3$, $P < 0.001$).

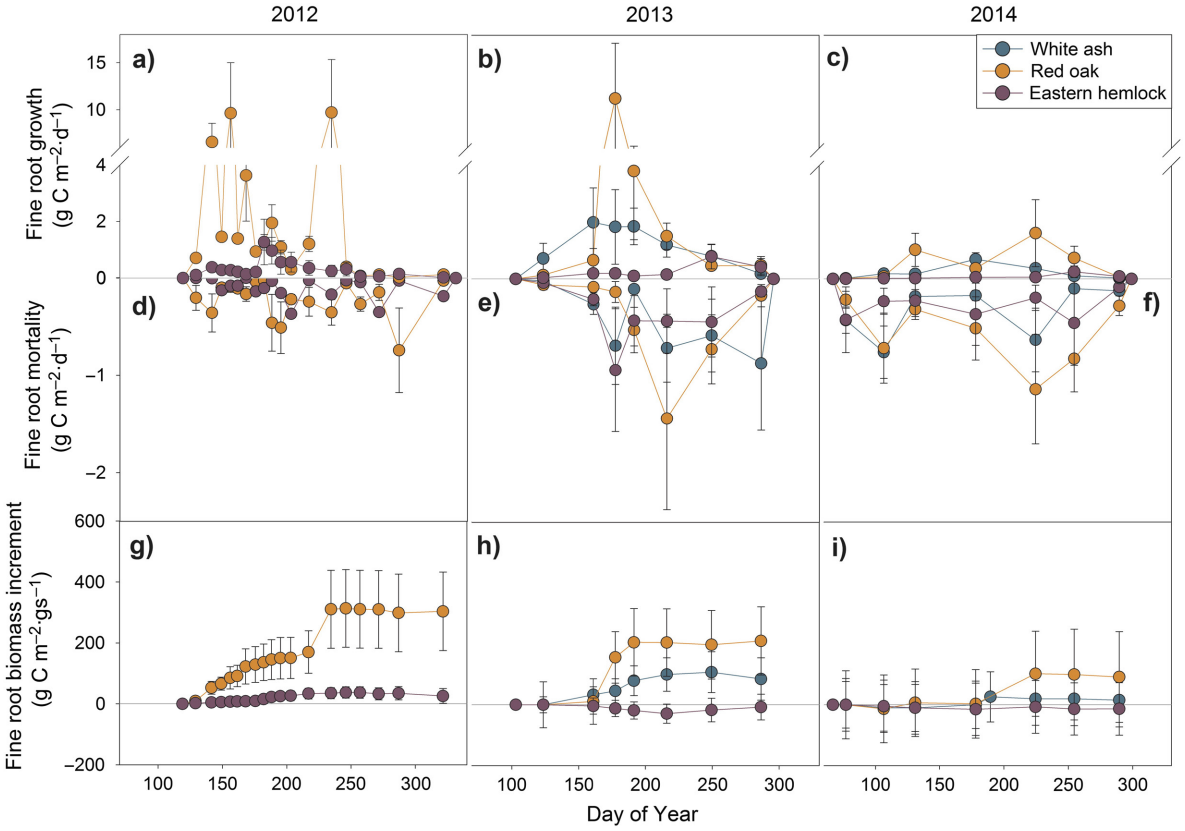

Fig. 1. Growth (a–c), mortality (d–f), and biomass increment (g–i) of fine roots. In 2012, $n = 4$ and $n = 9$ for red oak and eastern hemlock, respectively. In subsequent years, $n = 10$ for each stand. Error bars are standard error of the mean. There was a significant effect of year and stand on both growth (year: $F_{1,584} = 274.7$, $P < 0.001$; stand: $F_{2,584} = 147.7$, $P < 0.001$) and mortality (year: $F_{1,497} = 25.3$, $P < 0.01$; stand: $F_{2,497} = 106.5$, $P < 0.001$). The eastern hemlock stand had significantly less growth and mortality than the red oak and white ash stands ($P < 0.001$), but red oak and white ash stands were not different from each other (growth was only marginally different, $P < 0.1$).

Table 2. Median root biomass (25th percentile, 75th percentile) in g C/m^2 estimated from minirhizotron tubes binned into 5 cm depth increments averaged over the study period.

Depth	White ash[B]	Red oak[A]	Eastern hemlock[AB]
O horizon	–	119 (43.5, 399)	101 (22.2, 238)
0–5 cm	36.1 (19.6, 56.4)	109 (53.7, 284)	53.7 (12.4, 222)
5–10 cm	28.2 (18.4, 53.9)	59.9 (44.9, 140)	43.9 (14.3, 140)
10–15 cm	43.6 (26.3, 108)	26.9 (20.2, 158)	78.4 (29.8, 192)
15–20 cm	64.5 (37.1, 77.4)	71.2 (46.2, 348)	37.9 (8.0, 74.9)
20–25 cm	42.2 (30.2, 65.6)	162 (48.3, 218)	41.9 (11.6, 94.0)
25–30 cm	22.7 (20.6, 37.9)	16.5 (3.2, 21.5)	45.9 (5.0, 124)
30–35 cm	37.7 (14.3, 47.3)	–	–
35–40 cm	6.9 (4.0, 142)	–	–

Note: Uppercase letters indicate significant differences in root biomass between stands at the $P < 0.05$ level.

In all years, red oak and white ash stands allocated more C to fine roots compared to eastern hemlock (Fig. 1g–i). Averaged across growing seasons (gs), fine root production was 301 ± 76 g $C \cdot m^{-2} \cdot gs^{-1}$ and 133 ± 42 g C/gs in red oak and white ash stands, respectively. C allocation to fine root production in eastern hemlock was 42 ± 13 g $C \cdot m^{-2} \cdot gs^{-1}$.

Root respiration

The phenology of fine root respiration was similar between stands, with highest mass-specific respiration rates in midsummer (Fig. 2a). This follows from a positive correlation with soil temperature (Fig. 2b; $R^2_{adj} = 0.68$, $P < 0.001$). Mass-specific rates of fine root respiration were significantly higher in the white ash stand than in oak and hemlock stands (Fig. 2b). Arrhenius fits to mass-specific data indicate that white ash root respiration had a lower apparent activation energy (E_a = 20 kJ/mol) than that of red oak (E_a = 40 kJ/mol) with eastern hemlock intermediate between the two (E_a = 29 kJ/mol).

Mass-specific root respiration measured at constant temperature increased across the growing season (Fig. 3), in contrast to field measurements where respiration rate declined from its peak around DOY 150 despite increasing soil temperature beyond that date. Using the field observations, the average rate of fine root respiration was 229 ± 28, 242 ± 43, and 270 ± 37 g $C \cdot m^{-2} \cdot gs^{-1}$ for red oak, eastern hemlock, and white ash, respectively (Fig. 2c).

Nonstructural carbohydrates and root exudation

There was large interannual variability in the concentration of NSC in fine roots (Fig. 4). In 2012, there was a decline in NSC concentration midsummer relative to the spring and fall. In 2011, there was a slight but significant increase in NSC concentration across the growing season ($P < 0.001$). Red oak roots had significantly lower NSC concentration than white ash and eastern hemlock ($P < 0.001$; Fig. 4).

Exudation rate was highly variable and there was no clear stand-level difference or seasonal pattern, although there were significantly lower exudation rates in early spring compared to summer and fall (Table 3). Exudation rates for red oak, eastern hemlock, and white ash were 47 ± 24, 55 ± 25, and 46 ± 11 g $C \cdot m^{-2} \cdot gs^{-1}$, respectively.

Total belowground C flux

In the red oak stand, TBCF was 791 ± 94 g $C \cdot m^{-2} \cdot gs^{-1}$ based on $TBCF_{top}$, and 608 ± 85 g $C \cdot m^{-2} \cdot gs^{-1}$ based on $TBCF_{bottom}$. Of $TBCF_{bottom}$, 49% was allocated to root production, 38% to root respiration, 8% to exudation, and 5% to mycorrhizal fungi (Fig. 5, orange bars). In the eastern hemlock stand, $TBCF_{top}$ was 474 ± 29 g $C \cdot m^{-2} \cdot gs^{-1}$ and $TBCF_{bottom}$ was 380 ± 52 g $C \cdot m^{-2} \cdot gs^{-1}$. Approximately 11% of this flux was allocated to root production, 64% to root respiration, 14% to exudation, and 11% to mycorrhizal fungi (Fig. 5, purple bars). In the white ash stand, $TBCF_{top}$ was 415 ± 67 g $C \cdot m^{-2} \cdot gs^{-1}$, while $TBCF_{bottom}$ was 511 ± 58 g $C \cdot m^{-2} \cdot gs^{-1}$. Twenty-six percentage of $TBCF_{bottom}$ was allocated to root production, 53% to root respiration, 9% to exudation, and 12% to mycorrhizal fungi (Fig. 5, blue bars).

$TBCF_{top}$ was larger than $TBCF_{bottom}$ in the oak and hemlock stands (Table 4). In the red oak stand, $TBCF_{top}$ was 183 ± 34 g $C \cdot m^{-2} \cdot gs^{-1}$ larger than $TBCF_{bottom}$. Estimates of $TBCF_{bottom}$ were largest in the red oak and ash stands and smallest in the eastern hemlock stand. The phenology of $TBCF_{bottom}$ also differed between stands (Fig. 6). Red oak stands allocated more C belowground earlier in the growing season compared to white ash and eastern hemlock. The peak in $TBCF_{bottom}$ in red oak was coincident with the spring ramp-up of GPP. In eastern hemlock stands, the phenology of $TBCF_{bottom}$ was not pronounced. In both oak and hemlock stands, soil respiration peaked later in the season than either GPP or $TBCF_{bottom}$.

Discussion

Data on timing and partitioning of C to belowground processes are uncommon (Dohleman et al. 2012, Fahey et al. 2013). This study analyzes the seasonal dynamics of multiple root processes over 2–3 years in a midlatitude forest. We observed significant stand-level differences in belowground C flux and the partitioning of C to root growth, exudation, and respiration. The phenology of TBCF also differed broadly between stands, especially in the evergreen hemlock stand compared to the deciduous red oak and ash stands where the peak in TBCF was coincident with the peak in GPP. We found broad support

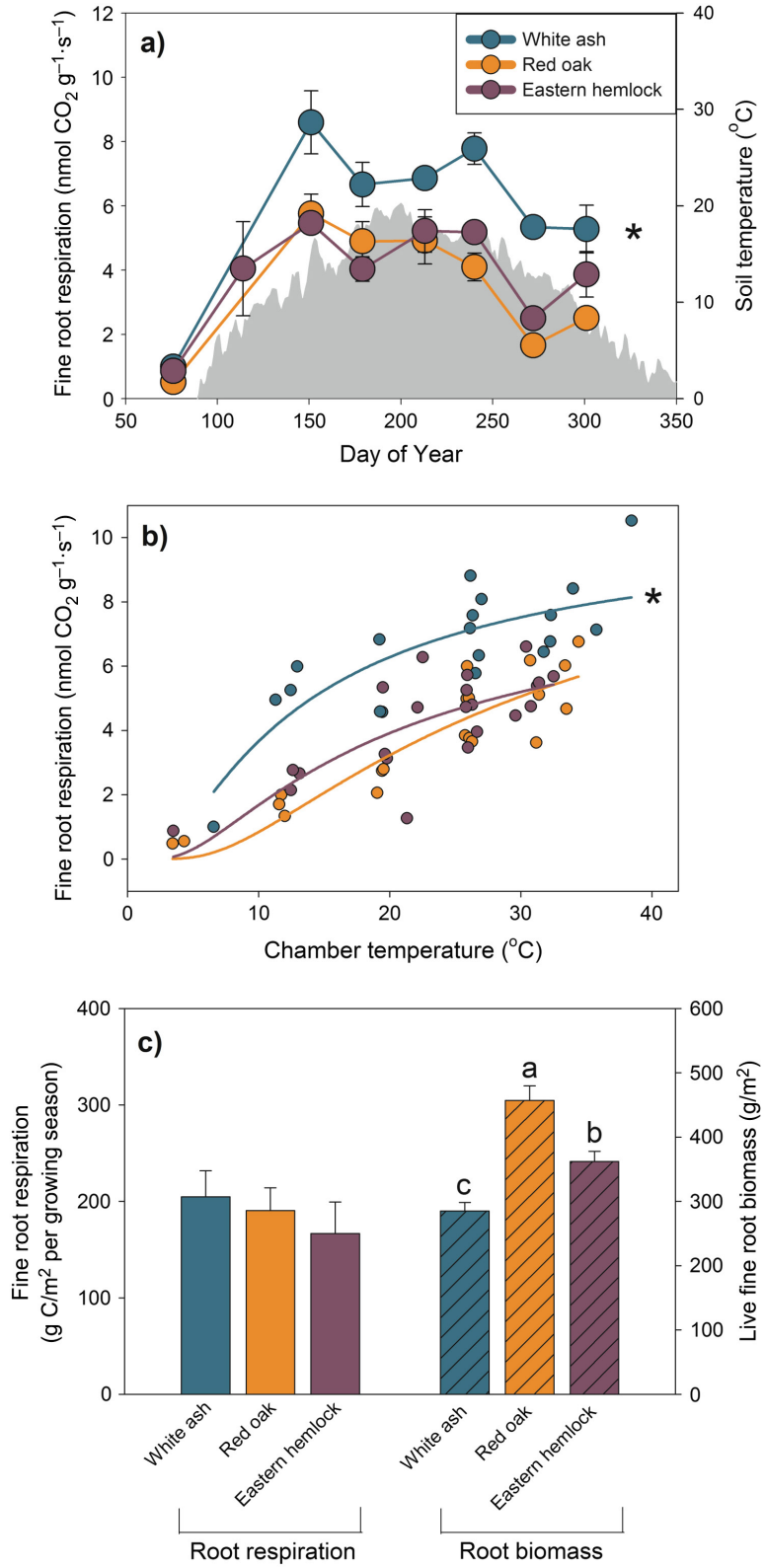
a)
White ash
Red oak
Eastern hemlock
Fine root respiration (nmol CO_2 g^{-1}·s^{-1})
Soil temperature (°C)
Day of Year
*
b)
Fine root respiration (nmol CO_2 g^{-1}·s^{-1})
Chamber temperature (°C)
*
c)
Fine root respiration (g C/m^2 per growing season)
Live fine root biomass (g/m^2)
a
b
c
White ash
Red oak
Eastern hemlock
Root respiration
Root biomass

Fig. 2. (a) Field measurements of monthly mean mass-specific fine root respiration (±SE) in 2013 (left axis). The shaded area represents soil temperature (right axis). (b) Relationship of fine root respiration to the temperature of the measurement chamber (n = 61). Lines are nonlinear fits of the Arrhenius function to data for each stand. (c) Fine root respiration (±SE) scaled to the growing season using fine root biomass from soil coring. In (a) and (b), the asterisk indicates that white ash had significantly higher respiration rates than did red oak and eastern hemlock. In (c), letters indicate significant differences between stands at the $P < 0.001$ level ($F_{2,57} = 13.1$, $P < 0.001$).

for two of our three hypotheses and a proximate explanation for the increase in the biomass of red oak throughout the Harvard Forest over the last two decades. The emergence of the invasive pest hemlock woolly adelgid during the course of this study may have negatively affected belowground C allocation and root production in hemlock from 2012 to 2014. The data reported here suggest that the phenology of belowground C flux depends on stand type, seasonal temperature variation, and C supply.

Phenology of root growth and mortality

We observed multiple flushes of fine root growth in the red oak stand in 2012 (Fig. 1a). Our direct observation of multiple root flushes supports the inference of Cardon et al. (2002) who assumed that a negative relationship between shoot elongation and soil respiration indicated red oak roots flush multiple times per growing season. In contrast to 2012, there were fewer flushes in the red oak stand root growth in 2013 and 2014, although this may reflect sampling intensity. The minirhizotrons were sampled biweekly in 2012 and monthly in 2013 and 2014, and the lower sampling frequency may have missed flushing episodes. Alternatively, there may have been fewer flushes in these years.

Consistent with our first hypothesis, we observed stand-level differences in fine root growth phenology. Fine root growth was initiated earlier in the growing season in the hardwood stands compared to the hemlock stand (Fig. 1a–c). The hardwood stands also had more

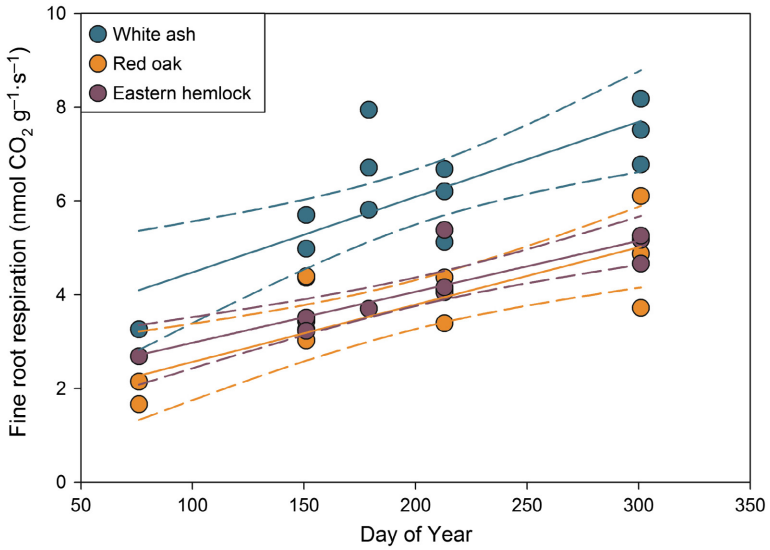

Fig. 3. Mass-specific fine root respiration measured in the laboratory at temperatures ranging between 16°C and 25°C, with a mean of 22.8 ± 0.5°C. There is a significant positive relationship between fine root respiration and sample date for white ash (β = 0.016, $F_{1,11} = 15.3$, $P < 0.01$, $R^2_{adj} = 0.54$), red oak (β = 0.012, $F_{1,9} = 17.6$, $P < 0.01$, $R^2_{adj} = 0.62$), and eastern hemlock (β = 0.011, $F_{1,9} = 32.1$, $P < 0.001$, $R^2_{adj} = 0.76$). Dashed lines are 95% confidence intervals around the regression fit.

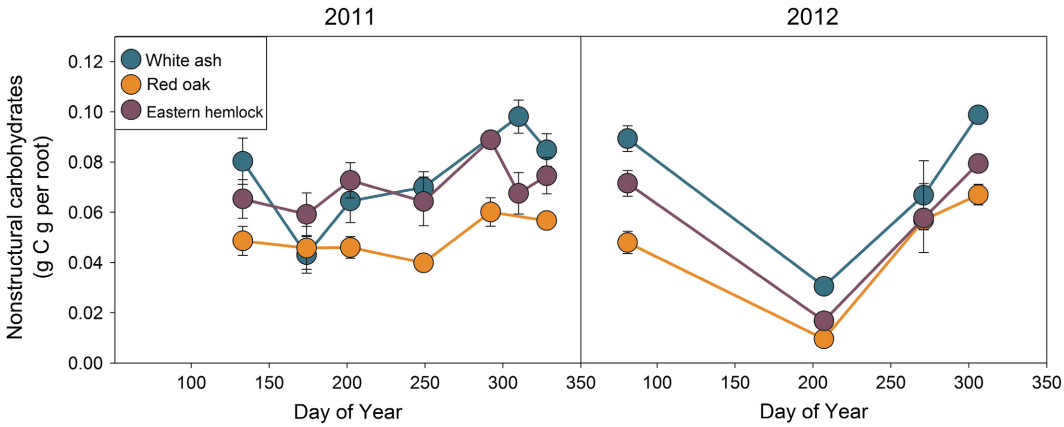

Fig. 4. Total nonstructural carbohydrates (±SE) from n = 6 samples from each stand. There are significant differences between years ($F_{1,1089}$= 23.7, P < 0.001), sample dates ($F_{1,1089}$= 66.9, P < 0.001), and stands ($F_{2,1089}$= 67.0, P < 0.001). Each stand is significantly different from the other two stands in both years.

synchronous above- and belowground phenology than the evergreen hemlock stand, a result consistent with our recent meta-analysis examining the phenology of root relative to shoot growth among biomes (Abramoff and Finzi 2015). The offset between maximum canopy greenness, a proxy for aboveground phenology, and root growth was about 30 d shorter in the deciduous compared to coniferous stands (Figs. 1 and 6).

Environmental controls over root growth and respiration

Root growth and respiration were positively correlated with soil temperature (data not shown). The positive correlation between root growth and temperature observed here has been recorded in a variety of ecosystems using both observational (Teskey and Hinekley 1981, Bevington and Castle 1985) and experimental approaches (Tryon and Chapin 1983, Lahti et al. 2005). This positive relationship between root growth and temperature reflects a number of processes including enhanced C supply due to photosynthesis, increases in the rate of cell division, and lower resistance to water uptake favoring cell expansion (Lambers et al. 2008). Although high temperatures (>30°C) can inhibit root growth, we did not observe soil temperatures greater than 24°C (Barney 1951, Graves et al. 1991).

The apparent temperature sensitivity (E_a) of mass-specific root respiration was lowest in the ash stands and highest in the red oak stand (Table 5). In ash, the rate of root respiration was high throughout the growing season, whereas respiration in red oak and eastern hemlock roots more closely followed the seasonal cycle of temperature (Fig. 2a). Similarly, the concentration of nitrogen was significantly higher in the fine roots of the ash stand than either red oak or hemlock. Given the correlation between tissue N concentration and root respiration (Reich et al. 1998, Burton et al. 2002), root chemistry may be a proximate cause for the consistently high mass-specific respiration rates observed in the ash stand. Notably at the plot scale, total fine root respiration (g C·m^{-2}·gs^{-1}) was highest in the ash

Table 3. Exudation rate (±SE) in mg C·g root^{-1}·d^{-1} for samples collected in 2012 and 2013.

Stand	June-12[a]	August-12[ab]	April-13[b]	May-13[ab]	July-13[a]	October-13[a]
White ash	1.35 (0.43)	1.14 (0.25)	0.29 (0.23)	1.03 (0.07)	2.69 (0.68)	1.39 (0.34)
Red oak	2.65 (1.34)	0.26 (0.10)	−0.47 (0.33)	0.52 (0.09)	1.37 (0.54)	0.70 (0.24)
Eastern hemlock	0.78 (0.35)	0.36 (0.07)	0.02 (0.17)	0.56 (0.25)	2.23 (0.99)	3.59 (1.77)

Note: Letters indicate significant differences between exudation rates on each date at the P < 0.05 level.

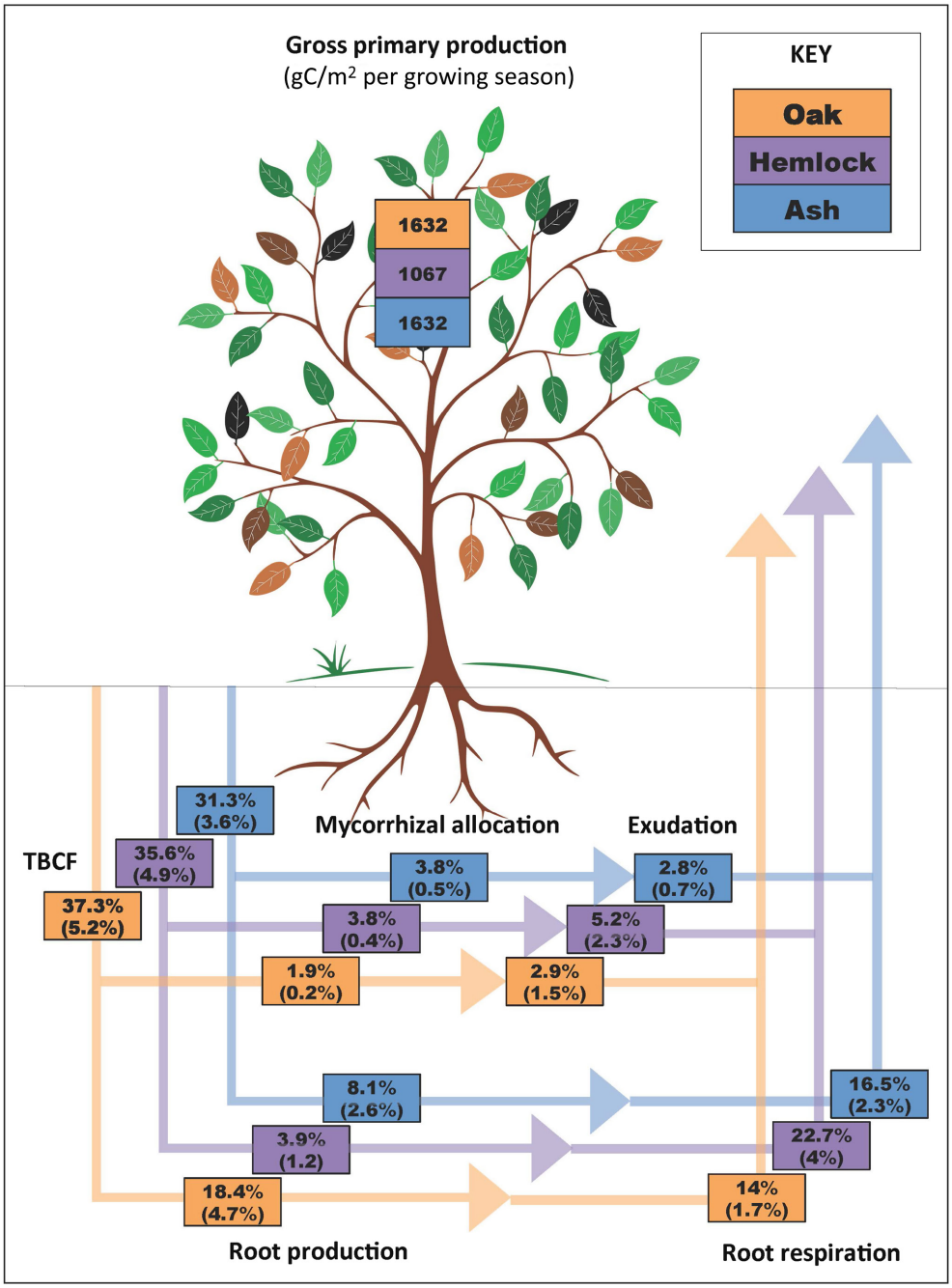

Fig. 5. Gross primary production and $TBCF_{bottom}$ (fine root production, root respiration, mycorrhizal allocation [production + respiration], exudation) for each stand. $TBCF_{bottom}$ and its components are expressed as a percentage of gross primary production. Standard error is reported in parentheses next to each pool or flux value, and all units are g $C \cdot m^{-2} \cdot gs^{-1}$. The growing season is defined as the six-month period from May to October.

Table 4. Belowground fluxes ($TBCF_{bottom}$ [fine root production, root respiration, exudation, mycorrhizal production, mycorrhizal respiration] and $TBCF_{top}$ [soil CO_2 efflux, leaching, litterfall, ΔC_{roots}]) for each stand.

Method	Flux or pool	White ash	Red oak	Eastern hemlock
$TBCF_{bottom}$	Root production	$133^{ab,\dagger}$ (42)	301^{a} (76)	42^{b} (13)
	Root respiration	270^{a} (37)	229^{a} (28)	242^{a} (43)
	Exudation	46^{a} (11)	47^{a} (24)	55^{a} (25)
	Mycorrhizal production	44^{a} (7)	3^{c} (0.4)	14^{b} (2)
	Mycorrhizal respiration	18^{b} (3)	28^{a} (4)	27^{a} (4)
	Total	511^{b} (58)	608^{a} (85)	380^{c} (52)
$TBCF_{top}$	Soil CO_2 efflux	530^{c} (38)	748^{a} (16)	696^{b} (15)
	Leaching	1 (0.1)	1 (0.1)	1 (0.1)
	Litterfall	193^{b} (6)	183^{c} (2)	224^{a} (8)
	ΔC_{roots}	77^{ab} (55)	225^{a} (93)	1^{b} (23)
	Total	415^{b} (67)	791^{a} (94)	$474^{b,\dagger}$ (29)

Notes: Standard error is reported in parentheses next to each pool or flux value, and all units are g $C \cdot m^{-2} \cdot gs^{-1}$. The growing season is defined as the six-month period from May to October.

$TBCF_{top}$ is equal to $F_{efflux} + F_{leaching} - F_{litter} + \Delta(C_{roots} + C_{soil})$. $TBCF_{bottom}$ is equal to $F_{roots} + F_{resp} + F_{exudates} + F_{fungi}$. Letters indicate significant differences between stands at the $P < 0.05$ level.

† Marginally significantly different ($P < 0.1$).

stand despite it having ~50% lower biomass than that in red oak.

When incubated at a common temperature, the rate of root respiration increased across the growing season in all three species (Fig. 3). This suggests an increase in photosynthate allocation to roots through time and that the decline of respiration rates in the fall is related to temperature

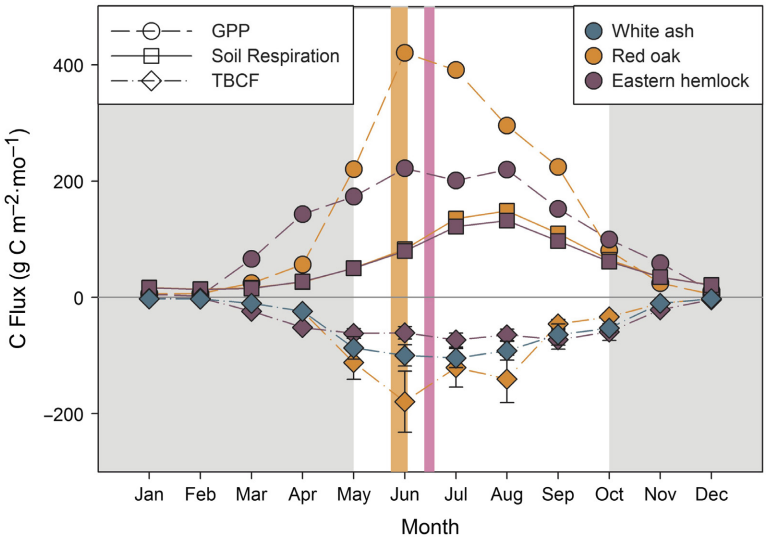

Fig. 6. Time series of gross primary production estimated using data from the Harvard Forest Environmental Measurement Station flux tower and the hemlock tower in 2012 (circles). Soil respiration for the eastern hemlock and red oak stands (squares) and root $TBCF_{bottom}$ (diamonds) for red oak, eastern hemlock, and white ash stands calculated using root GPP, respiration, and exudation (excluding mycorrhizal allocation) from May to October. For January–April and November–December when data were not available (shaded areas), we used the median ratio of TBCF:GPP to extrapolate TBCF from GPP data. This ratio (5th, 95th percentiles) was 0.42 (0.23, 0.5) for hardwoods and 0.36 (0.28, 0.57) for hemlock. The orange and pink bars show the period of maximum canopy greenness in red oak and eastern hemlock stands, respectively, for this study period determined using PhenoCam data (http://phenocam.sr.unh.edu/).

Table 5. Activation energy (E_a), pre-exponential constant (A), and R^2 of mass-specific rates of root respiration for each stand over the growing season.

Species	E_a (kJ/mol)	A (nmol $CO_2 \cdot s^{-1} \cdot g^{-1}$)	R^2
White ash	20	2.1×10^4	0.60
Red oak	40	3.6×10^7	0.81
Eastern hemlock	29	5.7×10^5	0.56

rather than acclimation of root respiration or substrate limitation. This observation is qualitatively similar to that of Burton and Pregitzer (2003) who did not find acclimation of root respiration across the growing season in sugar maple stands in Michigan. Both studies contrast with the apparent acclimation of root respiration in response to experimental soil warming at the Harvard Forest (Burton et al. 2008).

Nonstructural carbohydrates and root exudation

Root NSC concentrations varied significantly between stands ($F_{2,1089} = 67.0$, $P < 0.001$), between years ($F_{1,1089} = 23.7$, $P < 0.001$), and within each growing season ($F_{1,1089} = 66.9$, $P < 0.001$; Fig. 4). In 2012, there was a strong apparent seasonal decline in root NSC midsummer. In 2011, there was no evidence for a seasonal decline. The 2012 decline may reflect the metabolism of NSC for root growth and maintenance respiration (Lynch et al. 2013), and the difference between years may reflect interannual variation in allocation to root NSC pools. In contrast to stemwood NSC (Richardson et al. 2013), it is also possible that the seasonal trend in NSC concentration varies over timescales finer than the monthly sampling interval used here.

In contrast to root NSC, there was no significant difference in exudation rate among stands ($F_{2,106} = 0.89$, $P > 0.05$). Brzostek et al. (2013) similarly found no difference in the rate of root exudation among four stand types, including the hemlock and ash plots studied here. Exudation did, however, vary significantly at the seasonal timescale with the main distinction being significantly lower rates in the spring (DOY = 106) compared to summer and fall collection dates (Table 3). This pattern of exudation mirrors the seasonal increase in mass-specific rates of root respiration and supports the idea of a progressive increase in C allocation belowground across the growing season, which may occur as a result of a trade-off with leaf and stem growth earlier in the growing season (Fig. 3; Wolf et al. 2011).

Total belowground C flux

$TBCF_{bottom}$ varied between 31% and 37% of GPP, while $TBCF_{top}$ varied between 25% and 48% of GPP (Table 4). Consistent with our first hypothesis, red oak allocated C belowground earlier in the growing season compared to eastern hemlock. Supporting our second hypothesis, the red oak and ash stands had the highest rates of TBCF. In contrast to our third hypothesis, the hemlock stand did not allocate a high proportion of its GPP to TBCF, nor did it allocate a high proportion of TBCF to fine root production. The red oak stand had the greatest proportional allocation to fine root production (Fig. 5).

Eddy-covariance estimates of net ecosystem production at the Harvard Forest Environmental Measurement Station (EMS) site suggest a near doubling of C uptake from the atmosphere over the last 20 years (Urbanski et al. 2007, Keenan et al. 2012). The increase in C uptake is correlated with an increase in red oak productivity and biomass. Of the dominant species within the tower footprint, the concentration of N in red oak foliage is among the highest, and red oak has the most rapid rate of light-saturated net photosynthesis (Bassow and Bazzaz 1997). Given that belowground C allocation is required to acquire soil N, this analysis suggests that high TBCF in red oak has increased N uptake and facilitated its emergence as the species dominating C uptake within the EMS tower footprint (Fig. 5, Table 4).

The modest C investment in root production in the hemlock stand contrasts with evergreen trees in general, which tend to allocate a substantial fraction of C to roots (Gower et al. 2001, Clemmensen et al. 2013). Recently, this stand became infested by the invasive pest hemlock woolly adelgid (HWA), which became widespread at the Harvard Forest in 2012. The HWA feeds on phloem sap at the base of needles and progressively kills adult trees within 3–10 years of infestation (Orwig 2002, Orwig et al. 2008). Since 2012, there have been visible signs of crown thinning and HWA-induced tree mortality. Compared to 2012, the root biomass increment in the hemlock stand dropped by 96% in 2013 and 192% in 2014 relative to an

average decrease of 14% and 94% in the oak stand and 84% in the ash stand. Thus, it seems possible that low TBCF in this stand may reflect the negative effect of the HWA on photosynthesis and tree health. Surveys of hemlock roots in infested stands in Connecticut found that ectomycorrhizal colonization, bacterial abundance in the adjacent rhizosphere, and root C:N all declined (Vendettuoli et al. 2015). While we cannot exclude the possibility that hemlock has naturally low C investment to roots or that the three-year trend is due to chance, our direct observations of crown thinning in the area surrounding the minirhizotron tubes and measurements would seem to suggest otherwise (A. Finzi, *personal observations*). We are continuing measurements in this stand as the hemlock continue to decline.

The phenology of $TBCF_{bottom}$ differs between stands and relative to that of GPP or soil respiration (Fig. 6). In the deciduous red oak stand, the peak in $TBCF_{bottom}$ precedes the peak in soil respiration but coincides with the spring ramp-up of GPP and its peak in the early summer. This suggests that belowground C allocation in oak is strongly dependent upon the supply of photosynthate. This result is consistent with pulse-chase experiments demonstrating rapid transfer of C belowground (Högberg et al. 2008) and girdling experiments showing steep declines in springtime soil respiration owing to the absence of active roots (Högberg et al. 2001).

The phenology of $TBCF_{bottom}$ in white ash is similar to red oak. In contrast to the two deciduous stands, $TBCF_{bottom}$ remains low throughout the growing season in eastern hemlock. TBCF is dominated by root respiration in this stand, with root growth making up only 11% of $TBCF_{bottom}$. The phenology of soil respiration is similar in the oak and hemlock stand despite their differences in TBCF, possibly because the fluxes that contribute directly (root respiration) or indirectly (NSC accumulation and exudation) to soil respiration have similar phenology in both stands.

$TBCF_{top}$ was greater than $TBCF_{bottom}$ in two of the three stands. $TBCF_{top}$ may be consistently high due to an overestimate of ΔC_{root} in $TBCF_{top}$, an underestimate of root production, respiration, mycorrhizal allocation, or exudation in $TBCF_{bottom}$, or some combination of both. The largest difference between $TBCF_{top}$ and $TBCF_{bottom}$ (~183 g $C \cdot m^{-2} \cdot gs^{-1}$) was observed in the red oak stand. In this stand, ΔC_{root} estimated from minirhizotron images was 225 g C/m^2 (Fig. 1g–i), but there was no significant change in root biomass in soil cores collected across the growing season in 2012 (data not shown). Because of high spatial variability (Taylor et al. 2013), the absence of a change in root biomass in one year does not exclude the possibility of high root biomass increment. However, the three-growing-season average root biomass increment of 225 g C/m^2 estimated from minirhizotron imaging is about one-half of the standing crop (Fig. 2c), and should be measurable in soil cores. TBCF in red oak is therefore likely to reside between the two methods used to estimate this flux.

Implications

Climatic and atmospheric perturbations alter the magnitude of C inputs belowground (e.g., Drake et al. 2011), and thus at the interannual timescale, it is likely that changes in the quantity and phenology of belowground C inputs will influence soil biogeochemical cycling. Warming-induced increases in growing season length have significantly increased annual ecosystem C uptake at Harvard Forest, a large portion of which is hypothesized to be allocated belowground (Keenan et al. 2014). From this study, it is clear that stand-level characteristics, such as differences in dominant growth form and susceptibility to pests, can interact strongly with temperature to determine the phenology and partitioning of C belowground.

Acknowledgments

The authors thank Colin Averill, John Drake, William Munger, and Patrick Sorensen for use of data. The authors also thank Andrew Richardson (Harvard University) for making data from the PhenoCam network publicly available. Development of the PhenoCam network has been supported through the National Science Foundation (award EF-1065029). This research was supported by the American Association of University Women (AAUW) American Dissertation Fellowship, the Office of Science (BER), the U.S. Department of Energy (grant no. 10-DOE-1053), and the National Science Foundation (DEB-0743564).

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