esa ECOSPHERE

Fine root respiration is more strongly correlated with root traits than tree species identity

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Citation: Paradiso, E., F. Jevon, and J. Matthes. 2019. Fine root respiration is more strongly correlated with root traits than tree species identity. Ecosphere 10(11):e02944. 10.1002/ecs2.2944

Abstract. Carbon allocated to roots accounts for a large portion of net primary productivity, but the fate of that carbon is poorly understood. Absorptive fine roots are the primary way in which plants acquire nutrients. Previous studies have evaluated relationships among root morphological traits, including specific root length, root tissue density, and mycorrhizal colonization, across broad functional and taxonomic groups to test for the existence of a root economics spectrum (RES). Fine roots also release carbon dioxide through respiration, and other studies have found relationships between root morphological traits and root respiration within individual tree species. The objective of this study was to measure a suite of root traits in six co-occurring temperate tree species that represent a diverse set of aboveground traits to determine whether and how root characteristics influenced root respiration both within and among species. At the Harvard Forest in Petersham, Massachusetts, USA, we measured fine root respiration, root morphology, percent colonization for ectomycorrhizal species, and carbon and nitrogen concentrations on 292 roots from six tree species in June and July 2018. We found that most fine root morphological characteristics varied nearly as much within each tree species as they did among the six species. Root traits were dynamic over time during the two months of our study, where the magnitude of weekly mean trait values varied 32–95% across the study period. Strong correlations among traits suggested trade-offs on a spectrum from resource acquisition (long, thin, high-nitrogen roots) to resource conservation (thick, dense, low-nitrogen roots), and traits were not clustered by tree species within this spectrum. Along with temperature and weekly temporal variation, the resource acquisition strategy (long and thin roots that were high in nitrogen) was associated with higher root respiration, and this relationship was consistent among the six species. This study supported a strong link between the RES and respiration independent of species identity, which provides insight into functional axes for scaling root respiration from individual trees to the forest stand to better quantify belowground carbon flux.

Key words: belowground carbon flux; fine root morphology; root economics spectrum; root nitrogen; root respiration; specific root length; temperate forest.

Received 15 May 2019; revised 22 September 2019; accepted 8 October 2019. Corresponding Editor: Keirith Snyder.

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INTRODUCTION

Roots play an important role in the terrestrial carbon cycle: In addition to acquiring nutrients to support plant productivity, fine root production and turnover represent ~22% of global terrestrial net primary productivity (McCormack et al. 2015a) if not more (Jackson et al. 1997). However, the role that fine roots play within the carbon cycle of individual ecosystems remains less understood than more readily measured aboveground components (Erktan et al. 2018).

Total belowground carbon flux is the most variable component of forest carbon budgets (Litton et al. 2007), and roots remain understudied relative to other plant traits (Iversen et al. 2017, Laliberté 2017).

One proposed unifying theory for fine root traits is based on the leaf economics spectrum (LES) concept, a widely supported continuum of leaf investment strategies based on physiological trade-offs (Wright et al. 2004, Donovan et al. 2011). The most important traits in the LES include photosynthetic capacity, leaf mass per area, leaf nitrogen concentration, and leaf lifespan (Shipley et al. 2006). Tree species span the LES from thick, long-lived leaves with low photosynthetic capacity and nitrogen concentration (a resource conservation strategy), to thin, short-lived leaves with high rates of photosynthesis and nitrogen concentration (a resource acquisition strategy; Wright et al. 2004). The LES, which scales with demographic rates, helps to unify our understanding of plant traits and how they relate to plant community and ecosystem processes and environmental change (Wright et al. 2004, McMahon et al. 2011, Weemstra et al. 2016). More recently, evidence that root traits may follow a similar conservation/acquisition trade-off has gained support, leading to the suggestion of a root economics spectrum (RES; Roumet et al. 2016).

The hypothesized RES proposes that resource-acquisitive fine roots have high specific root length (SRL), high root nitrogen concentration, and low root tissue density (RTD), while resource-conservative roots have high RTD and low turnover (Roumet et al. 2016). This is based primarily on how these traits' analogs aboveground operate. While some studies appear to support coordination between aboveground and belowground traits (Freschet et al. 2010, Reich 2014, Valverde-Barrantes et al. 2017, de la Riva et al. 2018), others do not (Withington et al. 2006, Chen et al. 2013, Kramer-Walter et al. 2016, Liese et al. 2017). Correlations among root traits tend to be lower than those among leaf traits, suggesting that leaf traits are more strongly constrained by this trade-off (Roumet et al. 2016). This may be because of the strong local controls that soil moisture and soil nutrient content exerts on root morphology (de la Riva et al. 2018) or because some root traits (e.g., root diameter) are more phylogenetically structured than leaf traits (Valverde-Barrantes et al. 2017). In addition, mycorrhizal colonization provides a potential additional axis of variation in this trade-off. Plants that associate with arbuscular species tend to have lower branching intensity and thicker roots than those that associate with ectomycorrhizae (Comas et al. 2014, Eissenstat et al. 2015), but mycorrhizal type is only weakly correlated with most root morphological traits (Valverde-Barrantes et al. 2017). Regardless, the existence and importance of both a whole plant economics spectrum and a root economics spectrum remains equivocal.

There is some empirical evidence supporting root trait coordination consistent with an acquisition/conservation trade-off. In temperate trees, root lifespan is negatively related to SRL, nitrogen (N): carbon (C) ratio and positively related to root diameter (Luke Mccormack et al. 2012). Other work suggests that the spectrum may require multiple dimensions (Kramer-Walter et al. 2016). For example, there is support for a trade-off between high SRL (acquisitive) and high RTD (conservative) strategies, although root diameter may be independent of the trade-off (de la Riva et al. 2018). Similarly, Kong et al. (2016) found that thin fine roots (defined by the authors as <0.247 mm) follow a RES with an acquisition–conservation trade-off, but thicker fine roots did not. Additional empirical studies are needed to better understand the extent to which root traits adhere to this proposed framework.

Root traits may also be temporally and spatially dynamic. A recent meta-analysis revealed large variation within biomes in the synchronicity between aboveground and belowground growth (Abramoff and Finzi 2015). In temperate tree species, there is much wider variation in spring root phenology than leaf phenology (McCormack et al. 2015b), and seasonal patterns in root production vary by species (McCormack et al. 2014). Individual trees can have variable root turnover rates (Guo et al. 2008, Brunner et al. 2013, Kubisch et al. 2016). Changes in root traits over time have been linked to environmental changes (Montagnoli et al. 2012), and root tissue nitrogen concentration may be more responsive to temporal patterns than root morphology (Zadworny et al. 2015). Soil nutrient content (Farley and Fitter 1999, Hodge 2004) and other environmental gradients (de la Riva et al. 2016, 2018) also influence root traits, and the variation in root traits caused by temporal and spatial variation relative to other factors remains unclear.

Beyond understanding life history strategies, root traits may also directly influence terrestrial carbon cycling by controlling root respiration rate. Fine root respiration can account for anywhere from 10% to 90% of total soil respiration (Hanson et al. 2000), although values of 22–45% are typical in temperate forests (Savage et al. 2013). This wide range of estimates is likely due to the many exogenous factors that influence root respiration rates. For example, fine root respiration is highly sensitive to soil temperature (Zogg et al. 1996, Atkin et al. 2000, Pregitzer et al. 2000), and previous work at our study site suggests that temperature can account for 56–81% of the variation in fine root respiration (Abramoff and Finzi 2016). Additional exogenous factors include moisture (Pregitzer et al. 2000, Burton and Pregitzer 2003), soil fertility (Li et al. 2017), gross primary productivity (Hopkins et al. 2013), carbon availability to roots (Craine et al. 1998), phenology (Abramoff and Finzi 2015, 2016), and atmospheric CO_2 concentration (Clinton and Vose 1999).

Root morphology and chemical composition have previously been linked to root respiration. Root respiration rate decreases with ascending branch orders such that first-order roots have the highest root respiration rate (Jia et al. 2013, Rewald et al. 2014). Indeed, root characteristics associated with an acquisition strategy within the RES framework, such as high SRL and root nitrogen concentration, tend to correlate with higher root respiration (Makita et al. 2009, Roumet et al. 2016, Miyatani et al. 2018). However, gaining a better understanding of the relative importance of root morphology and root tissue nutrient composition will improve our understanding of the functional physiology of roots at the organ level and its control on this globally important carbon flux.

The goal of this project was to assess variation in fine root traits and respiration rates within and across six temperate tree species representing a diverse set of aboveground resource acquisition strategies. We tested (1) whether species with distinct leaf functional traits also had distinct root traits (SRL, RTD, mean diameter, mycorrhizal colonization rates, and root carbon and nitrogen concentrations), (2) whether these traits were temporally dynamic during June and July, and (3) the extent to which tree species identity, root traits, and/or environmental factors explained variation in root respiration rates. We used our data to evaluate the hypothesis that roots exhibit a resource conservation/acquisition trade-off by quantifying the relationships among root traits. We investigated whether the root morphology and chemical composition of individual tree species clustered on this spectrum, or whether fine roots within a species span this spectrum. Finally, we used model comparisons to explore the variation in root respiration over time, and assess whether temperature, timing within the growing season, root traits, and tree species were important drivers of root respiration.

We hypothesized that if there was support for root trait coordination along a resource conservation/acquisition axis, then including this axis of root trait variation in a statistical model would better explain variation in root respiration rates than species alone. To test this hypothesis, we compared four alternate models to explain variation in root respiration rates with different sets of independent variables: (1) environmental variables, (2) the conservation/acquisition axis of root trait variation, (3) tree species identity, and (4) the conservation/acquisition axis and tree species identity. Based on previous work in our system (Abramoff and Finzi 2016), we expected that soil temperature and timing (weekly variation) would also be critical drivers of root respiration, and we therefore included these variables in all four models. This model comparison yielded insight into the relative importance of root traits, species identity, and temporal variation for explaining measured variability in root respiration rates, an important belowground carbon flux within this ecosystem.

METHODS

Study site

We conducted this work at the 35-ha ForestGEO plot (Orwig et al. 2015) in the Prospect Hill tract of the Harvard Forest, a secondary growth forest in Petersham, Massachusetts, USA (42.5393° N, −72.1779°, elevation ~350 m). We selected six study species that represent the most abundant canopy tree species in the 35-ha ForestGEO plot: *Quercus rubra*, *Fagus grandifolia*, *Betula lenta*, *Acer rubrum*, *Tsuga canadensis*, and *Pinus strobus* (Orwig et al. 2015). Based on their leaf characteristics and growth rates, these six tree species span a wide range of the LES and life history strategies (Reich 2014) and represented a phylogenetically diverse sample (Comas and Eissenstat 2009).

We used data from the Harvard Forest meteorological station to calculate weekly precipitation (Boose 2018) in order to assess whether any seasonal trends were influenced by precipitation. We also used meteorological data from a tower adjacent to our field plots (Munger and Hadley 2018) to calculate the mean temperature that corresponded to the time of our field respiration measurements.

Field methods

To take root respiration measurements, fine roots were collected directly from individual trees. This process involved removing leaf litter around the tree and carefully digging up fine roots that were traced back to a specific tree (Yanai et al. 2008) and then cutting them from the tree using a knife and recording the time at which the root was severed from the tree. All roots collected were within 2–5 cm of the soil surface. Severed roots have been successfully used in several previous studies to assess fine root respiration (Zogg et al. 1996, Burton and Pregitzer 2003, Burton et al. 2012, Abramoff and Finzi 2016). Roots were cut into smaller functional root systems such that only root orders 1–3 were analyzed, as higher orders likely contain transport as well as absorptive fine roots (McCormack et al. 2015a). For each tree, we typically sampled four functional root systems. In total, we measured multiple functional root systems from 22 *A. rubrum* trees, 20 *B. lenta* trees, 24 *F. grandifolia* trees, 21 *P. strobus* trees, 20 *Q. rubra* trees, and 21 *T. canadensis* trees distributed across the study period (Appendix S1: Fig. S1).

Roots were measured in the field within two hours of collection (Burton and Pregitzer 2003, Abramoff and Finzi 2016). After roots were severed from the tree and subsampled, they were rinsed with deionized water and patted dry (Makita et al. 2009, Abramoff and Finzi 2016). The clean, dry root was then inserted into a glass chamber (475 mL in volume) connected to a Los Gatos Research Ultraportable Greenhouse Gas Analyzer (UGGA; 1 L/min flow rate) that collected CO_2 concentration measurements every five-seconds for a five-minute interval. We checked the calibration of the UGGA instrument before measurements, and we completed blank tests with a closed measurement system absent of roots to ensure that the system was not leaking and that the UGGA concentration response was stable at least twice daily. The UGGA analyzer does not control for temperature, and thus, we used temperature as a variable in subsequent respiration rate regression analysis.

Laboratory methods

Upon returning to the laboratory, we placed the root samples in a refrigerator until we scanned them (EPSON Perfection V600 Photo, Epson America, Inc., Long Beach, California, USA). We processed the images with ImageJ version 1.52a (Schneider et al. 2012) using the IJ_Rhizo macro that measures root morphological traits (Pierret et al. 2013). For scanning, we placed the root into a glass petri dish filled with deionized water. Each image was then manually assessed and, if necessary, edited to remove the edges of the petri dish and water ripples, and adjust the brightness and contrast to maximize the clarity of the image. Images were then run through the IJ_Rhizo macro using the default settings, which provided data for total root length (mm), total root volume (mm^3), and mean root diameter (mm) using methods described in Pierret et al. (2013).

After we scanned each root, the percent of root tips colonized by mycorrhizae (for the five ectomycorrhizal species only, all species excluding *A. rubrum*) was estimated visually in increments of 10 from 0% to 100% by counting the total number of root tips and the number that were colonized. Colonized tips were identified using their shape and color without the assistance of a microscope; thus, we refer to this metric as an estimate of colonization. Next, we dried the root at 60°C for a minimum of 24 h and measured dry root biomass using a microbalance. We cut root samples >10 mg into pieces <2.5 mm and randomly selected a subsample between 5 and

10 mg to measure total root carbon and nitrogen concentration on an elemental analyzer (Costech Analytical Model 041077; Costech Analytical Technologies, Valencia, California, USA). Root samples that were <10 mg were analyzed whole.

Data processing

We calculated root respiration rates by fitting a linear model to the relationship between time (s) and CO_2 concentration (ppm) within the root chamber, and we converted the slope of this linear model to CO_2 respiration flux in nmols carbon per second. We removed any calculated respiration values with an R^2 value <0.8 for the fit between the linear model and the actual concentration data. We also removed data where the dry root biomass was <1 mg. We ended up with complete root morphology, chemical composition, and respiration data for 48 *A. rubrum* roots, 46 *B. lenta* roots, 47 *F. grandifolia* roots, 53 *P. strobus* roots, 48 *Q. rubra* roots, and 50 *T. canadensis* roots. Sampling by species was relatively even during the study period (Appendix S1: Fig. S1).

We divided the root respiration measurements by the amount of dry root biomass in the sample to represent mass-specific root respiration (nmol $CO_2\ g^{-1}\cdot s^{-1}$). For subsequent statistical analysis, we calculated SRL (mm/g) by dividing total root length by root dry mass, RTD (g/mm^3) by dividing root dry mass by total root volume, mean root diameter (mm), percent mycorrhizal colonization (only for ectomycorrhizal species), root percent carbon content, root percent nitrogen content, and the ratio of root percent carbon to percent nitrogen (C:N). All data processing and analyses were performed in R (version 3.5.0).

Statistical analysis

We analyzed temporal patterns and species-level variation within each of the six measured root traits (SRL, RTD, mean diameter, percent nitrogen content, C:N, and percent mycorrhizal colonization) by constructing six linear mixed effects models, one model for each trait as the dependent variable. Each model included tree species, week, and their interaction as fixed effects, with a random intercept effect for individual tree.

To test the existence of a root trait economics spectrum, we fit a principal component analysis (PCA) model to the root morphology and chemical composition data to quantify the dominant dimensions of variation among the set of measured root traits. Because mycorrhizal colonization was only measured for the five ectomycorrhizal tree species, we conducted one PCA that included data from all six tree species and excluded mycorrhizal colonization, and a second PCA that only included data from the five ectomycorrhizal tree species and included mycorrhizal colonization. We examined whether tree species clustered within each PCA output by plotting the 95% confidence ellipses for each tree species onto the first two principal components.

We quantified the correlation among root respiration and individual root traits, components from the PCA results, and seasonal pattern. We evaluated covariation among the variables that potentially influenced root respiration by building a pairwise correlation matrix with the six root traits (excluding mycorrhizal colonization), temperature during respiration measurement, and root respiration. Based on the strong correlations among root traits found in previous studies (Roumet et al. 2016), we decided that if traits had pairwise correlation coefficients with an absolute value >0.3, we would use the coordinates (i.e., loadings) from the PCA root trait model that included all species, rather than the absolute values of each trait, within regression models. We also measured the correlation between respiration and principal components 1 and 2 and measurement temperature by calculating the correlation coefficients. We used each measurement week as a categorical factor to represent an integrated metric of temporal variation over our study period (June and July).

To assess the link between root respiration and root traits, measurement temperature, and phenology, we built a set of linear regression models. We fit four regression models to our root respiration data with different sets of independent variables capturing our hypotheses:

1. M1, Temperature and temporal pattern: measurement temperature + week
2. M2, Root economics spectrum: measurement temperature + week + PC1
3. M3, Tree species: measurement temperature + week + tree species
4. M4, Full model: measurement temperature + week + PC1 + tree species

Root respiration was the dependent variable for all models, which we log-transformed to follow a normal distribution, and all other independent variables followed a normal distribution. For all models, we included a random intercept effect of individual tree.

We fit all mixed effects models with the nlme package in R (Pinheiro et al. 2019). We quantified the ability of each regression model to capture variation in root respiration by calculating the model Akaike information criterion (AIC) value, and we used the MuMIn package to compare among the four model AIC values and coefficients (Bartoń 2018). We considered the model with the lowest AIC value to represent the best balance between the model fit and the number of model parameters. Both within and among linear regression models, we compared the regression coefficient estimates (effect sizes) and their standard errors, associated *P*-values, and the overall marginal and conditional R^2 values (Nakagawa and Schielzeth 2013). We used several additional packages within R for data manipulation and visualization, including dplyr (Wickham et al. 2018), lubridate (Grolemund and Wickham 2011), factoextra (Kassambara and Mundt 2017), corrplot (Wei and Simko 2017), and cowplot (Wilke 2019), which are represented within our code.

RESULTS

Traits by species across time

Variation within all six root traits was significantly explained by tree species identity and seasonal pattern (Fig. 1; Appendix S1: Table S1). Most traits had the largest departure from the growing season mean value in the last week of sampling. Root nitrogen concentration exhibited the most consistent temporal pattern among tree species, declining in all six species after mid-June. The weekly patterns in root traits were not influenced by the timing of sampling collection of particular species (Appendix S1: Fig. S1), nor by weekly patterns of precipitation (Appendix S1: Fig. S2).

Some traits varied more distinctly between species than others, but tree species identity explained significant variation within each of the six measured traits (Appendix S1: Table S1). Most strikingly, the RTD of *A. rubrum*, the only arbuscular mycorrhizal species in our study, was on average 57% lower than the RTD of the other five species (Fig. 1B). The mean diameter of *P. strobus* roots was on average 19% higher than the mean diameter of the other five species (Fig. 1A). While the RTD of all five ectomycorrhizal species was very similar, their SRL varied such that the conifers, *P. strobus* and *T. canadensis*, were generally lower than the three deciduous species.

Coordination of traits: the resource spectrum

Root traits had a high degree of interspecific overlap. The PCA for root traits conducted for all six species captured 72.5% of the variation in the first two PCA axes, with 52.8% variation on the first component (PC1; Fig. 2A). PC1 was positively related to RTD, mean diameter, and C:N and negatively related to root nitrogen concentration and SRL. The PCA model fit with just the ectomycorrhizal species was very similar, with percent mycorrhizal colonization related to the second PCA axis (Fig. 2B). Despite the differences in individual traits, across all species fine roots had high morphological variation and the different tree species did not cluster within either PCA.

Root respiration

Root respiration was significantly correlated with all root morphological characteristics, chemical traits, and temperature, with the strongest correlation to SRL and percent nitrogen (Fig. 3). Respiration was more strongly related to most of the root traits than it was to temperature. Root tissue density, diameter, and C:N were all positively correlated with one another and all negatively correlated with respiration. Due to the strong pairwise correlation among root characteristics and between individual root characteristics and respiration, we used the coordinates of the PCA model in subsequent respiration regression analysis rather than individual traits. The coordinates of the first component of the PCA model captured the dominant axes of synergistic variation among root characteristics (52.8% of total variation among the measured traits), while avoiding issues of multiple correlations among independent variables in a regression analysis.

Like root morphology, fine root respiration varied by species and through time (Fig. 4). We contrasted the ability of four different regression

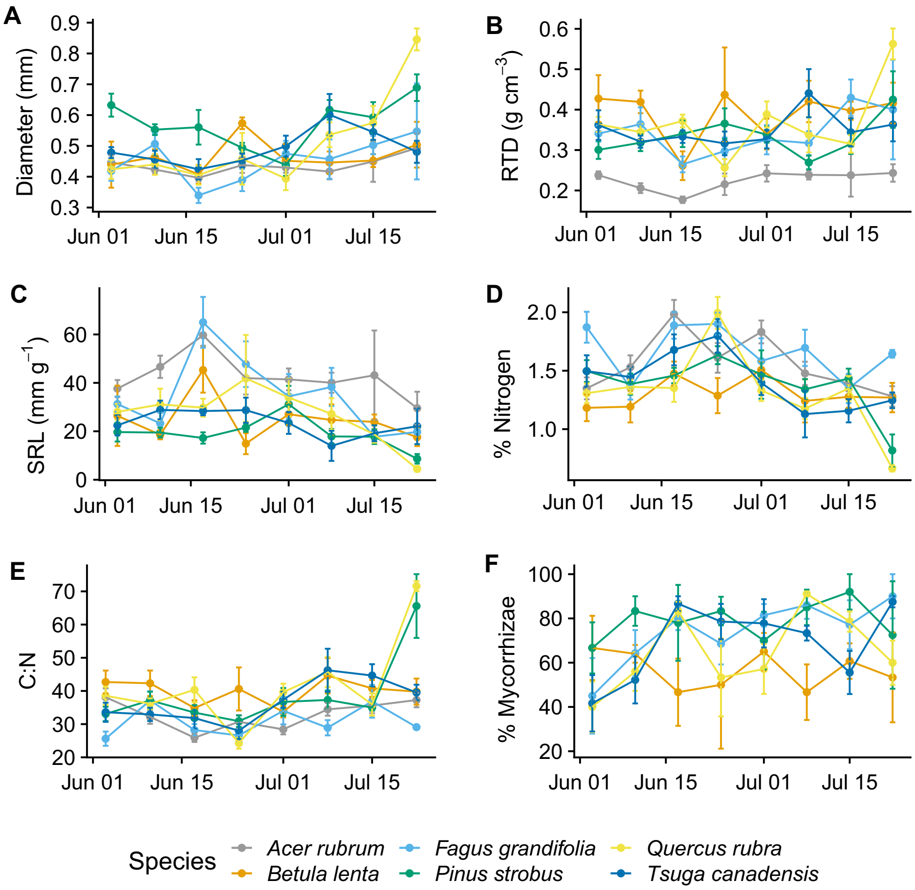

Fig. 1. Root trait values in each week sampled for (A) diameter, (B) root tissue density (RTD), (C) specific root length (SRL), (D) root percent nitrogen concentration, (E) root carbon to nitrogen ratio (C:N), and (F) percent mycorrhizal colonization (for ectomycorrhizal species only). Colors represent tree species. Points represent the mean trait value within each week and species, and error bars represent the standard error of the mean within each week.

models with a set of abiotic and biotic independent variables to describe this variation in our respiration measurements. The simplest regression model included independent variables for temperature and week (a proxy for temporal variation), and these variables captured 53% of the variation in root respiration measurements (Table 1). The best respiration model (the lowest AIC value) included the first principal component from the root characteristics PCA in addition to temperature and week (Table 1). Including the first component of the PCA captured an additional 16% of the variation in respiration measurements compared to the model with just temperature and week. We did not construct models with the second principal component from the PCA model due to the lack of a mechanistic explanation for a connection among these root characteristics and respiration rate in addition to the low correlation between this second component and respiration rate (Appendix S1: Fig. S3).

Including tree species identity, rather than the PCA coordinates, did not improve the AIC value, nor the R^2 value, compared to the model that included just temperature and week. The full model, which included temperature, week, the PCA coordinates, and tree species identity, had the second lowest AIC value among the models, but this model had the same R^2 value as the

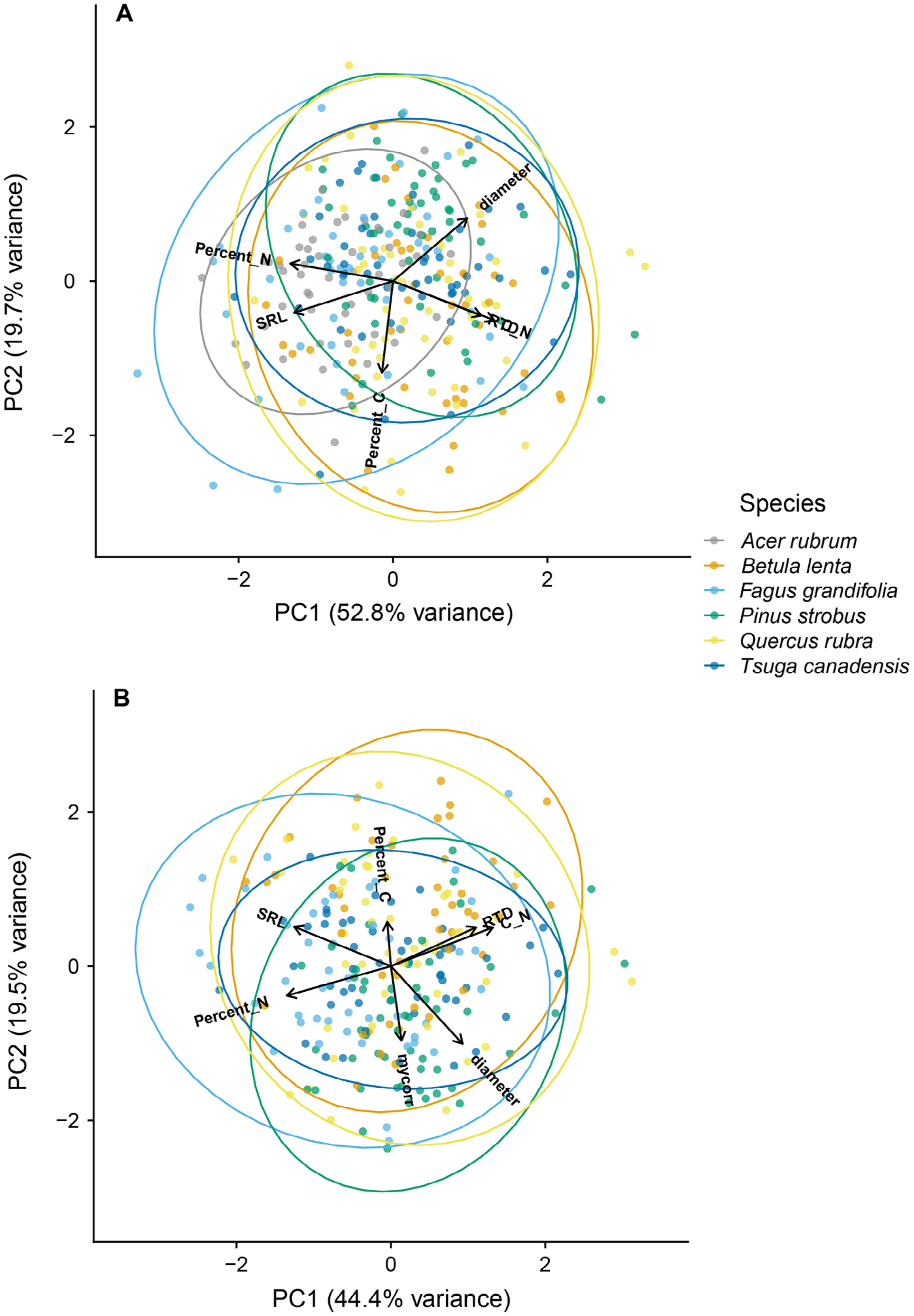

Fig. 2. Principal component analysis (PCA) of root traits. (A) Projection of six measured root traits in all six species (n = 292) onto the two main axes resulting from the PCA. (B) Projection of seven measured traits in the five ectomycorrhizal species (n = 243) on the plane defined by the two main axes resulting from the PCA model. Variables include percent nitrogen (Percent_N), specific root length (SRL), percent carbon (Percent_C), root tissue density (RTD), root C:N (C_N), mean diameter (diameter), and percent mycorrhizal colonization (mycorr). In both plots, RTD and C_N overlap.

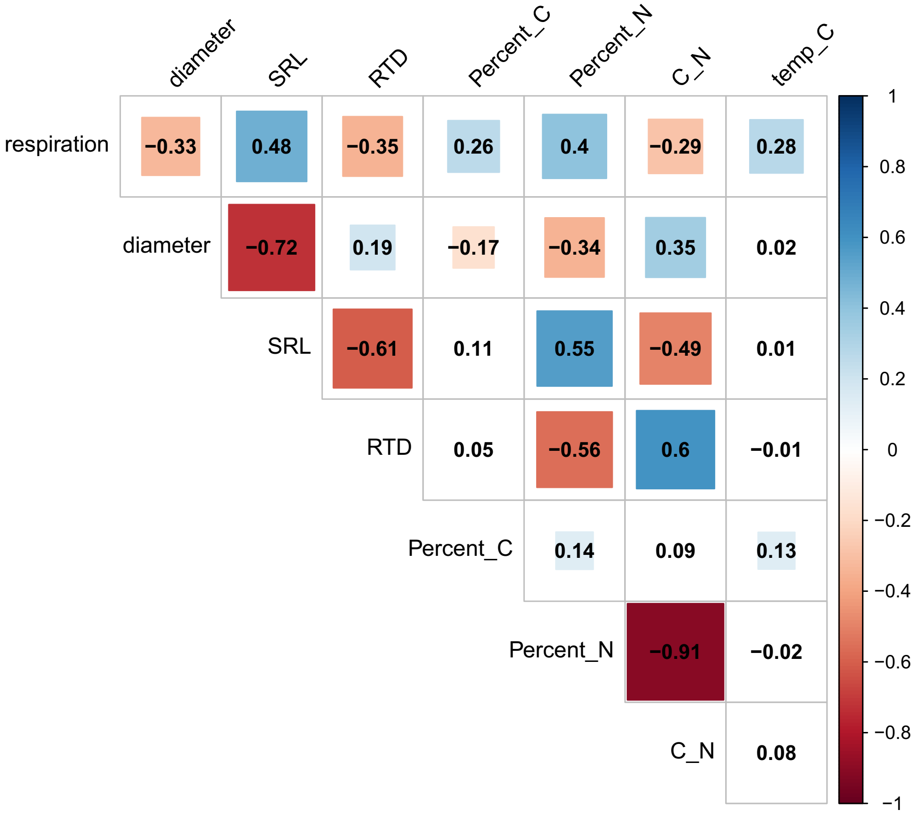

Fig. 3. Pairwise correlation matrix for root respiration, root characteristics, and temperature (°C) for all tree species (n_{species} = 6, n_{roots} = 292). Spearman's correlation coefficient is printed in each box, and darker blue boxes are traits that are more positively correlated with one another while darker red boxes represent traits that are more negatively correlated with one another. Boxes with no color were not significantly correlated ($P > 0.05$). Variable names as in Fig. 2.

model without tree species (Table 1). The model that included temperature, week, and species identity had the highest AIC value of the four models.

The estimated regression coefficient for the first principal component was the same for the two models that included this independent variable (Table 1). This suggested that the characteristics represented by this principal component—root percent nitrogen, SRL, RTD, and root C:N—played a strong and consistent role in explaining variation within root respiration rate, even when tree species was considered as an additional independent variable in the full model. The model coefficients for the fixed effects of week that captured the seasonal pattern were significant ($P < 0.05$), and effect sizes were consistent among the four models (Appendix S1: Fig. S4). The effects of week were uncorrelated with weekly precipitation (correlation coefficient = 0.13, $P > 0.05$). The random intercept effects for individual trees were similarly consistent among models. However, the model that included just temperature and week had a wider distribution of individual tree random effects, which suggested that some of the random effects were explained by the PCA coordinates (Appendix S1: Fig. S4).

DISCUSSION

Traits by species across time

We were somewhat surprised to find such a strong effect of temporal variation on measured

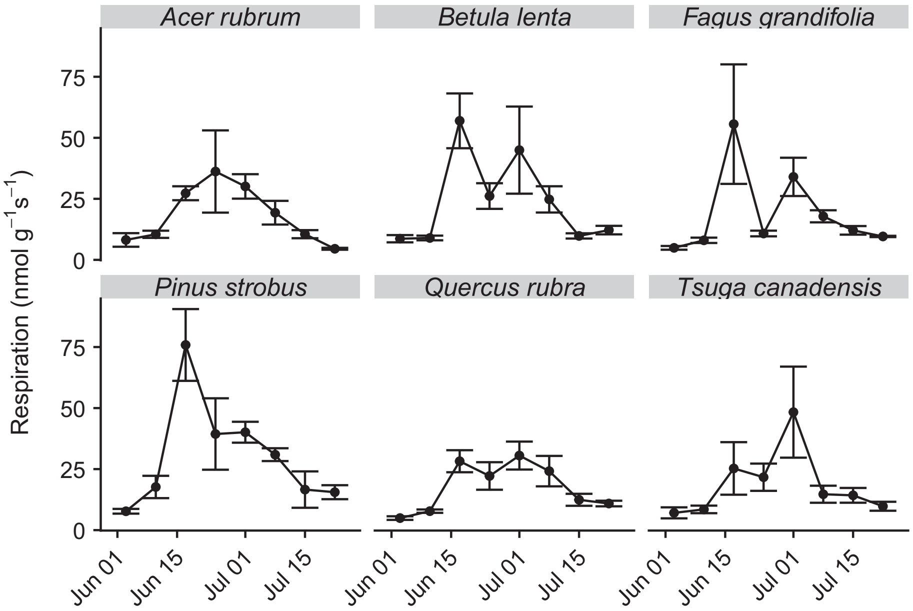

Fig. 4. Respiration CO_2 flux (nmol·g^{-1}·s^{-1}) by week (n = 8) for each tree species. Points represent the mean respiration flux for each species within each sampling week, and the error bars represent the standard error of the mean. The first peak in respiration rate was in the week of 15 June and the second peak in respiration was in the week of 1 July.

Table 1. Regression model metrics and coefficient estimates (standard error in parentheses) for the four candidate regression models.

Model	AIC	df	$R^2_{marginal}$	$R^2_{conditional}$	PC1	Temp
M1) Temperature + Week	516	11	0.53	0.70	n/a	0.044 (0.021)
M2) Temperature + Week + Traits	426	12	0.69	0.76	−0.20 (0.018)	0.041 (0.017)
M3) Temperature + Week + Species	518	16	0.58	0.70	n/a	0.047 (0.020)
M4) Temperature + Week + Traits + Species	447	17	0.69	0.77	−0.19 (0.019)	0.042 (0.017)

Notes: Metrics include model Akaike information criterion (AIC) values, model degrees of freedom, and the marginal (fixed effects) R^2 value and conditional (fixed plus random effects of individual tree) R^2 value. Regression coefficient estimates include root trait principal component 1 (PC1) and measurement temperature (Temp).

root characteristics, as our sampling took place over a relatively short time period (8 weeks). The observed weekly variation in SRL, RTD, and root diameter could be related to the phenological timing of fine root growth, the availability of soil nutrients, and/or carbon supply and demand from other tissues within the tree. Many other studies have found strong seasonal effects on leaf traits, particularly linking leaf nitrogen to photosynthetic rates (Field and Mooney 1983), but few studies have investigated the seasonal variation in root traits (see *Introduction*). We did not have a long enough sampling period to fully investigate this variation across the entire growing season and across species, but these results suggest that fine root morphology may vary dramatically over relatively short time periods. Small-scale spatial heterogeneity in nutrient availability could also be responsible for some of the variation we observed: Soil nutrient concentrations in forests can be highly variable over centimeters and meters (Farley and Fitter 1999), and root morphology often changes to take advantage of nutrient hotspots (Hodge 2004).

In addition to the observed temporal variation, we found that root diameter and RTD had some species specificity. The greatest species differences we saw were in RTD, where we saw a separation between the only measured arbuscular mycorrhizal species, *A. rubrum*, and the other five ectomycorrhizal species. Within the ectomycorrhizal species, we saw patterns that suggested differences between the conifers and the deciduous species. This is consistent with the finding that root traits may be phylogenetically conserved and that of all the root traits we measured, RTD was most affected by mycorrhizal type (Valverde-Barrantes et al. 2017). However, in spite of the large differences between the six species in the study in both their leaf habits and phylogeny, the majority of the variation in root traits occurred within species, rather than between them. This suggests that root characteristics and metabolic functions exhibit a high degree of plasticity among individuals in our six species. A fruitful future avenue of research will be to discover whether this variation is due to microenvironmental factors, genotypes, phenotypic variation, or some combination.

Coordination of traits: the resource spectrum

Our results supported the existence of a RES characterized by trait trade-offs between acquisition and conservation. One end of this spectrum captured thick and dense fine roots with high C:N, whereas the other end of the spectrum captured thin roots with low tissue density and high nitrogen content. These results are similar to previous studies which also supported trade-offs between root nitrogen content and RTD and root nitrogen content and mean root diameter (Roumet et al. 2016). Orthogonal to this trade-off was mycorrhizal colonization, which suggests that fully capturing the variation in root strategies does require a multidimensional axis, as is suggested by other studies (Kong et al. 2016, Kramer-Walter et al. 2016, de la Riva et al. 2018). Surprisingly, roots from these six phylogenetically distinct temperate tree species with very different leaf traits did not cluster along the spectrum, suggesting that roots of these species exhibit a variety of the strategies. This is consistent with the finding that plants may have a greater ability to adjust the morphology of their organs than allocation patterns (Poorter et al. 2012) and that intraspecific variation in plants traits can be high (Siefert et al. 2015). Our results illustrated the importance of capturing the intraspecific variation in fine root traits in addition to differences among species.

Root respiration

In this study, much of the variation in measured root respiration was explained by temperature and weekly variation (Table 1). The seasonal peak in respiration that we observed for *Q. rubra* and *T. canadensis* closely matched that of another study on fine root respiration at an adjacent site tracking the same two species (Abramoff and Finzi 2016). Similar temporal patterns of autotrophic respiration have been found within soil trenching studies at the Harvard Forest (Savage et al. 2013, 2018), although the respiration peak in our study was somewhat earlier. Future studies could try to better understand the mechanisms that influence the timing of the seasonal peak, which could include factors such as soil nutrient availability, weather patterns, and/or species-specific allocation patterns.

Root characteristics, as captured by the first principal component aggregating RTD, SRL, and root chemistry, played a critical secondary role in explaining variation in measured root respiration. This is also consistent with other studies which have found that fine root respiration correlated positively with root nitrogen (Desrochers et al. 2002, Roumet et al. 2016), and SRL (Roumet et al. 2016, Miyatani et al. 2018). Importantly, we show that root characteristics, but not tree species identity, explained variation in root respiration rates, in addition to temperature and temporal dynamics.

CONCLUSION

Previous work has focused on the variation in root traits either across many species, broad taxonomic groups and biomes, or within a single species. In this study, we illustrated the importance of understanding the relative importance of inter- and intraspecific variation in root morphology within the six most abundant tree species in a diverse temperate forest. Although these six species were phylogenetically diverse and captured a variety of leaf traits, root traits did not cluster by tree species. These root characteristics, but not tree species identity, explained significant variation within measured root respiration. Root respiration was strongly correlated with characteristics that fell along an acquisition/conservation trade-off that is similar to the mechanisms that describe the LES. We also found a strong effect of temporal variation in both root traits and associated respiration rates that was independent of temperature. Future research could address the mechanisms controlling temporal variation in belowground processes that are connected to both root characteristics and respiration, as well as the driver of within-species variation. The results from this study emphasized the importance of intraspecific variation in root traits, which are closely tied with rates of respiration, and could potentially serve as a mechanism for scaling form to ecological function in belowground processes.

ACKNOWLEDGMENTS

Funding for this project was provided by The Georgeanne Miller Mulhern Faculty/Student Research Fund in the Sciences at Wellesley College and the Harvard Forest REU program (National Science Foundation DBI-1459519). The authors would also like to thank Manisha Patel and everyone involved in the Harvard Forest REU program for supporting this project, and Cathy Summa for organizing the Wellesley Science Center Summer Research Program. We also thank William Werner for logistical support in the laboratory, and Ashley Lang for valuable conversations and advice about mycorrhizae. Finally, we would like to thank one anonymous reviewer and J. Aaron Hogan for constructive and comprehensive reviews. The authors have no conflicts of interest.

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DATA AVAILABILITY

The data associated with this paper, as well as the code for all analyses, are available on Mendeley data at https://doi.org/10.17632/bgszv9hxsh.1 and will be submitted to the Fine Root Ecology Database (https://roots.ornl.gov/).

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2944/full