Received: 3 November 2016 | Accepted: 19 April 2017

DOI: 10.1111/1365-2745.12800

RESEARCH ARTICLE

Journal of Ecology | BRITISH ECOLOGICAL SOCIETY

Root morphology and mycorrhizal type strongly influence root production in nutrient hot spots of mixed forests

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Funding information
US National Science Foundation, Grant/Award Number: IOS 1120482; DOE Terrestrial Ecosystems Program, Grant/Award Number: DE-SC0012003; Pennsylvania State University J. Lloyd Huck Dissertation Research Grant; US National Science Foundation, Grant/Award Number: IOS 1120482;

Handling Editor: Marcel van der Heijden

Abstract

1. Plants compete for nutrients using a range of strategies. We investigated nutrient foraging within nutrient hot-spots simultaneously available to plant species with diverse root traits. We hypothesized that there would be more root proliferation by thin-root species than by thick-root species, and that root proliferation by thin-root species would limit root proliferation by thick-root species.
2. We conducted a root ingrowth experiment in a temperate forest in eastern USA where root systems of different tree species could interact. Tree species varied in the thickness of their absorptive roots, and were associated with either ectomycorrhizal (EM) or arbuscular mycorrhizal (AM) fungi. Thus, there were thin- and thick-root AM and thin- and thick-root EM plant functional groups. Half the ingrowth cores were amended with organic nutrients (dried green leaves). Relative root length abundance, the proportion of total root length in a given soil volume occupied by a particular plant functional group, was calculated for the original root population and ingrowth roots after 6 months.
3. The shift in relative root length abundance from original to ingrowth roots was positive in thin-root species but negative in thick-root species ($p < .001$), especially in unamended patches (AM: +6% vs. −7%; EM: +8% vs. −9%). Being thin-rooted may thus allow a species to more rapidly recolonize soil after a disturbance, which may influence competition for nutrients. Moreover, we observed that nutrient additions amplified the shift in root length abundance of thin over thick roots in AM trees (+13% vs. −14%), but not in EM trees (+1% vs −3%). In contrast, phospholipid fatty acid biomarkers suggested that EM fungal hyphae strongly proliferated in nutrient hot-spots whereas AM fungal hyphae exhibited only modest proliferation.
4. We found no evidence that when growing in the shared patch, the proliferation of thin roots inhibited the growth of thick roots.
5. *Synthesis.* Knowledge of root morphology and mycorrhizal type of co-existing tree species may improve prediction of patch exploitation and nutrient acquisition in heterogeneous soils.

KEYWORDS
arbuscular mycorrhiza, ectomycorrhiza, ingrowth, nutrient foraging, plant–soil interactions, resource exploitation, root competition, root length density, root proliferation, soil heterogeneity

Paper previously published as Standard Paper

1 | INTRODUCTION

Terrestrial ecologists appreciate the extensive spatial patchiness of nutrients in the soil and its influence on below-ground competition for nutrients (Cahill et al., 2010; Casper & Jackson, 1997; Hodge, 2004). Root proliferation in nutrient 'hot-spots' has long been considered a competitive strategy when roots of species mixtures are sharing the same nutrient pools (de Kroon, Mommer, & Nishiwaki, 2003; Fitter 1977). Plant functional traits are hypothesized to drive plant–plant interactions, including resource depletion by competitive nutrient foraging (Grime, 1979). Thus, the use of plant functional traits, particularly root functional traits, to assess species variation in nutrient foraging may improve predictions of competitive interactions (Cahill, 2013; Hutchings, John & Wijesinghe, 2003; Teste et al., 2017).

However, most studies of nutrient foraging by coexisting plant species have been conducted using pots under glasshouse conditions with a limited numbers of plants (Cahill et al., 2010; Fransen & de Kroon, 2001; Fransen, de Kroon & Berendse, 2001; Hodge, Robinson, Griffiths & Fitter, 1999; McNickle, Deyholos & Cahill, 2016; Mommer et al., 2011; Robinson, Hodge, Griffiths & Fitter, 1999; Tamme, Gazol, Price, Hiiesalu & Pärtel, 2016). The few studies conducted under field conditions mainly focused on shrub and herbaceous species (e.g., Caldwell, Manwaring & Durham, 1991; Caldwell, Manwaring & Jackson, 1991; Eissenstat & Caldwell, 1988; Šmilauerová & Šmilauer, 2010). Studies on nutrient foraging by coexisting roots of different tree species are very rare, constraining our ability to predict below-ground competitive dynamics in forests. Moreover, because competitive success can be different in short-term and long-term studies (Fransen & de Kroon, 2001), long-term studies are particularly important in trees whose life span is often longer than those of herbaceous plants.

In addition to the lack of investigations on mature trees, few field studies have included the role of mycorrhizal fungi, despite their importance in understanding below-ground interactions and the patterns of nutrient acquisition (Fitter, 1977; Mommer, Kirkegaard & van Ruijven, 2016). Mycorrhiza type may influence the ability of plants to compete for nutrients (Teste, Veneklaas, Dixon & Lambers, 2014). Temperate tree species, for example, are frequently associated with either ectomycorrhizal (EM) fungi or arbuscular mycorrhizal (AM) fungi (a few species associate with both); whereas herbaceous species mostly associate with AM fungi (Smith & Read, 2008). Species with EM fungi may depend more on the hyphae to compete for organic nutrient hot-spots since EM fungi are generally better in mineralizing organic matter than AM fungi (Shah et al., 2015).

Effective competition for heterogeneously distributed nutrients may involve rapid exploitation of small volumes of root-free soil formed by disturbances such as root herbivory and bioturbation. For example, Stevens, Jones, and Mitchell (2002) proposed that root herbivory is the leading explanation for the 37% fine-root mortality in a longleaf pine stand. Another study showed that the 41% fine-root mortality in peach trees was caused by root herbivory (Wells, Glenn, & Eissenstat, 2002). On the other hand, bioturbations, including soil disturbance caused by animals (e.g. earthworms, rodents, badgers, foxes) burrowing for shelter or digging for food, are also widely distributed in forests (Kurek, Kapusta, & Holeksa, 2014). After a disturbance, the rate at which a soil volume is filled by mycorrhizal roots may determine the relative success of plant species to effectively compete for below-ground resources (Eissenstat & Caldwell, 1989; Hodge et al., 1999; Robinson et al., 1999). Previous studies of roots of tree species foraging in root-free patches were typically performed in monocultures or in root-isolating bags. Such studies suggest that thin-root AM tree species often exhibit faster root proliferation than thick-root AM tree species (Eissenstat, Kucharski, Zadworny, Adams & Koide, 2015; Liu et al., 2015). In contrast, EM tree species may depend more on mycorrhizal fungi in nutrient foraging (Chen et al., 2016; Cheng et al., 2016). However, whether the monospecific studies of root foraging can fully predict the behaviour of nutrient acquisition of plants experiencing interspecific competition remains largely untested (McNickle et al., 2016).

Because co-occurring plant species respond to nutrient heterogeneity in soils to varying rates and extents (Chen et al., 2016; Tamme et al., 2016), the species composition of roots filling newly formed root-free patches may shift dramatically from the root population that originally existed in that volume. Moreover, roots of one species may facilitate or suppress the growth of roots of other species (Bolte & Villanueva, 2006; Casper & Jackson, 1997; Dybzinski, Farrior, Wolf, Reich & Pacala, 2011; Meinen, Hertel & Leuschner, 2009; Messier, Coll, Poitras-Larivière, Bélanger & Brisson, 2009; Valverde-Barrantes, Smemo, Feinstein, Kershner & Blackwood, 2015). Thus, predicting below-ground root dynamics requires both the understanding of foraging strategies and the effects of interspecific root interactions.

A high-precision foraging strategy (preferential allocation to nutrient-rich patches over nutrient-poor patches) may be highly efficacious for some species and may confer a competitive advantage, at least in the short term (Bliss, Jones, Mitchell & Mou, 2002). Species that rapidly proliferate in nutrient-rich patches may preempt other species that proliferate more slowly (de Kroon et al., 2003; Hodge et al., 1999; Mommer et al., 2011; Ravenek et al., 2016; Robinson et al., 1999). Among temperate tree species that vary widely in root life span (McCormack, Adams, Smithwick & Eissenstat, 2012), there could be a trade-off between root foraging precision (RFP) and root turnover rates such that a preemptive strategy comes at the cost of rapid turnover of roots in the nutrient patch to avoid the maintenance cost of roots in a nutrient-depleted patch, which is a common strategy of thin-root tree species (Adams, Mccormack & Eissenstat, 2013). In contrast, species with thick roots may have a low-precision foraging strategy, but occupy nutrient-poor, less-crowded patches (Mommer, van Ruijven, Jansen, van de Steeg & de Kroon, 2012). The frequently longer life span of thick roots may be advantageous when nutrient availability is spatially uniform and temporally stable. This kind of spatio-temporal niche partitioning among thin- and thick-root species may allow their coexistence, but it requires further empirical testing.

In this study, we investigated the nutrient foraging patterns by plant species of diverse root functional types, including thin- and thick-root AM, and thin- and thick-root EM plant species. Our central goal was to determine the extent to which nutrient exploitation of a root-free patch by mixed tree species is related to root diameter

and mycorrhizal type. We hypothesized that in root-free patches, thin-root species would exhibit more root proliferation and higher foraging precision than thick-root species, and that proliferation by thin-root species would inhibit the proliferation by thick-root species. Moreover, we hypothesized that root proliferation would be more important than hyphal proliferation for AM species and that hyphal proliferation would be more important than root proliferation in EM species.

2 | MATERIALS AND METHODS

2.1 | Study site

This experimental site was located in a catchment of the Susquehanna-Shale Hills Critical Zone Observatory in central Pennsylvania, USA (http://criticalzone.org/shale-hills/). It is a humid, mixed, temperate forest with some trees greater than 110 years old. The dominant trees at Shale Hills include oak (*Quercus alba* L., *Quercus rubra* L., *Quercus prinus* L., *Quercus velutina* Lam.), hickory (*Carya glabra* Mill., *Carya tomentosa* Sarg., *Carya ovata* (Mill.) K. Koch), maple (*Acer rubrum* L., *Acer saccharum* Marshall), pine (*Pinus strobus* L., *Pinus virginiana* Mill.) and hemlock (*Tsuga canadensis* (L.) Carrière), with less abundant ash (*Fraxinus americana* L.), magnolia (*Magnolia acuminata* L.) and tulip poplar (*Liriodendron tulipifera* L.) (Smith, Eissenstat & Kaye, 2016).

2.2 | Ingrowth experiment

Because slope position may affect root distribution, and because thick-root AM species (*M. acuminata* and *L. tulipifera*) were only located on the valley floor of our experimental site, we positioned all 20 plots along the stream on the valley floor in which we inserted the ingrowth containers (Figure S1). Cylindrical root ingrowth containers were made from rigid plastic mesh (diameter = 3.5 cm, length = 10 cm, mesh = 4 × 4 mm square, Figure S1). Based on previous observations, more than half (*c.* 57%) of the absorptive root length on the valley floor in this forest is distributed in the top 10 cm of soil (Gaines et al., 2015). In early May 2015, in each square plot (2 m on a side), we installed 6 ingrowth containers. First, a soil auger was used to extract a 3.5-cm diameter, 10-cm deep, soil core. These soil cores were sieved and the roots were collected. Sieved, root-free soil was then placed back into each of the ingrowth containers, but half of the containers were first supplemented with 5 g ground, oven-dried, green leaves (amended treatment) and half were not (unamended treatment). Green leaves were harvested 1 week before the container installation from multiple species in a nearby common garden plantation (Chen et al., 2016). Leaves were dried, ground and well-mixed across species prior to filling into the ingrowth container, with 46.7 ± 0.1% total carbon and 1.77 ± 0.03% total nitrogen concentration in the dried leaves. To assess mycorrhizal fungal ingrowth, we also placed a small mesh bag (8 cm long, 3 cm width, 50 μm mesh) filled with 30 g sterilized silica sand in the centre of each ingrowth container (Figure S1). The ingrowth containers (with the mesh bag inside) were then installed in the same-size hole that was left from the soil coring. After harvest in early November 2015, about 6 months after installation, all root branches and mesh bags filled with sands in the containers were collected.

2.3 | Measurements of roots and mycorrhizal fungi

Roots collected from the ingrowth containers, as well as from the original core before the installation of ingrowth containers, were identified to plant functional group using a 40-x stereomicroscope, based on the root diameter, colour, rigidity, branching pattern and mycorrhizal colonization status. A subset of 96 random roots was collected and DNA barcoding techniques were used to verify the accuracy of microscopy-based species-group identification. Root DNA was amplified using the PCR primers rbcLaF (ATGTCACCACAAACAGAGACTAAAGC)/rbcLajf634R (GAAACGGTCTCTCCAACGCAT), following the protocol as suggested in Fazekas et al. (2008). The DNA of 86 samples were successfully amplified and sequenced at Genomics Core Facility of Pennsylvania State University. Root DNA sequence was blasted and compared to reference sequence (NCBI, http://blast.ncbi.nlm.nih.gov/) and the overall accuracy approximated 95% (Table S1). After verification, root length of each functional group (assessed microscopically) was separated and roots in the fourth or higher orders (*c.* 0–5% of total root length) were removed. The remaining first three orders of roots were considered absorptive roots (Guo et al., 2008) and they were measured for length using a desktop scanner and *WinRhizo* program (Regent Instruments Inc., Québec City, QC, Canada) for each root population. The first population is referred to as the 'original roots' (taken from the soil coring). The second population, recovered after 6 months of ingrowth into the containers, is referred to as the '6-month' roots.

Fungal biomass within the sand-filled mesh bags was determined for each sample using the phospholipid fatty acid (PLFA) method as previously described (Chen et al., 2016). Briefly, about 5 g of freeze-dried sand were used for fatty acid extraction, and PLFAs of different biomarkers were quantified by gas chromatography (Bossio, Scow, Gunapala & Graham, 1998). Hyphal biomass was estimated using the PLFA biomarker 16:1ω5c for AM fungi and 18:1ω6 + 18:2ω9 for EM fungi. The biomarker 16:1ω5c is also produced by some bacteria and the biomarkers 18:1ω6 and 18:2ω9 are also produced by saprotrophic fungi. Therefore, the use of these markers may overestimate EM and AM fungal biomass. Moreover, the error could be different in amended and unamended cores because saprotrophic bacteria and fungi may be more abundant in the amended cores because of the additional organic matter. However, within the sand-filled mesh bags, where there was no organic matter, we assumed that only a small amount of saprotrophic growth was possible (Kjøller, 2006; Wallander, Nilsson, Hagerberg & Bååth, 2001). For this reason, we assumed that PLFA biomarker 16:1ω5c was produced only by AM fungi and those PLFA biomarkers 18:1ω6 & 18:2ω9 were produced only by EM fungi. Moreover, we randomly selected 5–10 root branches of each plant functional group and examined the mycorrhizal status to ensure that mycorrhizal fungi were present in our ingrowth cores. The percentage of AM root length that was colonized by AM fungi was determined using the line-intersect method (McGonigle, Miller, Evans, Fairchild, & Swan, 1990).

2.4 | Statistical analysis

For each functional group shown in Table 1, the shift in relative root length abundance (Δ RLA) of each plant functional type was calculated by subtracting original root length abundance (RLA_{ori}) from the 6-month root length abundance ($RLA_{6\text{-}mon}$).

$$\Delta\ RLA(\%) = RLA_{6\text{-}mon}(\%) - RLA_{ori}(\%)$$

where RLA equals the percentage root length of a particular plant functional type relative to the total root length in the core.

For example, if thin AM roots represented 40% of total root length of the original roots and then represented 70% of total root length of the 6-month roots, this would represent a shift of +30% in root length abundance. We tested the influence of root morphology, mycorrhizal type and nutrient level on the relative shift of root length abundance with a three-way ANOVA. We also compared the change in richness of plant functional groups within a sample from original to 6-month roots using paired *t* tests. The mycorrhizal fungal biomass in the sand-filled hyphae ingrowth bags from the two treatments was also compared.

For a given soil core, the original root length density (RLD) was taken as a proxy for the density of meristems that can generate new roots into the newly formed patch. We tested whether original RLD was a good predictor of 6-month RLD and whether the prediction changed among plant functional groups and treatments (amended and unamended with green leaves) using linear regression analysis. In the linear regression, 6-month RLD was natural-log transformed. We also tested whether the root length of original thin roots (or thick roots) can influence the growth of thick roots (or thin roots) over the 6 months period by running a two-factor regression model. The calculation of a significant regression coefficient for contrasting root types (e.g. original thin-root length predicting 6-month thick-root length) indicated either suppression (negative coefficient) or facilitation (positive coefficient).

Finally, we examined RFP for each plant functional group to indicate the preferential root proliferation in nutrient amended containers compared to unamended containers. Root foraging precision ($RFP_{6\text{-}mon}$) was calculated for each site as the difference in 6-month RLD in the two nutrient types relative to averaged RLD.

$$RFP_{6\text{-}mon}(\%) = (RLD_{6\text{-}mon,amended} - RLD_{6\text{-}mon,unamended})/[0.5 * (RLD_{6\text{-}mon,amended} + RLD_{6\text{-}mon,unamended})]$$

We were also concerned that, by chance, the original root lengths may have been unequal for the amended and unamended treatments. In order to remove this potential bias, the adjusted RFP was calculated as the original root foraging precision (RFP_{ori}) subtracted from RFP after 6 months.

$$RFP_{ori}(\%) = (RLD_{ori,amended} - RLD_{ori,unamended})/[0.5 * (RLD_{ori,amended} + RLD_{ori,unamended})]$$

$$RFP_{adj}(\%) = RFP_{6\text{-}mon}(\%) - RFP_{ori}(\%)$$

We did not use $RLD_{unamended}$ as a denominator because RFP can be very large if $RLD_{unamended}$ is very small (which was not infrequent), so that the mean of RFP across sites was disproportionally influenced by low values of $RLD_{unamended}$. Unadjusted and adjusted RFP were compared among plant functional groups using ANOVA. All statistics were performed using R (R Core Team, 2016; version 3.3.0; R Foundation for Statistical Computing; www.r-project.org).

3 | RESULTS

Roots were recovered from all ingrowth containers. The overall RLD in individual containers after 6 months (2.02 cm cm^{-3}) was remarkably close to the original RLD (1.90 cm cm^{-3}). However, 6-month RLD was significantly lower than for the original roots specifically for AM thick roots ($p < .001$) and EM thick roots ($p < .001$) and significantly greater specifically for AM thin roots ($p = .012$). Root length density of EM thin roots did not differ significantly from that in the original roots ($p = .41$) (Table S2).

Relative root length abundance, both in the original roots and in the 6-month roots, differed significantly between thin vs. thick roots ($p < .001$) and between AM vs. EM ($p < .001$), with EM thin roots the most dominant and AM thick roots the least dominant in both unamended and amended ingrowth containers. Overall, thin-root species increased in relative abundance in the 6-month roots compared to the original roots, while thick-root species decreased (Figure 1) ($p < .001$). Amendment also influenced the shift between thick-root and thin-root species, and its influence differed between mycorrhizal types ($p = .02$). For AM species, the increase in the relative abundance of thin-root species and the decrease of thick-root species were stronger with amendment than without. In contrast, the positive response

TABLE 1 Tree species of different root functional types at Shale Hills Critical Zone Observatory (AM = arbuscular mycorrhizas, EM = ectomycorrhizas)

	AM	EM
Thin-root species[a]	*Acer rubrum*, *Acer saccharum*, *Fraxinus americana*	*Quercus alba*, *Quercus rubra*, *Quercus prinus*, *Quercus velutina*, *Carya glabra*, *Carya tormentosa*, *Carya ovata*
Thick-root species	*Magnolia acuminata*, *Liriodendron tulipifera*	*Pinus strobus*, *Pinus virginiana*, *Tsuga canadensis*

[a]The mean diameter of the first-three orders of roots (absorptive roots) was 0.28–0.37 mm for AM thin-root species, 0.65–0.83 mm for AM thick-root species, 0.19–0.21 mm for EM thin-root species and 0.40–0.64 mm for EM thick-root species.

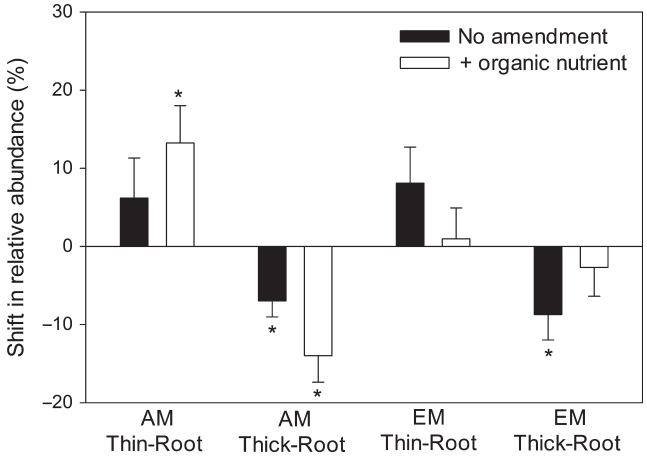

FIGURE 1 The shift of the relative root length abundance in the four plant functional groups from original roots to the root populations 6 months after ingrowth container installation. The functional groups were thin-root arbuscular mycorrhizas (AM), thick-root AM, thin-root ectomycorrhizas (EM) and thick-root EM. Ingrowth containers were either amended with organic matter (open bar) or not (black bar). Significant ($p < .05$) change between original abundance and 6-month abundance (shift deviate from 0) was indicated by '*'. Error bars represent SEM

of thin-root species and negative response of thick-root species was weaker for EM species with amendment than without (Figure 1).

The percentage of individual samples containing all four functional groups was reduced by more than half from the original root population to the 6-month root population (36% vs. 13%), while the percentage of individual samples containing two or fewer groups nearly doubled (29% vs. 51%, Figure S2). The changes in the richness of functional groups only slightly differed between amended and unamended containers (Figure S2). Among those individual samples in which the number of functional groups was reduced, 74% of them exhibited a reduction in the thick-root functional group while 12% exhibited a reduction in the thin-root functional group. Fourteen percent exhibited a reduction in both.

The original RLD was a significant predictor of 6-month RLD in the unamended containers for AM thick roots ($p = .006$), EM thick roots ($p = .014$) and EM thin roots ($p = .025$) but not for AM thin roots ($p = .26$). In amended containers, original RLD was a significant predictor only in the thin-root functional group ($p = .03$ for AM and $p = .04$ for EM) but not in the thick-root functional group ($p = .41$ for AM and $p = .37$ for EM) (Figure 2). The two-factor regression model showed that 6-month root length was only influenced by original root length of the same root functional type, except for 6-month thick roots in the amended treatment. In this treatment, original thick-root length was not predictive of 6-month thick-root length. Instead, thin roots exhibited a marginally significant facilitation effect on the growth of thick roots (Table 2).

Although we detected AM fungi in root tissues (Figure S3), the ingrowth of AM fungal hyphae into the sand was relatively low. Among the twenty plots, AM fungal PLFA (16:1ω5c) was detected in only four plots in unamended containers and eight plots in amended containers. In contrast, the EM fungal PLFA biomarkers 18:1ω6 and 18:2ω9

TABLE 2 Two-factor regression analyses for 6-month thin- and thick-root length (natural-log transformed) against original thin- and thick-root length in control (unamended) or organic (litter-amended) nutrient treatments

	Thin		Thick	
Predictor	Coefficient	*p*-value	Coefficient	*p*-value
Unamended				
Thin	0.71	.012	0.07	.78
Thick	0.18	.71	1.68	.004
+organic nutrient				
Thin	0.73	.008	0.48	.07
Thick	0.35	.46	0.50	.33

were found in most of the samples. If we assume all 18:1ω6 and 18:2ω9 PLFAs were produced by EM fungi only (but see Materials and Methods), EM fungal biomass was significantly higher than AM fungal biomass ($p < .001$). The biomass of both AM and EM fungi were significantly higher in amended containers than in unamended containers ($p = .001$), and the difference was larger for EM roots than for AM roots ($p = .004$) (Figure 3). However, it was not possible to assess the shift in fungal biomass relative to original levels simply because sterilized sand was not present in the original cores. For obvious reasons, we were also unable to separate the extramatrical fungal biomass associated with thin-root species from that associated with thick-root species within a single sample.

Root foraging precision varied widely across sites even for the same functional group (Figure 4). In general, there were no significant differences in foraging precision among the plant functional groups ($p = .33$). Even after removing the influence from unequal original root distribution between amended and unamended containers, foraging precision still did not differ significantly among functional groups ($p = .75$).

4 | DISCUSSION

4.1 | Proliferation of root and mycorrhizal hyphae by coexisting species

We found that when tree roots proliferate in a newly created nutrient patch in this temperate mixed forest, the diverse foraging strategies adopted by different plant functional groups could shift root community structure. First, the number of functional groups of the root mixtures tended to be smaller in the 6-month root population than in the original root population (Figure S2). Although there were thick roots recovered and recolonized into the ingrowth containers, the percentage of successful recovery was lower than for thin roots. The contrast was even higher in nutrient-amended patches, indicating nutrient hotspots stimulated proliferation of thin roots over thick roots (Table S3). Furthermore, in terms of RLD, the relative abundance of thin-root species increased but the relative abundance of thick-root species decreased in the 6-month roots compared to original root populations (Figure 1). These findings suggest that thin-root tree species

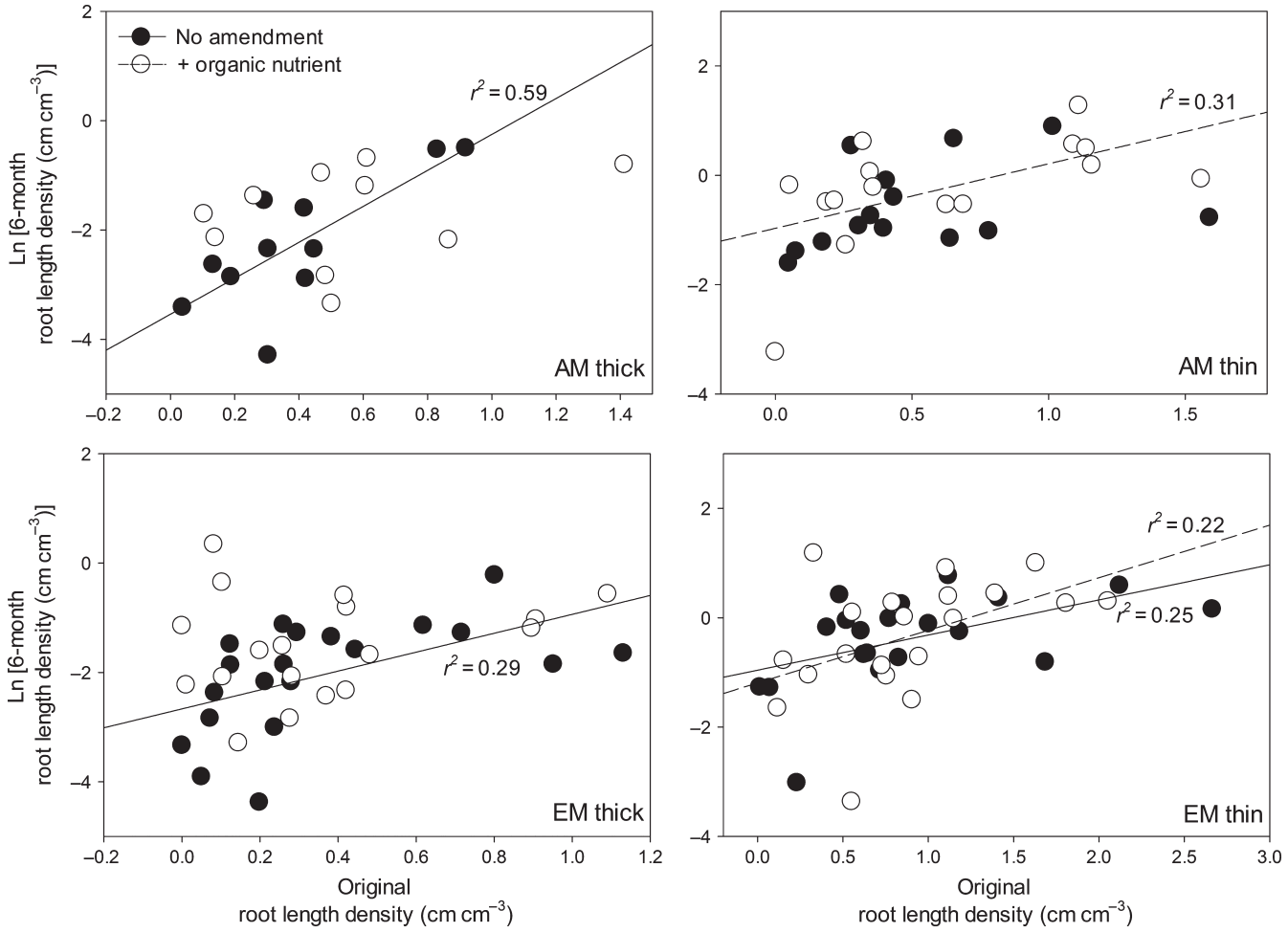

FIGURE 2 The relationship between original root length density and root length density 6 months after ingrowth container installation. Soils in the containers were either unamended (close circles) or amended with organic material (open circles). The 6-month root length density was natural-log transformed to produce equal variance. Significant (p < .05) linear regression lines are shown with the r-square values (solid line for no amendment treatment and dashed line for organic enrichment). Root length density was averaged for each site from three containers for each amendment treatment

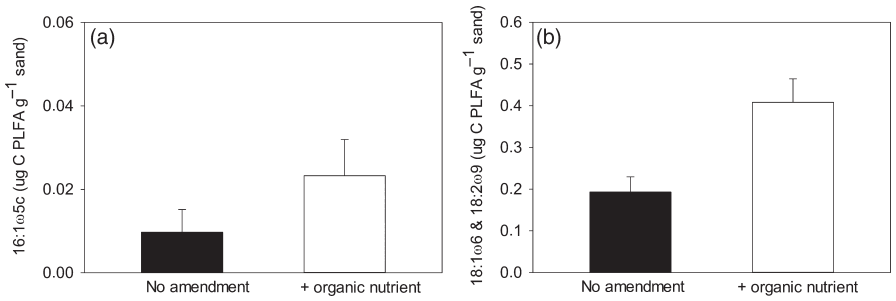

FIGURE 3 Ingrowth hyphal biomass indicated by phospholipid fatty acid (PLFA) biomarker for both arbuscular mycorrhizal (AM, 16:1ω5c) (a), and ectomycorrhizal fungi (EM, assuming all 18:1ω6 + 18:2ω9 PLFAs are from EM fungi) (b), within the sterile sand-filled mesh bag and for both amendment treatments. Data were averaged among sites for both AM and EM for each nutrient treatment and error bars represent SEM. AM biomass was significantly lower than EM biomass (p < .001). Mycorrhizal biomass was significantly higher in amended containers than in unamended containers (p = .001), and the difference was larger in EM than in AM (p = .004)

often respond more quickly to root-free patches than thick-root tree species, which is largely consistent with our hypothesis and previous research on single species with limited interspecific interactions (Chen et al., 2016; Cheng et al., 2016; Eissenstat et al., 2015; Liu et al., 2015).

Because RLD is an important predictor of nutrient acquisition (Hodge et al., 1999; Robinson et al., 1999) and competitive ability in both homogeneous and heterogeneous soils (Casper & Jackson, 1997; Mommer et al., 2011; Rajaniemi, 2007), our results suggest that the

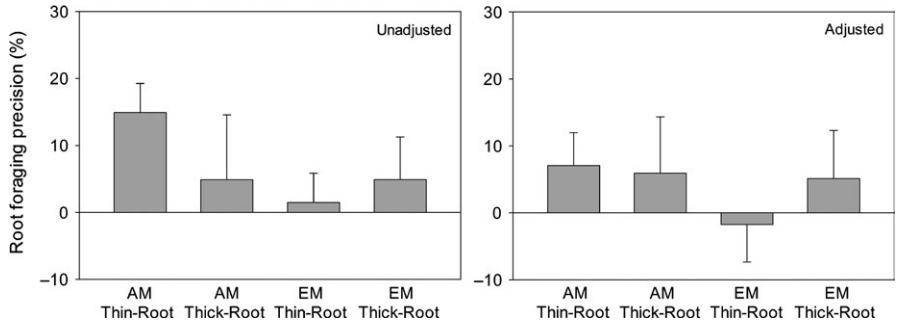

FIGURE 4 Root foraging precision in the four plant functional groups. Root foraging precision was calculated as the difference of root length density in the two nutrient types relative to total root length density. Precision was calculated using unadjusted (left) or adjusted (right) data for the 6-month roots (see Materials and Methods for details). No significant difference was detected among functional groups in both figures (p = .33 for unadjusted and p = .75 for adjusted). Error bars represent SEM

AM-colonized, thin-root, functional type may result in a competitive advantage in ephemeral nutrient hot-spots. It is possible that eventually the new root community will become similar to that of the original roots, but immediately following disturbance thin-root tree species may be able to preempt resources from thick-root species. When rapidly foraging for nutrients, the thin AM roots are also expected to turn over rapidly as the nutrient-rich patch becomes depleted (McCormack et al., 2012), which can be an efficient strategy to cope with ephemeral nutrient hotspots. This rapid-foraging strategy may also be efficient for annual herbaceous species that often associate with AM fungi, but further examination in grassland and desert ecosystems is needed. In contrast, EM thin-root tree species, as we hypothesized, only maintain a root proportion in the newly formed, nutrient-rich patches similar to that in the original community. However, they may compensate with increased hyphal proliferation (Figure 3), which would contribute to their competitiveness in such a situation (Bending & Read, 1995). The increased capability of EM hyphae to acquire nutrients from organic substrates than for AM hyphae (Shah et al., 2015) and the rapid growth of EM hyphae (Allen & Kitajima, 2013) may also contribute to EM tree species relying more on mycorrhizal hyphae than AM tree species in competitive nutrient foraging.

Although the roots of thin-root species may preempt the roots of thick-root species in nutrient hot-spots, there was no evidence of direct inhibition of thick-root species by thin-root species, which contradicted our hypothesis. Therefore, the dominance by thin-root species was apparently mainly the result of faster growth of thin-root species, not the result of their suppression of thick-root species. Instead, thin-roots appear to modestly facilitate the growth of thick roots in nutrient amended patches (Table 2). Thus, whether root mixtures exhibit overyielding relative to monocultures in a patch may depend somewhat on the nutrient concentration of the patch.

To a certain degree, RLD after 6 months could be predicted from original RLD. However, there were differences among plant functional groups and treatments (Figure 2). Nevertheless, our predictions can be useful in below-ground 'gap' models, which are similar to forest gap models (Bugmann, 2001), and they may be important in understanding root dynamics after a root-free patch of soil is created following disturbance. Moreover, the potential coordination between root proliferation rate and root turnover may serve as a mechanism for facilitating species coexistence in a forest community. Rapid root proliferation and fast turn over may be an efficient strategy to cope with temporally dynamic nutrient patches. On the other hand, slow root proliferation and longer life span may represent an alternative, more conservative strategy.

4.2 | Foraging precision of coexisting roots

We found that RFP was not significantly driven by root morphology or mycorrhizal type, in contrast with our hypothesis and results from previous monoculture studies (Chen et al., 2016; Cheng et al., 2016). This inconsistency may be the result of interspecific competition or of working in a more heterogeneous landscape of a mature forest. The large variation in RFP suggests considerable influence of unknown factors.

Also, although AM or EM species with similar root diameter may exhibit similar RFP, variation within a group still existed. For example, *Betula* species have higher foraging precision than *Quercus* species, despite their similarity in root diameter (Chen et al., 2016). In our study system, *Quercus* was much more dominant than *Betula*, which possibly led to an overall low RFP of the EM thin-root group. Also, *Tsuga* species were a relatively important EM thick-root species in our study area, but the foraging precision of *Tsuga* had not been studied previously. They may forage differently from other thick-root lineages in the Pinaceae family, such as *Pinus* and *Picea*, both of which were previously studied in a common garden setting (Chen et al., 2016). Finally, because we were unable to separate AM or EM hyphae from thin root and thick roots, it was difficult to link the potentially complementary hyphal foraging precision to RFP for thin-root and thick-root species as done previously in common gardens. Although a challenge to use for large trees, techniques such as isotopic labelling of PLFAs could possibly be used in future studies to examine the carbon allocation to mycorrhizal fungal hyphae from different host sources (Klein, Siegwolf & Körner, 2016; Treonis et al., 2004).

5 | CONCLUSIONS

Unlike the more widely known mechanisms that influence competition for light or water, the mechanisms that control competition

for nutrients in a spatially and temporally variable forest floor are poorly understood. Grouping species based on functional traits such as root diameter and mycorrhizal type may provide a simple but relatively accurate way to improve understanding of interspecific competition for nutrients. We found that thin-root species often increased their root length abundance in a root-free patch compared to thick-root species, especially for AM species in nutrient-rich patches. Moreover, EM species tended to rely more on hyphal proliferation than on root proliferation in the nutrient-rich patches. Differences in nutrient foraging strategies among plant functional groups may drive post-disturbance dynamics of root communities at a relatively small scale, and may facilitate species coexistence.

ACKNOWLEDGEMENTS

We thank Dr. Shuiqiang Yu for root processing in the lab. This project was supported by the US National Science Foundation (IOS 1120482) and the DOE Terrestrial Ecosystems Program (DE-SC0012003) to D.M.E. and R.T.K.; and the Pennsylvania State University J. Lloyd Huck Dissertation Research Grant to W.C. The field work was conducted in Penn State's Stone Valley Forest, which is supported and managed by the Penn State's Forestland Management Office in the College of Agricultural Sciences, and facilitated by NSF Critical Zone Observatory program grants to C. Duffy (EAR 07-25019) and S. Brantley (EAR 12-39285, EAR 13-31726).

AUTHORS' CONTRIBUTIONS

W.C. and D.M.E. conceived the ideas and designed methodology; W.C. collected and analyzed the data; W.C., D.M.E. and R.T.K. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.bh88s (Chen, Koide & Eissenstat, 2017).

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How to cite this article: Chen W, Koide RT, Eissenstat DM. Root morphology and mycorrhizal type strongly influence root production in nutrient hot spots of mixed forests. *J Ecol*. 2018;106:148–156. https://doi.org/10.1111/1365-2745.12800