Ecology, 97(10), 2016, pp. 2815–2823

Mycorrhizal fungi and roots are complementary in foraging within nutrient patches

Lei Cheng,[1,2,5] Weile Chen,[2] Thomas S. Adams,[2] Xing Wei,[2] Le Li,[2] Michael Luke Mccormack,[2] Jared L. Deforest,[3] Roger T. Koide,[2,4] and David M. Eissenstat[2]

[1]*College of Life Sciences, Zhejiang University, Hangzhou 310058 China*
[2]*Department of Ecosystem Science and Management, The Pennsylvania State University, University Park, Pennsylvania 16802 USA*
[3]*Department of Environmental and Plant Biology, Ohio University, Athens, Ohio 45701 USA*
[4]*Department of Biology, Brigham Young University, Provo, Utah 84602 USA*

Abstract. The roots of the majority of tree species are associated with either arbuscular mycorrhizal (AM) or ectomycorrhizal (EM) fungi. The absorptive roots of tree species also vary widely in their diameter. The linkages between root thickness, mycorrhiza type and nutrient foraging are poorly understood. We conducted a large root ingrowth experiment in the field to investigate how absorptive roots of varying thickness and their associated fungi (AM vs. EM) exploit different nutrient patches (inorganic and organic) in a common garden. In nutrient-rich patches, thin-root tree species more effectively proliferated absorptive roots than thick-root tree species, whereas thick-root tree species proliferated more mycorrhizal fungal biomass than thin-root tree species. Moreover, nutrient patches enriched with organic materials resulted in greater root and mycorrhizal fungal proliferation compared to those enriched with inorganic nutrients. Irrespective of root morphology, AM tree species had higher root foraging precision than mycorrhizal hyphae foraging precision within organic patches, whereas EM tree species exhibited the opposite. Our findings that roots and mycorrhizal fungi are complementary in foraging within nutrient patches provide new insights into species coexistence and element cycling in terrestrial ecosystems.

Key words: arbuscular mycorrhizal fungi; ectomycorrhizal fungi; functional complementarity; nutrient foraging; root morphology; species coexistence; tree species.

Introduction

Nutrient availability in soil is typically highly heterogeneous in space and time (Caldwell 1994). A range of traits in both plants (Eissenstat 1991, Eissenstat et al. 2015) and mycorrhizal fungi (Hart and Reader 2002, Weigt et al. 2012) have been identified that influence how efficiently foraging occurs for nitrogen (N) and phosphorus (P) in ephemeral nutrient hotspots. Among arbuscular mycorrhizal (AM) trees, those with thick absorptive roots and low branching intensity tend to have high mycorrhizal colonization but limited capacity to proliferate roots in nutrient-rich patches, both in temperate (Eissenstat et al. 2015) and subtropical (Liu et al. 2015) forests. However, we have only a very limited understanding of how ectomycorrhizal (EM) tree species forage in comparison to AM tree species or whether organic nutrient sources affect patterns of foraging differently from mineral sources. Yet in boreal and many temperate forests, EM species are the dominant trees and organic materials are frequently the dominant nutrient sources (Read and Perez-Moreno 2003).

Most plants have a complex, branched root system. Root branches can be classified into several orders according to their branch position with the thinnest, most distal roots identified as the 1st order (Pregitzer et al. 2002). Generally, the 1st and 2nd order roots function primarily in nutrient absorption and water uptake, while 3rd and higher order roots increasingly function in longitudinal nutrient and water transport (McCormack et al. 2015). Across diverse plant species, however, the thickness of absorptive roots varies greatly (Eissenstat 1992, Pregitzer et al. 2002, Comas and Eissenstat 2009). For instance, the minimum and maximum average diameters of the 1st and 2nd order roots of 25 co-existing woody species in a temperate North American forest were 0.2 and 0.9 mm, respectively (Comas and Eissenstat 2009). Moreover, this variation in root thickness has been linked to root functional traits such as longevity (McCormack et al. 2012, Adams et al. 2013) and growth rate (Eissenstat 1991, Eissenstat et al. 2015). Nonetheless, the linkage between root morphology and nutrient foraging is limited, especially in regards to ectomycorrhizal trees and the role of mycorrhizal extramatrical hyphal foraging.

The roots of greater than 90% of land plant species, especially tree species, are associated with either AM or EM fungi (Brundrett 2009). Mycorrhizal fungi have some advantages over roots in exploiting soil heterogeneity because their hyphae can provide a greater surface area per unit mass than absorptive roots. Mycorrhizal fungi acquire carbohydrates from their host plants, but they may also

Manuscript received 15 April 2016; revised 13 June 2016; accepted 13 June 2016. Corresponding Editor: J. D. Hoeksema.
[5]E-mail: lcheng@zju.edu.cn

transfer mineral nutrients, particularly N and P, to their hosts (Koide 1991, Smith and Read 2008, Koide et al. 2014). In a ^{15}N-labelling microcosm experiment, for example, Tu et al. (2006) demonstrated that fungal hyphae resulted in a 125% increase in plant ^{15}N acquisition from a nutrient patch. Several other recent studies have confirmed the role of mycorrhizal fungi in acquiring N and P from nutrient patches (Leigh et al. 2009, Cheng et al. 2012). Although these studies demonstrated the role of fungal hyphae in nutrient acquisition, they did not consider the interaction of hyphae with roots of varying morphology (thickness).

In this study, we investigated how AM and EM fungi exploit nutrient patches when in symbiosis with contrasting tree species possessing absorptive roots of varying thickness. We selected four tree species that constituted a 2 × 2 factorial combination of root thickness and mycorrhiza type. We set up one nutrient patch control, two levels of inorganic nutrient patches (low vs. high) and one organic nutrient patch, to simulate the natural field conditions where either AM- or EM- or both AM and EM-associated nutrient economies predominate (Phillips et al. 2013). We hypothesized that (1) thin-root tree species would proliferate their absorptive roots in nutrient patches more than thick-root tree species, and (2) mycorrhizal fungal hyphae associated with thick-root tree species would proliferate more in nutrient patches than those associated with thin-root tree species, and (3) both roots and mycorrhizal fungi would exploit more in nutrient-rich than in nutrient-poor patches, and more in organic nutrient patches than in inorganic nutrient patches.

Methods

Study site

The common garden was established in 1996, and is located in the Russell E. Larson Agricultural Research Center, about 10 miles southwest of the University Park campus of Pennsylvania State University (40°42′ N, 77°57′ W). Annual mean temperature is 8.9°C and annual mean precipitation is 1,010 mm. The field was a grass hayfield before 1995. The silt loam soil is a well-drained fine, mixed, semi-active, mesic Typic Hapludalf with a pH ranging from 6.1 to 6.5.

The common garden (Appendix S1: Fig. S1) is a randomized complete block design with 16 tree species randomly assigned into eight blocks (McCormack et al. 2012). Within each block, every tree species is represented by six individual trees in two rows of three. Spacing within and between individual trees in each plot is 3 m, with 5 m between plots. Each of these tree species coexist naturally in local forests. The tree species used in this study were *Acer negundo*, *Liriodendron tulipifera*, *Pinus strobus*, *Quercus alba*.

In-growth experiment

To test our hypotheses, we carried out a large-scale root in-growth experiment in the common garden in 2012. We selected four tree species and determined the proliferation of root and fungal hyphae in four different nutrient-patch treatments in the field. The four tree species constituted a 2 × 2 combination of root morphology and mycorrhiza type: *A. negundo* (thin-root, AM), *L. tulipifera* (thick-root, AM), *Q. alba* (thin-root, EM) and *P. strobus* (thick-root, EM) (Table 1). We constructed nutrient patches using 5 × 10 cm mesh envelopes (mesh size: 1 mm), which has been one of most often used methods in investigating root and mycorrhizal dynamics in microcosm and field experiments (Neill 1992, Wallander et al. 2001, Cheng et al. 2012, Addo-Danso et al. 2016). Each mesh envelope was filled with soil taken from the corresponding plot and was either unamended or amended with various nutrient additions. The four nutrient patch treatments were: (1) 90 g dry-weight soil (no nutrient addition, hereafter "control"), (2) 90 g soil plus 25 mg N (NH_4NO_3) kg^{-1} soil and 50 mg P (KH_2PO_4) kg^{-1} soil (Low inorganic N and P addition, hereafter "Low-NP"), (3) 90 g soil plus 100 mg N (NH_4NO_3) kg^{-1} soil and 200 mg P (KH_2PO_4) kg^{-1} soil (High inorganic N and P addition, hereafter "High-NP"), and (4) 81 g soil plus 9 g chopped dried fresh green leaves (ca. 1 cm length; Organic residue addition, hereafter "Organic"). The leaves were collected directly from the corresponding four species trees in the early-summer 2012, and then mixed at an equal mass per species to make a single homogenous leaf litter addition.

Table 1. The diameter (μm) of the first- and second-order roots of four tree species.

Species	*Acer negundo*	*Liriodendron tulipifera*	*Quercus alba*	*Pinus strobus*
Mycorrhiza	AM	AM	EM	EM
Root type	Thin	Thick	Thin	Thick
Harvest 1 (five weeks)				
1st order roots	265 ± 13	879 ± 12	184 ± 10	598 ± 39
2nd order roots	400 ± 29	995 ± 25	253 ± 15	854 ± 42
Harvest 2 (15 weeks)				
1st order roots	302 ± 9	860 ± 34	191 ± 10	762 ± 45
2nd order roots	410 ± 18	992 ± 44	281 ± 19	1,003 ± 52

Notes: AM, arbuscular mycorrhizae; EM, ectomycorrhizae. Values are means (n = 8) ± SEM.

We randomly buried the ingrowth envelopes at a10-cm soil depth within their corresponding tree plots in the mid-summer 2012. Each of the two AM tree plots received eight envelopes (4 nutrient patch types × 2 harvests), while every EM tree plot received 16 envelopes (4 nutrient patch types × 2 harvests × 2 root exclusion treatments). In order to exclude roots, we placed one set of mesh envelopes within root exclusion chambers made from PVC tubing (13 cm inner diameter, 60 cm long, with 50 μm mesh covering both openings). This allowed us to estimate the EM fungal biomass (as opposed to saprotrophic fungal biomass) accumulated within in-growth mesh envelopes by subtracting the hyphal biomass of the ingrowth bag with PVC root-exclusion chambers from that without exclusion chambers. In total, there were eight envelopes in each of eight replicate plots of two AM tree species (in total 128 envelopes) and 16 envelopes in each of eight replicate plots of two EM tree species (in total 256 envelopes). We then harvested ingrowth envelopes after five and 15 weeks. During this time, root production has been reported to be maximum at this site in most years (McCormack et al. 2014), and the period of maximal fungal growth was assumed to coincide with the maximal growth of absorptive roots (Wallander et al. 2001).

Measurements

Ingrowth envelopes were taken from the field and stored at 4°C until they were processed within 24 h. Root segments were removed from each envelope and root-free soil samples were frozen immediately at −20°C. Subsamples of soil (20 grams) were freeze-dried at −40°C for phospholipid fatty acids (PLFA) analysis. Fresh root samples were cleaned using tap water and stored at 4°C.

Root segments were scanned on an Epson Perfection 4,490 desktop scanner, and then analyzed with WinRhizo software (Regent Instruments, Quebec City, Quebec, Canada) for diameter, length and surface area (Comas and Eissenstat 2009). The length of absorptive roots was assessed as it is thought to be better predictor of plant nutrient uptake than root biomass (Hodge et al. 1999, Chapin et al. 2011). Subsamples of fresh root segments of the second harvest were taken randomly to determine AM and EM fungal colonization of roots. Briefly, the AM colonization was examined using the gridline-intersect method with cleared root segments stained with trypan blue (Sylvia 1994); and the EM colonization was determined by counting the number of root tips with and without a mantle and/or a Hartig net (Visser 1995).

Fungal biomass of each nutrient patch was estimated using PLFA analysis (Olsson 1999, Cheng et al. 2011). The protocol for the PLFA analysis was modified from Cheng et al. (2011). Only samples from the second harvest were used for PLFA analysis. PLFAs were extracted from 5 g of freeze-dried soil subsample using a solution containing 10 mL CH_3OH, 5 mL CH_3Cl and 4 mL PO_4^{3-} (K_2HPO_4 + KH_2PO_4) buffer at pH 7.0. Internal standards 21:0 PC (Avanti Polar Lipids, Alabaster, Alabama, USA), which are rarely present in soils, were also added to each sample to calculate the recovery rate of fatty acids. Solid phase extraction columns (SPE; Thermo Scientific, Waltham, Massachusetts, USA) were employed to separate phospholipids from neutral and glycol lipids. The obtained phospholipids were subjected to an alkaline methanolysis to generate fatty acids methyl esters (FAMEs). The resulting FAMEs were then separated and measured on a HP GC-FID (HP6890; Agilent Technologies, Santa Clara, California, USA). Peaks of fatty acids were identified using the Sherlock Microbial Identification System (MIDI Inc., Version 6.1, Newark, Delaware, USA). Biomass was estimated using external Newark, DE USA FAME standards (14:0, 16:0, 18:0, 20:0, 22:0, 24:0; K104 FAME mix; Grace, Deerfield, Illinois, USA).

The available N concentration within each nutrient patch after the second harvest was determined using the KCl-extraction method. Briefly, soil ammonium (NH_4^+) and nitrate (NO_3^-) in soil subsamples (5 g) were extracted with 50 mL of 2M KCl by shaking for 30 min. The concentrations of NH_4^+ and NO_3^- were then measured on a Lachat flow injection analyzer (Lachat Instruments, Milwaukee, Wisconsin, USA).

Data analyses

The biomarkers 16:1ω5c and 18:2ω6c were used to determine the AM (Olsson 1999, Cheng et al. 2012) and EM (Olsson 1999, Wallander et al. 2001) fungal biomass, respectively. Saprotrophic fungi are easily separated from AM fungi using PLFA analysis, so no exclusion tubes were used. For EM fungi, net proliferation of fungal hyphae in ingrowth envelopes during the experimental time was calculated using the total amount of each fungal biomarker in envelopes without root exclusions minus those in PVC tubes that prevented root penetration (Wallander et al. 2001). The subtractive method by which we estimated EM fungal biomass assumes that no interactions exist between EM and saprotrophic fungi. In fact, we do not know whether or not such interactions exist. Nevertheless, because there are no markers that allow us to distinguish between these two groups, the subtractive method, for the time being, remains the most suitable. Finally, the foraging precision (*FP*) of root length and hyphal biomass was calculated as the relative increase in nutrient amended patches (Low-NP, High-NP and Organic) compared to control patches as follows.

$$\mathrm{FP}_{\mathrm{root}}(\%) = \frac{100 \times \left(\mathrm{Root\ length}_{p_i} - \mathrm{Root\ length}_{\mathrm{control}}\right)}{\mathrm{Root\ length}_{\mathrm{control}}}, \quad (1)$$

$$\mathrm{FP}_{\mathrm{hyphae}}(\%) = \frac{100 \times \left(\mathrm{Hyphal\ PLFA}_{p_i} - \mathrm{Hyphal\ PLFA}_{\mathrm{control}}\right)}{\mathrm{Hyphal\ PLFA}_{\mathrm{control}}}, \quad (2)$$

where p_i denotes each of nutrient patches (Low-NP, High-NP and Organic).

All data were first subjected to analysis of variance (ANOVA) using the linear mixed-effects (LME) model as follows:

$$y_{ijk} = \mu + b_i + s_j + n_k + (s \times n)_{jk} + b_{ij} + \varepsilon_{ijk}, \quad (3)$$

where y_{ijk} is the specific measurement such as root length, fungal biomass and nutrient concentrations; μ the intercept, fixed effects; b_i the block, fixed effects; s_j the tree species treatment, fixed effects; n_k the nutrient patch treatment, fixed effects; $(s \times n)_{jk}$ the tree species and nutrient patch interaction, fixed effects; b_{ij} the tree species within block, random effects and ε_{ijk} the random experimental error. If a significant effect of the tree species treatment was detected, we redefined the LME model (1) by splitting the four tree species into a 2 × 2 factorial combination as follows:

$$y_{ijkl} = \mu + b_i + m_j + r_k + (m \times r)_{jk} + n_l + (m \times n)_{jl} + (r \times n)_{kl} + (m \times r \times n)_{jkl} + b_{ij} + b_{ijk} + \varepsilon_{ijkl} \quad (4)$$

where y_{ijkl} is the specific measurements such as root length, fungal biomass and nutrient concentrations; μ the intercept, fixed effects; b_i the block, fixed effects; m_j the mycorrhizal type, fixed effects; r_k the root morphology, fixed effects; $(m \times r)_{jk}$ the mycorrhizae and root interaction, fixed effects; n_l the nutrient patch, fixed effects; $(m \times n)_{jl}$ the mycorrhizae and nutrient patch interaction, fixed effects; $(r \times n)_{kl}$ the root morphology and nutrient patch interaction, fixed effects; $(m \times r \times n)_{jkl}$ the mycorrhizae, root morphology and nutrient patch interaction, fixed effects; b_{ij} the tree species within block, random effects; b_{ijk} the interaction of mycorrhizae and root morphology within block, random effects and ε_{ijkl} the random experimental error. The LME models were fit by the restricted maximum likelihood (REML) method using the "nlme" package (Pinheiro and Bates 2007). All statistical analyses were performed using the R program (Version 3.02, The R Foundation for Statistical Computing, 2013). Significant differences were accepted at a $P < 0.05$.

Results

Root proliferation

We first measured the diameter, length and surface area of roots that proliferated within in-growth envelopes after five and 15 weeks. The diameter of the 1st and 2nd order roots of both thin-root (*Acer negundo* and *Quercus alba*) and thick-root (*Liriodendron tulipifera* and *Pinus strobus*) tree species did not change between the two harvests ($P > 0.05$; Table 1). Consistent with our expectation, the diameters of the 1st and 2nd order roots of thick-root tree species were significantly larger than those of thin-root tree species ($P < 0.01$; Table 1). Across two harvests, the average diameters of the 1st and 2nd order roots of *L. tulipifera* were 3.1- and 2.5-fold thicker, respectively, than those of *A. negundo*; and *P. strobus* roots were 3.6- and 3.5-fold thicker, respectively, than those of *Q. alba* (Table 1).

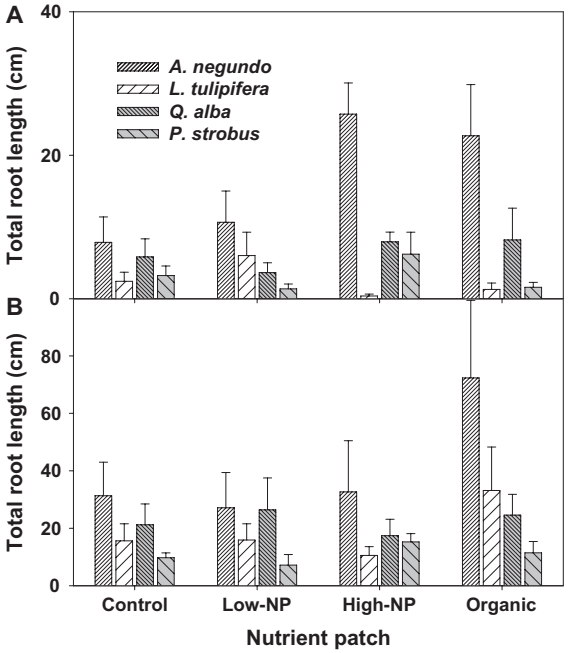

FIG. 1. The proliferation of absorptive roots of four tree species within different nutrient patches sampled after five weeks (A) and after 15 weeks (B). Values are means ($n = 8$) ± SEM. Nutrient treatments are: Control, no additional nutrients added to soil, Low-NP, low inorganic nitrogen (N) and phosphorus (P) addition; High-NP, high inorganic N and P addition; and Organic, the addition of a mixture of dried leaves. The four tree species were: *A. negundo*: arbuscular mycorrhizal, thin-root type; *L. tulipifera*: arbuscular mycorrhizal, thick-root type; *Q. alba*: ectomycorrhizal, thin-root type; and *P. strobus*: ectomycorrhizal, thick-root type.

Thin-root tree species developed significantly greater absorptive root lengths within ingrowth envelopes in comparison with thick-root tree species (Fig. 1 and Appendix S1: Fig. S2). Across mycorrhiza types and nutrient levels, the total root length of thin-root tree species within ingrowth envelopes were on average 11.6 and 30.5 cm at weeks five and 15, respectively, which were 3.1-fold (Fig. 1A; $F_{1,105} = 31.42$, $P < 0.001$) and 1.2-fold (Fig. 1B; $F_{1,105} = 9.12$, $P = 0.003$) longer than those of thick-root tree species over the same time periods. The total root length did not differ significantly across nutrient patch types at either week five (Fig. 1A; $F_{3,105} = 2.53$, $P = 0.06$) or week 15 (Fig. 1B; $F_{3,105} = 1.65$, $P = 0.18$). When we considered thin and thick roots separately, however, tree species with thin roots, especially *A. negundo*, tended to proliferate more in the organic nutrient patch.

The total average surface area of roots of thin-root tree species was significantly greater than that of thick-root tree species at week five (Appendix S1: Fig. S2A; $F_{1,105} = 5.77$, $P = 0.018$), but not at week 15 (Appendix S1: Fig. S2B; $F_{1,105} = 0.39$, $P > 0.1$). In addition, the total root surface area was not significantly affected by nutrient patch type at either five or 15 weeks (Appendix S1: Fig. S2; $P > 0.1$ for either harvest).

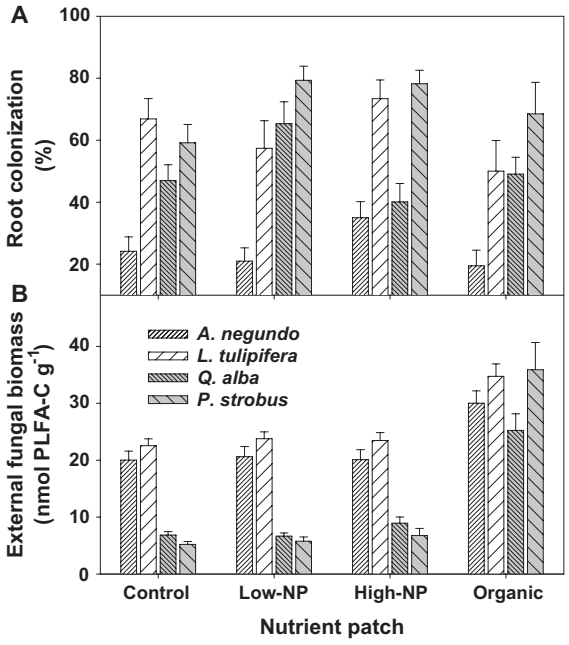

FIG. 2. Colonization of mycorrhizal fungi in roots collected from different nutrient patches at week 15 (A) and proliferation of external mycorrhizal fungal biomass within nutrient patches at week 15 (B). Values are means (n = 8) ± SEM. See Fig. 1 for abbreviations of nutrient patches.

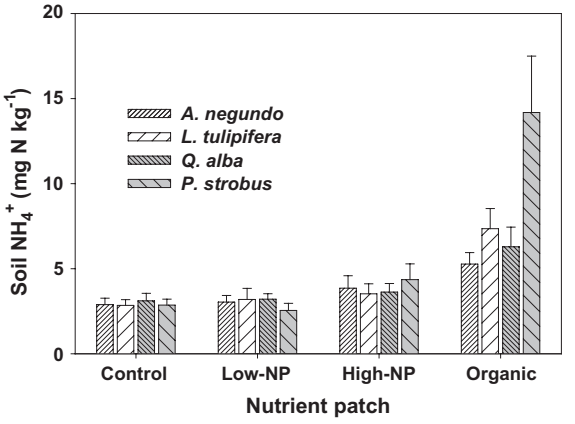

FIG. 3. Soil ammonium concentrations within different nutrient patches at week 15. Values are means (n = 8) ± SEM. See Fig. 1 for abbreviations of nutrient patches.

Fungal proliferation

Mycorrhizal colonization of roots of thin-root tree species, on average, was 40.8% lower than of thick-root tree species (Fig. 2A; $F_{1,105} = 9.16$, $P = 0.003$), but it was not significantly affected by the nutrient patch type (Fig. 2A; $F_{3,105} = 0.82$, $P > 0.1$). *A. negundo* had, overall, the lowest colonization of approximately 25% and *P. strobus* had the highest colonization of approximately 71%.

We used the two PLFA biomarkers 16:1ω5c and 18:2ω6c to represent the external fungal proliferation of AM and EM fungi within ingrowth envelopes, respectively. The external biomass of fungi associated with roots of thin-root tree species, on average, was 12.5% lower than that of thick-root tree species (Fig. 2B; $F_{1,105} = 7.94$, $P = 0.006$). Also, fungal biomass across the four nutrient patch types differed significantly (Fig. 2B; $F_{3,105} = 97.57$, $P < 0.001$). This was largely due to accumulation of fungal biomass within the organic patches (31.5 nmol PLFA-C g^{-1} soil), which was 2.2 times higher than the average of the other three patches (14.2 nmol PLFA-C g^{-1} soil; Fig. 2B).

Nutrient concentrations

Soil extractable N concentrations differed significantly across four nutrient patches, with the organic patches having a higher concentration of NH_4^+ and NO_3^- (Fig. 3; $F_{3,105} = 23.57$, $P < 0.001$; Appendix S1: Fig. S3; $F_{3,105} = 6.24$, $P < 0.001$). There was no significant difference in soil NO_3^- within the nutrient patches between thin- and thick-root tree species (Appendix S1: Fig. S3; $F_{1,105} = 0.03$, $P = 0.85$). However, soil NH_4^+ within the nutrient patches of thick-root tree species was, on average, 31% higher than in patches of thin-root tree species (Fig. 3; $F_{1,105} = 5.22$, $P = 0.02$), but this effect was primarily confined to organic patches.

Foraging precision

Foraging precision, on average, was significantly higher in organic patches than in Low-NP and High-NP patches ($P < 0.001$; Fig. 4). Within the organic patches, AM tree species exhibited higher root foraging precision than mycorrhizal hyphal foraging precision, irrespective of root morphology, whereas EM tree species exhibited the opposite.

DISCUSSION

We found that thin-root species more readily proliferated in nutrient-rich patches with their roots, and thick-root species more readily proliferated in nutrient-rich patches with their mycorrhizal hyphae. For instance, the ratio of root length to fungal biomass of thin-root species was, on average, two times that of thick-root species within three nutrient patches (Low-NP, High-NP, and Organic). These results suggest a possible evolutionary strategy in which roots and their associated mycorrhizal fungi function in a complementary fashion while foraging in nutrient patches. Because previous research has treated the role of roots and fungi separately, such findings have been largely overlooked in previous work on nutrient foraging (Koide 2000, Tibbett 2000). Studies that have ignored the role of mycorrhizal fungi have often shown that species with thinner and longer absorptive roots exploited nutrient-rich patches

more effectively than did those with thicker and shorter absorptive roots (Caldwell et al. 1985, Eissenstat 1991, Hutchings and Dekroon 1994, Hodge et al. 1999, Tibbett 2000, Ostonen et al. 2011, Pinno and Wilson 2013). A large number of studies have also documented that mycorrhizal fungi were capable of acquiring substantial mineral nutrients from nutrient patches (Koide 1991, Tu et al. 2006, Smith and Read 2008, Cheng et al. 2012, Johnson et al. 2013); however, few studies have explicitly considered whether proliferation by mycorrhizal hyphae could compensate for reduced proliferation of fine roots in nutrient patches. In the present study, we examined tree species that varied in root morphology and mycorrhiza type in a factorial experimental design in a tractable field system. Such a design led to the new finding that for thick-root species it is possible that mycorrhizal hyphal proliferation compensates for the relative inability to proliferate roots, especially in organic nutrient patches.

Mycorrhizal fungi are increasingly considered extensions of roots, playing a vital role in helping plants forage within nutrient hotspots (Koide 1991, Read and Perez-Moreno 2003, Smith and Read 2008, Cheng et al. 2012, Koide et al. 2014). For instance, it has been estimated that AM fungi can contribute up to 70% of P acquired by AM plants (Smith and Read 2008). Additionally, Leigh et al. (2009) calculated that AM fungi were able to provide 20% of their host plant N from an organic patch in a microcosm experiment. As such, understanding nutrient foraging strategies of plants requires a holistic approach that considers roots and their associated mycorrhizal fungi as the functional unit. In our study, thin-root tree species generally proliferated more of their roots but less associated fungal hyphae within nutrient patches, while thick-root tree species did the opposite, suggesting that efficient nutrient foraging involves a trade-off between carbon allocated to roots and carbon allocated to mycorrhizal fungi. Our data, therefore, provide supporting evidence for the emerging view that functional complementarity exists in the AM symbiosis (Koide 2000, Johnson 2010) and suggests that a similar complementarity exists in the EM symbiosis as well. Koide (2000) proposed that functional complementarity may occur among coexisting AM fungi within a single root system and between roots and their associated AM fungi. In the current work, we did not analyze the community of AM fungi, and it remains to be investigated whether AM fungi are functionally complementary within their communities on a single root. Even so, our results extend to a more generalized framework that the functions of both AM and EM fungi complement with those of roots with different morphology across different plant species.

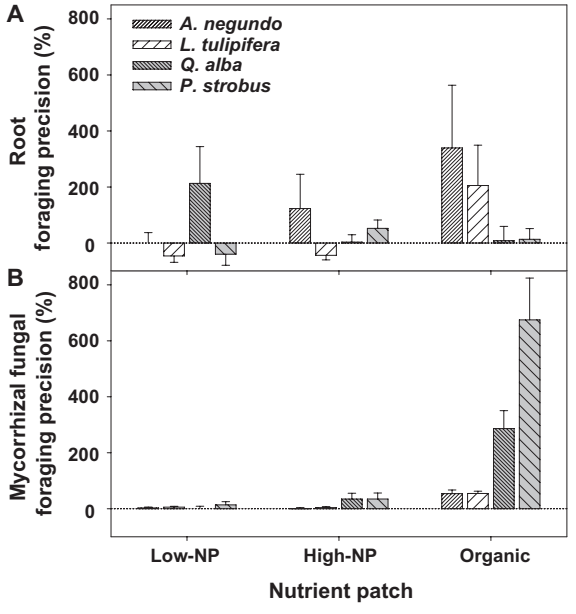

FIG. 4. Foraging precision of both root length (A) and mycorrhizal fungal hyphae (B) within different nutrient patches at week 15. Values are means (n = 8) ± SEM. See Fig. 1 for abbreviations of nutrient patches. The foraging precision was calculated as the relative increase in nutrient amended patches compared to control patches (see *Methods* for details).

Root and fungal proliferation exhibited distinct responses to organic and inorganic nutrient patches. One explanation for the higher proliferation of roots and fungi within organic patches is that mineralization of organic materials occurred over a protracted period of time, providing a long-lasting nutrient patch (Chapin et al. 2011). Although a patch of high concentrations of inorganic nutrients may initially cause root and hyphal proliferation as was observed in this study at week 5 (Figs. 1 and 2), such an effect may be short lived. Indeed, it was largely absent at week 15. Interestingly, organic patches in plots of thick-root tree species had a higher concentration of NH_4^+ than those in plots of thin-root tree species (Fig. 3), suggesting a discrepancy between the consequence of root and fungal exploitation in terms of N acquisition. While roots capture both N and P, it may be that mycorrhizal fungi are more efficient in capturing P than they are in capturing N. Nevertheless, the extent to which roots and their associated fungal hyphae use both N and P from mineralization on larger spatial and temporal scales requires further investigation.

Nutrient patch type also influenced foraging precision. All four tree species only exhibited foraging precision that was significantly different from zero in the organic nutrient patch. Moreover, this was achieved either mainly by the absorptive roots in AM tree species (Fig. 4A) or by the associated mycorrhizal fungal hyphae in EM tree species (Fig. 4B), suggesting that the way foraging precision is achieved differs between tree species of contrasting mycorrhiza type. Such results are consistent with the findings of a recent study conducted in the same area but included more tree species (Chen et al., *unpublished manuscript*). In contrast to the Chen et al. study, however, we did not observe thin roots to forage more precisely with their hyphae in the EM species. More study is needed to understand the potential links of hyphal foraging with root diameter in EM species.

Our results are not consistent with the scale-precision trade-off hypothesis for root foraging (Campbell et al. 1991). This hypothesis predicts that thin-root tree species (high scale) should exploit larger volumes of soil without exhibiting selectivity for high-nutrient hotspots (low precision), while thick-root tree species (low scale) prefer to selectively proliferate in nutrient-rich patches (high precision) to meet their nutrient demand. Instead, we found more preferential root proliferation in nutrient patches among the thin-root tree species (Fig. 1 and Appendix S1: Fig. S2). This observation is consistent with results by Rajaniemi and Reynolds (2004), who found a positive relationship, rather than a trade-off, between root foraging scale and precision. Kembel and Cahill (2005) similarly showed that across > 100 plant species the trade-off between root foraging scale and precision was not supported (Kembel and Cahill 2005). Taken together, these results suggest that the scale-precision trade-off hypothesis is not a good predictor of root foraging behaviors of plant species in the field, particularly when roots and their associated mycorrhizal fungi are considered together.

We recognize that the length of roots or the biomass of hyphae within a patch is the consequence of both finding the patch and proliferating within it. The odds of finding a patch, however, were likely similar among the four tree species in our study. The root length densities (RLD) in the same soil layer in which the nutrient patches were installed (0–10 cm) were ($cm/cm^3 \pm SE$): *Acer negundo*, 0.77 ± 0.08; *Liriodendron tulipifera*, 0.57 ± 0.05; *Pinus strobus*, 0.85 ± 0.09; and *Quercus alba*, 0.80 ± 0.07) with no significant differences in RLD among species ($P < 0.05$). The magnitude of our RLD suggest a high likelihood of a root encountering a nutrient patch of just a few square centimeters of surface area (Escamilla et al. 1991), and our patches had an area of 50 cm^2. Therefore, variation in growth within the patches observed in the current study very likely reflected variation in the ability to proliferate. The size of our nutrient patches (10×5 cm) was consistent with previous studies that examined roots and mycorrhizal fungi on similar time scales (Neill 1992, Wallander et al. 2001, Ostonen et al. 2011, Cheng et al. 2012, Addo-Danso et al. 2016). On time scales of weeks to months, root foraging usually occurs at the scale of centimeters to decimeters, and root proliferation in small nutrient-rich patches has been considered a major mechanism by which plants forage for nutrients (Eissenstat and Caldwell 1988, Neill 1992, Caldwell 1994, Rajaniemi and Reynolds 2004, Ostonen et al. 2011). While we did not explicitly examine the scale of spatial variability of nutrients in our plots, tree species should often experience significant variation in nutrient availability on a spatial and temporal scale comparable to our nutrient patches due to leaf fall and movement on the forest floor, and small scale disturbances associated with the activities of organisms such as earthworms as well as the activities of other invertebrates and small vertebrates (Chapin et al. 2011, Garcia-Palacios et al. 2014).

Our finding that roots and fungi are functionally complementary in terms of foraging within nutrient patches has two important implications. First, increasing recognition of functional complementarity between roots of different morphology together with their associated fungi provides insight into the co-evolution of tree species and mycorrhizal fungi (Brundrett 2002, Hoeksema 2010). Species of more basal lineages such as those in the Magnoliales (e.g., *L. tulipifera*) are typically highly colonized by AM fungi and have thick roots whereas plant species that diverged from these basal lineages evolved diverse strategies of nutrient foraging including more specialized mycorrhizal associations (e.g., ectomycorrhizas) or thinner roots that typically are less dependent on mycorrhizas for nutrient uptake (Wang and Qiu 2006). While complementarity of root morphology and mycorrhizas in nutrient foraging of mineral nutrients has been observed in AM trees (Eissenstat et al. 2015, Liu et al. 2015), the linkages of root diameter in EM species with root foraging had not been previously explored, especially in organic nutrient-rich patches. Here we provide evidence that complementarity between roots and mycorrhizas applies in both AM and EM tree species, but with important differences. In organic matter patches, while thin-root species of both AM and EM species foraged more than corresponding thick-root species, overall root foraging (Fig. 1) and root foraging precision (Fig 4) was much higher in AM than EM tree species, suggesting that EM species rely more on selective hyphal foraging for their nutrient acquisition. In terrestrial ecosystems where nutrients are primarily complexed in organic materials, use of EM hyphae may provide distinct advantages compared to AM hyphae (Read and Perez-Moreno 2003).

Second, the result that roots and fungal hyphae proliferated within organic patches in a complementary way may have a fundamental influence on soil C and nutrient cycling in forest ecosystems, depending of patch duration. Fine root litter constitutes a large fraction of organic C and nutrients in many forest ecosystems. But the turnover of fine roots is slower than that of hyphae of either AM fungi (Matamala et al. 2003, Staddon et al. 2003) or EM fungi (Koide et al. 2011), suggesting potential differences for soil C and nutrient cycling under thin-root and thick-root species. For example, in a field study, Koide et al. (2011) demonstrated that hyphae of EM fungi decomposed more rapidly than fine roots of *Pinus resinosa*. This indicates that C and nutrient cycling within patches may be more rapid when proliferation of hyphae occurs (thick-root species) as opposed to when proliferation of roots occurs (thin-root species). Eventually, however, if the patches are sufficiently long-lived, species with thick roots should eventually colonize the patch (e.g., compare *L. tulipifera* at 15 weeks to five weeks in Fig. 1). Because thick absorptive roots typically live longer than thin absorptive roots across species (McCormack et al. 2012), these different patterns of patch colonization and patch retention with different types of

root systems may cause shifts in the competitive advantage and patterns of nutrient cycling, depending on the degree nutrients are in an organic form and the relative permanence of the nutrient patch. Thus, in forests with wide variation in the spatial and temporal patterns of nutrient-rich patches, there may be multiple niches where a diversity of tree species can coexist with important consequences for belowground nutrient and carbon cycling.

Acknowledgments

This research was supported by the National Science Foundation of USA (NSF IOS-1120482) awarded to D.M.E and R.T.K. Lei Cheng was partially supported by National Science Foundation of China (31370487, 31422010), the Zhejiang Provincial Funds for Distinguished Young Scientists (LR14C030001) and the Fundamental Research Funds for the Central Universities.

Literature Cited

Adams, T. S., M. L. McCormack, and D. M. Eissenstat. 2013. Foraging strategies in trees of different root morphology: the role of root lifespan. Tree Physiology 33:940–948.

Addo-Danso, S. D., C. E. Prescott, and A. R. Smith. 2016. Methods for estimating root biomass and production in forest and woodland ecosystem carbon studies: a review. Forest Ecology and Management 359:332–351.

Brundrett, M. C. 2002. Coevolution of roots and mycorrhizas of land plants. New Phytologist 154:275–304.

Brundrett, M. C. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil 320:37–77.

Caldwell, M. M. 1994. Exploiting nutrients in fertile soil microsites. Pages 325–347 *in* M. M. Caldwell and R. W. Pearcy, editors. Exploitation of environmental heterogeneity by plants: ecophysiological processes above- and belowground. Academic Press Inc, San Diego, California, USA.

Caldwell, M. M., D. M. Eissenstat, J. H. Richards, and M. F. Allen. 1985. Competition for phosphorus: differential uptake from dual-isotope labeled soil interspaces between shrub and grass. Science 229:384–386.

Campbell, B. D., J. P. Grime, and J. M. L. Mackey. 1991. A trade-off between scale and precision in resource foraging. Oecologia 87:532–538.

Chapin, F. S., P. A. Matson, and H. A. Mooney. 2011. Principles of terrestrial ecosystem ecology. Second edition. Springer-Verlag, New York, New York, USA.

Cheng, L., F. L. Booker, K. O. Burkey, C. Tu, H. D. Shew, T. W. Rufty, E. L. Fiscus, J. L. Deforest, and S. Hu. 2011. Soil microbial responses to elevated CO_2 and O_3 in a nitrogen-aggrading agroecosystem. PLoS One 6:e21377.

Cheng, L., F. L. Booker, C. Tu, K. O. Burkey, L. Zhou, H. D. Shew, T. W. Rufty, and S. Hu. 2012. Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2. Science 337:1084–1087.

Comas, L. H., and D. M. Eissenstat. 2009. Patterns in root trait variation among 25 co-existing North American forest species. New Phytologist 182:919–928.

Eissenstat, D. M. 1991. On the relationship between specific root length and the rate of root proliferation—a field study using citrus rootstocks. New Phytologist 118:63–68.

Eissenstat, D. M. 1992. Costs and benefits of constructing roots of small diameter. Journal of Plant Nutrition 15:763–782.

Eissenstat, D. M., and M. M. Caldwell. 1988. Seasonal timing of root growth in favorable microsites. Ecology 69:870–873.

Eissenstat, D. M., J. M. Kucharski, M. Zadworny, T. S. Adams, and R. T. Koide. 2015. Linking root traits to nutrient foraging in arbuscular mycorrhizal trees in a temperate forest. New Phytologist 208:114–124.

Escamilla, J. A., N. B. Comerford, and D. G. Neary. 1991. Soil-core break method to estimate Pine root distribution. Soil Science Society of America Journal 55:1722–1726.

Garcia-Palacios, P., F. T. Maestre, M. A. Bradford, and J. F. Reynolds. 2014. Earthworms modify plant biomass and nitrogen capture under conditions of soil nutrient heterogeneity and elevated atmospheric CO_2 concentrations. Soil Biology & Biochemistry 78:182–188.

Hart, M. M., and R. J. Reader. 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytologist 153:335–344.

Hodge, A., D. Robinson, B. S. Griffiths, and A. H. Fitter. 1999. Why plants bother: root proliferation results in increased nitrogen capture from an organic patch when two grasses compete. Plant Cell and Environment 22:811–820.

Hoeksema, J. D. 2010. Ongoing coevolution in mycorrhizal interactions. New Phytologist 187:286–300.

Hutchings, M. J., and H. Dekroon. 1994. Foraging in plants: the role of morphological plasticity in resource acquisition. Advances in Ecological Research 25:159–238.

Johnson, N. C. 2010. Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. New Phytologist 185:631–647.

Johnson, N. C., C. Angelard, I. R. Sanders, and E. T. Kiers. 2013. Predicting community and ecosystem outcomes of mycorrhizal responses to global change. Ecology Letters 16:140–153.

Kembel, S. W., and J. F. Cahill. 2005. Plant phenotypic plasticity belowground: a phylogenetic perspective on root foraging trade-offs. American Naturalist 166:216–230.

Koide, R. T. 1991. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. New Phytologist 117: 365–386.

Koide, R. T. 2000. Functional complementarity in the arbuscular mycorrhizal symbiosis. New Phytologist 147:233–235.

Koide, R. T., C. W. Fernandez, and M. S. Peoples. 2011. Can ectomycorrhizal colonization of *Pinus resinosa* roots affect their decomposition? New Phytologist 191:508–514.

Koide, R. T., C. Fernandez, and G. Malcolm. 2014. Determining place and process: functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. New Phytologist 201:433–439.

Leigh, J., A. Hodge, and A. H. Fitter. 2009. Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. New Phytologist 181:199–207.

Liu, B., H. Li, B. Zhu, R. T. Koide, D. M. Eissenstat, and D. Guo. 2015. Complementarity in nutrient foraging strategies of absorptive fine roots and arbuscular mycorrhizal fungi across 14 coexisting subtropical tree species. New Phytologist 208:125–136. http://dx.doi.org/10.1111/nph.13434

Matamala, R., M. A. Gonzalez-Meler, J. D. Jastrow, R. J. Norby, and W. H. Schlesinger. 2003. Impacts of fine root turnover on forest NPP and soil C sequestration potential. Science 302:1385–1387.

McCormack, M. L., T. S. Adams, E. A. H. Smithwick, and D. M. Eissenstat. 2012. Predicting fine root lifespan from plant functional traits in temperate trees. New Phytologist 195:823–831.

McCormack, M. L., T. S. Adams, E. A. H. Smithwick, and D. M. Eissenstat. 2014. Variability in root production,

phenology, and turnover rate among 12 temperate tree species. Ecology 95:2224–2235.
McCormack, M. L., et al. 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. New Phytologist 207:505–518.
Neill, C. 1992. Comparison of soil coring and ingrowth methods for measuring belowground production. Ecology 73: 1918–1921.
Olsson, P. A. 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiology Ecology 29:303–310.
Ostonen, I., et al. 2011. Fine root foraging strategies in Norway spruce forests across a European climate gradient. Global Change Biology 17:3620–3632.
Phillips, R. P., E. Brzostek, and M. G. Midgley. 2013. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon-nutrient couplings in temperate forests. New Phytologist 199:41–51.
Pinheiro, J., and D. Bates. 2007. Linear and nonlinear mixed effects models.
Pinno, B. D., and S. D. Wilson. 2013. Fine root response to soil resource heterogeneity differs between grassland and forest. Plant Ecology 214:821–829.
Pregitzer, K. S., J. L. DeForest, A. J. Burton, M. F. Allen, R. W. Ruess, and R. L. Hendrick. 2002. Fine root architecture of nine North American trees. Ecological Monographs 72:293–309.
Rajaniemi, T. K., and H. L. Reynolds. 2004. Root foraging for patchy resources in eight herbaceous plant species. Oecologia 141:519–525.
Read, D. J., and J. Perez-Moreno. 2003. Mycorrhizas and nutrient cycling in ecosystems—A journey towards relevance? New Phytologist 157:475–492.
Smith, S. E., and D. J. Read. 2008. Mycorrhizal symbiosis. Second edition. Academic Press, San Diego, California, USA.
Staddon, P. L., C. B. Ramsey, N. Ostle, P. Ineson, and A. H. Fitter. 2003. Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of ^{14}C. Science 300: 1138–1140.
Sylvia, D. M. 1994. Vesicular-arbuscular mycorrhizal fungi. Pages 351–378 *in* R. W. Weaver, editor. Methods of soil analysis: microbiological and biochemcial properties. Soil Science Society of America, Madison, Wisconsin, USA.
Tibbett, M. 2000. Roots, foraging and the exploitation of soil nutrient patches: the role of mycorrhizal symbiosis. Functional Ecology 14:397–399.
Tu, C., F. L. Booker, D. M. Watson, X. Chen, T. W. Rufty, W. Shi, and S. J. Hu. 2006. Mycorrhizal mediation of plant N acquisition and residue decomposition: impact of mineral N inputs. Global Change Biology 12:793–803.
Visser, S. 1995. Ectomycorrhizal fungal succession in jack pine stands following wildfire. New Phytologist 129:389–401.
Wallander, H., L. O. Nilsson, D. Hagerberg, and E. Baath. 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. New Phytologist 151:753–760.
Wang, B., and Y. L. Qiu. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16: 299–363.
Weigt, R. B., S. Raidl, R. Verma, and R. Agerer. 2012. Exploration type-specific standard values of extramatrical mycelium—a step towards quantifying ectomycorrhizal space occupation and biomass in natural soil. Mycological Progress 11:287–297.

Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.1514/suppinfo