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Research

Complementarity in nutrient foraging strategies of absorptive fine roots and arbuscular mycorrhizal fungi across 14 coexisting subtropical tree species

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Summary

- In most cases, both roots and mycorrhizal fungi are needed for plant nutrient foraging. Frequently, the colonization of roots by arbuscular mycorrhizal (AM) fungi seems to be greater in species with thick and sparsely branched roots than in species with thin and densely branched roots. Yet, whether a complementarity exists between roots and mycorrhizal fungi across these two types of root system remains unclear.
- We measured traits related to nutrient foraging (root morphology, architecture and proliferation, AM colonization and extramatrical hyphal length) across 14 coexisting AM subtropical tree species following root pruning and nutrient addition treatments.
- After root pruning, species with thinner roots showed more root growth, but lower mycorrhizal colonization, than species with thicker roots. Under multi-nutrient (NPK) addition, root growth increased, but mycorrhizal colonization decreased significantly, whereas no significant changes were found under nitrogen or phosphate additions. Moreover, root length proliferation was mainly achieved by altering root architecture, but not root morphology.
- Thin-root species seem to forage nutrients mainly via roots, whereas thick-root species rely more on mycorrhizal fungi. In addition, the reliance on mycorrhizal fungi was reduced by nutrient additions across all species. These findings highlight complementary strategies for nutrient foraging across coexisting species with contrasting root traits.

Introduction

Absorptive fine roots (i.e. non-woody roots in woody plants; Pregitzer *et al.*, 2002; Guo *et al.*, 2008) and mycorrhizal fungi both play key roles in soil resource acquisition (Robinson *et al.*, 2003). Recent studies have shown that traits of absorptive fine roots can vary widely across plant species (Pregitzer *et al.*, 2002; Tjoelker *et al.*, 2005; Guo *et al.*, 2008; Comas & Eissenstat, 2009; Holdaway *et al.*, 2011). This variation in root traits may reflect key belowground foraging behaviour, such as reliance on mycorrhizal fungi (Graham & Syvertsen, 1985; Rillig *et al.*, 2003; Kong *et al.*, 2014), and may be linked to plant performance, such as growth rate (Wahl & Ryser, 2000; Comas *et al.*, 2002; Comas & Eissenstat, 2004). The identification of patterns of root trait variation across species is thus valuable for understanding the nutrient foraging behavior of absorptive fine roots and mycorrhizal fungi, and the diversity of belowground resource acquisition strategies (Bardgett *et al.*, 2014; Iversen, 2014; McCormack *et al.*, 2014).

The variation of absorptive fine root traits and mycorrhizal colonization may be related to plant phylogeny (Brundrett, 2002; Kong *et al.*, 2014). Species of basal clades tend to produce thick and sparsely branched root systems (thick-root species), whereas species of recently diverged clades tend to produce thin and densely branched root systems (thin-root species) (Baylis, 1975; St John, 1980; Pregitzer *et al.*, 2002; Comas & Eissenstat, 2009; Chen *et al.*, 2013). Furthermore, there is evidence that thin-root species have greater rates of root growth (Eissenstat, 1991), whereas thick-root species are generally more densely colonized by arbuscular mycorrhizal (AM) fungi (Fitter, 2004; Smith & Read, 2008). This may be because thick-root species have a limited intrinsic ability to acquire soil resources (Bates & Lynch, 2001), and need to increase absorptive surfaces by relying more on finely structured AM fungal hyphae (Raven & Edwards, 2001). Together, this implies that there exists a complementarity between absorptive fine roots and associated mycorrhizal fungi in nutrient foraging across species with contrasting root functional traits.

Given that roots and mycorrhizal fungi represent alternative strategies in the construction of belowground absorptive surface area, it is important to understand how roots and mycorrhizal fungi respond to heterogeneity in soil nutrient availability, which is ubiquitous in nature. Root growth within nutrient-rich patches has been widely reported to increase plant nutrient capture (Robinson, 1994; Hodge *et al.*, 1998), albeit with a few exceptions in which no root proliferation was observed in nutrient-rich patches (Jackson & Caldwell, 1989; Li *et al.*, 2014). By contrast, mycorrhizal fungal colonization and external hyphal production often decrease under nutrient additions, mostly via broadcast fertilization (Johnson *et al.*, 2003; Treseder, 2004; Liu *et al.*, 2012; Grman & Robinson, 2013; Johnson *et al.*, 2015; but see localized addition of phosphorus (P) and nitrogen (N) from Duke *et al.*, 1994). Overall, previous studies have evaluated how either roots or mycorrhizal fungi respond to nutrient additions, but few studies have examined the responses of both roots and mycorrhizal fungi in the field, especially with respect to mineral nutrient patches.

It is necessary to examine roots and mycorrhizal fungi simultaneously because tree species differ in their root and mycorrhizal traits, particularly in subtropical and tropical forests, in which thick-root species are very common and coexist with thin-root species. Because root traits are strongly correlated with mycorrhizal colonization and also potentially with hyphal density, particularly in AM woody species (Kong *et al.*, 2014), roots and mycorrhizal fungi may respond to changes in soil nutrients in a coordinated manner. Moreover, different nutrient elements may influence the responses of roots and mycorrhizal fungi (Robinson, 1994; Johnson *et al.*, 2008) in a different manner. For example, Drew and coworkers reported that roots of non-mycorrhizal barley (*Hordeum vulgare*) plants showed increased root length and number of laterals under phosphate, ammonium or nitrate addition, but not under potassium (K) addition (Drew, 1975; Drew & Saker, 1975). In three grassland systems, internal AM colonization and external hyphal density responded strongly and consistently to soil P availability, but not to soil N availability (Johnson *et al.*, 2015). It is likely that N-rich patches are less effective than P-rich patches in reducing internal colonization and external hyphal production, particularly in AM plant species, given that the major function of AM fungi is to facilitate P acquisition (Smith & Read, 2008). However, other studies showed no definitive responses of internal AM colonization and external hyphal production to nutrient additions (Sylvia & Neal, 1990; Treseder & Allen, 2002; Johnson *et al.*, 2003; Egerton-Warburton *et al.*, 2007; Grman & Robinson, 2013). Therefore, an understanding of root and mycorrhizal fungal responses to nutrient-rich patches and the root–mycorrhiza trade-off requires that we consider the attributes of the nutrient patch itself.

Finally, responses of roots and mycorrhizal fungi to nutrient availability may be reflected more strongly in some traits than in others. Both root morphological and architectural traits may be altered by nutrient availability, but architectural traits may be more responsive (Hodge, 2004), because root morphological traits, such as root diameter, have been shown to be phylogenetically conserved (Kong *et al.*, 2014), and may have limited plasticity as soil conditions vary. More studies are needed to clarify how root morphological and architectural traits differ in their responses to nutrient availability.

Here, we selected 14 AM tree species that coexist in a subtropical forest. We measured a variety of traits of the first two root branch orders (termed absorptive fine roots here, *sensu* McCormack *et al.*, 2015) under four nutrient addition treatments using a root-bag approach (Comas & Eissenstat, 2004). The studied species encompassed a wide variety of root morphology and architecture, with an almost four-fold range in root diameter of the finest lateral roots (or first-order roots) based on previous studies (D. L. Guo, unpublished data). The absorptive fine root and fungal traits measured covered several key aspects of foraging behavior, including root morphology and architecture, carbon (C) and N concentrations, length and mass proliferation, AM colonization and extramatrical hyphal length. The localized nutrient addition treatments consisted of unfertilized control, N addition, P addition and multi-nutrient (NPK) addition. We aimed to test the following hypotheses: (1) patterns of production in absorptive fine roots and their associated mycorrhizal fungi are complementary across species in nutrient foraging: thin-root species forage nutrients primarily using absorptive fine roots, whereas thick-root species forage with more reliance on mycorrhizal fungi; (2) plants increase root length but reduce mycorrhizal colonization in response to nutrient-rich patches; and (3) root architectural traits (e.g. root branching intensity or ratio) exhibit more phenotypic plasticity to nutrient-rich patches than do root morphological traits (e.g. root diameter or specific root length).

Materials and Methods

Study sites and species selection

The study site was located in the Jiulianshan National Nature Reserve (24°29′18″–24°38′55″N, 114°22′50″–114°31′32″E) in Jiangxi province, China. The site belongs to the typical subtropical climatic zone with mean January, July and annual temperatures of 6.8, 24.4 and 16.4°C, respectively, and with mean annual precipitation of 2156 mm (Li, 2006). Soils in this area are laterites rich in iron and aluminum, and soil texture is mainly clay loam according to the China Soil Scientific Database (www.soil.csdb.cn).

Soil samples were collected and measured for their physical and chemical properties from 0 to 20 cm depth in early March 2013. The average soil gravimetric water content was $24.8 \pm 0.7\%$ (mean ± SE) (using oven drying) during the study period. Soil total C and total N were $21.88 \pm 1.15\ g\ kg^{-1}$ and $1.67 \pm 0.07\ g\ kg^{-1}$, respectively (using an elemental analyzer; Vario MAX CN; Elementar, Hanau, Germany). Soil available N (KCl-extractable ammonium and nitrate) was $11.91 \pm 0.58\ mg\ kg^{-1}$ (using an AutoAnalyzer 3; Bran & Luebbe, Hamburg, Germany). These soils are characterized by relatively high total and available phosphorus (P) ($(NH_4)_2CO_3$-extractable available P), with $0.41 \pm 0.02\ g\ kg^{-1}$ and $24.53 \pm 1.79\ mg\ kg^{-1}$, respectively (using an inductively coupled

plasma-optical emission spectrometer (ICP-OES); Perkin Elmer, Norwalk, CT, USA). Soil pH was 4.5 ± 0.1 (soil to water mass ratio of 1 : 2.5).

At this site, we chose 14 coexisting dominant tree species (Table 1) that expressed a large degree of interspecific variation in the diameter and related root traits of first-order roots based on our preliminary observations. All 14 species at this site may be colonized by AM fungi (Wang & Qiu, 2006; Kong *et al.*, 2014).

Root bag installation, fertilization and harvesting

A root-bag approach was used to isolate roots of different trees in a mixed forest (Comas & Eissenstat, 2004). In early March 2013, a *c.* 5-mm-diameter woody root was traced back to an identified tree. The distal end of the root was trimmed of all fine lateral roots using scissors and *c.* 25 cm of length was inserted into a root bag. Root bags were constructed from polyester fabric (30×30 cm^2), with a mesh size of 0.5 mm, and were filled with 3 kg of sieved fresh soil collected from the forest surface (0–20 cm depth). The bags containing the pruned woody roots were reburied with the original forest soil, covered with the original litter layer, and watered. To ensure that a statistically sufficient number of root bags with well-developed root systems can be harvested (Supporting Information Fig. S1), we installed 48 root bags under four separate individual trees (12 bags per tree) for each species, with a total of 672 bags for all 14 species (Table S1).

In early June 2013, the four nutrient addition treatments were randomly assigned to 12 root bags per tree (three bags for each treatment), and then sprayed with deionized water, 300 ml per bag. These four treatments included unfertilized control (Unfert.), N addition (+N; 0.13 g N per bag, in the form of slow-release urea containing 40% N), P addition (+P; 0.29 g P per bag, in the form of NaH_2PO_4 containing 20% P) and multi-nutrient addition (+NPK; 0.13 g N, 0.07 g P and 0.10 g K per bag, in the form of Osmocote, which is a slow-release compound fertilizer containing 40% N, 22.5% P, 30% K and essential micronutrients, as well as calcium and magnesium). The amount of fertilizer given was approximately four times 'available' soil background N or P concentration, and was chosen according to the results of Adams *et al.* (2013), who observed no increases in root length growth at threefold available soil N concentration, but increases at higher levels of N fertilization.

In late September 2013, root bags were harvested from the field by cutting the woody roots at the entrance of the bags. The intact bags were immediately placed into a cooler and transported to the laboratory. In the laboratory, the intact root samples were gently washed with tap water to remove the soil adhered to roots. Three intact root segments containing the first five root orders were spread out in water with minimal overlap and scanned in gray scale at 400 dpi using automatic threshold settings (Comas & Eissenstat, 2004). Afterwards, these samples were placed into formalin-aceto-alcohol (FAA) solution (90 ml of 50% ethanol, 5 ml of 100% glacial acetic acid, 5 ml of 37% methanol) for the measurement of AM colonization later. The remaining samples were frozen at −20°C until the measurement of root traits.

Root trait measurements

Root recovery from pruning for a given species was expressed as the percentage of all bags for each of the four treatments that recovered from pruning by exhibiting new root growth. Averaged across all 14 species, *c.* 70% of root bags installed contained newly growing root systems (Table S1), with the remaining 30% of bags containing no new roots. In addition, for all the root bags that contained new roots, the amount of new root growth showed some variation (Fig. S1) and we separated these bags into two categories; we classified more than two-thirds of root bags with new root growth as well-developed root bags and the remaining less than one third as poorly developed root bags (Table S1). To reduce the labor requirements associated with the dissection of

Table 1 Absorptive fine root trait mean (SE) for four main variables measured from recovery after root pruning under unfertilized control for 14 arbuscular mycorrhizal (AM) tree species from a subtropical forest, China

Species	Abbreviation	Family	Life form	Diameter (mm)	SRL (m g^{-1})	BrIntensity (cm^{-1})	AMC (%)
Acer fabri	Acfa	Aceraceae	EB	0.19 (0.00)	190.3 (16.7)	2.23 (0.51)	21.26 (3.21)
Schima superba	Scsu	Theaceae	EB	0.21 (0.01)	141.0 (16.5)	1.25 (0.09)	32.66 (1.86)
Choerospondias axillaris	Chax	Anacardiaceae	DB	0.25 (0.01)	87.3 (5.7)	1.08 (0.20)	47.02 (0.35)
Acer cinnamomifolium	Acci	Aceraceae	EB	0.26 (0.01)	107.7 (8.3)	1.10 (0.18)	33.21 (2.34)
Liquidambar formosana	Lifo	Hamamelidaceae	DB	0.26 (0.01)	89.4 (6.2)	0.67 (0.08)	52.08 (1.68)
Elaeocarpus glabripetalus	Elgl	Elaeocarpaceae	EB	0.30 (0.01)	68.6 (9.4)	0.85 (0.18)	54.47 (0.85)
Alniphyllum fortunei	Alfo	Styracaceae	DB	0.31 (0.02)	73.8 (7.1)	0.22 (0.07)	28.45 (4.35)
Cinnamomum porrectum	Cipo	Lauraceae	EB	0.33 (0.03)	66.7 (12.0)	0.50 (0.10)	47.88 (3.86)
Cinnamomum austrosinense	Ciau	Lauraceae	EB	0.41 (0.03)	38.6 (5.7)	0.87 (0.35)	28.31 (0.34)
Machilus oculodracontis	Maoc	Lauraceae	EB	0.51 (0.02)	23.2 (3.4)	0.28 (0.05)	58.95 (4.34)
Manglietia yuyuanensis	Mayu	Magnoliaceae	EB	0.57 (0.02)	30.2 (1.6)	0.23 (0.04)	65.73 (1.80)
Neolitsea phanerophlebia	Neph	Lauraceae	EB	0.55 (0.03)	31.7 (3.4)	0.55 (0.17)	90.68 (1.57)
Cunninghamia lanceolata	Cula	Taxodiaceae	EN	0.64 (0.02)	17.5 (0.7)	0.16 (0.04)	76.06 (0.68)
Taxus chinensis	Tach	Taxaceae	EN	0.86 (0.07)	12.0 (0.8)	0.41 (0.02)	84.18 (2.14)

Variables included root diameter, specific root length (SRL), root branching intensity (BrIntensity) (first-order root number per unit length of second-order roots) and AM colonization (AMC) of first- and second-order roots combined. Life form: evergreen broadleaf (EB), deciduous broadleaf (DB) and evergreen needle (EN).

roots to each branch order and to enhance comparability across species with different root morphology and architecture, we randomly selected four bags from the well-developed root bag category for each treatment (including unfertilized control, +N, +P and +NPK) for each species, with a total of 224 bags for all 14 species. Samples of each bag were separately assessed for root traits, including morphology, architecture and chemistry, root length and mass proliferation, and AM colonization and extramatrical hyphal length in soils (Table 2). These parameters were assessed on a root order basis following the root order classification suggested by Pregitzer *et al.* (2002).

Following the dissection, samples from each root order were arranged in water with minimal overlap and scanned on an Epson Expression 10 000 XL desktop scanner (resolution of 400 dpi, document type set to 'film mode'). Root samples were then oven dried at 60°C for 48 h and weighed. After weighing, each sample was ground to a fine powder, and root C and N concentrations were determined using an elemental analyzer (Vario EL Cube; Elementar). From the scanned images, the average root diameter, root total length, volume and root number for each root order were measured using WinRHIZO software (Regent Instruments Inc., Quebec City, QC, Canada).

The specific root length (SRL) was calculated as the root total length divided by its dry mass for each root order. The root tissue density was calculated as the ratio of root dry mass to its volume. The root branching intensity was calculated as the number of first-order roots divided by the total root length of second-order roots. The root branching ratio was calculated as the number of first-order roots divided by the number of second-order roots. The total branch order was obtained by recording the highest root order from the newly growing root branching system after pruning in each root bag. The root total length for each root order within each root bag was measured for all roots, including root samples stored in FAA. The root total dry mass for each root order was calculated as the root total length divided by its SRL for each root bag.

Here, we combined the first two root orders for our analyses and defined them as absorptive fine roots (*sensu* McCormack *et al.*, 2015). Furthermore, previous studies have shown that the first two or three root orders for most tree species have an intact cortex, are frequently colonized by mycorrhizal fungi and mainly perform the function of resource acquisition (Pregitzer *et al.*, 2002; Guo *et al.*, 2008; Xia *et al.*, 2010; Gu *et al.*, 2014). Our assessment on mycorrhizal colonization also confirmed that the first two root orders in all 14 species were non-woody and mycorrhizal, consistent with these previous studies.

Mycorrhizal colonization

Root samples stored in FAA solution were washed carefully with deionized water and the first two root orders were selected for the measurement of AM colonization using acid fuchsin staining (Giovannetti & Mosse, 1980). Root segments were cleared in 10% (w/v) KOH solution at 90°C for 50 min, acidified in 2% HCl at room temperature for 5 min and stained with 0.05% (w/v) acid fuchsin (1.2 g acid fuchsin mixed with glycerin, lactic acid (10%) and water in proportions of 1 : 1 : 1 by volume) at 90°C for 20 min. Then 50 1-cm-long root segments were randomly selected for the measurement of AM colonization at ×200 magnification (Leica DM 2500; Leica Mikrosysteme Vertrieb GmbH, Bensheim, Germany) using the line-intersect method described by McGonigle *et al.* (1990). Arbuscules, vesicles, non-

Table 2 Abbreviations and descriptions of absorptive fine root morphological, architectural and chemical traits, and root proliferation and mycorrhizal colonization

Parameter	Abbreviation	Units	Description
Morphological traits			
Average root diameter	Diameter	mm	Average diameter of combined first two order roots
Average root length	Length	cm	Average individual root length of combined first two order roots
Specific root length	SRL	$m\ g^{-1}$	Length per unit dry mass of combined first two order roots
Root tissue density	RTD	$g\ cm^{-3}$	Mass per unit root volume of combined first two order roots
Architectural traits			
Branching intensity	BrIntensity	cm^{-1}	Number of first-order roots per unit length of second-order roots
Total branch order	TBO		Highest root branching order contained within a root bag
Branching ratio	BrRatio		Number of first-order roots divided by the number of second-order roots
Chemical traits			
Root carbon concentration	Root C	%	Average root carbon concentration of combined first two order roots within a root bag
Root nitrogen concentration	Root N	%	Average root nitrogen concentration of combined first two order roots within a root bag
Root proliferation			
Pruning recovery	PR	%	Percentage of a given species that recovered from pruning by proliferating new absorptive roots
Root length growth	RL	cm	Total absorptive fine root (combined first two order roots) length proliferation within a root bag
Root mass growth	RM	mg	Total absorptive fine root (combined first two order roots) dry mass proliferation within a root bag
Mycorrhizal colonization			
Arbuscular mycorrhizal colonization	AMC	%	Percentage of absorptive root length colonized by arbuscules, vesicles or coils within a root bag
Extramatrical hyphal length	EHL	m	Extramatrical hyphal length within a root bag

septate and pink-coloured hyphae within the roots were all considered as evidence of AM colonization, and overall colonization was expressed as the percentage of the total root segments scored.

After all roots from a root bag had been carefully picked, a fresh soil subsample containing no roots was taken from each bag and extramatrical hyphae were extracted from this soil sample by the membrane filter technique (Miller *et al.*, 1995; Rillig *et al.*, 1999). Specifically, a fresh soil subsample of 4.0 g from each root bag was mixed by hand with 100 ml deionized water and 12 ml sodium hexametaphosphate (35 g l^{-1}). The blended suspensions were shaken for 30 s and left on the bench to settle for 30 min. The supernatant was poured through a 38-μm sieve to retain hyphae, roots and other organic matter. The materials on the sieve were sprayed gently with deionized water to remove clay particles, and then transferred to a 250-ml flask with deionized water to reach a volume of 200 ml. The flask was shaken vigorously by hand for 5 s and left on the bench for 1 min. Duplicate 2-ml aliquots were pipetted onto 25-mm-diameter, 1.2-μm Millipore filters. The filters were covered with 1% acid fuchsin for 5 min and observed at ×200 magnification (Nikon 80i; Nikon, Tokyo, Japan) using the line-intersect method described by McGonigle *et al.* (1990). Hyphal length was determined using the modified formula by Tennant (1975). Because extramatrical hyphae may have rapid decomposition rates (Fernandez & Koide, 2012) and require a relatively long period of time to be extracted, we were not able to measure extramatrical hyphae for all treatments. We instead focused our analyses on the unfertilized control samples and the +NPK samples, a comparison that should show the strongest contrast.

Data analysis

Testing of our first hypothesis required the determination of whether the absorptive fine root diameter across species was positively correlated with its proliferation (root recovery from pruning, root length and mass proliferation) and negatively correlated with mycorrhizal colonization (AM colonization and extramatrical hyphal length per unit length of absorptive fine roots within a root bag). We used linear regression to examine the relationship between root diameter vs root proliferation and between root diameter vs mycorrhizal colonization. The slopes of the linear regressions under all nutrient addition treatments were compared in the R 3.0.3 statistical platform (R Development Core Team, 2014) with the R package SMART (Higdon *et al.*, 2004). Parameters of root proliferation and mycorrhizal colonization were logarithmically transformed to meet a normal distribution and homogeneity of variance.

To test the second hypothesis, we determined whether plants tended to increase absorptive fine root length proliferation as opposed to mycorrhizal colonization across 14 species in response to nutrient-rich patches. In addition, to test the third hypothesis, we determined whether root architectural traits (e.g. root branching) exhibited more phenotypic plasticity than root morphological traits (e.g. root diameter and SRL) in response to nutrient-rich patches. We used two-way factorial ANOVA to test the influence of species and fertilization treatments on root traits, root proliferation and mycorrhizal colonization, and, when appropriate, *post hoc* means comparisons were made using least square difference (LSD) tests in SPSS 17.0 (SPSS Inc., Chicago, IL, USA). The mean values and coefficient of variation (CV) of all traits averaged across 14 species for each of the four treatments were calculated. Pairwise trait relationships were calculated using Pearson's correlations in SPSS 17.0 (SPSS Inc.). In addition, trait relationships were analyzed after removing the influence of phylogeny via phylogenetic independent contrasts (PICs) using the R package *picante* (Kembel *et al.*, 2010).

Results

Root trait variation across species

We observed wide variation in root morphology and architecture in the lateral fine root branches across 14 coexisting subtropical AM tree species (Fig. S2). These traits were measured on new roots growing in root bags and may differ in specific values from naturally growing roots, but the species differences were the same between roots in root bags and roots in the field (Fig. S3). The mean diameter of absorptive fine roots based on the first two root orders combined varied 4.5-fold across the 14 species, ranging from 0.19 mm in *Acer fabri* to 0.86 mm in *Taxus chinensis* ($P < 0.001$, CV = 49%, Tables 1, 3 and S2). SRL varied almost 16-fold with a CV of 73% (from 12.0 m g^{-1} for *T. chinensis* to 190.3 m g^{-1} for *A. fabri*). Root branching intensity varied nearly 14-fold with a CV of 75% (from 0.16 cm^{-1} for *Cunninghamia lanceolata* to 2.23 cm^{-1} for *A. fabri*). By contrast, variations in root tissue density, total branch order and root branching ratio across species were smaller, with CVs of 16%, 21% and 27%, respectively (Table S2).

Correlations of root traits across species

Across 14 species, root diameter and SRL were significantly correlated with most root morphological and architectural traits (Table S3). Root diameter was negatively correlated with SRL, root tissue density, branching intensity and total branch order, but positively correlated with individual root length. SRL was negatively correlated with root diameter, but unrelated to root tissue density (Table S3), indicating that the variation in SRL across species was predominantly controlled by root diameter and its influence on root volume per unit length, rather than tissue density (mass per volume). These correlations remained robust under all treatments (Table S3), but were greatly reduced after removing the influence of phylogeny via PICs (Table S4), demonstrating a strong phylogenetic influence. In addition, root morphological and architectural traits were almost unrelated to measured root chemical traits (e.g. root C and N concentrations) under all treatments (Table S3).

Mycorrhizal colonization was also significantly correlated with many morphological and architectural traits. Specifically, AM colonization showed a strong positive ($r > 0.60$) relationship with root diameter and individual root length (Table S3). At the same time, AM colonization was strongly and negatively ($r < -0.60$)

Table 3 Results of ANOVA for the absorptive fine root traits, root proliferation and mycorrhizal colonization among 14 tree species and four nutrient addition treatments (see Table 2 for abbreviations of parameters)

	Species		Treatment		Species × treatment	
Parameter	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value
Morphological traits						
Diameter	292.49	< 0.001	2.31	0.078	1.81	0.006
Length	72.29	< 0.001	4.02	0.009	4.18	< 0.001
SRL	97.88	< 0.001	1.71	0.168	0.98	0.509
RTD	23.15	< 0.001	1.82	0.145	1.17	0.244
Architectural traits						
BrIntensity	70.20	< 0.001	9.44	< 0.001	2.41	< 0.001
TBO	24.48	< 0.001	25.15	< 0.001	1.15	0.274
BrRatio	8.39	< 0.001	16.54	< 0.001	1.68	0.013
Chemical traits						
Root C	18.56	< 0.001	4.26	0.006	2.19	< 0.001
Root N	150.94	< 0.001	6.08	0.001	1.70	0.012
Root proliferation responses						
PR	2.59	0.010	1.77	0.164	ND	ND
RL	13.70	< 0.001	3.27	0.023	1.06	0.396
RM	6.76	< 0.001	2.74	0.045	0.49	0.995
Mycorrhizal colonization						
AMC	63.01	< 0.001	70.49	< 0.001	10.49	< 0.001
EHL/RL[1]	6.98	< 0.001	93.80	< 0.001	1.41	0.172

Note that root recovery from pruning (PR) was measured for a given species, and so there is no interaction effect of species and treatment. EHL/RL, which was transformed on a $\log_{10}$ scale, included unfertilized control and multi-nutrient (NPK) addition treatments.
[1]EHL/RL represents extramatrical hyphal length per unit length absorptive fine roots within a root bag.
ND, no data.

related to SRL, branching intensity and total branch order, but was generally unrelated to root tissue density or branching ratio (Table S3).

Complementarity between roots and mycorrhizal fungi across species

Average root recovery from pruning across species decreased linearly with absorptive fine root diameter averaged across treatments ($R^2 = 0.46$, $P < 0.01$, Fig. 1a), suggesting that thin-root species are better equipped for recovery from disturbance to roots. Moreover, root length proliferation across all species decreased linearly with absorptive fine root diameter ($R^2 \geq 0.28$, $P < 0.05$, Fig. 1b) under three of the treatments, other than the N addition (+N) treatment, whereas AM colonization under all four treatments and extramatrical hyphal length per unit length of absorptive fine roots under unfertilized control and multi-nutrient addition (+NPK) treatments increased linearly with absorptive fine root diameter ($R^2 \geq 0.4$, $P < 0.05$, Fig. 1d,e). There was no evidence that thin-root species were differentially affected by the attributes of the nutrient patch itself relative to thick-root species (slopes of regression lines across different fertilization treatments did not differ, $P > 0.05$, Fig. 1b,d,e).

Responses of root proliferation and mycorrhizal colonization to fertilization

Fertilization treatments significantly affected root length proliferation ($P = 0.023$) and root mass proliferation ($P = 0.045$), as well as mycorrhizal colonization ($P < 0.001$), averaged across all 14 tree species (Table 3). Compared with the unfertilized control, only the +NPK treatment significantly ($P < 0.05$) increased root length and reduced AM colonization (Fig. 2). Although the +N treatment tended to increase root length and mass proliferation (Fig. 2a, data of root mass not shown), both N and P additions (+P) tended to reduce AM colonization compared with the unfertilized control (Fig. 2b).

We found little evidence that thin-root species exhibited a greater response than thick-root species of root proliferation and mycorrhizal colonization to fertilization based on the observation that slopes running across all species did not differ significantly across fertilization treatments (Fig. 1b,d,e). In addition, we observed that variation in root length proliferation responses could be linked to root morphology in most species, but there were also exceptions. For example, *Acer cinnamomifolium* has relatively thin and densely branched roots, but unexpectedly exhibited relatively low root length proliferation (Table 1; Fig. 2a), probably because the roots of this species were not fully developed (i.e. reaching a steady-state architecture), as indicated by the fact that the average individual root length of *A. cinnamomifolium* in root bags was much shorter than that of other thin-root species, although all bags were harvested at the same time.

Responses of root traits to fertilization

Fertilization treatments had significant effects on root architectural traits ($P < 0.001$) and chemical traits ($P = 0.006$ for root C concentration and $P = 0.001$ for root N concentration), but not

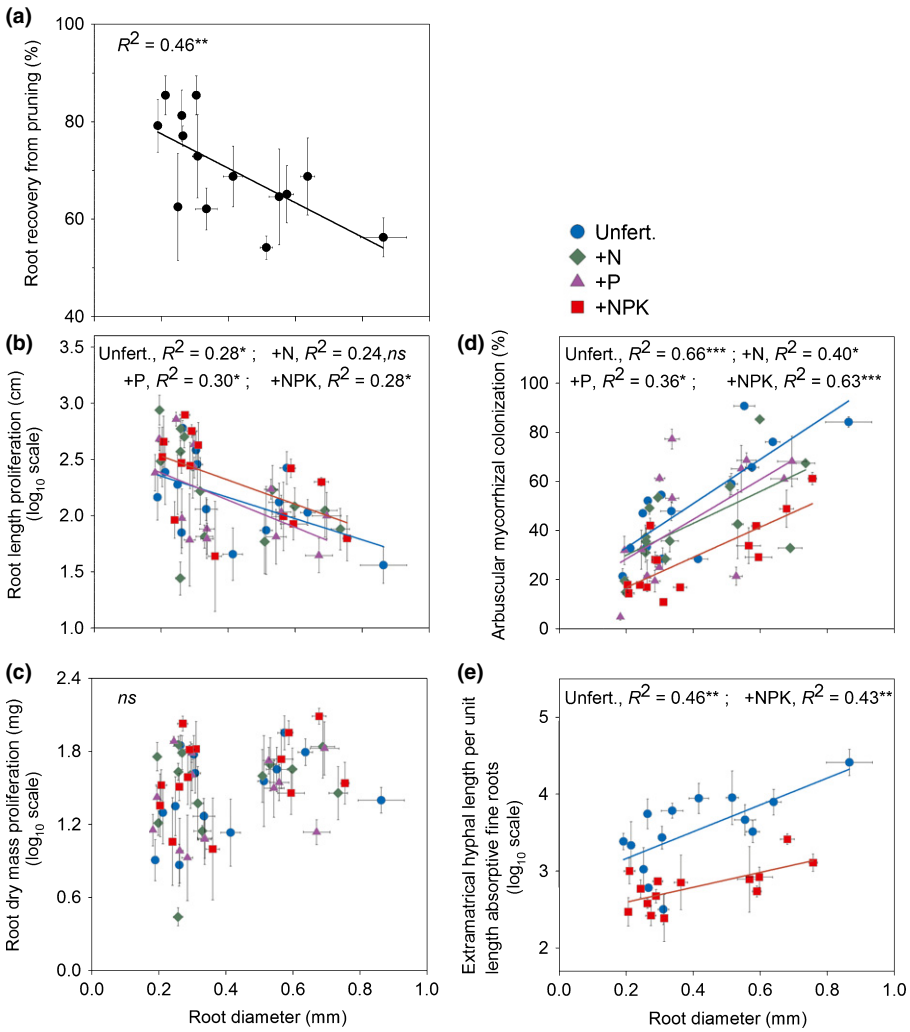

Fig. 1 The relationship of (a) root recovery from pruning averaged across different treatments, (b) absorptive fine root length proliferation, (c) absorptive fine root dry mass proliferation, (d) arbuscular mycorrhizal (AM) colonization and (e) extramatrical hyphal length per unit length absorptive fine roots in each bag (EHL/RL) with mean diameter of the first two order roots across 14 AM tree species under four treatments (Unfert., unfertilized control; +N, nitrogen addition;+P, phosphate addition; +NPK, multi-nutrient addition). Error bars, ± SE of the mean. Note that the *y* axes of (b), (c) and (e) are presented at log_{10} scale. Solid lines of (b), (d) and (e) represent the linear correlations across species for different treatments. Differences between the slopes of the regression lines of all graphs were not significant ($P > 0.05$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; *ns*, not significant.

on root morphological traits ($P > 0.05$), except for individual root length ($P = 0.009$) (Table 3). For root architectural traits, root branching intensity and total branch order tended to increase under all fertilization treatments, but the root branching ratio tended to decrease with +N or +P treatment, and exhibited no significant change with +NPK treatment, compared with the unfertilized control (Table S2). For root chemical traits, root C and N concentrations increased with +N or +P treatment compared with the unfertilized control, but there was no effect of +NPK treatment (Table S2). Similarly, individual root length decreased only with +N or +P treatment compared with the unfertilized control (Table S2). Other morphological traits (diameter, SRL and root tissue density) were unaffected by nutrient additions. Overall, root architectural traits expressed the greatest phenotypic plasticity in response to nutrient-rich patches, followed by root chemical traits, and then root morphological traits.

Discussion

We studied 14 AM tree species that varied widely in their absorptive fine root traits and subjected them to various types of nutrient addition to examine the complementarity between absorptive fine roots and mycorrhizal fungi in nutrient foraging behavior. These tree species ranged from highly branched, thin-root species to sparsely branched, thick-root species (Tables 1, S5; Fig. S1). We specifically examined how species of markedly different root morphology responded to different nutrient-rich patches, and how the responses of roots themselves might differ from the responses of mycorrhizal fungi. We obtained three key findings. First, thin-root species mostly produced absorptive fine roots for resource foraging, whereas thick-root species showed greater reliance on mycorrhizal fungi. Second, root length increased, but mycorrhizal colonization decreased, significantly with the +NPK treatment across 14 species. Third, root architectural traits (e.g. branching intensity or ratio) exhibited greater phenotypic plasticity than root morphological traits (e.g. root diameter or SRL) in response to nutrient-rich patches.

Complementarity between roots and fungi in nutrient foraging strategies

Increasing evidence supports the idea that traits of absorptive fine roots exhibit wide variation across species and that the

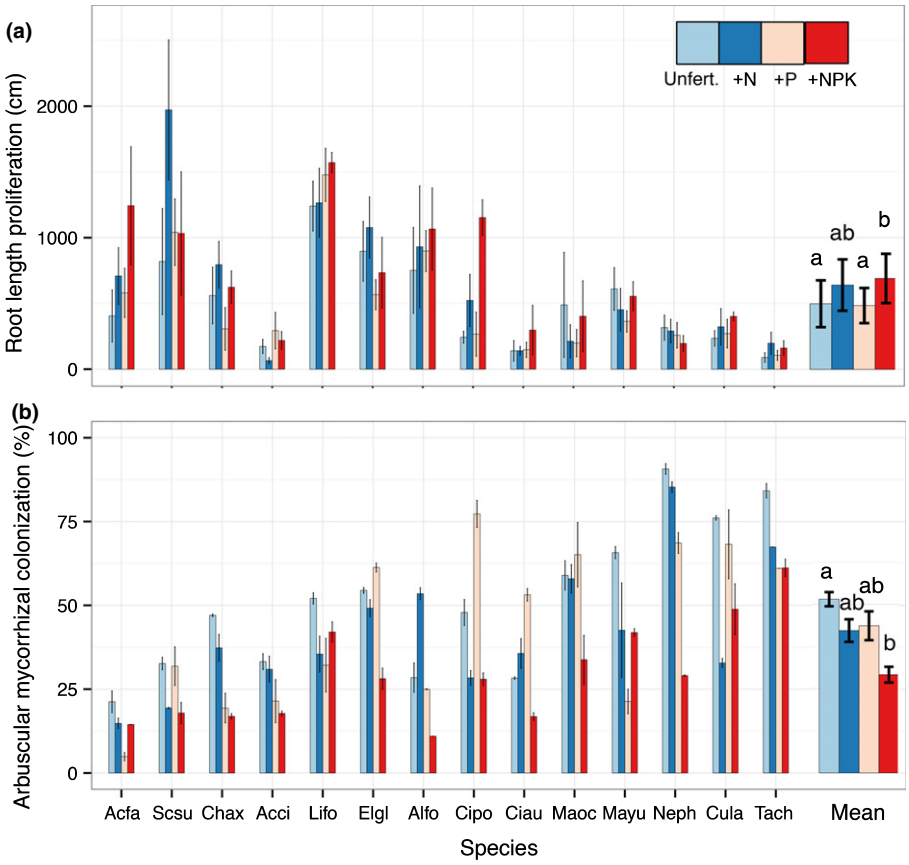

Fig. 2 (a) Absorptive fine root length proliferation and (b) arbuscular mycorrhizal (AM) colonization for 14 AM tree species and averages across 14 species under four treatments (Unfert., unfertilized control; +N, nitrogen addition; +P, phosphate addition; +NPK, multi-nutrient addition) (see Table 1 for abbreviations of tree species). Error bars, ± SE of the mean; different letters show statistically significant differences among the fertilization treatments ($P < 0.05$).

colonization by AM fungi is strongly influenced by the root traits (e.g. root diameter) of their host plants (Baylis, 1975; Fitter, 2004; Holdaway *et al.*, 2011; Kong *et al.*, 2014). Species with thick and sparsely branched roots generally depend more on AM fungi than do species with thin and densely branched roots (Brundrett, 2002; Kong *et al.*, 2014). In this study, we used a root-bag approach to test the complementarity between roots and fungi in nutrient foraging strategies. This approach has both shortcomings and tremendous potential value. For example, different species may have different recovery rates from root pruning, and some species, such as *A. cinnamomifolium*, showed low regrowth capacity from root pruning. Nevertheless, this approach is valuable for quantitative research on root production by isolating roots of different trees in a mixed forest (Comas & Eissenstat, 2004). Our results showed that, compared with thick-root species, thin-root species had a better ability to regrow roots following pruning (Fig. 1a) and showed more root length proliferation (Figs 1b, 2a), but had lower AM colonization and extramatrical hyphal length per unit root length (Fig. 1d,e). These results indicate that a distinct complementarity in belowground resource foraging exists between absorptive fine roots and their associated mycorrhizal fungi across these species: thin-root species forage mainly by using absorptive fine roots, whereas thick-root species forage with more reliance on mycorrhizal fungi. In a similar study using six temperate AM tree species, Eissenstat *et al.* (2015) also observed that thick-root species showed more reliance than thin-root species on mycorrhizal fungi for foraging after root pruning and following nutrient additions ($P < 0.01$).

The complementarity between absorptive fine roots and mycorrhizal fungi in resource foraging across species may be explained by the trade-off between root construction costs and resource acquisition benefits. From a cost–benefit perspective, the most successful plants should use root and mycorrhizal foraging to maximize benefits, whilst minimizing costs for soil resource acquisition (Eissenstat & Yanai, 1997). One important cost is tissue construction costs for building absorptive surfaces for water and soil nutrients. Thin-root species have lower construction costs per unit root length (as indicated by their high SRL values, Table 1); thus, it is not surprising that they show more root length proliferation to increase nutrient acquisition from soil (Fig. 2a; Eissenstat, 1991; Hodge, 2004). Conversely, thick-root species have higher construction costs per unit root length (low SRL values, Table 1); thus, the production of root length (thick root diameter) may not be a very efficient way of generating absorptive surfaces. Instead, investing carbon in much finer mycorrhizal fungal hyphae with low construction costs per unit length (hyphae typically have diameters of more than ten-fold thinner than even the finest roots in most tree species) (Friese & Allen, 1991) may be more efficient. Although the high construction costs of thick-root species may be compensated by increasing the root lifespan (McCormack *et al.*, 2012; Adams *et al.*, 2013), increasing mycorrhizal hyphal production is clearly a strategy frequently adopted by these thick-root species (Kong *et al.*, 2014;

Lee *et al.*, 2014). Indeed, if thick-root species are to produce an absorptive length comparable with that of thin-root species, the production of more mycorrhizal hyphae would seem to be the only viable strategy.

Thus, our results are consistent with the hypothesis that thin-root species are generally efficient in producing root length, whereas thick-root species rely more on mycorrhizal fungi for the acquisition of nutrients. This finding suggests that thin- and thick-root species use markedly different nutrient foraging strategies. A recent meta-analysis by Maherali (2014) argued that coarse root architecture is not necessarily a predictor of plant growth response to AM fungal colonization (see also Veresoglou & Rillig, 2014), which seems to contradict our results. However, our study differs from this meta-analysis in that we focused on root and mycorrhizal length production rather than on the whole-plant growth response. Indeed, our results also showed that, despite the marked differences in root length proliferation and mycorrhizal colonization/hyphal production across species, total root biomass growth did not differ significantly across species. Thin-root species produced much more root length than thick-root species (Fig. 2a), but with similar investments in root biomass (Fig. 1c). For example, *Liquidambar formosana*, which had a thinner root diameter for combined first- and second-order roots (0.26 ± 0.01 mm), showed root length proliferation *c.* 12-fold greater than that of *Taxus chinensis,* which had a much thicker root diameter for combined first- and second-order roots (0.86 ± 0.07 mm), across different nutrient addition treatments (Table 1; Fig. 2a). However, the total root biomass corresponding to these contrasting root length responses only differed by *c.* three-fold between the two species (data not shown). Thus, total carbon allocation to root and hyphal production may not differ as markedly across different species. This is consistent with a recent study by Valverde-Barrantes *et al.* (2014), who showed that different canopy species allocated similar root biomass in high-resource patches in natural forests. Moreover, although thin-root species may be efficient in producing root length, they may derive little benefit (commensalism, *sensu* Johnson *et al.*, 2015) and may even be negatively impacted by AM fungi (parasitism, *sensu* Johnson *et al.*, 2015), whereas thick-root species may consistently gain benefits from their mycorrhizal fungi, thus reducing the advantage of thin-root species, and placing both types of species on a balance in terms of overall plant performance. In fact, thin-root species tend to have a high percentage of passage cells (Zadworny & Eissenstat, 2011). Because most mycorrhizal hyphae may enter roots exclusively through passage cells (Sharda & Koide, 2008), it may be unavoidable for thin-root species to contain a certain degree of mycorrhizal colonization even if no benefits can be gained, particularly when soil available P is high, as in our study (soil available P concentration averaged 24.53 ± 1.79 mg kg^{-1}) and a recent study in grasslands (available P concentration reaching 46.0 ± 2.1 mg kg^{-1}; Johnson *et al.*, 2015). Future studies should further examine these potential mechanisms related to soil N/P stoichiometry, root anatomy and mycorrhizal fungal behavior to fully understand how mycorrhizal fungi influence plant growth performance and plant competition in natural communities.

Responses of roots and mycorrhizal fungi to different types of nutrient-rich patch

Our results support the hypothesis that plants will increase root length, but reduce mycorrhizal colonization, in response to nutrient-rich patches. The responses of roots and mycorrhizal fungi found here are consistent with those in previous studies. For example, many studies have shown increased root growth (Drew, 1975; Drew & Saker, 1975; Hodge *et al.*, 1998; Hodge, 2004), but decreased mycorrhizal colonization (Koide & Li, 1991; Nilsson & Wallander, 2003; Nilsson *et al.*, 2007; Sharda & Koide, 2010) , with increasing nutrient availability. The contrasting responses between roots and mycorrhizal fungi to nutrient-rich patches may be caused by the extra costs of maintenance and proliferation for hyphae, coupled with potential decreases in the relative benefits (i.e. nutrient acquisition), as nutrients become more freely available and are no longer limiting (Graham & Eissenstat, 1998; Gavito & Olsson, 2003). The finding that mycorrhizal colonization was reduced in all species to a similar degree (Fig. 1d,e) differs somewhat from a similar study using six temperate AM tree species (Eissenstat *et al.*, 2015), in which thin-root species showed a greater reduction than thick-root species in mycorrhizal colonization in nutrient-rich patches. These differences may again be related to the fact that, in our study, soil P availability was high and mycorrhizal colonization may offer little benefit to thin-root species, whereas, in Eissenstat *et al.* (2015), soil available P was probably in short supply and thin-root species derived substantial benefits from mycorrhizal associations when soil was not amended with fertilizer. It should also be noted that the findings in our study and in Eissenstat *et al.* (2015) apply only to mineral nutrient additions. Root and mycorrhizal fungal response to organic patches may differ as mycorrhizal fungi may have an advantage in utilizing organic patches by secreting enzymes, such as phosphatases (Koide & Kabir, 2000).

Despite the general pattern of root proliferation being favored over mycorrhizal colonization in nutrient-rich patches, the degrees and intensities of the responses were not uniform across different species and types of nutrient-rich patch (Fig. 2). Root proliferation was stimulated and mycorrhizal colonization was suppressed for most species in nutrient-rich patches, but overall responses of average all species were significant only in +NPK patches, but not in +N or +P patches (Fig. 2). This may be a result of the differential sensitivity of species to different types of nutrient-rich patch, and root growth may be constrained by highly imbalanced N and P in the patches. These differential responses have also been observed in previous studies. For example, Jackson & Caldwell (1989) observed marked root proliferation of *Artemisia tridentata* in NPK- and N-rich patches, but only slight root growth in P-rich soil, probably because plants can regulate the degree of root proliferation according to their demands for different soil resources. Liu *et al.* (2012) found that *Elymus nutans* roots showed a significant reduction in AM colonization with high fertilizer inputs (19.1 g N and 21.1 g P m^{-2} yr^{-1}), but no significant responses to low fertilizer inputs (12.7 g N and 14.1 g P m^{-2} yr^{-1}), in comparison with the

unfertilized control, suggesting that nutrient addition doses may also influence the responses of AM fungi. Overall, the question of if and how a plant alters patterns of root proliferation or mycorrhizal colonization in nutrient-rich patches depends on both the degree and types of nutrient limitation and nutrient capture ability of different species.

Plasticity of root morphological and architectural traits to nutrient-rich patches

Plant roots often proliferate when they encounter nutrient-rich patches (Drew, 1975; Drew & Saker, 1975). However, which types of root traits are primarily responsible for root proliferation in nutrient-rich patches have not been firmly established. In our study, root architectural traits (e.g. root branching intensity or ratio, and total branch order) responded significantly to fertilization treatments, whereas root morphological traits (e.g. root diameter, SRL and root tissue density) did not show significant responses to fertilization treatments averaged across 14 AM tree species (Table 3).

The lack of response of root morphological traits to nutrient-rich patches may be a result of the phylogenetic conservatism of root morphology. Key root morphological traits, such as root diameter, may have high phylogenetic conservatism and thus have limited capacity to respond to environmental changes (Chen *et al.*, 2013; Kong *et al.*, 2014). When encountering nutrient-rich patches in the soil, thick-root species of basal clades may have limited capacity for high root growth rates compared with the thin-root species of more recently diverged clades. In our study, AM tree species from more basal families, including Lauraceae (e.g. *Cinnamomum porrectum*, *Machilus oculodracontis* and *Neolitsea phanerophlebia*) and Magnoliaceae (e.g. *Manglietia yuyuanensis*), had thicker root diameters, lower root branching intensities and higher mycorrhizal colonization than other broadleaf tree species from more recently diverged families (Table 1; see also Kong *et al.*, 2014). Therefore, although the apparent cause of patterns of root proliferation and reliance on mycorrhizal fungi may be related to trade-offs associated with root diameter, more basic patterns of plant phylogeny could be the underlying factor.

In contrast with the high conservatism of root diameter, root branching has been shown to have relatively low phylogenetic conservatism and may be more sensitive to nutrient patches (Kong *et al.*, 2014). Other studies have also shown that root morphological traits are less plastic than architectural traits to nutrient additions. For example, Fitter and coworkers showed that roots were much less variable in morphology than in architecture in the efficient exploration of soil resources (Fitter, 1982, 1987; Fitter & Stickland, 1991). It should be noted that plants may also employ other strategies to increase nutrient uptake in fertile patches, potentially including adjustments in root anatomy (e.g. minimizing secondary development) (Lynch, 2011; Gu *et al.*, 2014; Kong *et al.*, 2014) or through plant signaling mechanisms responding to limiting resources (Bisseling & Scheres, 2014; Tabata *et al.*, 2014). Overall, the root length proliferation in nutrient-rich patches observed in our study was mainly achieved by the production of greater numbers of individual roots rather than an alteration in basic root morphology.

Conclusion

In this study, we found that there exists distinct complementarity between absorptive fine roots and mycorrhizal fungi in belowground resource foraging: thin-root species forage by using primarily absorptive fine roots, whereas thick-root species rely more on mycorrhizal fungi. Furthermore, our finding that plants significantly increase root length, but reduce mycorrhizal colonization, in response to the +NPK patches suggests that roots are favored over mycorrhizal fungi when foraging in nutrient-rich patches. These findings suggest that both root traits and soil nutrient conditions regulate root–mycorrhizal interactions. Future studies may incorporate additional comparisons using ectomycorrhizal species and other plant forms to determine how root traits, mycorrhizal type and soil conditions together mediate belowground resource foraging strategies.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Images of representative examples of fine root systems of the two selected species (*Acer fabri* and *Neolitsea phanerophlebia*), with the former representing thin-root species and the latter representing thick-root species (see Table 1 for absorptive fine root diameters of the two species).

Fig. S2 Scanned images of representative examples of the branching fine root systems of 14 coexisting subtropical arbuscular mycorrhizal tree species.

Fig. S3 Frequency distribution of root diameter for absorptive fine roots without root pruning (green solid line, samples from naturally growing roots) and from recovery after root pruning (red dashed line) across 14 coexisting subtropical arbuscular mycorrhizal tree species (see Table 1 for abbreviations of tree species).

Table S1 Information on root bags installed and root recovery from pruning under different treatments

Table S2 Summary of the absorptive fine root traits averaged across 14 tree species under four treatments

Table S3 Pearson's correlations for pairwise traits with original data for the absorptive fine root traits, root proliferation and mycorrhizal colonization under four treatments

Table S4 Pearson's correlations for pairwise traits with phylogenetic independent contrasts (PICs) for the absorptive fine root traits, root proliferation and mycorrhizal colonization under four treatments

Table S5 Mean (SE) of root morphological and chemical trait values for 14 arbuscular mycorrhizal (AM) tree species in this study (see Table 1 for abbreviations of tree species)

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