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Different responses of absorptive roots and arbuscular mycorrhizal fungi to fertilization provide diverse nutrient acquisition strategies in Chinese fir

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ABSTRACT

Absorptive roots and arbuscular mycorrhizal fungi (AMF) constitute two related pathways for plant nutrient acquisition. However, if and how soil nutrient availability can regulate the integrated pattern of roots and AMF remain unclear. We analyzed the abundance and morphology of absorptive roots and AMF as well as the intraradical AMF community composition in response to four-year nitrogen (N) and phosphorus (P) additions in a Chinese fir (*Cunninghamia lanceolata*) plantation. Absorptive root biomass, length density and tissue density significantly decreased, and specific root length significantly increased with P addition. However, none of these root characteristics were significantly affected by N addition. In contrast, extraradical hyphal length density in the rhizosphere soil and the ratio of hyphal length density to mycorrhizal colonization rate significantly decreased with N addition, but remained unchanged under P addition while extraradical hyphal length density in the ingrowth mesh bags significantly decreased with both N and P additions. The relative abundance of Acaulosporaceae and Gigasporaceae increased and that of Glomeraceae decreased with P, while N addition did not significantly shift AMF community composition. Our findings indicate that the responses of absorptive roots and AMF to N addition in the Chinese fir plantation differ from their responses to P addition, suggesting diverse strategies for a single tree species to adjust to multiple soil nutrient conditions.

1. Introduction

Within the arbuscular mycorrhizal symbioses formed in most land plants, absorptive roots and their fungal partners constitute two related pathways of soil nutrient uptake (Smith and Smith, 2011). Hence nutrient acquisition strategies for plants in different environments should depend on adjustments in both absorptive roots and their fungal symbionts. In the context of enhanced atmospheric nutrient deposition (e.g., nitrogen and phosphorus) (Smil, 2000; Galloway et al., 2004), numerous studies have separately examined the responses of roots or arbuscular mycorrhizal fungi (AMF) to experimental fertilization from aspects such as their abundance (biomass and length density for roots; intraradical mycorrhizal colonization and extraradical hyphal length for AMF) and morphology (Johnson et al., 2003; van Diepen et al., 2007; Poorter et al., 2012; Wurzburger and Wright, 2015), as well as AMF community composition (Camenzind et al., 2014; Sheldrake et al., 2018). However, results of these studies are far from universal. For example, the responses of both root and AMF abundance to nitrogen (N) addition can vary from positive to negative (Treseder, 2004; Peng et al., 2017). Given the recent emphasis on the complementary reliance of different tree species on roots and mycorrhizal fungi for nutrient foraging (Eissenstat et al., 2015; Liu et al., 2015; Chen et al., 2016) and the multidimensional aspects of plant adjustments to their environments (Bardgett et al, 2014; Kleyer and Minden, 2015; Weemstra et al., 2016), determining how separate responses of multiple characteristics of absorptive roots and AMF are integrated remains key to understanding these discrepancies and overall plant strategies for soil resource acquisition. Indeed, it is still unclear if and how multiple changes in soil nutrient conditions can result in integrated responses of roots and AMF within a single tree species.

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According to the functional equilibrium model (Brouwer, 1983), plants should allocate more photosynthate to the organs responsible for acquiring the most limiting resources. Therefore, relative allocation to absorptive roots or AMF should be higher in less fertile soils (Johnson, 2010; Poorter et al., 2012). However, it is unclear whether increased allocation belowground would increase both absorptive root and AMF abundance similarly, or if one of them should be the favored pool over the other. Because AMF are thought to be more efficient at garnering limiting soil resources, especially phosphorus (P), than absorptive roots due to their higher surface-area-to-volume ratios (Smith and Smith, 2011), resource allocation to AMF may experience greater relative increases compared to absorptive roots when soil nutrients begin to limit plant growth. However, at very low levels of soil fertility, the growth of AMF may also become limited (Treseder and Allen, 2002; Teste et al., 2016; Lambers et al., 2018). In this case, the fungal partner may retain absorbed nutrients for their own growth and reduce the favorability of resource exchange between plants and fungi (Johnson et al, 1997; Johnson, 2010; Antunes et al., 2012). Consequently, plants may adopt other strategies to alleviate nutrient limitation, such as increasing the allocation to absorptive roots (Poorter et al., 2012). Overall, the combined, yet potentially distinct responses of AMF and absorptive root abundance to changes in soil nutrient availability have not been fully investigated in the field.

In addition to changes in abundance, shifts in morphological traits of roots may also alter belowground resource strategies. For instance, higher specific root length (SRL) is thought to be associated with more efficient soil exploration, shorter root lifespans and a more acquisitive strategy (McCormack et al., 2012; Weemstra et al., 2016). However, root morphological variation along soil fertility gradients is poorly understood. Ostonen et al. (2007) synthesized data across different tree species throughout Europe and reported that SRL tends to decrease with fertilization, which may then result in lower total root length. At the same time, tremendous variation is often observed among studies, even for the same tree species. In two recent studies, Scots pine (*Pinus sylvestris*) showed opposing patterns of variation in absorptive root morphological traits along similar latitudinal gradients which paralleled gradients in nutrient availability (Ostonen et al., 2017; Zadworny et al., 2017). Understanding the functional contribution of adjustments in root abundance and morphology as well as their interactions with mycorrhizal fungi may help to explain these discrepancies (Poorter and Ryser, 2015).

Like root morphology, variation in AMF morphological traits (i.e., the relative allocation to extraradical versus intraradical AMF structures) can reflect differences in soil exploration and nutrient acquisition efficiency. Higher relative allocation to extraradical hyphae, for example, is related to higher rates of P transport to the host and hence promotes nutrient acquisition at a lower carbon cost (Avio et al., 2006). However, as AMF morphology is simultaneously determined by the abundance of intraradical and extraradical AMF structures and it has been suggested that fertilization can affect allocation to both intraradical and extraradical AMF structures depending on soil nutrient conditions (Treseder and Allen, 2002; Johnson et al., 2003), the general patterns of AMF morphology in response to fertilization remain unclear. Importantly, the modification of AMF morphology in response to changing soil nutrient availability may be related to the shifts in their community composition as AMF taxa differ in morphology (Hart and Reader, 2002) as well as within species responses to nutrient manipulations (Camenzind et al., 2014; Sheldrake et al., 2018). Shift in community composition together with their morphology may be integrated as another aspect of plant nutrient acquisition strategies.

Chinese fir (*Cunninghamia lanceolata*) is one of the most widely cultivated tree species in China, and forms associations with AMF. To determine the integrated adjustments of plant roots and AMF in different soil nutrient conditions, we assessed abundance and morphological characteristics of both absorptive roots and AMF, as well as the intraradical AMF community composition after four-year N and P additions in a 16-year-old subtropical Chinese fir plantation. Recent studies focusing on root responses in young Chinese fir plantations and potted saplings have shown that the abundance, allocation and morphology of the Chinese fir roots can exhibit significant plasticity across soil resource availability gradients (Zou et al., 2014; Dong et al., 2016; Wang et al., 2016; Wang et al., 2017), though little has been done to assess coincident responses by AMF. Aboveground, additions of N and P in this experiment have previously been shown to accelerate stem growth and differentially alter N:P ratios in leaves and branches (Chen et al., 2015). In turn, we anticipate that belowground strategies for resource acquisition by absorptive roots and AMF would respond to fertilization treatments, and that the integrated responses of the two partners would differ between N and P treatments since the N:P ratio of plant tissues may be a good indicator of the structure and function of arbuscular mycorrhizas (Johnson, 2010). We hypothesized that 1) allocation to absorptive roots and AMF would decrease with fertilization, but the extent that each partner contributes to the total decrease would depend on the specific fertilization treatment; 2) morphology of absorptive roots and AMF would respond to fertilization, acting as an additional nutrient acquisition strategy, without a priori prediction on their change directions; and 3) AMF community composition would shift under fertilization and the changes in relative abundance of the dominant AMF families would be closely related to the shifts in AMF morphology.

2. Materials and methods

2.1. Study site and experimental design

This study was conducted at the Shixi forest farm (26°41′26″N, 115°03′35″E) in southeast China's Jiangxi Province. The climate is subtropical humid monsoon, with an annual precipitation of 1500 mm and a mean annual temperature of 17.9 °C. The soil is a Typic Hapludult Ultisol and is locally referred to as red soil developed from Quaternary Red Clay. The Chinese fir was planted from one-year-old seedlings spaced at 2 m × 2 m intervals in 2000. The seeds used to grow the seedlings were collected from 32 parent trees in a nearby second-generation Chinese fir seed plantation. These planted seedlings have since dominated the site with very minor contributions of other local tree species (e.g., *Pinus massoniana* and *Liquidambar formosana*).

The nutrient addition experiment was initiated in November 2011 as a randomized block experimental design consisting of five replicate blocks encompassing 30 total plots. The full study design was described in detail previously (Chen et al., 2015). Briefly, each block contained six 20 m × 20 m plots, including one control and five fertilization treatments. The plots were randomly assigned to individual plots which received no fertilization, or either $5\ g\ N\ m^{-2}\ yr^{-1}$ (N_5) or $10\ g\ N\ m^{-2}\ yr^{-1}$ (N_{10}) as NH_4NO_3, $5\ g\ P\ m^{-2}\ yr^{-1}$ (P_5) as NaH_2PO_4, or the combination of P with each of the levels of N. Soil and leaf chemical characteristics and stem growth under each treatment in the second year of fertilization are shown in Table S1.

2.2. Field sampling

Root samples for estimation of root abundance and morphology were obtained using soil cores. Twelve intact soil cores (10 cm in diameter, 20 cm in depth) were randomly taken from each of the 30 plots in May 2016 (360 soil cores in total). Additionally, at four out of the twelve locations we cored consecutively to a depth of 20–50 cm to estimate the root abundance in the deeper soil. All roots were immediately picked out from the soil by hand and samples were then stored in Ziploc bags in a freezer at −20 °C until further processing. During the same collection period, root samples for estimation of AMF colonization and community composition were dug out by shovel from surface soils (0–20 cm) at 10 randomly selected locations in each plot. Each of the 10 sampling locations and soil cores was located at least 2 m

apart. Five to eight intact root branches from each of the 10 locations (a total of about 60 branches) were pooled as one composite sample per plot. From this, one sample of about 30 g of loosely adhering soil was collected by hand-shaking the composite root sample yielding rhizosphere soil which was used to estimate AMF extraradical hyphal length for each plot. These samples were also stored in Ziploc bags and stored in a freezer at −20 °C until further processing. This was repeated in each plot with the exception of a single, P addition plot where insufficient material was recovered to accommodate the AMF analyses.

After collection of the rhizosphere soil from the roots dug from bulk surface soils, we then used these roots and selected the distal first and second branch orders of live Chinese fir roots, considered as absorptive roots (McCormack et al., 2015), for additional analyses of mycorrhizal colonization and DNA extraction. Higher order roots were not processed in this study. Chinese fir roots were distinguished from the roots of other species based on color and morphology (diameter and branching), and white or light brown roots with turgescent tissues were identified to be live. Following collection, all absorptive root samples were gently washed free from soil by tap water and then deionized water. We selected *ca.* 100 absorptive root segments for estimation of mycorrhizal colonization and *ca.* 60 absorptive root segments for DNA extraction from each of the 30 composite root samples collected by shovel. The root samples for mycorrhizal colonization estimation were then stored in tightly sealed plastic vials containing 50% ethanol and samples for DNA extraction were stored in plastic Ziploc bags containing silica gel at room temperature, respectively.

2.3. Root abundance and morphology

All the first and second order root segments from each core were mixed and arranged on a transparent plate and scanned using an Epson Expression 10000XL scanner at a resolution of 400 dpi. Roots were then oven-dried at 65 °C for 48 h and weighed to estimate dry mass (M). Scanned images were analyzed for total length (L) and mean diameter ($\bar{D}$) using WinRHIZO software (Regent Instruments Inc., Quebec, Canada). Absorptive root biomass and length were determined by extrapolating the dry mass and total length per soil core to a ground area basis, respectively. We also measured the stand basal area at the time of root sampling, and then calculated absorptive root biomass and length per stand basal area as proxies of relative allocation to absorptive roots (Ostonen et al., 2017). Specific root length (SRL) for each core was calculated as L/M. Root tissue density (RTD) was estimated as $M/[\pi(\bar{D}/2)^2 L]$. Core-specific values of absorptive root biomass (in g m^{-2}), root length density (RLD, in m m^{-2}), mean diameter, SRL and RTD were averaged for each plot.

2.4. Hyphal abundance and morphology

Extraradical hyphae were extracted from 8.0 g of rhizosphere soil collected from each plot according to Miller, Reinhardt and Jastrow (1995). Soil was suspended in mixture of 100 ml deionized water and 12 ml sodium hexametaphosphate solution (35 g l^{-1}) for 1 h. The supernatant was decanted on a 38 μm sieve, and the materials retained on the sieve were transferred to 200-ml deionized water in a flask using a plastic washing bottle. Then the flask was shaken by hand for 5 s and left to settle for 1 min. Four 2-ml aliquots for each sample were filtered with 1.2-μm Millipore membranes, with one aliquot per membrane. The membranes were stained with 1% acid fuchsin on slides and observed at 200× magnification using a compound microscope. Fifty fields of view were scored for each membrane using the gridline intercept method and hyphal length density in rhizosphere soil (HLD, in cm g^{-1} dry soil) was estimated following the modified method of Tennant (1975). We identified AMF hyphae based on the criteria of nonseptate walls, irregular branches and red staining (Treseder and Allen, 2002; Camenzind and Rillig, 2013). However, based on the criteria used here we cannot guarantee that the identified hyphae exclusively originated from AMF.

As a complementary approach to distinguish the AMF hyphae from that of nonmycorrhizal fungi (e.g., saprotrophic fungi), we also measured hyphal length using the ingrowth mesh bag method. Nylon mesh bags (5 cm × 4 cm, 50 μm mesh size) were filled with 20 g acid-washed silica sand (0.5–1 mm particle size). Use of sand can help to minimize the ingrowth of saprotrophic fungi owing to the lack of organic substrates utilized by these fungi (Wallander et al., 2001). In each of the 30 plots, six mesh bags were buried in surface soil (0–20 cm depth) in November 2016. All 180 bags were harvested after one year in November 2017. As the extraradical hyphae of AMF are usually expected to turn over in less than one year, with the observed residence time varying from several days to months (Staddon et al., 2003; Treseder et al., 2010), our one-year observation was likely to reflect the standing pool rather than the total production of hyphae. Although use of sand as a growth substrate in mesh bags may lead to underestimation of hyphal production (Hendricks et al., 2006), this method provides another measure to compare the effects of fertilization treatments on extraradical AMF hyphae. Harvested mesh bags were transferred to the laboratory within a few hours and stored at −20 °C until further processing. Sand from the six mesh bags per plot was pooled into one composite sample for each plot and extraction and measurement of hyphae from sand in the ingrowth bags followed the same method used for rhizosphere soil.

Mycorrhizal colonization rate, defined as the proportion of root length colonized by AMF structures, was estimated as a measure of intraradical abundance of AMF. For this measure, *ca.* 50 absorptive root segments for each plot were rinsed with deionized water, cleared by autoclaving in 10% KOH for 30 min, acidified in 2% HCl for 30 min at room temperature, and stained in 0.1% acid fuchsin for 60 min at 60 °C. All stained root segments of each plot were arranged on slides. Two hundred root intersections for each plot were randomly observed using a compound microscope at 200× magnification, according to the line intercept method (McGonigle et al., 1990). Arbuscules, vesicles, hyphae and coils were recorded separately.

The total amount of rhizosphere soil in a given plot should be positively related to the RLD meaning that there may be changes in total (i.e. plot level) rhizosphere hyphal length that accompany changes in RLD even in the absence of changes in rhizosphere HLD. Therefore, HLD in the rhizosphere soil is given as a relative value of soil hyphae against RLD. We then calculated the ratio of HLD in the rhizosphere soil to mycorrhizal colonization rate to estimate changes in AMF morphology (i.e., the relative allocation of extraradical versus intraradical AMF structures). AMF morphology was then independently estimated by calculating the ratio of HLD in the mesh bags to the colonized root length density.

2.5. DNA extraction and sequencing

The AMF community was analyzed by next generation amplicon sequencing. DNA was extracted from 300 mg of dried roots for each of the 29 available plot samples using the FastDNA® SPIN Kit for Soil (MP Biomedicals, USA) according to the manufacturer's instructions. We amplified the partial small subunit (SSU) region of 18S rDNA (c. 260 bp) by nested PCR. The first PCR was conducted using the primer pair AML1 and AML2 (Lee et al, 2008). The second PCR was carried out using the primer pair AMV4.5NF and AMDGR (Sato et al., 2005) with Illumina index adapters. Both PCR reactions were performed in a 20 μl reaction volume with 4 μl 5 × PCR Buffer, 2 μl 2.5 mM dNTPs, 0.8 μl of each primer (5 μM stock) and 10 ng of DNA and 0.4 μl TransStart FastPfu DNA polymerase (TransGen Biotech, China). The reactions were run on an ABI GeneAmp 9700 thermal cycler (Applied Biosystems) under the following conditions: 95 °C for 3 min; 32 (first PCR) or 30 cycles (second PCR) of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s; followed by 72 °C for 10 min and 10 °C until halted by user. The PCR products were extracted from a 2% agarose gel, further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA), and

quantified using QuantiFluor™-ST (Promega, USA). Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq platform (Illumina, USA) by Majorbio Technology Co. Ltd.

2.6. Sequence data processing

Raw reads were de-multiplexed and quality-filtered with an average quality score threshold of 20 and a 50 bp sliding window using Trimmomatic (Bolger et al., 2014). Paired-end reads with more than 10 bp overlaps were merged using FLASH (Magoč and Salzberg, 2011). The resulting sequences were clustered into Operational Taxonomic Units (OTUs) using UPARSE at 95% identity, and chimeric sequences were identified and removed using UCHIME (Edgar et al., 2011; Edgar, 2013). To identify the obtained AMF OTUs, representative sequences of each OTU were subjected to a BLAST search against the MaarjAM database (Öpik et al., 2010) with the following criteria for a match: sequence similarity ≥97%; query coverage ≥95%; and e-value < $1 \times e^{-50}$. As AMF morphology often vary across different families (Hart and Reader, 2002), we calculated the relative abundance (proportion of reads) of each AMF family in each plot.

2.7. Statistical analyses

Two-way analysis of variance (ANOVA) with LSD *post hoc* tests among the three N treatments was performed to explore N and P additions and their interactive effects on absorptive root and AMF abundance and morphology, total AMF richness, as well as the relative abundance of Glomeraceae, Gigasporaceae and Acaulosporaceae (the three most studied and dominant AMF families). We also analyzed effects of N additions at each P treatment level by one-way ANOVA followed by LSD post hoc test for further interpretations and graphical illustrations. Normality of data and homogeneity of variance were tested using Shapiro-Wilks's and Levene's tests, respectively. When necessary, data were log-transformed prior to variance analyses. Variance analyses were performed using SPSS v22 (IBM Corp, USA).

Rarefaction analysis was used to assess the OTU number estimates using MOTHUR (Schloss et al., 2009). The variation of OTU composition of AMF was examined in the R environment (R core Team, 2017). The effect of fertilization on AMF community at the OTU level was analyzed by permutational multivariate ANOVA based on Bray-Curtis distances and using the function adonis() from R package vegan with 999 permutations (Oksanen et al., 2017). Dissimilarities of AMF community composition across N and P treatments were visualized separately using nonmetric multidimensional scaling (NMDS) in the vegan package.

3. Results

3.1. Absorptive root abundance and morphology

Across the unfertilized plots, both absorptive root biomass and RLD in the surface soil (0–20 cm) accounted for 51% of their total amount in the top 50 cm soil. Fertilization significantly decreased absorptive roots in the surface soil (0–20 cm), but not those in the deeper soil (20–50 cm) (Table S2). Responses of absorptive roots in the surface soil to fertilization varied between N and P additions. Absorptive root biomass and length declined with P addition by 40% ($F_{1,24} = 11.09$, $P = 0.003$) and 33% ($F_{1,24} = 10.06$, $P = 0.004$), respectively (Fig. 1a,b). Reductions in absorptive root biomass and length per stand basal area were even greater with declines of 53% ($F_{1,24} = 10.95$, $P = 0.003$) and 47% ($F_{1,24} = 10.68$, $P = 0.003$), respectively (Fig. 1c,d). At the same time, P addition led to a 13% increase in SRL ($F_{1,24} = 10.05$, $P = 0.004$) and a 10% decrease in RTD ($F_{1,24} = 5.23$, $P = 0.031$), but no significant change in root diameter ($F_{1,24} = 0.01$, $P = 0.92$) (Fig. 2; Table S2). In contrast to the responses to P addition, neither abundance nor morphology of absorptive roots in the surface soil significantly responded to N additions (for RLD, $F_{2,24} = 0.64$, $P = 0.54$; for biomass, $F_{2,24} = 0.24$, $P = 0.79$; for SRL, $F_{2,24} = 0.90$, $P = 0.42$; for RTD, $F_{2,24} = 0.27$, $P = 0.77$). Additionally, there were no significant N × P interactions (Table S2).

3.2. AMF abundance and morphology

In contrast to absorptive roots, HLD in the rhizosphere soil did not significantly respond to P addition ($F_{1,23} = 1.20$, $P = 0.29$), but was decreased with the addition of N at both moderate and high levels ($F_{2,23} = 10.60$, $P = 0.001$; *post hoc* test, $P = 0.002$ and $P = 0.001$, respectively) (Fig. 3a). However, HLD in the mesh bags decreased with both N ($F_{2,24} = 4.20$, $P = 0.027$) and P ($F_{1,24} = 6.51$, $P = 0.018$) additions (Fig. 3b, Table S1). Mycorrhizal colonization rate remained stable across all treatments at *ca.* 63% and showed no response to N ($F_{2,23} = 0.09$, $P = 0.92$) or P additions ($F_{1,23} = 0.01$, $P = 0.917$) (Fig. 3c; Table S2). Similarly, the colonization of specific intraradical AMF structures was also unchanged by fertilization (Table S2). Across all treatments, absorptive roots were primarily colonized by intraradical hyphae (51%) and coils (26%). Only 3% and less than 1% of absorptive root length was colonized by arbuscules and vesicles, respectively. The ratio of HLD in the rhizosphere soil to mycorrhizal colonization rate was significantly decreased with N addition ($F_{2,23} = 7.83$, $P = 0.003$) but was unchanged with P addition ($F_{1,23} = 1.02$, $P = 0.32$) (Fig. 4a). The ratio of HLD in the mesh bags to colonized root length density was similarly unchanged with P addition ($F_{1,23} = 0.03$, $P = 0.87$) and marginally decreased with N addition ($F_{2,23} = 2.99$, $P = 0.07$) (Fig. 4b). Similar to absorptive roots, there were no significant N × P interactions relating to AMF abundance and morphology (Table S2).

3.3. AMF community composition

A total of 851,987 reads representing 40 OTUs and seven families matched the AMF database (Table S3). The highest numbers of reads were found in the Glomeraceae, followed by Acaulosporaceae and Gigasporaceae, with their relative abundances of 75.2%, 14.7% and 9.8%, respectively. Rarefaction curves suggested adequate sampling depth for this site and sequencing depth for each sample (Fig. S1).

Neither the OTU richness nor the community composition (at the OTU level) of AMF was significantly affected by N or P addition (for OTU richness, ANOVA, $F_{2,23} = 1.13$, $P = 0.34$ and $F_{1,23} = 1.28$, $P = 0.27$, respectively; for community composition, adonis, $P = 0.19$ and $P = 0.43$, respectively, Fig. S2). However, the three most abundant families differentially responded to P addition. The relative abundance of Glomeraceae significantly decreased ($F_{1,23} = 14.86$, $P = 0.001$) while that of Acaulosporaceae and Gigasporaceae increased with P addition ($F_{1,23} = 4.67$, $P = 0.041$ and $F_{1,23} = 4.33$, $P = 0.049$, respectively) (Fig. 5; Table S2).

4. Discussion

4.1. Different responses of absorptive roots and AMF

Although tree growth and soil nutrient availability were improved by N and P additions in our study site (Table S1), the high leaf N:P ratio across all treatments (> 16) suggests that trees continue to be limited by P or co-limited by N and P (Koerselman and Meuleman, 1996; Güsewell, 2004). Therefore, we expect that AMF continue to provide benefit to host trees with improved nutrient acquisition across all treatments (Johnson, 2010). Still, the combined responses of significantly decreased HLD in both rhizosphere soil and mesh bags, unchanged mycorrhizal colonization rate, and unchanged RLD with N addition indicate (1) that total allocation to intraradical and extraradical AMF structures are decreased with N addition, and therefore (2) that plants may adapt to N addition by preferentially modifying

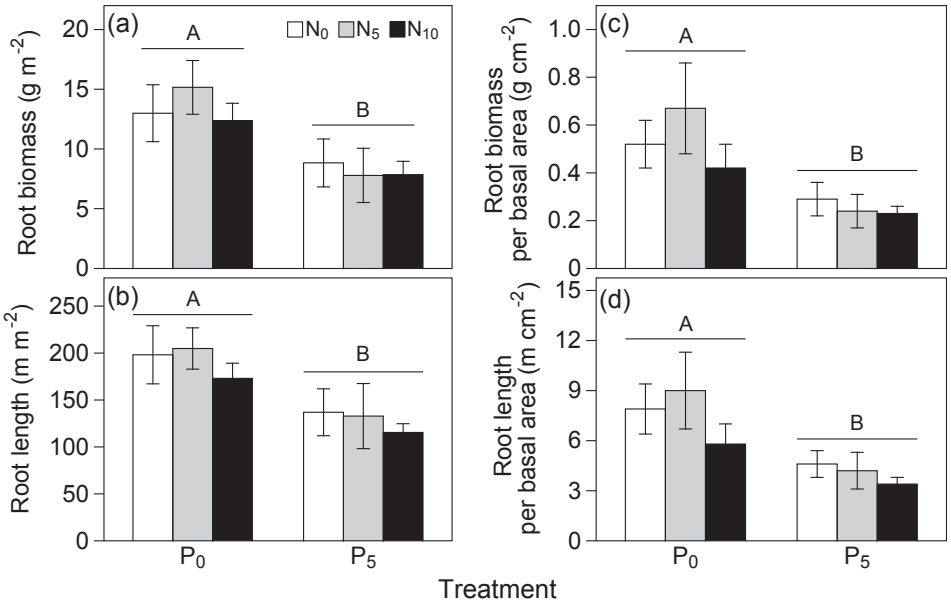

Fig. 1. Abundance of absorptive roots (a, b) in surface soils (0–20 cm depth) and their relative values against aboveground stand basal area (c, d) in plots without nitrogen (N_0) or phosphorus additions (P_0), or amended with 5 g N m^{-2} yr^{-1} (N_5), 10 g N m^{-2} yr^{-1} (N_{10}) or 5 g P m^{-2} yr^{-1} (P_5). Bars represent means ± SE ($n = 5$). Groups of bars with different letters above them are significantly different ($P < 0.05$).

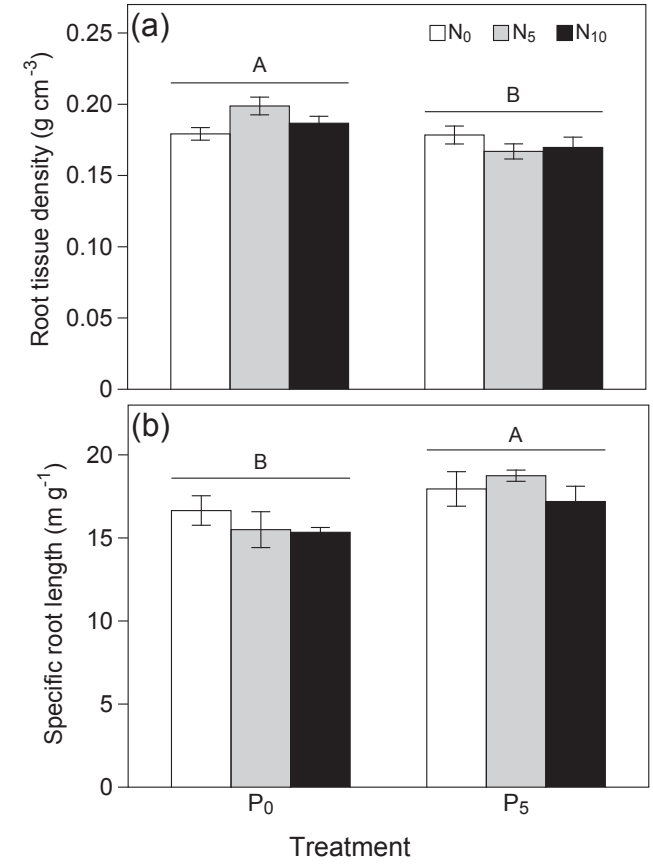

Fig. 2. Tissue density (a) and specific root length (b) of absorptive roots in surface soils (0–20 cm depth) in plots without nitrogen (N_0) or phosphorus additions (P_0), or amended with 5 g N m^{-2} yr^{-1} (N_5), 10 g N m^{-2} yr^{-1} (N_{10}) or 5 g P m^{-2} yr^{-1} (P_5). Bars represent means ± SE ($n = 5$). Groups of bars with different letters above them are significantly different ($P < 0.05$).

associations with AMF. A similar tradeoff between roots and AMF has also been observed in northern temperate forests where the biomass of fungal hyphae rather than that of fine roots declined with N addition (van Diepen et al., 2007; Burton et al., 2012). Meanwhile, the decline of HLD in both rhizosphere soil and mesh bags despite stable root biomass and RLD may further suggest that a higher proportion of N is taken up directly by roots in the N fertilization plots given that roots and hyphae may have similar N uptake efficiencies due to the high mobility of N in soil (Smith and Smith, 2011). In contrast to N addition, the root and fungal responses to P addition suggest more stable rates of resource allocation to AMF per unit root length (i.e. unchanged mycorrhizal colonization rates and stable HLD in rhizosphere soil) despite significant declines in overall RLD and HLD in the mesh bags. It is possible that the differing responses of HLD in rhizosphere soil vs. the sand mesh bags may be due to each capturing a different amount of non-target (i.e. non-AMF) hyphae. However, it is also likely due in part to the placement of the sand mesh bags in bulk soil which necessarily links changes in HLD in the mesh bags to the broader changes in RLD. This occurs as less roots growing near the mesh bags (i.e. lower RLD) would necessitate a reduction in root-associated hyphal growth occurring within the bags even if hyphal growth per unit root remained unchanged. In either case, the decrease in total allocation to the mycorrhizal symbiosis in response to either N or P additions is consistent with the functional equilibrium model (Brouwer, 1983; Johnson, 2010) and our first hypothesis.

The lack of response of mycorrhizal colonization rate to fertilization is not consistent with previous work indicating that N and P additions typically decrease AMF root colonization (Treseder, 2004). Two possible explanations include, first, mycorrhizal colonization rate alone does not always determine the nutrient uptake of AMF as the amount of extraradical hyphal length may still vary widely (Sawers et al., 2017). This has been highlighted by a *meta*-analysis showing that differences in mycorrhizal colonization rate explained only a fraction of variation in plant growth and P content (Treseder, 2013). Second, maintaining a certain percentage of roots colonized by AMF provides other important benefits to the host plants beyond nutrient acquisition such as protection against pathogens (Sikes et al, 2009; Lambers et al., 2018). Therefore, maintaining relatively high levels of colonization, *ca.* 63% in this study, may be beneficial even if the role of AMF in nutrient acquisition declines with fertilization.

4.2. Shifts in absorptive root abundance and morphology

Although both root biomass and morphological traits (i.e., SRL) are positively related to total absorptive root length, biomass is often more plastic and contributes more to changes in total absorptive capacity than morphological traits (Freschet et al, 2015; Kramer-Walter and

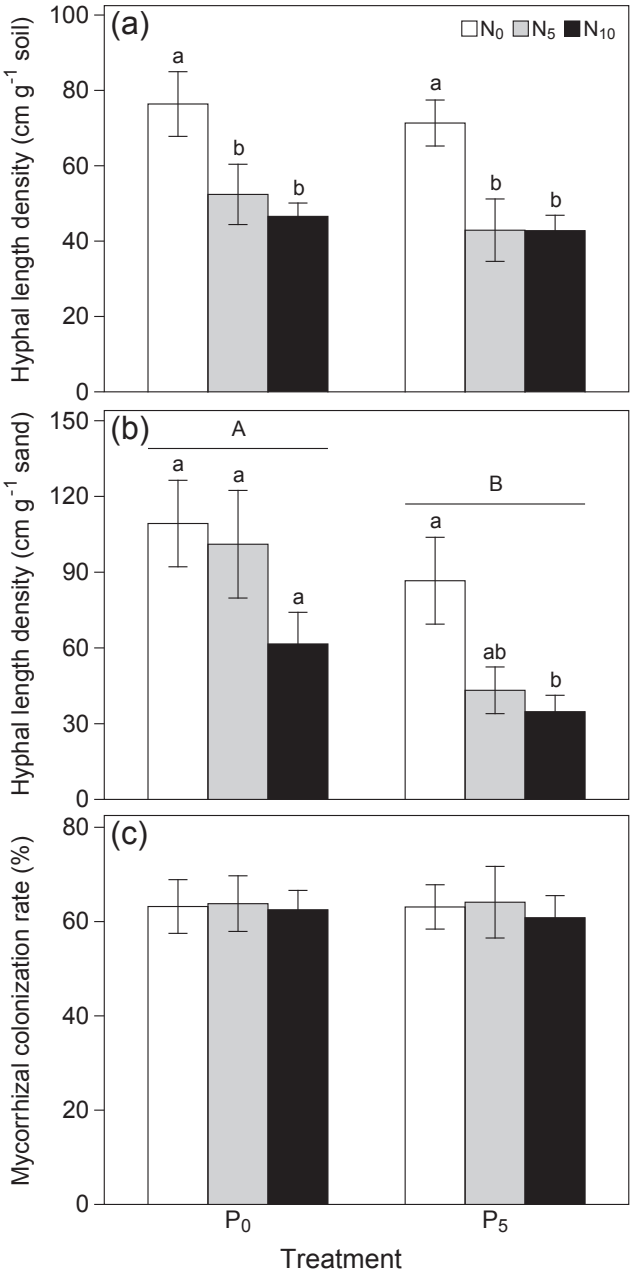

Fig. 3. Hyphal length density in the rhizosphere soil (a) and sand filled mesh bags (b), and mycorrhizal colonization rate (c) in plots without nitrogen (N_0) or phosphorus additions (P_0), or amended with 5 g N m^{-2} yr^{-1} (N_5), 10 g N m^{-2} yr^{-1} (N_{10}) or 5 g P m^{-2} yr^{-1} (P_5). Bars represent means ± SE (n = 5). Groups of bars with different letters above them are significantly different (P < 0.05). Within each group of P treatments, bars with different letters above them are significantly different (P < 0.05).

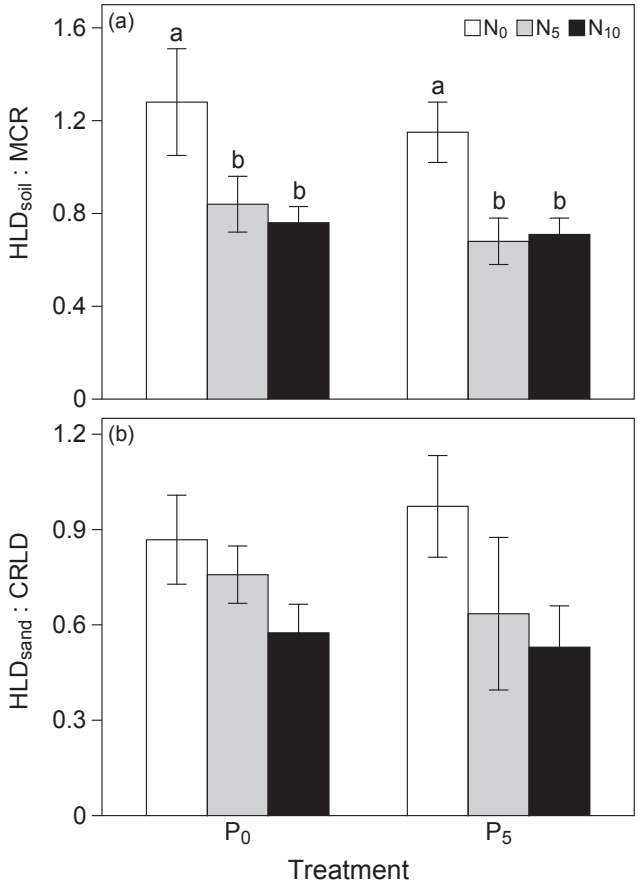

Fig. 4. Morphology of AMF in surface soils (0–20 cm depth) in plots without nitrogen (N_0) or phosphorus additions (P_0), or amended with 5 g N m^{-2} yr^{-1} (N_5), 10 g N m^{-2} yr^{-1} (N_{10}) or 5 g P m^{-2} yr^{-1} (P_5). Within each group of P treatments, bars with different letters are significantly different (P < 0.05). Morphology of AMF was estimated by the ratio of hyphal length density in the rhizosphere soil (HLD_{soil}) to mycorrhizal colonization rate (MCR) (a) and the ratio of hyphal length density in the sand filled mesh bags (HLD_{sand}) to colonized root length density (CRLD) (b). Bars represent means ± SE (n = 5).

Laughlin, 2017). Accordingly, we observed similar decreases in absorptive root length density and biomass in response to P addition (Fig. 1), despite the potential for greater RLD associated with increased SRL under P addition (Table S2; Fig. 2b). On the other hand, from the whole plant perspective, the higher SRL may have a positive feedback to further reduce biomass allocation to absorptive roots and therefore enhance allocation to other structures (e.g., stem) to compete for additional limiting resources (e.g., light). In general, the increased SRL with P addition observed here supports our second hypothesis that modifications in absorptive root morphology can, at least partly, adjust belowground acquisition strategies.

Responses of root abundance and morphology to soil nutrient availability can also be affected by the relationship between root morphology and turnover (McCormack and Guo, 2014). Decreased RTD with P addition in this study may reflect lower investment in root structural defenses and faster root turnover (Eissenstat et al., 2000). Increased turnover rate, which gives rise to a root population that is younger on average, may improve the nutrient acquisition efficiency and therefore reduce the need for root biomass (Volder et al., 2005). Nevertheless, increased root turnover also contributes to a potential increase in annual root production (Nadelhoffer, 2000) and respiration (Volder et al., 2005), which may then reduce the nutrient acquisition efficiency. Discerning the balance among the multifaceted root responses in terms of abundance, morphology, annual production and turnover warrants further study.

4.3. AMF morphology and community composition

The responses of AMF morphology and community composition to fertilization ran counter to our second and third hypotheses. The significantly decreased ratio of HLD in the rhizosphere to mycorrhizal colonization rate with N addition, likely indicating a relative reduction in allocation to extraradical hyphae and soil N scavenging, agrees with predictions based on functional equilibrium theory for AMF structures (Johnson et al., 2003). While the results based on HLD in the mesh bags were not significant, they demonstrated a qualitatively similar trend. Here, the lack of significant change in the ratio of HLD in the mesh bags

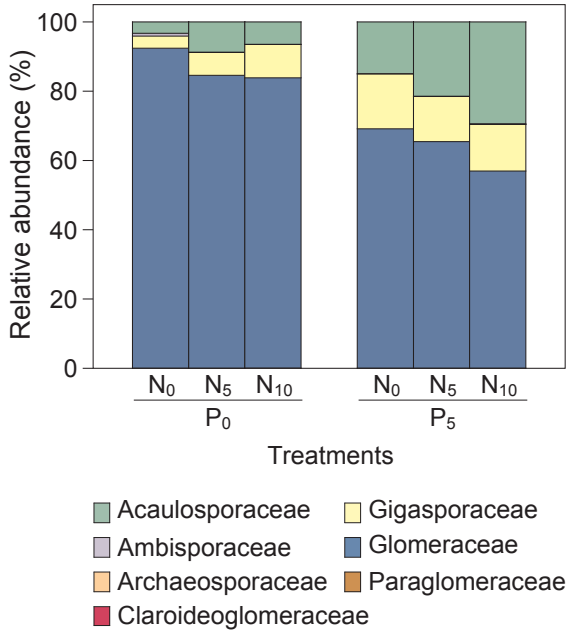

Fig. 5. Mean relative abundance of AMF families ($n = 5$) detected in roots from plots without nitrogen (N_0) or phosphorus additions (P_0), or amended with 5 g N m^{-2} yr^{-1} (N_5), 10 g N m^{-2} yr^{-1} (N_{10}) or 5 g P m^{-2} yr^{-1} (P_5).

to colonized root length density with N addition may be due to high variation inherent among mesh bags and a need for greater replication (Figs. 3b, 4b). Alternatively, it is possible that the significant result based on the rhizosphere soil may have been due to inadvertent inclusion of some non-mycorrhizal hyphae. Regardless, the general pattern of reduced allocation to external HLD relative to internal fungal structures may further indicate decreasing allocation and plant reliance on AMF with N addition. On the other hand, while there were significant shifts in AMF community composition with P addition (Fig. 5), these shifts were not associated with a significant change in AMF morphology (Fig. 4). The inconsistency may result from the decoupling of AMF morphology and function (e.g., rate of nutrient transport) as well as a strong likelihood for morphological plasticity within AMF families or even species (Koch et al., 2017). Furthermore, use of sand in the mesh bags, the limited distances which AMF may grow into soil from the root surface, and the different time of mesh bag and root samplings in our study may also be responsible for additional uncertainties (Wallander et al., 2013).

Despite apparent decoupling of AMF community composition and morphology, the responses of some AMF taxa in our study may be predictable. For example, the increased relative abundance of Acaulosporaceae (considered as a stress tolerator) can be expected according to the C-S-R framework developed for AMF, which suggests that potential stresses associated with fertilizations (i.e., carbon limitation due to reduced plant allocation belowground, and a trend toward lower soil pH, *not significant*, Table S1) will favor more stress tolerant members in AMF community (Chagnon et al., 2013). Although it has often been reported that Glomeraceae becomes more dominant with N (Treseder et al., 2018) and P addition (Alguacil et al., 2010), reduced relative abundance of some species from Glomeraceae in the AMF community under N addition (Li et al., 2015) and P addition (Sheldrake et al., 2018) has also been documented. The increased relative abundance of Gigasporaceae with P addition in our study is inconsistent with the findings or predictions in previous studies (Johnson et al., 2003; Chagnon et al., 2013). In addition to the intraspecific variation within AMF families, the context- or host-dependent AMF responses to environmental changes may also contribute to the different shifts in AMF community composition (van der Heyde et al., 2017; Zheng et al., 2018).

5. Conclusions

By assessing multiple characteristics of the arbuscular mycorrhizal symbiosis in response to N and P additions in a subtropical Chinese fir plantation, we provided novel evidence supporting the view that absorptive roots and mycorrhizal fungi together define plant strategies for belowground resource acquisition (Liu et al., 2015; Chen et al., 2016). Different responses of absorptive roots and AMF to N and P additions were observed in this monoculture forest plantation. These results show that while root diameter provides a useful first approximation of mycorrhizal reliance across species (Eissenstat et al., 2015; Liu et al., 2015), there is still substantial plasticity within a single tree species that allows for discrete modifications of root and mycorrhizal fungi to generate multiple nutrient acquisition strategies in response to changing environment. In other words, the degree of dependence by a single tree species on absorptive roots and AMF is not constant, but can be mediated by multiple soil nutrients. In addition to shifts in absorptive root and hyphal abundance, the responses of root and AMF morphology represent further aspects mediating plant nutrient acquisition strategies, although they are less pronounced than the responses of abundance. Community composition of AMF in our study was not linked with AMF morphology. While this conclusion is preliminary, future studies should be cautious when assuming that shifts in AMF community are related to predictable shifts in patterns of intraradical and extraradical hyphal production in diverse field settings. Importantly, our results highlight the necessity of connecting multiple aspects of absorptive root and mycorrhizal fungal responses to different soil nutrient conditions, as they may in turn differently affect plant and ecosystem functioning (Bardgett et al, 2014).

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2018.10.055.

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