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Research

Linking root traits to nutrient foraging in arbuscular mycorrhizal trees in a temperate forest

David M. Eissenstat[1,2], Joshua M. Kucharski[1], Marcin Zadworny[1,3], Thomas S. Adams[2] and Roger T. Koide[1,4]

[1]Intercollege Graduate Degree Program in Plant Biology, Penn State University, University Park, PA 16802, USA; [2]Department of Ecosystem Science and Management, Penn State University, University Park, PA 16802, USA; [3]Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland; [4]Department of Biology, Brigham Young University, Provo, UT 84602, USA

Author for correspondence:
David M. Eissenstat
Tel: +1 814 863 3371
Email: dme9@psu.edu

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Summary

- The identification of plant functional traits that can be linked to ecosystem processes is of wide interest, especially for predicting vegetational responses to climate change. Root diameter of the finest absorptive roots may be one plant trait that has wide significance. Do species with relatively thick absorptive roots forage in nutrient-rich patches differently from species with relatively fine absorptive roots?
- We measured traits related to nutrient foraging (root morphology and architecture, root proliferation, and mycorrhizal colonization) across six coexisting arbuscular mycorrhizal (AM) temperate tree species with and without nutrient addition.
- Root traits such as root diameter and specific root length were highly correlated with root branching intensity, with thin-root species having higher branching intensity than thick-root species. In both fertilized and unfertilized soil, species with thin absorptive roots and high branching intensity showed much greater root length and mass proliferation but lower mycorrhizal colonization than species with thick absorptive roots. Across all species, fertilization led to increased root proliferation and reduced mycorrhizal colonization.
- These results suggest that thin-root species forage more by root proliferation, whereas thick-root species forage more by mycorrhizal fungi. In mineral nutrient-rich patches, AM trees seem to forage more by proliferating roots than by mycorrhizal fungi.

Introduction

There has been much interest in identifying plant functional traits that can be used both to understand plant physiology and to scale ecosystem properties such as carbon, water, and nutrient cycling for regional or global predictions (Lavorel & Garnier, 2002; Cornelissen *et al.*, 2003; Westoby & Wright, 2006; Osnas *et al.*, 2013). While traits such as leaf thickness, leaf mass per unit area (LMA), and leaf longevity have been shown to follow an economics spectrum associated with tradeoffs between rapid assimilation and longevity, similar patterns in roots have proved to be elusive (Comas & Eissenstat, 2004; Tjoelker *et al.*, 2005; Withington *et al.*, 2006; Espeleta *et al.*, 2009; Holdaway *et al.*, 2011), although some patterns have been found using constrained phylogenies (Ryser, 1996; Comas *et al.*, 2002; Craine *et al.*, 2005). One explanation is that the root traits of too few plant species have been characterized to develop a sound understanding of the economics spectrum in this important organ. Another explanation may be that roots, unlike leaves, are usually engaged in a symbiosis with mycorrhizal fungi, rendering indistinct the relationships between functional attributes such as nutrient acquisition and simple root traits such as thickness, specific root length (SRL) and other aspects of root construction (Eissenstat *et al.*, 2000; Comas *et al.*, 2012).

The thickness of absorptive roots (e.g. first-, second-, and sometimes third-order; *sensu* Pregitzer *et al.*, 2002; McCormack *et al.*, 2015) varies enormously among plant species, with the finest roots of some species in the Magnoliales exhibiting roots >1 mm in diameter, while in some graminoid and ericaceous species roots may be <0.05 mm in diameter, at least a 20-fold range (Eissenstat, 1992; Chen *et al.*, 2013). While investigators have speculated on linkages of root traits to plant function (Chapin, 1980; Eissenstat, 1992), there have been surprisingly few studies that have directly explored these links, especially outside of container studies or garden plantings. Often a root morphological trait like root thickness only partially contributes variation to a plant functional trait like mycorrhizal colonization, root longevity, or root proliferation. For example, mycorrhizal colonization in arbuscular mycorrhizal (AM) species tends to be higher in species with thick absorptive roots and few root hairs (Baylis, 1975), but other factors can also play roles. For example, in citrus rootstocks in a mature field planting, rootstocks that previously exhibited the greatest growth responsiveness to mycorrhizal colonization under controlled conditions were more rapidly colonized by mycorrhizal fungi than less dependent rootstocks (Graham *et al.*, 1991). Consistent with theory (Koide, 1991), mycorrhizal dependency in these rootstocks was a function of both their ability to acquire phosphorus (P, with thick roots being less effective

if nonmycorrhizal) and their demand for P resulting from differences in whole-plant potential growth rate. Thickness of the absorptive fine roots, along with other traits, has also been associated with root longevity. In a common garden study of 12 temperate forest trees, median root life span was more than threefold longer in the species with the thickest absorptive roots than in the species with the finest absorptive roots (McCormack *et al.*, 2012). Other factors contributing to species variation in root longevity in this study included tree potential growth rate and root nitrogen (N) concentration. While root diameter is quite conserved phylogenetically (Comas & Eissenstat, 2009; Chen *et al.*, 2013), there is evidence that in related species, faster-growing species often have finer root diameters. Using congeneric contrasts of trees of different above-ground potential growth rate in an *c.* 65-yr-old forest, Comas & Eissenstat (2004) found that when constrained by phylogeny, faster-growing trees had finer roots, longer SRL (length per unit mass) and greater branching intensity (number of first-order roots per unit length of second-order root) than slower-growing species. Thus, linkages of root traits with plant functions such as life span, mycorrhizal colonization and whole-plant potential growth rate may be at least partly influenced by root morphology. If it is possibly to characterize below-ground ecosystem functions by readily measured plant traits, this can be a great asset in modeling the feedbacks of terrestrial vegetation with global changes (Smithwick *et al.*, 2013; Warren *et al.*, 2015).

There has emerged over the last few decades a large body of research on various aspects of root foraging for heterogeneous soil resources (reviewed by Hodge, 2004, 2006). Some studies that have examined the linkages of root morphology with root proliferation provide evidence that suggests that plant species with thick absorptive roots (low SRL) may have a reduced ability at scale-based foraging (total length produced in a patch), presumably because of both the higher direct costs of root construction of root length production in the patches (Eissenstat,1991; Fitter, 1994; George *et al.*, 1997; Mou *et al.*, 1997; Fransen *et al.*, 1998; Farley & Fitter, 1999; van Vuuren *et al.*, 2003), as well as a whole-plant strategy that might lead species with thick root systems to exhibit lower plasticity in root foraging (Grime *et al.*, 1986). However, the links between root foraging in nutrient-rich patches (scale-based) and traits such as whole-plant growth rate are often muddled because of factors such as growing conditions, plant developmental stage and phylogenetic effects (de Kroon & Mommer, 2006). Moreover, most root foraging studies have been performed either with seedlings in containers or in common gardens with limited species interactions below ground. Rarely have observations been made of individual root behavior in mixed communities with mature plants that are interacting with neighbors of different species (but see Liu *et al.*, 2015).

Here we report on a study where we use a root bag approach (Comas & Eissenstat, 2004) to examine root proliferation of mature canopy-dominant AM trees in a mixed-hardwood forest in central Pennsylvania. The objectives of the experiment were to determine: whether there is a syndrome of absorptive root traits linked to variation in root diameter; if species with thin absorptive roots exhibit faster root proliferation in nutrient-rich patches but lower mycorrhizal colonization than thick-root species; and whether thin-root species tend to be more plastic in root growth and mycorrhizal colonization in nutrient-rich patches, or in recovering from root pruning, than species that produce thick absorptive roots.

Materials and Methods

Field location and tree species selection

The study was conducted in central Pennsylvania at the Penn State Stone Valley Experimental Forest (40°39′ N 77°54′W) in Huntingdon County from April to October 2005. Within *c.* 1 km^2 of the forest, seven co-occurring, AM hardwood tree species were selected, representing a wide range of first-order mean root diameters (order terminology *sensu* Pregitzer *et al.*, 2002). The species chosen were *Acer rubrum* L., *Fraxinus americana* L., *Juglans nigra* L., *Liriodendron tulipifera* L., *Magnolia acuminata* L., *Ulmus americana* L. and *Ulmus rubra* Muhl. *Ulmus americana* and *U. rubra* were combined into a single group because of their very similar root characteristics and shared phylogenetic background. At 27 different locations of varying topographical positions in the forest, pairs of co-occurring species of divergent root diameters with trunks located within 15 m of each other were selected. Trees had a healthy appearance, were canopy dominants or codominants, and had similar diameters at breast height (1.3 m from the ground).

Root bag installation

Because the forest soil contains a matrix of roots of numerous plant species and absorptive roots of a known species are difficult to isolate, a root bag technique was utilized (Comas & Eissenstat, 2004). In April–early May 2005, a period when typically minimal root growth occurs in this region (D. M. Eissenstat, pers. obs.), woody lateral roots were traced from the trunk of an identified tree to the point where the root had tapered to *c.* 4 mm in diameter. Usually, woody roots of the appropriate diameter were found within the top 20 cm of forest soil. The 4-mm-diameter woody root was cut and *c.* 25 cm was inserted into the root bag. Before inserting into the bag, the root was pruned of any residual absorptive roots, ensuring that future absorptive roots arising from the woody root were new growth. In most cases, pruning was minimal. Three root bags were installed for each tree for a total 162 individual bags. Root bags were constructed from fine-mesh polyester fabric, 26 × 30 cm, with holes of *c.* 0.5 mm. After inserting the woody root into the root bag, the bag was filled with *c.* 1600 cm^3 of sieved forest soil and watered. The bag containing the woody root was reburied with forest soil, covered with the original duff layer, and again watered.

Growth assessment, fertilization, and watering

After allowing *c.* 14 wk for the woody roots within the installed root bags to initiate new absorptive root growth, bags were amended with and without a broad-spectrum fertilizer to

simulate a patchy nutrient environment. To ensure similar initial root growth of the bags to be fertilized with that of unfertilized controls, bags were uncovered and new root growth was visually assessed and photographed. This was necessary as some root bags failed to contain new growth. The fertilizer treatment, 37 g of Osmocote 19-6-12 slow-release fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, WA, USA) was randomly assigned to one bag. In a preliminary laboratory experiment, this amount, when scaled to the coverage area of the dimensions of the root bag, resulted in a fourfold increase in ammonium and nitrate release relative to that of unamended forest soil, based on KCl extraction of ammonium and nitrate. This amount was chosen as a sufficient amount of fertilizer to gain a root proliferation response without excessively fertilizing the soil. The fertilizer was mixed in with the soil and duff layer above the root bag without disturbing the roots. Then, all the bags were watered. Weekly watering was resumed for both fertilized and unfertilized bags in early August because of a lack of rain (1.3 l for each bag wk^{-1}) until the completion of the experiment. On average, the roots were exposed to fertilizer under moist soil conditions for 9.5 wk.

Root bag harvesting and processing

From mid-September to mid-October 2005, *c.* 23 wk after the initial placement in the ground, the entire root samples contained within the root bags were recovered by severing the large-diameter woody root where it entered the root bag and gently removing the intact root sample from the soil contained within the root bags. Root bags were harvested from the field in the same sequence as they were installed so as to keep duration of roots in the bag constant. The thin absorptive roots still attached to the large-diameter woody root were placed into sealable plastic bags and transported back to the laboratory where they were stored at 4°C, thus preserving the absorptive root architecture.

Once in the laboratory, the fine absorptive roots, still attached to the large-diameter woody root, were gently washed with tap water to remove residual soil particles before measurements of morphology and architecture. Root orders were described and dissected by branching order using the morphometric method, where the finest laterals with no branch roots were order 1 (Fitter, 1982; Pregitzer *et al.*, 2002).

Root morphology, architecture, and proliferation

Manual measurements Total root order for the absorptive root samples was obtained by recording the highest root order contained within each root bag. Recovery from root pruning was expressed as the percentage of total root bags of a given species with absorptive root growth (27 bags per species). Next, the branching intensity of the first-order roots was measured and calculated (number of first-order roots per length of second-order root) using a dissecting microscope (Model SZ-4045, Olympus, Tokyo, Japan) and an ocular micrometer. Lastly, root diameter (control roots only) was determined for the first-, second-, and third-order roots by taking three measurements of diameter along the length of the root with the dissecting microscope.

Scanner image measurements Root subsamples were analyzed using WinRHIZO (Regent Instruments, Quebec, Canada) by dividing a given subsample into two separate scans. The first scan contained only first- and second-order roots, while the second scan contained third-order and higher roots. By dividing a subsample in this manner, the first two root orders could be compared across species for the morphological measures of diameter, SRL, and tissue density. Roots were scanned in distilled water using a clear water tray in grayscale at 500 dpi. Afterwards, the root samples were oven-dried at 65°C for 48 h and weighed. While root diameter, length, and root volume can be directly obtained through the software output, SRL (length/first- and second-order DW) and tissue density (first- and second-order DW/root volume) were calculated. Then, by combining the two scans, the entire subsample could be used to estimate the total root length for a sample. From the total root length and mass estimates, growth rates were determined. Root growth rate responses to fertilizer were calculated by subtracting the average fertilizer growth rate from the average unfertilized growth rate for each species. The descriptions and abbreviations of the various morphological, architectural, mycorrhizal, and proliferation responses investigated are described in detail in Table 1.

Mycorrhizal colonization

Owing to insufficient sample material, *Magnolia* roots were not included in mycorrhizal colonization measurements. To visualize mycorrhizal colonization across the five remaining species, dry root samples were placed in 10% KOH solution overnight at room temperature, and then after several washes in water, roots were placed in a solution of 3% H_2O_2 and 10% ammonia for 20 min in order to remove color from the roots. After bleaching, roots were washed three times in water. Staining was performed using 0.05% Trypan Blue in a solution of glycerin, lactic acid, and distilled water in a 1 : 1 : 1 ratio by volume (Brundrett *et al.*, 1996) at a temperature of 80°C. To avoid destruction of fragile root samples, whole roots were placed on microscope slides without cutting. Observations were performed with an Olympus light microscope. Mycorrhizal colonization was assessed according to Trouvelot *et al.* (1986) using the computer program 'MYCOCALC' (http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.html). Estimation of the following parameters were obtained: AM_F (%), relative frequency of mycorrhiza in the root system (100 × number of colonized roots divided by total root number); and AM_I (%), intensity of mycorrhizal colonization based on the following classes: 0% for 0 class; < 1% for first; 1–10% for second; 10–50% for third; 50–90% for fourth; and finally > 90% for the fifth class. The mean of each class was then used in calculating a mean percent intensity of all the roots examined using the program 'MYCOCALC' (AM_I).

Statistics

For a given root trait, all data were tested for normality and homogeneity of variance. Data not meeting the earlier assumptions were transformed using either logarithmic or square-root

Table 1 Abbreviations, definitions, and descriptions of the various morphological and architectural traits, and proliferation and mycorrhizal responses examined

Trait	Abbreviation	Units	Description
Morphological traits			
first- + second-order diameter	$Diam_{1st+2nd}$	mm	Diameter of combined first- and second-order absorptive roots
Specific root length	SRL	$m\ g^{-1}$	Length per unit dry mass of combined first- and second-order absorptive roots
Tissue density	TissDen	$g\ cm^{-3}$	Mass per unit root volume of combined first- and second- order absorptive roots
Architectural traits			
Branching intensity	BranInt	cm^{-1}	Number of first-order absorptive roots per length of second-order absorptive root
Total order	TO	none	Highest root branching order contained within a root bag
Proliferation responses			
Pruning recovery	PR	%	Percentage of woody roots of a given species that recovered from wounding by proliferating new absorptive roots
Length growth rate	GR_L	$cm\ d^{-1}$	Total absorptive root length produced d^{-1} standardized for a 20 cm length of woody root
Mass growth rate	GR_M	$mg\ d^{-1}$	Total absorptive root mass produced d^{-1} standardized for a 20 cm length of woody root
Fertilization response on length growth rate	$Fert_L$	$cm\ d^{-1}$	Fertilized minus unfertilized length growth rate
Fertilization response on mass growth rate	$Fert_M$	$mg\ d^{-1}$	Fertilized minus unfertilized mass growth rate
Mycorrhizal responses			
Arbuscular mycorrhizal colonization – frequency	AM_F	%	Relative frequency of mycorrhiza in the root system (number of colonized roots divided by total root number)
Arbuscular mycorrhizal colonization – intensity	AM_I	%	Intensity of mycorrhizal colonization (estimated based on intensity classes) of all roots examined

transformation, depending on the structure of the variance. Species and treatment differences were analyzed by ANOVA, and when appropriate, *post hoc* means comparisons were made using Tukey tests. Treatment by covariate interactions were assessed by ANCOVA. Trait correlations were calculated using Pearson product-moment correlations and Spearman rank order correlations. Pearson correlations (r) were used for all correlations except pruning recovery, in which the Spearman rank correlation (ρ) was used. Differences at $P < 0.05$ were considered significant, while differences between $P > 0.05$ and $P \leq 0.10$ were considered marginally significant. All statistics were performed using JMP (SAS, Cary, NC, USA).

Results

Root morphology and architecture

This field study compared six tree 'species' (the two species of *Ulmus* were treated as a single 'species') of different absorptive root morphology (Figs 1, 2) in which the root diameters varied widely. For instance, the finest lateral first-order roots (Dia_{1st}) exhibited continuous variation across species with an almost fourfold difference between the smallest diameter (0.20 mm, *Ulmus*) and the largest (0.78 mm, *Magnolia*) ($P < 0.01$, Fig. 2). This range was similar for the combined diameters of the first two root orders that were based on scanner images ($Dia_{1st+2nd}$) (Table 2). Overall, the absorptive roots among the six hardwood tree species differed considerably in both morphological traits and architectural traits, suggesting the existence of suites of correlated traits ($P < 0.01$, Table 2). For instance, the morphological trait, SRL, and the architectural trait, branching intensity, both displayed continuous variation across species. Species exhibited a 6.4-fold difference in SRL (*Magnolia*, $15.1\ m\ g^{-1}$; *Ulmus*, $97.0\ m\ g^{-1}$), and a 3.8-fold difference in branching intensity (*Magnolia*, 0.28 roots cm^{-1}; *Ulmus*, 1.07 roots cm^{-1}). By contrast, fewer individual species exhibited significant differences in tissue density; instead, patterns reflected differences in clustered groups of species at the extremes, with only 1.7-fold difference between the lowest (*Magnolia*, $0.13\ g\ cm^{-3}$) and the highest (*Ulmus*, $0.23\ g\ cm^{-3}$) values. There was a high predictability that absorptive roots of thinner diameter would have higher SRL (Dia_{1st}, $r = -0.97$, $P < 0.05$; $Dia_{1st+2nd}$, $r = -0.98$, $P < 0.05$), higher branching intensity (more laterals cm^{-1} of second-order root; $r = -0.90$, $P < 0.05$), and more total root orders ($r = -0.96$, $P < 0.05$) than species that produce thicker diameter roots (Table 3; Supporting Information Fig. S1). Overall, the evidence supports our first hypothesis of the existence of a syndrome of absorptive root traits linking root morphology with root architecture.

Root diameter and tissue density are the two components of SRL (Eissenstat, 1991). While diameter was negatively correlated with SRL, tissue density (dry mass per fresh volume) was not significantly correlated with SRL ($r = 0.65$, $P = 0.16$). This indicated that root diameter is the primary factor influencing SRL variation among species, and not tissue density. However, there was marginally significant evidence that tissue density was negatively correlated with root diameter (Dia_{1st}, $r = -0.80$, $P \leq 0.10$; $Dia_{1st+2nd}$, $r = -0.79$, $P \leq 0.10$). Thus, thicker first- and second-order roots tended to have lower tissue density, which would not be consistent with their long life span (McCormack

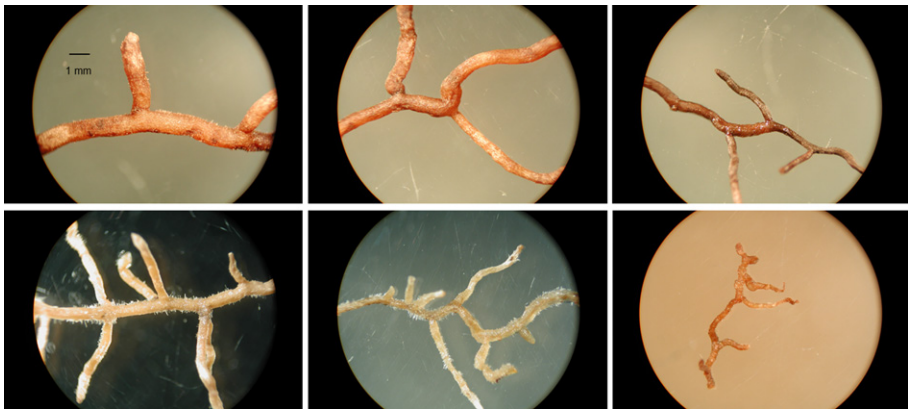

Fig. 1 Micrographs of the finest absorptive root orders from the six co-occurring arbuscular mycorrhizal (AM) tree species. From left to right: top, *Magnolia acuminate*, *Liriodendron tulipifera*, *Juglans nigra*; bottom, *Fraxinus americana*, *Acer rubrum*, *Ulmus americana*.

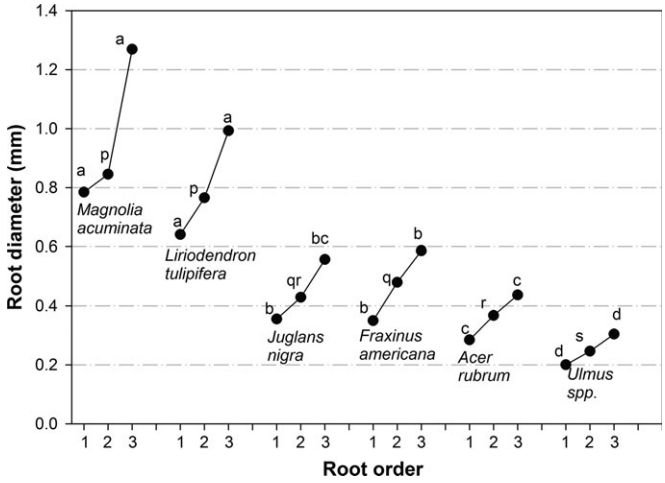

Fig. 2 Mean root diameter for the first three orders of roots from six co-occurring arbuscular mycorrhizal (AM) trees. Within a given root order, different letters indicate significant differences ($P < 0.05$) across all species. Data represent unfertilized controls only ($n = 4$–9 except for third-order *Magnolia acuminata*, for which $n = 3$).

et al., 2012; Adams *et al.*, 2013) and a stress-tolerant strategy (*sensu* Grime, 1977; Ryser, 1996; Garnier *et al.*, 2004).

Species responses to root pruning

The percentage recovery of the woody roots that were pruned in early spring (PR) differed significantly across the species ($\chi^2 = 35.63$, $P < 0.01$). For half the species (*Juglans*, *Acer*, and *Liriodendron*), recovery was in the 70–75% range, while *Fraxinus* and *Ulmus* roots recovered at *c.* 90%. At the low end of the recovery response, *Magnolia* roots only recovered *c.* 30% of the time. Both root diameter (first order; Table 3; Fig. S2) and branching intensity (Table 3) tended to be correlated ($P < 0.10$) with pruning recovery. Thus, compared with thin-root species, species with thick roots of lower branching intensity tended to be less able to recover from root pruning, a common proxy for herbivory.

Root proliferation

Root proliferation responses varied widely among species ($P < 0.01$, Table 2). Growth rate of roots on a mass basis (GR_M) in the bag varied 31-fold from the lowest (*Magnolia*) to the highest (*Ulmus*) species-specific response. The species represented a rather continuous pattern of variation, with *Ulmus* and *Fraxinus* occupying the high end of the spectrum and *Magnolia* occupying the low end (Table 2). Moreover, when comparing the lowest (*Magnolia*) with the highest (*Ulmus*) species on a length basis, root growth rate on a length basis (GR_L) showed a remarkable 89-fold variation. Root diameter and branching intensity were negatively and positively correlated with root growth rate, respectively, on either a length or mass basis (Figs 3, 4). Root morphological traits were less highly correlated with the root proliferation responses than root architectural traits (Table 3). Generally, when the proliferation responses are expressed on a length basis instead of a mass basis, the correlations were stronger. Root length responses were a result of both differences among species in SRL and differences in GR_M.

Root responses to fertilization

Our data provided mixed support for our third hypothesis that thin-root species of higher branching intensity would be more responsive (i.e. difference in growth rate between fertilized and unfertilized patches) to nutrient-rich patches than thick-root species. While species exhibited large differences in responsiveness of root proliferation to fertilization (Table 2), the correlation was not significant for diameter with either mass or length response to fertilization (Table 3). However, there was a significant correlation ($P < 0.05$) of branching intensity with mass and length responses to fertilization (Table 3). These relationships are explored further in Figs 3 and 4. As indicated by the steeper slopes, there was a tendency for growth rate to be more affected by fertilization in thin-root species with higher branching intensity than thick-root species of lower branching intensity. However, using ANCOVA (control + fertilized) with diameter or branching intensity as a covariate, we did not detect a significant treatment × covariate interaction ($P > 0.16$; data not shown).

Fertilization had a relatively small effect on root morphology (e.g. diameter) and architecture (e.g. branching intensity). When averaged across all species, $Dia_{1st+2nd}$ increased an average of only 11% with fertilization ($P < 0.05$; Table 2), with some species responding more than others (species × treatment interaction,

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Table 2 Traits associated with morphology, architecture, and proliferation for six co-occurring arbuscular mycorrhizal (AM) tree species (top), and ANOVA of traits (bottom)

	$Diam_{1st+2nd}$ (mm)*		SRL (m g^{-1})		TissDen (g cm^{-3})		BranInt (no. cm^{-1})		Total order (no.)		PR (%)	GR_L (cm d^{-1})		GR_M (mg d^{-1})	
Species	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized + fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized
Magnolia	0.73 ab	0.93 a	17.5 f	13.1 f	0.15 cd	0.12 cd	0.36 d	0.21 d	2.7 f	3.0 def	29	0.2 e	0.2 e	0.16 e	0.18 de
Liriodendron	0.67 b	0.68 b	18.9 f	21.5 ef	0.15 cd	0.13 d	0.36 d	0.32 d	3.7 cdef	4.0 bcde	75	1.7 bcde	2.5 bcde	1.37 bcde	1.58 abcde
Juglans	0.41 d	0.51 c	40.7 cd	32.0 de	0.19 bcd	0.16 cd	0.43 d	0.48 cd	4.3 bcde	3.4 df	70	1.4 cde	1.1 de	0.73 cde	0.57 cde
Fraxinus	0.41 d	0.45 cd	32.4 de	31.0 de	0.23 ab	0.21 abc	0.74 bc	0.78 b	4.2 bcde	4.2 bce	90	7.7 abc	17.5 a	3.16 abc	7.79 ab
Acer	0.33 e	0.33 e	69.3 ab	63.2 bc	0.17 cd	0.19 abcd	0.80 b	0.82 b	4.9 ab	4.6 abc	73	6.7 abcd	12.4 ab	1.51 bcde	3.25 abcd
Ulmus	0.24 f	0.24 f	88.5 ab	106 a	0.25 a	0.20 abc	1.22 a	0.93 ab	5.3 a	5.6 a	92	9.6 abc	37.6 a	2.53 abcd	10.37 a

	Species		Treatment		Species × treatment Interaction	
Trait	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value
First- + second-order diameter	196.24	**< 0.01**	13.65	**< 0.01**	2.61	**0.03**
SRL	95.18	**< 0.01**	0.98	0.32	1.77	0.13
Tissue density	14.11	**< 0.01**	6.44	**0.01**	1.60	0.17
Branching intensity	59.40	**< 0.01**	3.74	*0.06*	2.49	**0.04**
Total order	28.81	**< 0.01**	0.15	0.70	2.30	*0.05*
Pruning recovery	35.63†	**< 0.01**	n/a	n/a	n/a	n/a
Length growth rate	22.55	**< 0.01**	2.77	*0.10*	1.03	0.41
Mass growth rate	13.41	**< 0.01**	3.78	*0.05*	1.10	0.36

Within a particular trait or response, means not sharing a letter are significantly different ($P < 0.05$). Bold *P*-values were < 0.05; italicized *P*-values were < 0.10.
*$Diam_{1st+2nd}$, diameter of combined first- and second-order roots, a scanner-based estimate; SRL, specific root length; TissDen, root tissue density of first- and second-order roots combined; BranInt, branching intensity or number of first-order laterals cm^{-1} of second-order root; total orders, highest root branching order in root bag; PR, pruning recover; GR_L, length growth rate; GR_M, mass growth rate.
†Analyzed using chi-squared.

Table 3 Correlation matrix (Pearson *r*) of six root morphological and architectural traits with root proliferation responses (top), with other morphological and architectural traits, and with arbuscular mycorrhizal (AM) colonization across six co-occurring tree species of widely differing root morphology (bottom)

Morphological and architectural trait	Root proliferation response				
	Pruning Recovery	GR_L	GR_M	Length response to fertilization ($Fert_L$)	Mass response to fertilization ($Fert_M$)
First-order diameter	*−0.76*	**−0.85**	*−0.76*	−0.72	−0.70
$Diam_{1st+2nd}$*	−0.71	**−0.82**	−0.72	−0.72	−0.72
SRL	0.61	*0.76*	0.63	0.65	0.66
Tissue density	*0.77*	*0.76*	*0.76*	*0.75*	*0.73*
Branching intensity	0.67	**0.86**	*0.78*	**0.89**	**0.93**
Total order	**0.85**	**0.89**	**0.83**	*0.75*	0.65

	Root morphological and architectural traits					Mycorrhizal colonization†	
	First- + second-order diameter	SRL	Tissue density	Branching intensity	Total order	Unfertilized AM colonization frequency	Fertilized AM colonization frequency
First-order diameter	**0.99**	**−0.97**	*−0.80*	**−0.90**	**−0.96**	0.79	–
$Diam_{1st+2nd}$	–	**−0.98**	*−0.79*	**−0.92**	**−0.94**	0.79	**0.96**
SRL		–	0.65	**0.87**	**0.93**	−0.70	*−0.81*
Tissue density			–	**0.82**	0.70	−0.80	−0.78
Branching intensity				–	**0.83**	**−0.92**	**−0.93**
Total order					–	−0.75	−0.72

Data represent unfertilized controls only except for pruning recovery, response to fertilization (length and mass) and AM colonization frequency. Root morphological and architectural traits and proliferation responses were analyzed using transformed values with the exceptions of tissue density and pruning recovery (see the Materials and Methods section for details). Coefficients in bold type were significant at $P < 0.05$, while coefficients in italic type were significant at $P \leq 0.10$ ($n = 6$).

*$Diam_{1st+2nd}$, diameter of combined first- and second-order roots, a scanner-based estimate; SRL, specific root length; PR, pruning recover; GR_L, length growth rate; GR_M, mass growth rate; $Fert_L$, root length response to fertilization; $Fert_M$, root mass response to fertilization.

†Only five species were compared for mycorrhizal colonization, as *Magnolia* roots had insufficient material.

$P < 0.05$). *Juglans nigra* was the only species significantly different between control and treatment (25% increase) and thus appeared to be the main driver of the overall response. SRL decreased an average of 4% in response to fertilization (treatment, $P = 0.32$; interaction, $P = 0.13$; Table 2). This nonsignificant directional response was observed in two-thirds of the species, with only *Liriodendron* and *Ulmus* increasing in SRL. Tissue density of the fertilized roots was *c.* 16% lower than that of unfertilized roots for every species except *Acer*, which exhibited a 15% increase in tissue density (treatment, $P < 0.05$; interaction, $P = 0.17$; Table 2). The root architectural trait, branching intensity (BranInt), was on average diminished 10% by fertilization ($P \leq 0.10$; Table 2), with half the species responding to the nutrient-rich patch through increased BranInt, and the other half decreasing BranInt in response to the patch. There was no evidence that species with thinner roots and higher BranInt shifted more in BranInt in response to fertilization ($P = 0.83$, data not shown) than thick-root species.

Mycorrhizal colonization

Across all species, mycorrhizal colonization was primarily observed in only the first- and second-order roots, with mycorrhizal colonization in third-order roots being either very low or absent (Fig. 5). Fertilization significantly decreased both colonization frequency (AM_F) and intensity (AM_I) in the first- and second-order roots of two thin-root species (*Acer* and *Ulmus*), but had little to no effect on the moderate- to thick-root species across any root order (Fig. 5). Mycorrhizal colonization frequency (AM_F) increased with root diameter across the five species investigated, and decreased with branching intensity (Fig. 6). The tendency for thin-root species to show a greater reduction in mycorrhizal colonization frequency or intensity than thick-root species was not significant (i.e. differences in slopes, $P > 0.10$).

Discussion

In our study of AM temperate tree species, which varied in first-order root diameter by almost fourfold, we supported our hypothesis that thin-root species more readily proliferated in both mass- and length-based growth but had lower mycorrhizal colonization than thick-root species. Additionally, there were significant correlations among the individual root traits investigated, suggestive of a syndrome of traits associated with root thickness in AM trees. There was a strong negative correlation of root diameter with branching intensity, so that species with the finest root morphology (*Acer* and *Ulmus*) had the greatest branching intensity. These results are consistent with the complementary roles of roots and mycorrhizal fungi in foraging for nutrients, where thick-root species seem to rely more on mycorrhizal fungi

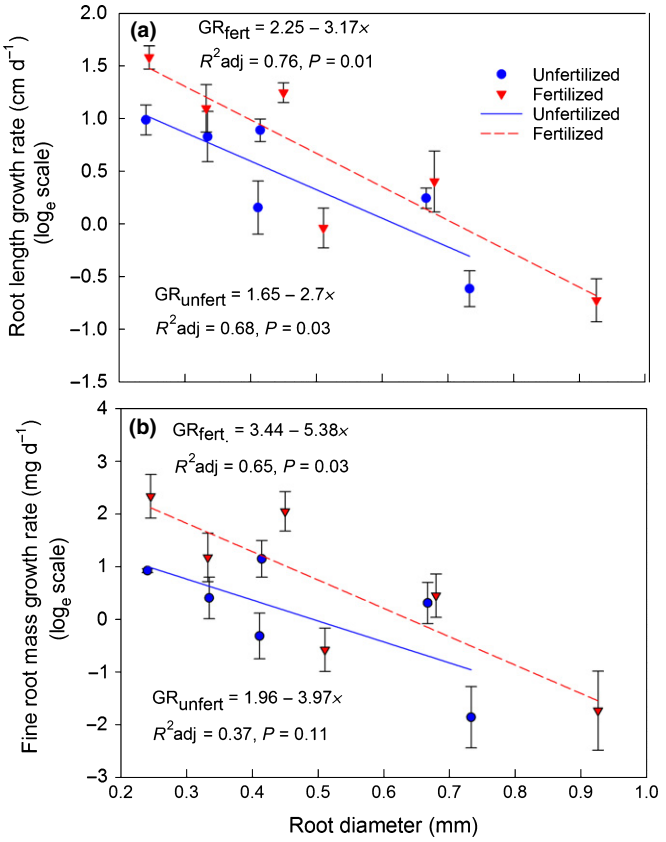

Fig. 3 Relationship of daily root length growth rate (top, GR_L, cm d^{-1}) and daily root mass growth rate (bottom, GR_M, mg d^{-1}) for a 20 cm length of woody root with average first- + second-order root diameter in unfertilized (GR_{unfert}) and fertilized (GR_{fert}) treatments. Error bar represents ± 1 SE. Note that the *y*-axis is on a $\log_e$ scale. Regression lines represent the best-fit linear relationship for unfertilized and fertilized treatments across species. Slopes of regression lines were not significantly different for either length or mass growth rate (ANCOVA, $P > 0.10$)

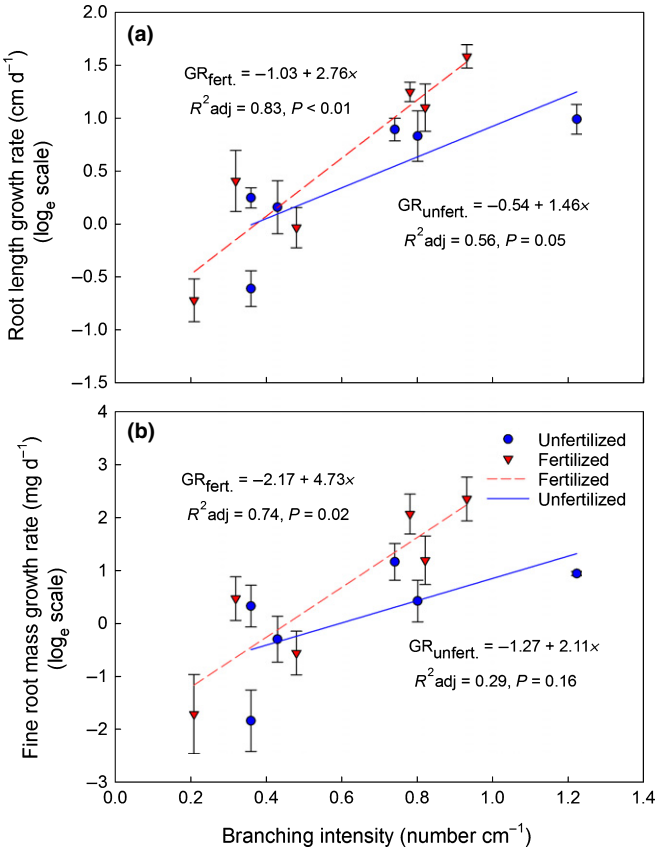

Fig. 4 Relationship of daily root length growth rate (top, GR_L, cm d^{-1}) and daily root mass growth rate (bottom, GR_M, mg d^{-1}) for a 20 cm length of woody root with root branching intensity (number of first-order laterals cm^{-1} of second-order root) in unfertilized ($GR_{unfert.}$) and fertilized ($GR_{fert.}$) treatments. Error bar represents ± 1 SE. Note that the *y*-axis is on a $\log_e$ scale. Regression lines represent the best-fit linear relationship for unfertilized and fertilized treatments across species. Slopes of regression lines were not significantly different for either length or mass growth rate (ANCOVA, $P > 0.10$)

for foraging and thin-root species rely more on root growth. More broadly, this suggests that, at least in canopy AM trees, root thickness, which tends to be quite stable under different environmental conditions, is a strong indicator of root foraging strategy.

Our hypothesis that thin-root species would show greater plasticity in response to fertilization, both in increasing root growth rate and in reducing mycorrhizal colonization, had mixed and generally nonsignificant support. Fertilization generally reduced the frequency of AM colonization across all species (also see Koide & Li, 1991; Nilsson & Wallander, 2003; Nilsson *et al.*, 2007; Sharda & Koide, 2010), with the two species with the thinnest roots showing the greatest decline. Nonetheless, we were unable to show that thin-root species overall had greater declines in mycorrhizal colonization than thick-root species. Similarly, the evidence that thin-root species were more plastic in root proliferation than thick-root species was mixed. While there was a tendency for thin-root species to be more responsive in root length proliferation than thick-root species, the results were often not significant. A similar lack of evidence of greater plasticity in thin- than in thick-root species was also observed in a subtropical forest (Liu *et al.*, 2015). In AM trees, it appears that foraging in inorganic nutrient-rich patches is achieved primarily through increased root growth rather than increased mycorrhizal colonization independent of root diameter. Thus, because of their slower root growth rate, it appears that thick-root species are less capable of quickly exploiting nutrient-rich patches, as they too seem to be foraging more by root rather than by hyphal growth (also see Liu *et al.*, 2015). However, thick-root species may still be capable of exploiting soil nutrient heterogeneity if nutrient patches are temporally stable, partly because of their long root life span (McCormack *et al.*, 2012).

There appears to be clear tradeoffs associated with constructing roots of different morphology. Constructing thick roots of low SRL is an inefficient allocation of biomass for producing absorptive surface area, if root life span does not differ among species (Eissenstat & Yanai, 1997). Thus, the greater investment in mycorrhizal fungi for nutrient foraging as an alternate strategy by thick-root species is predictable from a cost–benefit perspective. One advantage of thick first- and second-order roots is their tendency to be longer-lived than first- and second-order roots of thin-root species

(McCormack *et al.*, 2012; Adams *et al.*, 2013). However, there may be a number of costs associated with thick roots. One appears to be the ability to proliferate new root length (Fig. 4). There was also a marginally significant ($P < 0.10$) response that species with thick first-order roots were less able to recover from root pruning, a proxy for herbivory (Table 3; Fig. S2). In a similar study using 14 subtropical AM tree species, Liu *et al.* (2015) also observed that thick-root species recovered from root pruning less frequently than thin-root species ($P < 0.01$). Collectively, these data suggest that root losses by herbivory and other soil disturbance may be less disruptive to thin-root species than thick-root species. It also suggests that in mixed species forests, root disturbance by methods such as ingrowth cores may selectively favor thin-root species.

Our results are broadly consistent with recent studies (Comas *et al.*, 2012; Adams *et al.*, 2013; Kong *et al.*, 2014) and support earlier work by Baylis (1970, 1975) that a plant's potential dependence on mycorrhizal symbioses is related to root morphology. Baylis argued that plants of more basal lineages ('magnolioid species') tend to have thick roots of limited root hair development, and have greater reliance on mycorrhizal fungi when foraging for nutrients (Hetrick, 1991). A recent meta-analysis by Maherali (2014) of published studies using AM mycorrhizal inoculation to examine the relation between root architecture and mycorrhizal growth response (MGR) found no significant relationship between SRL, root diameter or root hair length, or density with MGR. These results led to the conclusion that thick roots alone do not indicate a greater potential plant growth benefit from AM colonization. Other factors that influence the MGR (Koide, 1991) and mycorrhizal colonization (Graham *et al.*, 1991) include soil nutrient availability and whole-plant demand for nutrients, which can be very high in some species, such as *Liriodendron tulipifera* (McCormack *et al.*, 2012). It is probable that the importance of morphology of absorptive roots in influencing the growth response is context-dependent and only one of many factors that act in concert to influence whole plant growth. In our study, conducted in natural forest stands with trees in the forest canopy, the fine roots of all of the tree species investigated had some degree of mycorrhizal colonization, and corresponding tree-level growth responses were not considered. Rather, we investigated the relationship between root traits and the degree of mycorrhizal colonization under different nutrient concentrations. Under these conditions, thick-root species of low branching intensity generally had higher AM colonization frequency and intensity than thin-root species.

Patterns of mycorrhizal colonization with root order were fairly consistent across species, with the second-order roots typically showing the highest mycorrhizal colonization. We suspect the first-order roots may have had somewhat lower colonization because of their younger age. In the case of the third-order roots, colonization was generally very low, probably because many of these roots may have been originally pioneer roots where mycorrhizal colonization can be very limited (Zadworny & Eissenstat, 2011).

Our work presented here appears to have broad relevance to forests with AM trees. Using a similar approach in a subtropical forest, Liu *et al.* (2015) also found among 14 subtropical trees that species with thin roots and high branching intensity had faster root length growth rate and lower mycorrhizal colonization than did thick-root species of low branching intensity. Unlike this study, however, they did not observe that root mass growth rate was negatively correlated with root diameter or positively correlated with branching intensity. They also observed that full-spectrum fertilizer addition increased root growth but decreased mycorrhizal colonization and extramatrical hyphal length per unit root length. Overall, the broad similarity of functional attributes associated with absorptive root diameter and branching intensity in temperate forest species with those of subtropical species suggests that, at least in well-established AM trees, there appears to be strong linkages of root morphology and architecture with root foraging strategy. This may have considerable value in the identification of plant traits that can be used in scaling to terrestrial biosphere models (Warren *et al.*, 2015). Care should be taken, however, in extrapolating these results to other life forms and other types of mycorrhiza (e.g. ectomycorrhizal tree species), as the foraging strategies may be much more complex. Moreover, the effects of foraging will depend both on the inorganic nutrient form (e.g. P or N; Liu *et al.*, 2015) and on whether the nutrient-rich patches are of organic or inorganic origin (Hodge, 2004, 2006).

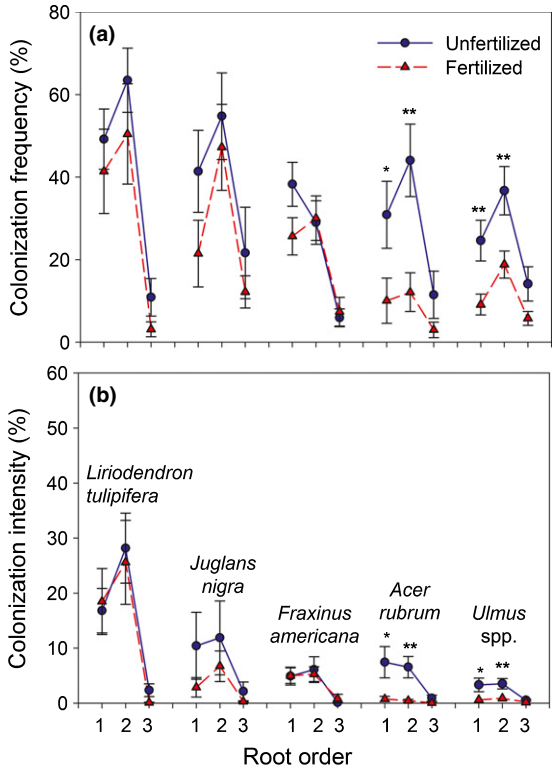

Fig. 5 Colonization frequency (AM_F) (a) and colonization intensity (AM_I) (b) of the first-, second- and third-order roots of trees in control and fertilized conditions. Error bar represents ± 1 SE. Asterisks indicate that root colonization of the studied order was significantly different when comparing control and fertilized plants. Student's *t*-test: *, $P < 0.05$; **, $P < 0.01$.

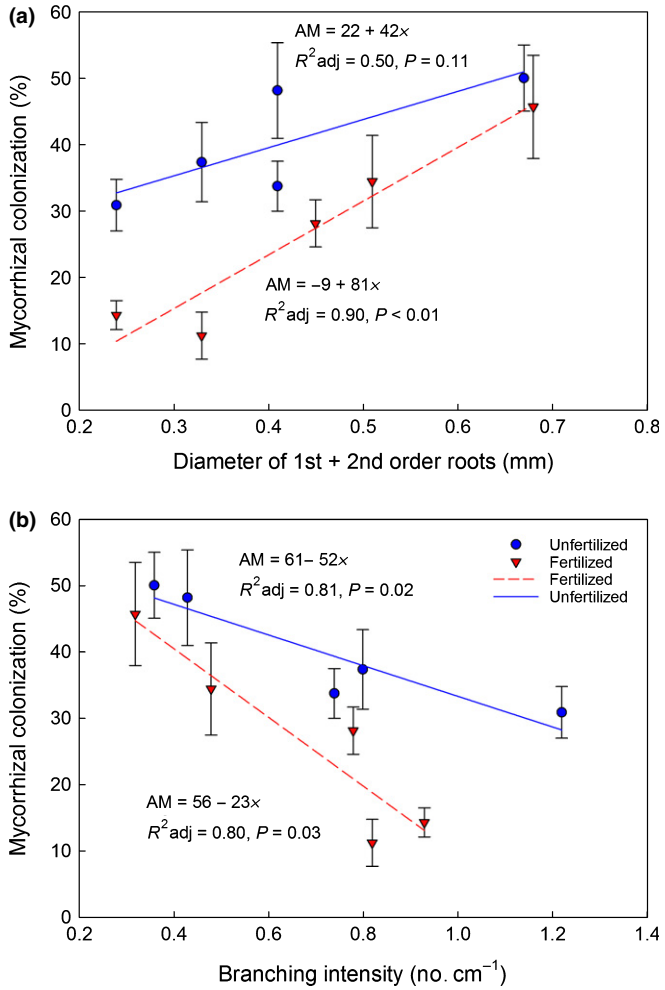

Fig. 6 The relationship of percentage arbuscular mycorrhizal (AM) colonization frequency (AM_F) (± SE) with average first- and second-order root diameter (a) and root branching intensity (number of first-order roots cm^{-1} of second-order root) (b) in five co-occurring temperate tree species in fertilized and unfertilized soil. Least-squares regression lines and corresponding equations, adjusted R^2, and *P*-values are also shown. Slopes of regression lines were not significantly different for either diameter or branching intensity (ANCOVA, $P > 0.10$)

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Supporting Information

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

Fig. S1 Relationship of root diameter with root branching intensity and with total root order.

Fig. S2 Relationship of root diameter with pruning recovery.

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