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Patterns in root trait variation among 25 co-existing North American forest species

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Summary

• Ephemeral roots have essential roles in plant and ecosystem functioning. In forests, roots account for a major component of carbon cycling, yet few studies have examined ranges of root trait variation and how different species vary in root form and function in these communities.

• Root branching intensity, specific root length (SRL; root length per unit dry mass), root diameter, tissue density, phenolic concentration and nitrogen concentration were determined for the finest two root orders of 25 co-existing North American woody species sampled from mature plants in a single forest community. Trait correlations and multivariate patterns were examined to evaluate the most important trait differences among species.

• Branching intensity, SRL, and phenolic concentration varied most widely among species (coefficient of variation (CV) = 0.42, 0.57 and 0.58, respectively). Species predominately forming ectomycorrhiza (EM) had a higher branching intensity than those forming arbuscular mycorrhiza (AM) with mycorrhizal types correctly predicted in *c*. 70% of individual observations by branching intensity alone. There was notably no correlation between SRL and nitrogen. Variation in SRL among species mapped partially along phylogenetic lines (consistency index (CI) = 0.44), with remaining variation attributable to differences in species' ecological specialization.

• Variation found in root traits suggests different nutrient acquisition strategies within this community, which could have potential species-level effects on carbon and mineral nutrient cycling.

Introduction

Belowground systems remain one of the most poorly understood areas in terrestrial ecology. While the importance of fine root functions in these systems is recognized, understanding of root trait variation and its effects on ecological processes remains limited (Westoby & Wright, 2006). The effects of plants on ecosystem processes have been partly attributed to variation in leaf traits (Reich *et al.*, 1999, 2005) and mycorrhizal symbioses (Cornelissen *et al.*, 2001). Broad investigations have found convergent patterns of leaf trait co-variation and trade-offs in functions among species (Reich *et al.*, 1999), but investigations of root traits are still few and focused almost entirely on herbaceous species (e.g. Craine *et al.*, 2002; Levang-Brilz & Biondini, 2003; Tjoelker *et al.*, 2005; Roumet *et al.*, 2006). In forest communities, ephemeral fine roots were found to show extensive trait diversity in a few studies that examined trait co-variation (Brundrett *et al.*, 1990; Reich *et al.*, 1998b; Pregitzer *et al.*, 2002; Withington *et al.*, 2006) or linked patterns of variation to plant growth strategies (Comas *et al.*, 2002; Comas & Eissenstat, 2004). Critical questions remain as to whether general trade-offs exist among root traits of woody species, and whether these are linked to different plant strategies, especially nutrient acquisition.

Plant growth strategy theories (e.g. Grime, 1977; Chapin *et al.*, 1993) supported by broad examinations of aboveground traits have suggested that there are plant syndromes optimized for quick resource exploration that associate traits such as thin tissue with high surface area, metabolic activity, and nitrogen (N) concentration (Reich *et al.*, 1999), short lifespan and low chemical defenses (Coley, 1988).

Belowground traits have shown similar associations as those found in aboveground traits, supporting syndromes of fast versus conservative growth between annual and perennials in herbaceous plants (Roumet *et al.*, 2006). Although comparisons across families showed no patterns, congeneric comparisons of root morphology, architecture and chemical defenses between fast- and slow-growing tree species have suggested similar syndromes at both seedling and mature stages (Comas *et al.*, 2002; Comas & Eissenstat, 2004). Before generalizations about belowground trait patterns can be made, we need more information on broad patterns of root trait variation (i.e. which traits vary among which species), especially from studies comparing root material selected by branching order to avoid the confounding issues that can arise when roots with different functions are compared (Comas *et al.*, 2002; Pregitzer *et al.*, 2002; Withington *et al.*, 2006; Guo *et al.*, 2008).

Root systems have a complexity that is often underappreciated, with different root branching orders serving different functions. In an ordering system where fine unbranched terminal roots are first order and those at the next level of branching are second order (Fitter, 1982), the finest two orders primarily serve functions of nutrient acquisition, with the third order typically being transitional between the functions of absorption and transport, and higher order roots serving functions of structural support and transport once they undergo secondary development (Guo *et al.*, 2008). An important metric of how roots deploy biomass for nutrient acquisition is specific root length (SRL; root length per unit dry mass), because nutrient acquisition is improved more by increasing root length and surface area than by increasing mass (Eissenstat, 1991; Fitter, 1991). Root branching intensity governs exploration through the soil matrix and thus may also affect nutrient acquisition (Fitter, 1991). Universal patterns in leaf, stem and root functioning can be found correlating dark respiration and N concentration (Reich *et al.*, 2008). However, root functions of soil resource exploration have additional complexity because roots of temperate tree species typically form symbiotic associations (mycorrhizas) with fungi to acquire nutrients, which may improve resource capture at a cost to the plant (Peng *et al.*, 1993). It is widely accepted that the type of mycorrhiza affects the amount and type of resources directly available to plants (Brundrett, 2002; Smith & Read, 2008), although both arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) plants can indirectly access nutrients through common mycorrhizal networks independently of their mycorrhizal types (He *et al.*, 2006).

In the work presented here, we investigated six root traits related to important functional differences among species in terms of nutrient acquisition: SRL, root diameter, tissue density, branching intensity, total phenolic concentration, and N concentration. Traits were measured on clusters of the finest two orders of roots so that roots serving similar functions were compared among species. Traits were examined in 25 coexisting woody species common to mesic temperate forests of North America, which included species forming the two most widespread symbiotic associations (arbuscular mycorrhizas (AM) and ectomycorrhizas (EM)) and spanned a broad range of phylogeny, including both angiosperms and gymnosperms. We aimed to answer the following fundamental questions: Which root traits are the most variable among these species? Which traits, if any, co-vary? To what extent do ecological factors, phylogenetic factors or perhaps a combination of the two contribute to root trait variation? We examined the hypotheses that: morphological and architectural traits are most variable among co-existing species, assuming that variation in N and phenolics is associated with adaptations to environments of different resource availability or different life-forms (e.g. annual versus perennial); species with roots of high SRL, thin diameter and low tissue density are associated with root chemistry of low phenolic and high N concentration; and root trait differences are greatest between deciduous angiosperms and evergreen gymnosperms as a consequence of ecological and phylogenetic differences.

Materials and Methods

Species selection and field site

The species sampled included 25 woody species common to mesic forests of North America (Table 1). The selected species represented a broad range of taxa of the most common woody species in these forests and included 20 trees and five shrubs. Eleven of these species predominantly form EM; 12 predominantly form AM; and two regularly form both EM and AM (Harley & Harley, 1987; Wang & Qiu, 2006; L. H. Comas, pers. obs.).

Samples were collected from two forest stands located *c.* 2.25 km apart in Penn State Stone Valley Experimental Forest (Barree Township, Huntingdon County, Pennsylvania, USA) in two different sub-basins along northwest-facing slopes of two different tributaries. Stands were *c.* 65 yr old, even-aged and predominately hardwoods. Soil in one stand was an Ernest silt loam (fine-loamy, mixed, superactive, mesic Aquic Fragiudult) and that in the other was a Newark silt loam (fine-silty, mixed, active, nonacid, mesic Aeric Fluvaquent). All measurements were taken from individuals of each species that were either in the upper canopy or in open canopy. Trunk diameters of sampled trees ranged from 7 to 75 cm at breast height. All shrubs were taller than 1 m. Trunk diameter was not a significant covariate in root trait differences among species (P ranged from 0.36 to 0.94).

A sampling transect was oriented parallel to the stream through each stand. A random point on each transect was picked as the central point of that site and the nearest trees of each species to that site center along the transect were selected. All 25 species were sampled from both stands except for *Lindera benzoin* (L.) Blume, which was only found in one stand.

Table 1 The 25 species examined in this study along with the abbreviations used in this paper, common name, and family membership.

Species	Abbreviation	Common name
Acer negundo L.	AN	Box elder
Acer saccharum March.	AS	Sugar maple
Betula lenta L.	BL	Sweet birch
Carya glabra (Mill.) Sweet	CG	Pignut hickory
Carya ovata (Mill.) K. Koch	CO	Shagbark hickory
Cercis canadensis L.	CC	Redbud
Crataegus L. spp.	CP	Hawthorn
Fagus grandifolia Ehrh.	FG	American beech
Fraxinus americana L.	FA	White ash
Hamamelis virginiana L.	HV	Witch hazel
Juglans nigra L.	JN	Black walnut
Lindera benzoin (L.) Blume	LB	Spice bush
Liriodendron tulipifera L.	LT	Tulip tree
Pinus pungens Lamb.	PP	Mountain pine
Pinus strobus L.	PA	White pine
Pinus virginiana Mill.	PV	Virginia pine
Populus grandidentata Michx.	*PG*	Bigtooth aspen
Prunus serotina Ehrh.	PS	Black cherry
Quercus alba L.	QA	White oak
Quercus rubra L.	QR	Red oak
Rhus typhina L.	RT	Staghorn sumac
Sassafras albidum (Nutt.) Nees	SA	Sassafras
Tilia americana L.	TA	American basswood
Tsuga canadensis (L.) Carrière	TC	Eastern hemlock
Ulmus rubra Muhl.	UR	Slippery elm

The most common type of mycorrhiza (Myc) formed by each is listed (AM, arbuscular mycor reviews (Harley & Harley, 1987; Wang & Qiu, 2006). When information was not available in t observations.

Root collection and measurement

Three root samples of each species were collected from independent plants (three plants each sampled once) over 6 wk in June and July 1999. Because one stand was larger than the other, two plants were sampled at the larger stand and one plant at the smaller stand. Six of the 75 samples collected were omitted from analysis because roots in those samples had many dry sections that hindered trait quantification. Two additional samples were too small to allow chemical analysis but yielded samples for morphological analyses.

Roots were excavated from the top 20 cm of soil and were traced back to the trunk for species identification. Up to six plants of different species were sampled each day until all species were sampled once. Fine nonwoody roots were left attached to large-diameter woody roots (0.5–1 cm), sprayed with deionized water, and kept on ice in a cooler until they could be cleaned. Roots were washed with tap water. The two terminal orders of roots were collected together as a cluster and placed in distilled water. Healthy first and second terminal orders of roots typically have a fully intact cortex and mycorrhizal formations and display primary development, compatible with activity for nutrient acquisition (Guo *et al.*, 2008; Valenzuela-Estrada *et al.*, 2008; L. H. Comas, unpublished data). Thus, we collected both first- and second-order roots together. One pool of roots collected from each plant was considered a single sample and subdivided for different analyses.

Subsamples for morphological and architectural measurements were imaged with a desktop scanner. Roots were scanned in grayscale at 450 dpi with a filter of 1.0 mm^2 and an automatic threshold (brightness) method appropriate for each (automatic methods specific for pale, normal or dark roots) (Bouma *et al.*, 2000). Root samples were then dried and weighed. The mass of mycorrhizal fungi was included in the weight of the mycorrhizal roots. WinRhizo software (Regent Instruments, Inc., Quebec, Canada) generated tip counts, length, average diameter, and volume of roots in each image. Branching intensity was calculated from the number of root tips divided by the total root length. This simple measure could be used to quantify branching intensity because only the two most terminal branches of roots were analyzed. Specific root length was calculated from root length divided by mass, and tissue density from mass divide by turgid tissue volume.

Subsamples used for phenolic and N analysis were immediately freeze-dried after cleaning and then ground using a mortar and pestle. The stele that could not be ground was cut into 1 mm or smaller pieces with scissors. Ground tissue was stored at 4°C. Total phenolic concentrations were determined by

Table 2 Descriptive statistics for six root traits characterizing the root architecture (branching intensity), morphology (specific root length (SRL), root diameter and root tissue density), and chemistry (total phenolic concentration per unit dry weight (TA_M) and percentage of nitrogen per unit root dry weight (N_M)) of the 25 woody species measured in this study

Root trait	Abbreviation	Units	Minimum	Maximum	Mean	Median	CV
Branching intensity	–	cm^{-1}	0.71	5.33	2.79	2.88	0.42
Length per dry mass	SRL	$m\ g^{-1}$	10.9	115.1	46.3	38.7	0.57
Average root diameter	–	cm	0.022	0.090	0.045	0.043	0.29
Root tissue density	–	$g\ cm^{-3}$	0.065	0.368	0.180	0.165	0.37
Total phenolic concentration	TA_M	$mg\ g^{-1}$	0.010	0.152	0.052	0.045	0.58
Nitrogen concentration (%)	Root N_M	$g\ g^{-1}$	1.00	3.12	1.55	1.48	0.25

The minimum and maximum values, mean, median, and coefficient of variation (CV) are given for each trait.

quantifying tannic acid concentration from acetone extraction (30 min extraction at 4°C) with the Folin–Ciocalteau assay (Waterman & Mole, 1994). Per cent N concentration was determined with an elemental analyzer (EA 1108 CHNS-O; Fisons Instruments, Mt. Pleasant, NJ, USA).

Statistical analyses

All traits were tested for normality with the Komogorov–Smirnov test across species (SAS Institute, Inc., Cary, NC, USA). All traits except branching intensity were log-transformed to correct departures from normality. Multivariate normality was assessed with the Mardia test (SAS Institute). The multivariate standard error of skewness was 0.28 and kurtosis 0.55 when all traits were included in the analysis, and indicated no serious departure from multivariate normality. General correlations between traits were examined with Pearson correlation coefficients. Correlations were considered statistically significant if $P \leq 0.05$. Correlations among root traits indicated that no trait was collinear with another ($R < 0.90$ for all). Principal component analysis was completed with PROC PRINCOMP performed on correlations among traits (SAS Institute). Eigenvalues > 1 were considered significant (Tabachnik & Fidell, 1996). Eigenvalues > 0.95 were considered marginally significant. Species were ordinated by their score on each principal component. Observations of root traits were grouped by EM gymnosperms, EM angiosperms, and AM angiosperms with stepwise discriminant function analysis performed to identify traits that maximized the centroid distances of these groups. Trait variances were calculated for these three groups and found to be homogeneous. Standardized discriminant coefficients for each independent variable are partial correlation coefficients (partial r^2) expressing the unique contribution of each variable. A jackknifed (leave-one-out) classification following discriminant analysis was used to cross-validate group separations and verify that no one case overly influenced the analysis (SPSS, Chicago, IL, USA). Discriminant functions were considered significant at $P \leq 0.05$.

A consistency index (CI) was determined from linear parsimony of SRL mapped onto phylogeny of the 25 species, treating SRL as an unordered character (Fitch optimization) (MacClade 4.06; Sinauer Associates, Sunderland, MA, USA). The SRL of internal nodes was calculated with SRL as a continuous character with squared-change state reconstruction (MacClade 4.06).

Analyses of single trait evolution, especially of continuous characters, need to be interpreted carefully because these analyses can be sensitive to unbalanced phylogenetic sampling (Oakley & Cunningham, 2000). The 25 species sampled in this study are phylogenetically broad and include at least one member of the most common clades with woody plants found in temperate northeastern US forests, and thus are appropriate for assessing the broad phylogenetic distribution of traits in this community, although 25 species is a relatively small sample size with which to determine broad evolutionary patterns. Among the assumptions made in the phylogenetic analyses of single traits, erroneous assumptions of the likelihood of gains and losses in traits lead to erroneous conclusions but are more of an issue for complex traits that are more easily lost than gained (Cunningham *et al.*, 1998). Specific root length is a trait for which the assumption of equal chances of losses and gains is likely to be valid because anatomical changes needed to cause differences in SRL can be attributed to additional cell layers in root cortical or stele regions (Eissenstat & Achor, 1999) that can be controlled by single genes regulating development (Shi & Stanley, 2006). Ultimately, trends mapped along phylogenetic lines can be compared to trends in fossil records to identify spurious conclusions.

Results

General patterns of root trait variation

There was nearly an order of magnitude of variation in most of the six traits used to assess differences in root morphology, architecture, biochemical defenses and metabolic activity in the 25 plant species (Table 2). Branching intensity, SRL, and phenolic concentration had the greatest proportional variation among observations (CV = 0.42, 0.57 and 0.58, respectively). Variation in traits was generally much higher among species

Table 3 Pearson's correlation matrix for six root traits characterizing the root architecture, morphology, defense chemistry, and metabolic activity of the woody species measured in this study

	Branching intensity	SRL	Diameter	Tissue density	TA_M	Root N_M
Branching intensity	1.00					
SRL	**0.55**	1.00				
Diameter	**–0.63**	**–0.79**	1.00			
Tissue density	0.09	**–0.39**	**–0.26**	1.00		
TA_M	0.21	0.07	–0.10	0.05	1.00	
Root N_M	**–0.29**	–0.07	**0.30**	**–0.35**	–0.13	1.00

SRL, specific root length per unit dry mass; TA_M, total phenolic concentration per unit dry weight; N_M, percentage of nitrogen per unit root dry weight.
The number of observations (*n*) varied from 67 to 69 for each trait. Significant correlations ($P < 0.05$) appear in bold type.

than within species across both sites (Supporting Information Table S1), indicating that variation was not confounded across sites and that across-species variation accounted for the majority of the total variation measured.

Among traits, the strongest general correlations occurred among morphological and architectural traits (Table 3). In particular, branching intensity increased as SRL increased ($r = 0.55$, $P < 0.05$) and decreased as root diameters increased ($r = -0.63$, $P < 0.05$). Within morphological traits, high SRL was more strongly correlated with small root diameter ($r = -0.79$, $P < 0.05$) than low tissue density ($r = -0.39$, $P < 0.05$). Root phenolic and N concentrations were notably not strongly correlated with any of the other root traits measured (Table 3). Weak correlations of increased N concentration associated with an increase in root diameter and with a decrease in tissue density ($r = 0.30$ and -0.35, respectively, $P < 0.05$) resulted in no general correlation between N concentration and SRL ($r = -0.07$, $P = 0.60$).

Principal components analysis (PCA) identified three significant axes of variation (Table 4). The first and strongest principal component was mainly associated with species differences in branching intensity, SRL and root diameter. The second principal component was associated primarily with tissue density and a portion of its variation that covaried with SRL and N concentration (high SRL and N concentration associated with low tissue density). The third principal component was almost entirely associated with phenolic concentration. Examination of this third component indicated few distinguishable patterns among different types of species (data not shown). Species with the highest values for this component included *L. benzoin*, *Juglans nigra*, *Hamamelis virginiana*, *Rhus typhina*, *Pinus strobus*, *Tsuga canadensis*, *Quercus rubra*, *Fagus grandifolia* and *Carya glabra*.

Table 4 Results of principal component analysis on the six root traits from 25 woody species measured in this study, including the proportion of variation explained (top section) and loading scores of traits on each component (bottom section)

Component	Eigenvalue	Difference	Proportion	Cumulative
1	2.44	0.94	0.41	0.41
2	1.50	0.52	0.25	0.66
3	0.98	0.33	0.16	0.82
4	0.65	0.21	0.11	0.93
5	0.44	0.44	0.07	1.00
6	0.00	0.00	1.00	

Variable	Component 1	Component 2	Component 3
Branching intensity	**0.53**	–0.01	0.07
SRL	**0.51**	**0.46**	–0.07
Root diameter	**–0.59**	0.01	0.21
Tissue density	0.08	**–0.73**	–0.20
Root N_M	–0.28	**0.50**	0.03
TA_M	0.16	–0.14	**0.95**

Components with eigenvalues greater than 1 are typically considered significant (Tabachnik & Fidell, 1996). Component 3 was considered significant because of its proximity to 1. Variable loading scores with the greatest load on each component appear in bold. Species are listed in Table 1.
SRL, specific root length per unit dry mass; TA_M, total phenolic concentration per unit dry weight; N_M, percentage of nitrogen per unit root dry weight.

Root trait variation among groups of species

Ordination of species by PCA suggested that root traits of EM gymnosperms, EM angiosperms, and AM angiosperms grouped separately (data not shown). Classifying observations of traits by these three groups, using stepwise discriminant function analysis, indicated that only two traits, branching intensity and SRL, were needed to distinguish the three groups along two orthogonal axes (partial $r^2 = 0.53$ and 0.44 for branching intensity and SRL, respectively, $P < 0.05$; Fig. 1). Jackknife (leave-one-out) analysis verified the strength of group separations, indicating that individual observations of EM gymnosperms, EM angiosperms, and AM angiosperms were correctly predicted between 67 and 73% of the time. For most species, replicates were generally relatively close in proximity in discriminant function analysis ordinations,

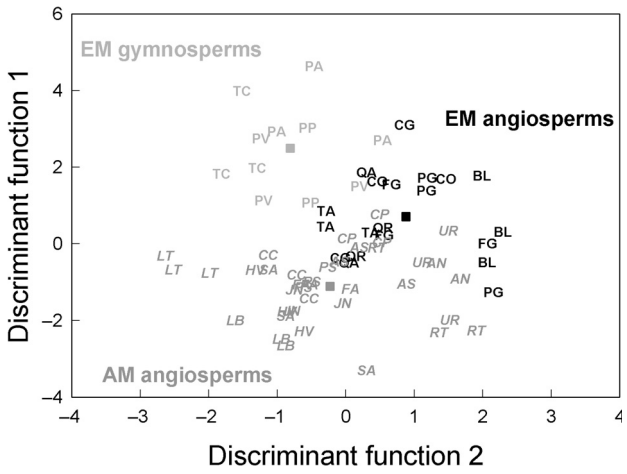

Fig. 1 Ordination of three groups of species, namely ectomycorrhizal (EM) gymnosperms (light gray), EM angiosperms (black), and arbuscular mycorrhizal (AM) angiosperms (dark gray italic), in root trait space assessed by stepwise discriminant analysis. Solid squares mark the centroid of each group. Stepwise discriminant analysis was conducted on six root traits from 25 species classified into the three groups. Of the six variables describing root architecture, morphology and chemistry, only two (specific root length and root branching intensity) were needed to separate the three groups of species ($P = 0.05$). Branching intensity primarily contributed to the first discriminant function. Specific root length primarily contributed to the second discriminant function. Plant species and their abbreviations are listed in Table 1. A complete list of the root traits analyzed is given in Table 2.

suggesting that measurements of these traits were repeatable and that broad variation in SRL and branching intensity was mainly attributable to among-species differences (Fig. 1), which was consistent with the smaller CV in traits within-species as compared with among-species (Table S1).

Along the first discriminant axis, branching intensity was primarily responsible for separating root systems between EM- and AM-forming species, with EM species having higher branching intensity (high range in discriminant function 1) than that of deciduous AM species ($P < 0.05$; Fig. 1). An overlapping zone between AM- and EM-forming angiosperms along discriminant function 1 was found among species with the greatest SRL (AM and EM groups overlapped in function 1 at the high range of discriminant function 2). This overlap mainly included AM angiosperms, such as *Ulmus rubra*, *Prunus serotina*, *Crataegus* spp., *Acer saccharum* and *Acer negundo*, and EM angiosperms, such as *Q. rubra*, *Quercus alba*, *Tilia americana* and *Populus grandidentata*.

Along the second discriminant functional axis, SRL was primarily responsible for species separation with a small contribution of branching intensity ($P < 0.05$). The spread of species along this axis showed wide variation within both EM and AM groups (Fig. 1). Within EM-forming species, discriminant function 2 separated gymnosperms and angiosperms, with gymnosperms having lower SRL than EM-forming angiosperms ($P < 0.05$), although AM-forming angiosperms had lower SRL than these gymnosperms. The broad variation in SRL within AM and EM species appeared to sort along phylogenetic lines, compelling the exploration of this pattern more directly.

Variation in SRL associated with phylogeny

Examination of SRL with phylogenetic analyses indicated that variation in SRL was moderately parsimonious with phylogenetic relationships among the 25 species (CI = 0.44; Fig. 2). A strong phylogenetic signal was prevented as a consequence of within-clade variation in SRL, especially among eurosid clades (i.e. Fagaceae, Rosaceae and Aceraceae, and their relatives).

Discussion

Traits related to root architecture, morphology, and defense chemistry had the greatest variation among co-existing mature woody species in this community. Variation in branching intensity separated AM and EM plants, corresponding to structural differences in the forms of these symbioses for nutrient acquisition. Large variation in SRL within AM and EM groups may imply diverse strategies for soil resource acquisition among these co-existing species. Total phenolic concentration also widely varied among these species, with potentially important implications for differences in tissue turnover among them. However, variation in total phenolic concentration was large in phylogenetically closely related species without corresponding to any other traits or patterns. Assays of total phenolics not only include very different classes of phenolics used for tissue defense but also may include compounds that serve functions other than defense. More specific assays of root phenolics may yield clearer patterns of sources of species variation, although assays of specific phenolics may be difficult to compare among diverse species. There was comparatively little variation in root N concentration among these species, suggesting either that various root strategies for resource acquisition have similar metabolic costs or that additional factors need to be considered before making comparisons among these species.

Correlations among measured traits indicated a negative relationship between SRL and root diameter among these species and no relationship between SRL and root tissue density as previously found in a more restricted comparison of tree species (Comas *et al.*, 2002; Comas & Eissenstat, 2004). The association of SRL and root diameter among these species could imply that differences in SRL among woody plants are associated with differences in mycorrhizal strategies if species with thin roots of high SRL ultimately have less cortical area to form mycorrhizal symbioses (Guo *et al.*, 2008) or are otherwise less dependent on mycorrhizas (Graham *et al.*, 1991). The positive correlation between root branching intensity and

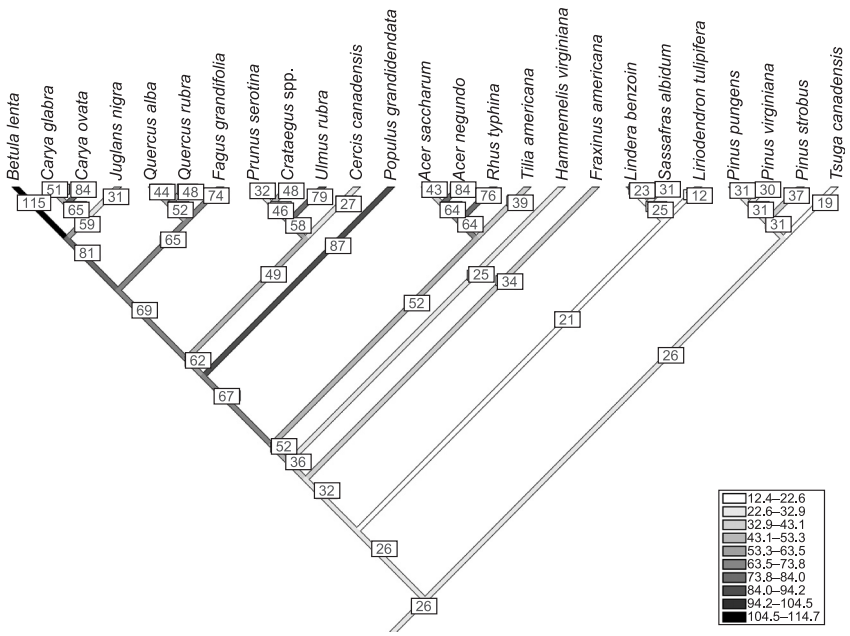

Fig. 2 Specific root length (SRL; m g^{-1}; given in boxes) mapped onto phylogenetic relationships among the 25 species examined in this study. The SRL of terminal branches is the average SRL for each species. The SRL of internal nodes was calculated using species averages with squared-change state reconstruction for a continuous character (MacClade 4.06; Sinauer Associates, Sunderland, MA, USA). The consistency index (CI), determined by linear parsimony of SRL as an unordered character (Fitch optimization), indicated moderate consistency between SRL and species phylogeny (CI = 0.44), with SRL increasing in groups of species with more species radiations but with greater variation within these groups. Species phylogeny is arranged so that taxonomic groups with more species radiations are to the left and those with fewer radiations are to the right. Shading of branches indicates average SRL for each branch, with darker shading indicating longer SRL and lighter shading indicating shorter SRL. The length of branches does not indicate the proximity of relationships among species.

SRL (and the associated negative correlation between branching intensity and diameter) may imply potential constraints on the length of root segments with small diameter and high SRL, and warrant further investigation. Incidentally, correlations with branching intensity among these species were not an artefact of sampling because samples included only first and second root orders, as opposed to including all roots under an arbitrary diameter size, which would result in more branching levels being sampled in species with small root diameters and high SRL.

The lack of an association between SRL and N concentration across these species contrasted with associations found elsewhere, such as between SRL and N concentration in woody seedlings (Reich *et al.*, 1998a) and many general correlations found in leaves between specific leaf area (SLA) and N concentration (Reich *et al.*, 1999). It is possible that roots of species with greater SRL here could have proportionally greater stele to cortex area (e.g. Guo *et al.*, 2008), and thus a greater proportion of anatomical area with low N concentration. The lack of correlation here is consistent with other studies of mature trees (Comas & Eissenstat, 2004; Withington *et al.*, 2006). This suggests another possible explanation: that traits such as N concentration that are linked to tissue metabolism may also be linked to whole-plant physiology, which may be more similar among mature trees in a common community (Comas & Eissenstat, 2004). Additionally, both root and leaf N concentrations here were less variable than SRL (CV = 0.25, $n = 67$ for both root and leaf N), although EM angiosperms among these species tended to have proportionally lower root N relative to their leaf N while EM gymnosperms and AM angiosperms had similar root and leaf N concentrations (see Fig. S1).

Ectomycorrhizal plants exhibited higher branching intensity than AM plants. On EM plants, fine root clusters with more root tips and branching could allow for greater colonization by EM fungi, as EM primarily form on short root tips (Brundrett, 2002). Strikingly, quantification of branching intensity here could alone distinguish between AM and EM species in *c.* 70% of observations. Overlap in branching intensity among AM and EM angiosperms, such as AM *U. rubra*, *P. serotina*, *Crataegus* spp., *A. saccharum* and *A. negundo* and EM *Q. rubra*, *Q. alba*, *T. americana* and *P. grandidentata*, occurred among species that have the capacity to form both AM and EM symbioses or have close relatives that can form both AM and EM symbioses (Harley & Harley, 1987; Dickie *et al.*, 2001; Egerton-Warburton & Allen, 2001; Wang & Qiu, 2006).

Mycorrhizal fungi have been seen to affect root branching intensity in both AM- and EM-forming species when they are exposed to mycorrhizal fungi (Balestrini *et al.*, 1992; Karabaghli-Degron *et al.*, 1998). However, when we compared field root architecture from this study for seven AM and EM species

with architecture from a glasshouse study where these seedlings showed no evidence of mycorrhizal colonization (Comas *et al.*, 2002; L. H. Comas, unpublished data), we found that regression suggests little plasticity of architecture to colonization with greater among-species than within-species differences in architecture (the 95% confidence limit of the *y*-intercept and slope from the regression overlapped with the 1 : 1 reference line of branching intensity among species assessed in both of these studies; data not shown). EM plants have a dimorphic root system (a combination of short and long roots) and high branching densities irrespective of colonization (Wilcox, 1968; Brundrett *et al.*, 1990), suggesting that these plants have experienced positive selection pressure by EM fungi for more tips and increased branching among first- and second-order roots through enhanced plant nutrient status. By contrast, AM plants may not experience similar selection pressures because AM colonization occurs in cortical tissue along the root axis. The overlap in root branching intensity among species having the capacity to form both AM and EM or having relatives that form both AM and EM may indicate intermediate phenotypes, possibly shaped by selection pressures acting on AM and EM, or may be attributable to plant species originally forming AM that are transitioning to form EM. More research comparing the root branching intensities of colonized and uncolonized roots would provide further evidence by which to evaluate these hypotheses.

Root morphological traits such as SRL also have important implications for soil exploration. We found large variation in SRL within both EM and AM groups and interesting patterns in this variation. Across-species variation in SRL mapped moderately closely along phylogenetic lineages, with basal clades having shorter SRL than distal clades, supporting the idea that coarse roots of low SRL are basal features, as suggested by the fossil record (Baylis, 1972; Li & Edwards, 1995; Brundrett, 2002). A strong phylogenetic signal was prevented as a consequence of SRL variation within subclades, especially in more distal clades. However, variation within clades also revealed interesting patterns. In several cases, within-clade variation was consistent with known congeneric species differences in growth rate, including variation between *Q. rubra* and *Q. alba*, *C. glabra* and *Carya ovata*, and *A. negundo* and *A. saccharum* (Comas *et al.*, 2002; Comas & Eissenstat, 2004). Additionally, *Cercis canadensis* and *Juglans nigra*, two plant species in distal lineages with especially low SRL, stood out as potentially having different strategies for acquiring soil nutrients. *Cercis canadensis* is a leguminous species forming symbioses with *Rhizobium* bacteria for nitrogen fixation, and thus may not face similar selection pressures to optimize root morphology as nonleguminous species. *Juglans nigra* produces allelopathic compounds in its roots (Ponder & Tadros, 1985), and thus may not face the same selection pressures to optimize root morphology as nonallelopathic species. Interestingly, because basal angiosperms had low SRL (lower than gymnosperms), SRL did not separate gymnosperms from angiosperms. Low SRL of gymnosperms and basal angiosperms may indicate high dependences of both on mycorrhizas. Alternatively, basal angiosperms may use other strategies to compete belowground, such as increasing root length density per plant or allelopathy.

Resource deployment in fine roots and variation of root traits among species could have many potential implications for their functional ecology and species-level effects on ecosystem-level processes such as carbon and mineral nutrient cycling. In addition to known differences in rhizosphere effects between AM and EM plants, such as EM plants having higher net N mineralization and phosphatase activity (Phillips & Fahey, 2006), differences in root architecture between AM and EM plants may be linked to differences in how these plants explore the soil profile, with ecosystem-level consequences. EM roots with short terminal branches may not directly explore as large a volume of soil through root growth as AM roots with long terminal branches. However, the fungal mycelium in EM associations extends further into the soil and persists longer during the season than that in AM associations (Querejeta *et al.*, 2007), which may allow EM plants to invest in proportionally fewer fine roots and lower turnover of these roots. Differences in SRL within EM and AM plants may also be associated with different nutrient acquisition strategies, linked to differences in root length density per plant or dependence on mycorrhizas, which could have different implications for carbon and nutrient budgets.

This is a first attempt to sort root trait diversity among woody plants by examining community-level patterns in a relatively diverse northeastern American temperate forest. There was large variation in fine root architecture and morphology within a single community, suggesting that diverse strategies for acquiring resources exist within this community. As root traits can be plastic in response to environmental factors, caution should be exercised in extrapolating these species-level patterns to other communities. The patterns found here suggest the hypotheses that selection pressures associated with different types of mycorrhiza may shape root morphology and architecture for soil resource foraging, and that selection pressures shaping SRL may shift with species diversification. Trait patterns found among species in this community, however, need to be compared against patterns in other ecosystems and biomes before broad generalizations can be made.

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Supporting Information

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

Fig. S1 Root nitrogen (N) concentration plotted against leaf N concentration for samples obtained from the same arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) plants.

Table S1 Trait averages and their coefficient of variation (CV) within and among species, with CV within species appearing in parentheses next to the trait average for that species

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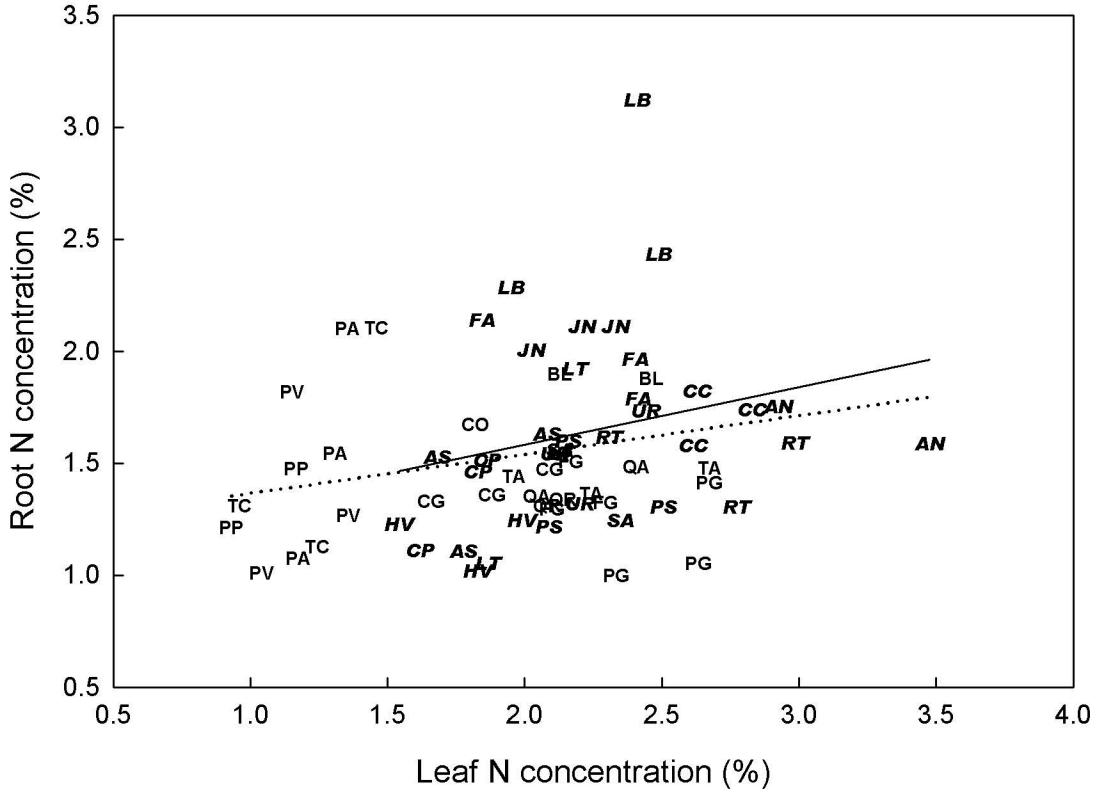
Supporting Information

Fig. S1 Root nitrogen (N) concentration plotted against leaf N concentration sampled from the same plants in AM (bold font) and EM (normal font) plants (leaf data from Comas, 2001). Among all 25 species, root N concentration was correlated with leaf N concentration (r = 0.23, $P < 0.05$; dotted line). Correlation between root and leaf N was marginally significant among AM species (r = 0.25, P = 0.07; solid line) but not significant among EM species due to lower root N in EM angiosperms relative to their leaf N (r = 0.00001, P = 0.50). The coefficient of variation of leaf N concentration was similar to that of root N concentration (CV = 0.25, n = 67).


Root N concentration (%)
Leaf N concentration (%)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
LB
LB
LB
FA
JN JN
JN
FA
BL
LT
CC
FA
UR
CC
AN
CO
AS
RT
CC
RT
AN
PA TC
PV
PA
PP
AS
CP
TA
CG
QA
TA
PG
TC
PP
PV
CG
CG
HV
HV
PS
SA
PS
RT
PA
TC
PV
CP
AS
LT
HV
PG
PG

Supporting Information Table S1 Trait averages and their coefficient of variation (CV) within and among species with CV within species appearing in parentheses next to trait average for that species. Among-species CV was calculated from among-species means and standard deviations and does not include variation within species. Trait abbreviations are explained in Table 2.

Species	Branching intensity	SRL	Diameter	Tissue density	TA_M	Root N_M
Acer negundo	3.18 (0.01)	83.8 (0.1)	0.036 (0.012)	0.117 (0.133)	0.025 (0.314)	1.67 (0.07)
Acer saccharum	2.65 (0.07)	48.0 (0.3)	0.042 (0.033)	0.158 (0.176)	0.033 (0.619)	1.42 (0.20)
Betula lenta	4.29 (0.22)	109.7 (0.0)	0.027 (0.271)	0.184 (0.548)	0.062 (0.215)	1.89 (0.01)
Carya glabra	3.89 (0.33)	50.8 (0.2)	0.033 (0.218)	0.259 (0.367)	0.071 (0.228)	1.38 (0.06)
Carya ovata	4.61 -	83.8 -	0.032 -	0.145 -	0.050 -	1.67 -
Cercis canadensis	1.85 (0.10)	27.1 (0.1)	0.055 (0.062)	0.159 (0.077)	0.039 (0.104)	1.71 (0.07)
Crataegus spp.	3.17 (0.10)	47.9 (0.1)	0.033 (0.135)	0.245 (0.114)	0.059 (0.446)	1.36 (0.16)
Fagus grandifolia	1.93 (0.09)	33.5 (0.2)	0.048 (0.110)	0.173 (0.332)	0.021 (0.204)	1.96 (0.09)
Fraxinus americana	3.75 (0.12)	73.6 (0.4)	0.034 (0.072)	0.165 (0.252)	0.061 (0.229)	1.37 (0.08)
Hammemelis virginiana	1.33 (0.22)	25.2 (0.2)	0.048 (0.153)	0.226 (0.212)	0.103 (0.640)	1.16 (0.11)
Juglans nigra	1.60 (0.17)	31.0 (0.2)	0.049 (0.072)	0.177 (0.206)	0.092 (0.464)	2.08 (0.03)
Lindera benzoin	0.74 (0.06)	22.6 (0.2)	0.077 (0.167)	0.100 (0.126)	0.036 (0.169)	2.61 (0.17)
Liriodendron tulipifera	1.14 (0.10)	12.4 (0.2)	0.077 (0.091)	0.180 (0.210)	0.022 (0.336)	1.51 (0.29)
Pinus pungens	3.73 (0.22)	31.4 (0.0)	0.048 (0.000)	0.176 (0.006)	0.041 (0.074)	1.34 (0.14)
Pinus strobus	4.69 (0.14)	37.2 (0.4)	0.051 (0.163)	0.145 (0.325)	0.069 (0.171)	1.57 (0.33)
Pinus virginiana	3.47 (0.16)	29.9 (0.4)	0.040 (0.025)	0.295 (0.381)	0.033 (0.531)	1.36 (0.30)
Populus grandidendata	3.99 (0.17)	87.0 (0.3)	0.034 (0.152)	0.136 (0.087)	0.032 (0.557)	1.15 (0.20)
Prunus serotina	2.00 (0.13)	31.9 (0.1)	0.041 (0.104)	0.244 (0.218)	0.055 (0.288)	1.37 (0.14)
Quercus alba	3.30 (0.33)	44.1 (0.1)	0.043 (0.072)	0.157 (0.039)	0.054 (0.107)	1.42 (0.07)
Quercus rubra	3.01 (0.15)	47.9 (0.1)	0.040 (0.060)	0.166 (0.012)	0.083 (0.252)	1.32 (0.01)
Rhus typhina	2.53 (0.17)	75.9 (0.3)	0.045 (0.050)	0.090 (0.283)	0.082 (0.550)	1.51 (0.11)
Sassafras albidum	1.07 (0.14)	35.5 (0.4)	0.048 (0.345)	0.180 (0.302)	0.021 (0.802)	1.40 (0.16)
Tilia americana	3.07 (0.05)	39.3 (0.2)	0.044 (0.050)	0.172 (0.156)	0.042 (0.236)	1.42 (0.04)
Tsuga canadensis	3.52 (0.22)	19.3 (0.1)	0.050 (0.071)	0.272 (0.274)	0.072 (0.072)	1.51 (0.34)
Ulmus rubra	3.12 (0.22)	79.1 (0.1)	0.037 (0.097)	0.122 (0.200)	0.031 (0.356)	1.53 (0.14)
CV among species:	0.40	0.5	0.270	0.297	0.456	0.20