

The distribution of below-ground traits is explained by intrinsic species differences and intraspecific plasticity in response to root neighbours

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Summary

1. Large variation in tree root architecture and morphology has been reported for temperate forest communities. However, it is not clear whether this variation represents adaptation of species to specific soil properties, alternative resource acquisition strategies among co-occurring species, or canalized traits without a strong impact on the success of individuals in different environments. Here, our goal was to test these alternative hypotheses and quantify how community-aggregated and intraspecific root trait variations are explained by biotic versus abiotic mechanisms in a temperate deciduous forest.

2. We conducted our study in an *Acer-Fagus*-dominated forest in north-east Ohio, USA. Using molecular barcoding techniques, we identified 738 root systems belonging to 14 tree species. We measured seven functional root traits related to root architecture and morphology at the species and community-aggregated levels.

3. Although we found significant relationships between soil resource gradients and root trait distributions, intrinsic differences between coexisting species were more important than soil factors in explaining the distribution of root traits in the community. Additionally, root trait variation at the species level was also influenced by the presence of other species within cores.

4. Community-aggregated variation was more influenced by the combination of species present than soil properties in each sample, suggesting that biotic interactions play an important role in controlling community root trait distribution.

5. *Synthesis.* We propose that root trait differentiation between coexisting species is the result of inherent differences between species and plasticity-mediated responses to neighbours. Hence, the large variation in root traits reported in temperate forest seems to reflect alternative evolutionary pathways that allow individuals to exploit distinct niches in relatively close proximity.

Key-words: community structure, community-aggregated traits, plant–soil (below-ground) interaction, root diameter, root plasticity, root traits, specific root length

Introduction

Fine roots are crucial to ecosystem processes, consuming up to 40% of all carbon fixed by photosynthesis in terrestrial ecosystems and supplying most of the nutrients and water to above-ground tissues (Litton, Raich & Ryan 2007). Although below-ground niche partitioning is regarded as a fundamental factor explaining plant species coexistence (Hutchings, John & Wiljesinghe 2003; Silvertown 2004), few *in situ* ecological studies have evaluated the role of

below-ground components structuring plant communities (Casper, Schenk & Jackson 2003) and only a handful of recent studies have resolved species-specific root patterns in mixed forest communities (Comas & Eissenstat 2009; Espeleta, West & Donovan 2009; Meinen, Hertel & Leuschner 2009; Holdaway *et al.* 2011). As a consequence, fine root trait variation among and within species in natural conditions is still largely unknown, hindering the scaling of root traits from individual plants and species to the community level. This lack of understanding about below-ground community dynamics also precludes inferences about the role of fine root traits in plant community assembly (Westoby & Wright 2006).

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A central goal in plant ecology is to understand the factors associated with morphological trait variation in plant populations and communities (Mooney & Dunn 1970; Grime 2006). Variation in traits among species is often larger than trait variation within species, resulting in phylogenetically determined, canalized trait differences. At the population level within species, traits can be affected by intraspecific genetic variation, but an individual may also vary its phenotype to cope with environmental heterogeneity (i.e. plasticity *sensu* Valladeres, Gianoli & Gómez 2007). The magnitude of both interspecific and intraspecific trait variation in natural communities has been rarely quantified (Lake & Ostling 2009). At the community level, community-aggregated traits (i.e. trait values averaged across individuals or species in a community, weighted by abundance; Shipley 2010) reflect major environmental constraints on plant traits. They are also important starting points for predicting how the accumulation of alternative trait sets from competing species can maximize community-wide efficiency in the uptake of limiting resources (Westoby & Wright 2006). Deterministic (niche) models predict that plant community assembly is the result of interaction between environmental filtering effects and interspecific competition, with both forces defining the realized niche of the species (Weiher, Clarke & Keddy 1998; Cornwell & Ackerly 2009). Neutral theory assumes ecological equivalence among individuals and a stochastic partitioning of resources, implying that traits could have a random distribution with respect to both the environment and community composition (Hubbell 2001). However, even under neutral dynamics, if species differ in their traits, community composition can determine community-aggregated trait values.

Previous research, focused mostly on leaf and stem traits, has found that deterministic (i.e. competitive and filtering mechanisms) rather than stochastic processes are involved in the landscape-scale distribution of community traits (Ackerly & Cornwell 2007; Kraft, Valencia & Ackerly 2008). Abiotic factors, such as moisture availability, were correlated with above-ground community-aggregated trait shifts along environmental gradients, and this change was correlated with species turnover (Cornwell & Ackerly 2009). These results suggest that filtering effects are more important than competitive interactions or intraspecific trait plasticity. The distribution of below-ground plant traits with respect to environmental gradients remains mostly uninvestigated (Schenk 2006). Although there are clearly canalized differences between plant lineages in root traits (Baylis 1975; Brundrett 2002), roots are also highly plastic and interactive organs, capable of responding physiologically and morphologically to both biotic and abiotic factors (Hodge 2004; Wijesinghe, John & Hutchings 2005). In fact, they often express traits, particularly among woody plants, not necessarily correlated with leaf trait syndromes (Comas & Eissenstat 2004; Withington *et al.* 2006; McCormack *et al.* 2012), which has limited our understanding of the factors affecting root trait trade-offs.

Relatively few hypotheses have been formally proposed to link root traits and soil properties at the community level. Several previous studies have examined species-specific root

traits within natural communities (Comas & Eissenstat 2004, 2009; Espeleta, West & Donovan 2009; Alvarez-Uria & Körner 2011; Holdaway *et al.* 2011). However, the ability to test for environmental filtering versus competitive interactions has been hampered by focusing on only a few species or lack of information about soil properties or neighbour species where roots were found. A few studies have shown alternative root morphological patterns between communities established in contrasting environmental conditions (Taub & Goldberg 1996; Holdaway *et al.* 2011), indicating strong filtering effects shaping the distribution of community-aggregated root traits at large scales. Nevertheless, community responses to environmental gradients may be mediated by population-level root trait plasticity. Trees can modify root morphology as a response to nutrient availability and moisture gradients (Coleman 2007; Richter *et al.* 2007; Kazda & Schmid 2009). Finally, the existence of highly contrasting root traits among co-occurring species has also been demonstrated (Roumet, Urcelay & Diaz 2006; Comas & Eissenstat 2009; Meinen, Hertel & Leuschner 2009), suggesting some degree of niche segregation within communities.

In addition to environmental effects, the distribution of below-ground traits could also be influenced by the outcome of interactions among species. Although the ability of roots to react to interspecific competition has been recorded under controlled conditions (Cahill & Casper 2000; Gersani 2001; Bartelheimer, Steinlein & Beyschlag 2006), the importance of this interaction has not been quantified under natural conditions. It has been predicted that competitive displacement would limit similarity among coexisting species, producing a nonrandom, even spacing of traits among species in direct competition (Stubbs & Wilson 2004). This pattern could be generated through competitive exclusion from a site, or trait plasticity in response to competition (Cornwell & Ackerly 2009). Thus, it is still not clear whether soil properties limit the range of morphological variation among plants that co-occur in the same habitat or whether competitive interactions lead coexisting species to exploit distinct niches, enhancing morphological differentiation between them (Fitter 1991).

To distinguish between these scenarios, we had three primary objectives.

- 1 Examine intraspecific root trait variation in multiple tree species under a range of natural conditions and determine whether the observed variation was related to soil properties and/or the identity of root neighbours.
- 2 Determine whether, at a given location, the distribution of root traits among species is most often consistent with environmental filtering, competitive displacement or neutral assembly. If environmental filtering due to soil properties is the main driver in root trait assembly, we would expect species to show alternative root morphologies associated with distinct ranges of soil properties. A potential correlation between root traits and soil properties could arise through either species turnover or intraspecific root plasticity in response to soil properties. Under neutral assembly, species distributions would be unrelated to soil properties, and root

traits could only be correlated with soil properties through plasticity.

- Finally, we wanted to quantify the response of community-aggregated traits to soil properties and species composition. This response was then interpreted in the context of results from our other objectives regarding community assembly and intraspecific plasticity.

Materials and methods

STUDY SITE AND DATA COLLECTION

We conducted the study in Jennings Woods, a 30-ha forest in north-east Ohio, USA, which was subjected to selective timber extraction during the 1920–30s and has been unmanaged for ~65 years. The forest includes well-drained areas of upland forest, poorly drained bottomland forest and riparian forest within 50 m of the West Branch of the Mahoning River. Soils in the area are predominantly Alfisols (Typic Hapludalf) with Inceptisols (Fluvaquentic Endoaquepts) more common along riverbeds. Typical soil profiles are comprised of an 11- to 16-cm-deep A horizon, 12- to 16-cm-deep B horizon and 18- to 27-cm-deep C horizon with no significant differences in horizon thickness across forest types. *Acer saccharum*, *Fagus grandifolia* and *Acer rubrum* are the dominant tree species, comprising nearly 50% of the forest tree cover. Other common canopy tree species are *Quercus rubra*, *Carya ovata*, *C. cordifolia* and *Prunus serotina*, whereas the understorey is dominated by *Carpinus caroliniana*, *Hamelis virginiana* and *Lindenia benzoin*.

Samples were collected from 130 plots (2.5 × 5 m) arranged to maximize the detection of ecotones and ensure good spatial coverage of the different environments. One large soil core (10 cm diameter × 15 cm deep) from each plot was extracted in late August 2008. Most cores did not have a significant O layer, so we sampled the uppermost mineral layer, removing all litter material before sampling. Cores were soaked for at least 12 h in deionized water and sieved through a # 20 mesh with a water jet. All fine root segments were carefully separated from the soil, cleaned with deionized water and classified as live or dead based on appearance. Live roots were divided into very fine (< 1 mm in diameter) and fine (1–3 mm in diameter) categories. Very fine root clusters < 10 cm in length that generally included 4 orders of roots were selected for species identification and trait measurements. On average, 7 ± 2 root system clusters were extracted from each core and represented 21.6 ± 10.8% of the total very fine roots from each core (See Appendix S1 for more details).

Each root sample was scanned using a high-resolution flatbed scanner (800 DPI resolution, 256-level gray-scale, TIFF format; Epson Scanner Perfection V700 Photo, USA) and WinRhizo software (2007 Pro version, Instrument Regent, Quebec, Canada, details in Appendix S1). After scanning, all samples were oven-dried for 48 h at 65 °C and then weighed. We analysed seven different root traits: four morphological traits typically associated with trade-offs between organ cost per potential return in trees [specific root length (SRL), specific root surface area (SRA), root tissue density (RTD) and average diameter; Comas, Bouma & Eissenstat 2002]; and three traits associated with plant root foraging strategies [fractal dimension (D, Eshel 1998, Wang *et al.* 2009), specific root tip abundance (SRTA, Hertel, Leuschner & Hölschner 2003; Meinen, Hertel & Leuschner 2009) and average link length (Pregitzer 2002; Dupuy *et al.* 2010)]. On average, nonwoody segments comprised 65–80% of the total length of each root sample (Table S2). Further details of root analysis procedures are included in Appendix S1.

Root identification started with the construction of a molecular barcoding library (Appendix S1). Reference barcodes were generated by amplifying the plastid gene *trnL* and the noncoding spacer region *trnH-psbA* (Kress *et al.* 2005), from leaves of 33 canopy species and some abundant understorey species such as *Hammamelis virginiana*, *Podophyllum peltatum*, *Symplocarpus foetidus* and *Thelypteris noveboracensis*. We combined RFLP profiles of both genes using GelCompar II software (version 4.5, Applied Maths BVBA, Belgium) to generate a library with enough resolution to distinguish most species, at least to the genus level. RFLP patterns obtained from unknown roots (Appendix S1) were compared with reference patterns using a cluster analysis based on Jaccard distances. In cases of doubt among congeneric species, identity was inferred only when one of the possible species was near the sampling location. Following this procedure, we were able to identify 738 root samples, including 14 canopy tree species present in at least three cores. This pool was subsequently used for estimations of trait plasticity for each species and community-aggregated root traits in each core. In addition to the root samples analysed as described above, the rest of the root biomass in each core was also carefully removed from the soil and all biomass > 1 mm in diameter was dried and weighed to determine total fine root biomass per sample. Biomass by species was estimated using the proportional abundance of root samples identified by barcoding in each core.

Soil data were obtained from 5- to 10-cm-deep soil cores taken from each plot in May 2008. Samples from each plot were pooled. Fourteen soil variables were quantified, including % clay, % silt, % sand, % moisture, pH, total soil carbon (C) and nitrogen (N), soil C:N ratio, particulate organic matter C (POM C, %), particulate organic matter N (POM N, %), POM C: POM N ratio, and organic, inorganic and total bicarbonate-extractable phosphorus (Table S1). To reduce the number of soil variables, we performed principal components analysis (PCA) for subsets of variables that showed $r > 0.85$ in pairwise correlation analysis. One subset included seven variables (soil C, soil N, POM C, POM N, organic P, inorganic P and total P). From this PCA, we selected the first 2 PCA axes; the first PCA axis explained 79% of the variance, similarly loaded for all variables and was interpreted as a nutrient availability index (PCA_fertility). The second axis explained 10% of the variance, loaded more heavily on P variables (PCA_phosphorus) and thus was used as a surrogate of additional variation in P not explained by the first axis. A second PCA was performed for texture variables (% clay, % silt and % sand); in this case, we selected the first PCA (PCA_texture), which summarized 98% of the variance.

STATISTICAL ANALYSIS

Root plasticity under natural conditions

Our first objective was to examine how variation in root traits was related to soil properties, whether these relationships were similar across tree species and whether traits of some species were also affected by the identity of neighbouring roots. Initially, we used an ANCOVA to determine whether the direction and/or intensity of plasticity varied among species. For this analysis, we calculated trait averages for each species separately for each soil core. We used the Akaike Information Criterion (AIC) in a forward selection procedure in which a new predictor is added to the model as long as AIC would be decreased by including the additional predictor and the *P*-value for the factor is < 0.05 (Burnham & Anderson 2002). First, this procedure was used to identify the soil factors that created the best fitting

linear model predicting root traits, with tree species identity included as an intercept effect without species*soil factor interactions. Then, the model with selected soil variables was compared with a model that incorporated interactions between species identity and the previously selected soil variables. The direct effect of soil variables was interpreted as a common level of plasticity across all species after correction (via the intercept effect) for inherent root trait differences between species, whereas the interaction between species and soil properties was interpreted as variation between species in the strength and directionality of intraspecific root plasticity.

The importance of species interactions on root plasticity in each species was then evaluated in separate redundancy analyses (RDA) for each species (See Appendix S1 in Supporting Information). Briefly, we created a matrix of root trait response variables that included all morphological trait values for a species for each core where the species was present. Additionally, for each species, two predictor variable matrices were created. One predictor variable matrix was a species presence–absence matrix for the other 13 most common species identified in the soil cores, which we refer to as the ‘root community matrix’. Additionally, a soil condition matrix was created using soil variables described above. To remove the effects of differing scales, the soil factor and response matrices were standardized to have a mean equal to zero and standard deviation equal to one. Parsimonious subsets of competitor species and soil factors as explanatory variables were then selected following the procedure of Blanchet, Legendre & Borcard (2008). First, a global analysis was performed testing the importance of each factor in the matrix separately (ter Braak 1986). If the global analysis was significant, a step-wise RDA was performed, adding variables in order of declining explanatory power and using both the significance of the added variable and adjusted R-squared as stopping criteria (Blanchet, Legendre & Borcard 2008). Variance partitioning between soil abiotic factors and root neighbours was performed as described in Peres-Neto *et al.* (2006).

Root community assembly processes

For the second objective, we initially tested whether environmental filtering and species turnover explained root trait shifts along soil gradients by regression of species mean root traits against species mean locations in the soil gradients. Mean trait values for each species were calculated using all root samples identified in the study. Soil variables used to represent soil gradients in this analysis were the ones shown to have an important effect on root traits in the analysis described above. The mean location along a soil gradient for a given species was estimated as the weighted average soil gradient value across all soil cores occupied by the species, with weights provided by the species relative abundance of roots in each core. Species tolerance was defined as the range of soil properties where a single species was observed (Ackerly & Cornwell 2007).

We also assessed the relative signal of environmental filtering and competitive trait displacement in trait distributions within each soil core. Because environmental filtering should limit the types of traits able to establish in the community (Cornwell, Schwikl & Ackerly 2006), we predicted that under environmental filtering, the observed range and variance of traits in each core should be significantly smaller than the null expectation obtained under random community assembly. Likewise, competitive displacement will affect the spacing of the traits, producing more evenly distributed traits among coexisting species than expected by chance (Pacala & Tilman 1994). Therefore, we would expect lower nearest neighbour standard deviation

(NNSD) and lower kurtosis values than those estimated from a null distribution. As the NNSD and kurtosis tests do not differentiate between competitive trait displacement due to plasticity shifts and species exclusion, we tested for patterns of root co-occurrence consistent with checkerboard patterns (Gotelli 2000). This pattern would indicate that species exclude each other from the same soil volume and that competitive trait displacement is due to patterns of species presences and absences rather than trait plasticity. The range, variance, NNSD, kurtosis and checkerboard tests are described in the supplementary text (Appendix S1).

Community-aggregated traits

Community-aggregated traits were calculated by averaging the values for all root samples obtained from a given soil core, whereas community-aggregated biomass was the sum of all fine root biomass (i.e. all roots < 1 mm in a core). The effect of soil properties and the root community matrix on community-aggregated root traits and biomass was estimated using RDA as described above, except that the response variable matrix was comprised of community-aggregated traits instead of individual species traits. All species were included in the root community matrix.

Results

ROOT TRAIT PLASTICITY AND SPECIES DIFFERENCES

Morphological parameters differed significantly between species (Tables 1 and 2). Our community encompassed an array of fine root morphologies ranging from species with relatively scarce branches and thick roots (*Liriodendron tulipifera*) to highly branched systems with thin roots (*Ulmus* sp.) within ranges of morphological variation present in similar forest types (Table 1; Comas & Einssestat 2009; Meinen, Hertel & Leuschner 2009). Species exhibited a relatively broad tolerance (as defined by Ackerly & Cornwell 2007) to varying soil properties, with only a few genera, such as *Ulmus* and *Liriodendron*, restricted to relatively narrow ranges of soil properties.

Intrinsic trait differences between species (i.e. intercept differences reflecting a degree of canalization) were the most important factor explaining fine root trait variation at the individual plant scale (ANCOVA test, $P < 0.001$, Table 2 and Figs 1a and S1). In addition to the species intercept effect, PCA_fertility was also significantly related to variation in all traits except fractal dimension (related to per cent moisture) and average link length (related to POM C) (AIC-based factor selection; *post hoc* ANCOVA test $P < 0.05$, Table 2 and Figs 1a and S1). With the exceptions of SRL and average link length, the interactions between species and the relevant soil properties were not significant (Table 2), indicating that intraspecific plasticity in response to soil properties was similar across species, despite their broad differences in morphology, mycorrhizal association and phylogenetic background (Figs 1a and S1).

Trait variation within each species was also analysed separately to incorporate the possible effects of neighbouring roots of other species due to competitive displacement. In addition

Table 1. Root trait mean (standard deviation) for eight variables measured and determined for 14 canopy tree species in a temperate deciduous forest. Variables included specific root length (SRL), specific root surface area (SRA), root tissue density (RTD), fractal dimension (Fractal), specific root tip abundance (SRTA), average root diameter and average link length for each species. Values were estimated from fine root clusters (N) which included the four most terminal root orders. Diameter values were limited to first-order roots. Mycorrhizal types: arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal (ECM)

Species	N	Mycorrhizal type	Root traits						
			SRL (m g^{-1})	SRA ($\text{cm}^2 \text{g}^{-1}$)	RTD (g cm^{-3})	Diameter (mm)	SRTA (tips mg^{-1})	Fractal	Length (cm)
<i>Acer rubrum</i>	159	AMF	26.55 (14.18)	460.76 (234.23)	0.20 (0.11)	0.40 (0.08)	3.32 (2.04)	1.48 (0.06)	0.26 (0.11)
<i>Acer saccharum</i>	219	AMF	30.47 (16.54)	528.55 (272.58)	0.18 (0.12)	0.41 (0.06)	3.71 (2.20)	1.46 (0.05)	0.29 (0.10)
<i>Betula alleghaniensis</i>	5	ECM	26.49 (15.23)	432.15 (213.13)	0.22 (0.16)	0.37 (0.04)	2.80 (1.73)	1.54 (0.06)	0.28 (0.24)
<i>Carpinus caroliniana</i>	16	ECM	20.89 (17.32)	381.23 (267.17)	0.21 (0.07)	0.39 (0.05)	2.78 (2.50)	1.51 (0.05)	0.22 (0.05)
<i>Carya cordifolia</i>	20	ECM	23.88 (20.24)	441.28 (372.94)	0.22 (0.11)	0.38 (0.05)	4.06 (3.97)	1.52 (0.04)	0.19 (0.04)
<i>Carya ovata</i>	99	ECM	20.73 (10.79)	366.39 (168.16)	0.24 (0.12)	0.36 (0.06)	2.88 (1.74)	1.50 (0.07)	0.22 (0.08)
<i>Fagus grandifolia</i>	65	ECM	17.97 (11.73)	317.25 (165.78)	0.28 (0.16)	0.37 (0.07)	2.09 (1.35)	1.47 (0.05)	0.28 (0.09)
<i>Fraxinus americana</i>	45	AMF	19.07 (11.12)	394.08 (207.72)	0.18 (0.08)	0.48 (0.08)	1.95 (1.11)	1.49 (0.06)	0.35 (0.11)
<i>Fraxinus nigra</i>	27	AMF	26.99 (15.19)	478.30 (260.47)	0.21 (0.14)	0.45 (0.09)	1.67(0.73)	1.43 (0.06)	0.51 (0.21)
<i>Liriodendron tulipifera</i>	7	AMF	10.46 (3.89)	306.31 (115.69)	0.16 (0.08)	0.73 (0.05)	0.68 (0.30)	1.50 (0.04)	0.74 (0.41)
<i>Platanus occidentalis</i>	5	AMF	27.91 (20.21)	516.87 (316.93)	0.17 (0.08)	0.40 (0.10)	2.75 (2.24)	1.45 (0.03)	0.34 (0.06)
<i>Prunus serotina</i>	30	AMF	19.60 (9.81)	381.05 (162.00)	0.20 (0.09)	0.52 (0.10)	1.57 (0.89)	1.43 (0.06)	0.55 (0.20)
<i>Quercus rubra</i>	23	ECM	21.17 (10.81)	355.45 (164.00)	0.26 (0.15)	0.36 (0.07)	2.50 (1.25)	1.46 (0.04)	0.24 (0.07)
<i>Ulmus sp.</i>	18	AMF	49.28 (31.46)	868.58 (521.32)	0.11 (0.06)	0.37(0.05)	6.35 (4.91)	1.48 (0.04)	0.25 (0.08)
Entire community	738		25.86(15.48)	459.86 (257.86)	0.20 (0.12)	0.40 (0.09)	3.44 (2.17)	1.47 (0.06)	0.30 (0.15)

Table 2. ANCOVA results describing effects of species identity and fine root trait plasticity along soil gradients ($n = 234$)

Factor	Biomass	SRL	SRA	Diameter	RTD	SRTA	Fractal	Length
Soil factor [†]	***	**	**	**	**	***	**	*
Species (intercept)	NS	***	***	***	***	***	***	***
Soil factor*Species	NS	*	NS	NS	NS	NS	NS	*
Model adjusted R^2	0.38	0.24	0.19	0.30	0.12	0.32	0.25	0.50

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[†]Biomass, SRL, SRA, Diameter, RTD and SRTA were significantly related to PCA_fertility. Fractal was related to % moisture and Length was related to Proportional POM Carbon.

to the expected influence of soil properties on trait values (affecting 6 of 14 species), the presence of other species also affected root trait variation in 8 of the 14 species analysed ($P < 0.05$). In fact, some species such as *Quercus rubra* were influenced more by biotic interactions than soil factors, suggesting that coexisting species can differentially affect the level of trait plasticity among neighbours (Figs 2a and S3). Results were species specific, with variance explained by either neighbouring roots or soil factors ranging from 0 to 24% (Figs 2a and S3).

ROOT COMMUNITY ASSEMBLY PROCESSES

Mean trait values by species were not related to soil properties for average root diameter, RTD, fractal dimension and average link length (black dots in Figs 1a and S1, Table 3). Trait values for SRL, SRA and SRTA were significantly asso-

ciated with the soil properties gradient; however, these trends were highly influenced by *Ulmus sp.* (standardized residual value > 1 for all cases). Once *Ulmus sp.* was removed, regressions were not significant ($P = 0.15, 0.34, 0.23$, respectively). This suggests that species were not constrained by soil properties with the possible exception of *Ulmus sp.*, which presented the highest SRL and SRTA and lowest RTD of all species. Furthermore, most of our cores showed higher trait variance and larger trait ranges than expected from a null model (Table 4), indicating no support for the hypothesis of environmental filtering shaping the community structure.

However, across the entire forest, the distribution of root traits within cores showed lower kurtosis values than the null expectation for all traits ($P < 0.01$). Six of seven traits had lower mean NNSD values than the null model expectation ($P < 0.1$). These results suggest that, on average, co-occurring species traits were more evenly distributed than expected by

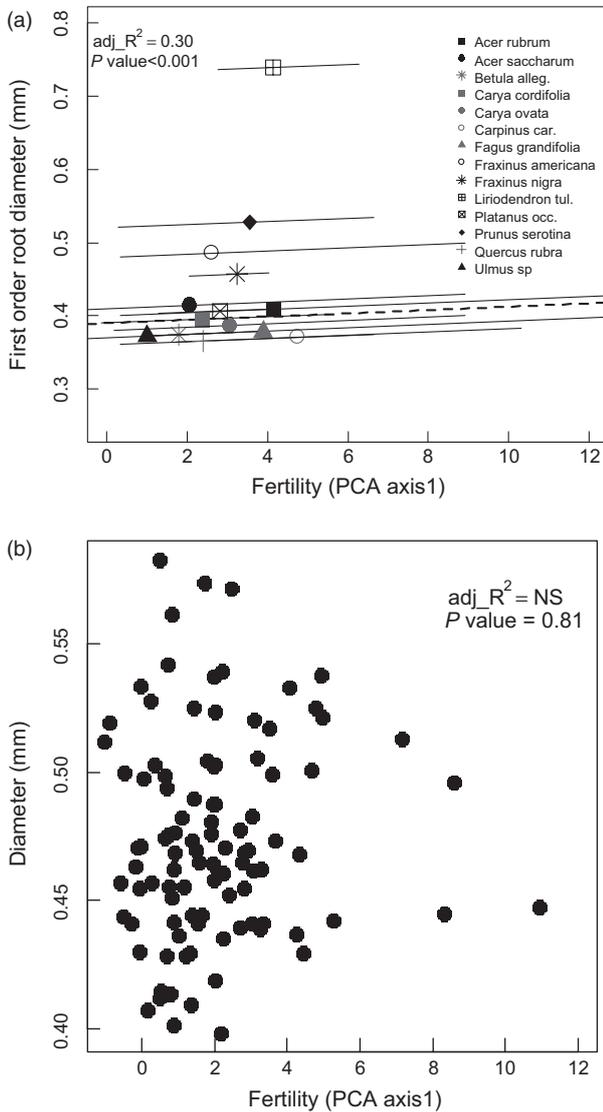


Fig. 1. Relationship between first-order root diameter and soil nutrient availability (PCA_fertility) for tree canopy species in a temperate deciduous forest. (a) Symbols indicate species-weighted mean diameter values for the 14 most abundant species. Lines represent results of the ANCOVA model (Table 2), where the species intercept effect and overall relationship with soil fertility were significant ($P < 0.05$). The soil fertility*species interaction was not significant (i.e. the slopes for all species are not significantly different). Solid lines show the range of soil fertilities for each species, and the dashed line shows the effect of soil fertility across all roots sampled. (b) Dots indicate average root diameter for each core (community-aggregated level) along the fertility gradient.

chance. Additionally, we found no evidence of checkerboard patterns in the root community ($P > 0.1$). The C-score and CHECKER index P -values were 0.6 and 0.73, respectively, indicating low species segregation (Gotelli 2000). Our results support the idea that the shifts associated with competitive trait displacement within cores are a product of root plasticity in response to neighbouring root species identity rather than species segregation. The results also indicate that the distribution of root traits within cores is influenced not only soil properties, but also the presence or absence of other species.

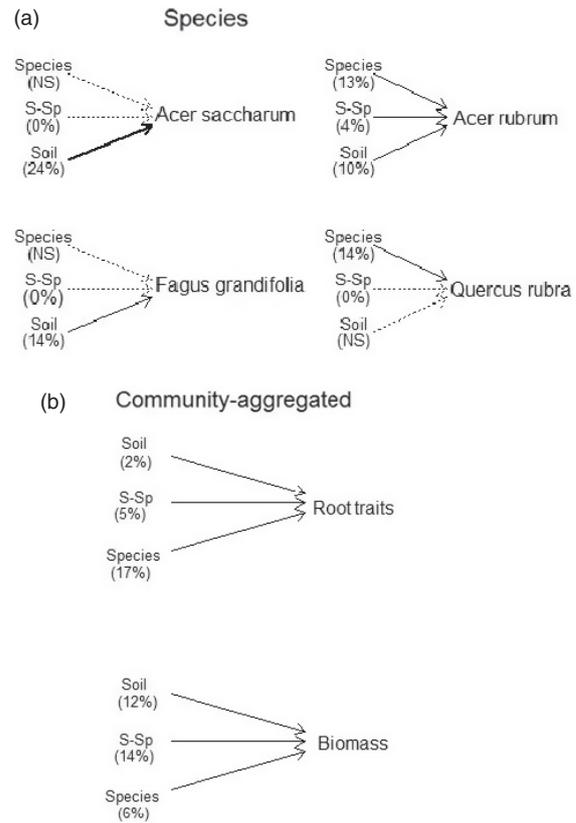


Fig. 2. Multivariate variance partitioning for seven root traits in individual species and the tree community. Arrows summarize the amount of variation in all root traits examined in this study that can be accounted for by soil properties (Soil), root community matrix (Species) or the overlap (S-Sp) between these factors. Arrow thickness represents strength of relationship with dashed arrows expressing no significant relationship. (a) Partial contribution of root community composition and soil properties to the plasticity of root traits in the four most abundant tree species at Jennings Woods. Results for other species are shown in Fig. S3. (b) Community-aggregated analysis included root biomass and root traits separately.

Table 3. Least square mean fit using species means and soil fertility scores (Fertility PCA axis) for each species in Jennings Woods ($n = 14$)

Trait	Intersection	Slope	R^2	R^2 (adj)	F	$P (>F)$
SRL	23.53	4.05	0.43	0.38	8.92	0.01
SRA	430.08	56.26	0.35	0.30	6.47	0.03
Diameter	0.63	-0.03	0.17	0.10	2.41	0.15
RTD	0.21	0.00	0.00	0.00	0.02	0.89
Fractal	1.48	0.00	0.01	0.00	0.06	0.80
SRTA	2.73	0.71	0.38	0.33	7.43	0.02
Length	0.34	-0.05	0.15	0.07	2.04	0.18

COMMUNITY-AGGREGATED TRAITS

Community-aggregated traits showed shifts along soil condition gradients; although in many cases, the patterns were relatively weak [adjusted R^2 ranged from 0 to 0.11 (Fig. S2) compared with 0.12 to 0.50 for trait variation at the species level (Table 2)]. The community showed a tendency to

Table 4. Testing the effects of environmental filtering and competitive trait displacement on the distribution of root traits at the soil core level. Values indicate the proportion of individual soil cores that deviate significantly from a null model[‡]. Study-wide *P*-values determined using Stouffer's method (Whitlock 2005) to combine *P*-values from all plots. **P* < 0.05; †*P* < 0.1

Hypothesis Trait	Soil filtering [‡]		Competitive trait displacement [§]	
	Variance	Range	NNSD [¶]	Kurtosis
SRL	0.25	0.41	0.70*	0.81*
SRA	0.23	0.42	0.71†	0.80*
Average Diameter	0.18	0.44	0.58†	0.83*
RTD	0.24	0.46	0.60†	0.83*
Fractal	0.23	0.37	0.73*	0.79*
SRTA	0.24	0.42	0.65†	0.81*
Length	0.23	0.37	0.57	0.84*

[‡]In the case of filtering effects, the hypothesis tested for each core was that the species showed lower dispersion than expected in a random assemblage. Values for variance and range represent the proportion of cores that had significantly lower variance and range values in comparison with 999 random associations (see Appendix S1). Stouffer's method was used to combine results across all cores to give a study-wide *P*-value.

[§]For competitive displacement, the hypothesis tested was that species in cores showed a more even trait distribution than expected by chance. Values for NNSD and Kurtosis represent the proportion of cores that had a significantly more even distribution of traits compared with 999 random associations (see Appendix S1). Stouffer's method was used to combine results across all cores to give a study-wide *P*-value.

[¶]NNSD: nearest neighbour standard deviation. It was estimated by sorting species trait values within the core and considering 'neighbour distance' to be the difference between two adjacent members in the sorted list.

increase biomass and decrease root tip abundance along the soil fertility gradient (Fig. S2), as well as decrease SRL and SRA and increase RTD with per cent moisture, whereas soil factors had no effect on root diameter, fractal dimension or average link length (Figs 1b and S2). Moreover, in pooled analyses of root morphological traits by RDA, variation was mainly influenced by the combination of species present in each core (17% of variation), with little evidence of soil properties (2%) affecting core root trait distribution (Fig. 2b).

Discussion

The primary goal of this study was to understand, under natural conditions, the effect of both soil nutrient availability and species interactions on the variation in root traits at both the species and community levels. Although we did find that under natural conditions fine roots can be plastic with respect to environmental conditions (Table 2), we also found a level of canalization that constrains the range of trait variation within species (Tables 1 and 2), which is consistent with previous descriptions of root morphologies for trees in temperate areas (Brundrett, Murase & Kendrick 1989; Comas & Einssestat 2009; Meinen, Hertel & Leuschner 2009). Remarkably, we found that root trait plasticity in the field was not only a

response to soil properties, but also a reaction to the presence of neighbour species (Table 4, Figs 2 and S3). Moreover, community-aggregated traits were primarily structured by species composition (Fig. 2b), because species with intrinsically different root traits were distributed across the entire soil gradient and responded to other species within a soil core (Table 4). Our results also highlight the utility of using molecular methods to measure root neighbourhood composition under natural conditions (Jones 2011).

PLASTICITY-MEDIATED COMPETITIVE TRAIT DISPLACEMENT BELOW-GROUND

Our results support the hypothesis that alternative root morphologies function as a mechanism of coexistence at relatively small spatial scales. This hypothesis is supported by the lack of evidence of a relationship between species root traits and soil properties, the parallel trends in most root trait values with respect to soil gradients and the lack of evidence for competitive exclusion. The responses of some species to the identities of root neighbours, and the even distribution of traits within cores, suggest that some degree of plasticity in root systems may help plants to avoid trait overlap. In contrast, previous studies have linked different root morphologies as adaptations to alternative soil properties (Taub & Goldberg 1996; Neatrou, Jones & Golladay 2007; Espeleta, West & Donovan 2009) or alternative mycorrhizal associations (Guo *et al.* 2008; Comas & Einssestat 2009). Therefore, contrasting root morphologies have been regarded as adaptations that will segregate species to specialized soil properties, thus predicting a stabilizing coexistence by filtering effects (Fitter 1991; Hutchings, John & Wiljesinghe 2003; Kembel *et al.* 2008). We suggest that the variation in root traits within communities can be explained also as a response to competitive interactions, possibly reducing interspecific competition for resources and maximizing species coexistence (Caldwell, Manwaring & Durham 1996; Bezemer 2010).

The ability of roots to recognize the identity of neighbouring root systems, and in turn modifying allocation and morphology, has been reported previously under controlled conditions (Caldwell, Manwaring & Durham 1996; Gersani 2001; Callaway 2002; Dudley & File 2008), but rarely evaluated against the effect of soil gradients and neighbour interactions in natural conditions. Our results suggest that competitive trait displacement is an important process underlying below-ground species coexistence in the same location. This contradicts previous studies focused on above-ground trends, where species turnover had a strong effect on leaf trait distribution (Kraft, Valencia & Ackerly 2008; Cornwell & Ackerly 2009). This disagreement suggests either that contrasting processes shape the distributions of above- and below-ground community traits (such as greater symmetry of competition below-ground compared with above-ground (Cahill & Casper 2000)) or that there is unmeasured variation in above-ground traits at a scale smaller than what has been considered in previous studies (as suggested by Lake & Ostling 2009). It also implies that diverse fine root morpho-

logies could play an important role in differentiating resource use below-ground, complementing the reported above-ground trait similarity among coexisting species (Kraft, Valencia & Ackerly 2008). Hence, the role that root–root interactions play in regulating species coexistence and structuring plant communities below-ground should be addressed.

Besides direct competition, alternative mechanisms may be controlling root trait distribution. For example, intraspecific trait variation could be related to fine structuring of genotypes within populations (Nicotra 2010). Moreover, substantial changes in root morphology between soil horizons have been observed, especially between the organic forest floor and the underlying mineral soil (Hendrick & Pregitzer 1993). Hence, alternative root morphologies may be segregated vertically, implying that the observed even trait distributions could be the result of avoidance and partitioning by soil horizon. Similarly, although our study included a wide range of soil properties, more extreme variation in soil properties could represent a stronger filter to species and potentially reveal a higher contribution of filtering effects and/or species turnover on the distribution of traits below-ground. Future studies addressing soil filtering or vertical trait distributions could help elucidate the main mechanisms involved in root trait distributions in forest communities.

CONTROLS ON COMMUNITY-AGGREGATED ROOT TRAITS

We found that community-aggregated traits were better explained by species composition than soil properties, although a substantial amount of variation in community-aggregated root traits was left unexplained (Fig. 2b). We also found that alternative root trait syndromes may increase the probability of coexistence among competitors residing in the same location. Our results help explain the lack of correspondence between community-aggregated root characteristics and soil properties observed in other studies (Finér *et al.* 2011; Holdaway *et al.* 2011), as well as the fact that canopy tree root systems largely overlap (Jones 2011). Furthermore, our study highlights the need to expand the description of root traits within and between species in diverse ecosystems to understand how root trait differences can affect competitive interactions. Because community-aggregated root traits (i.e. biomass, SRL, etc.) are a common measurement in ecosystem studies related to below-ground processes such as root mortality, carbon allocation and soil carbon turnover (Litton, Raich & Ryan 2007; Klumpp & Soussana 2009), more detailed studies of interspecific interactions and intraspecific plasticity may be necessary. For example, our study suggests that more detailed descriptions of below-ground species interactions could improve the understanding of diversity effects on below-ground productivity (Cadotte *et al.* 2009; Mommer 2010).

Conclusion

Our study suggests that variation in fine root morphological traits among woody species enhances coexistence at small

spatial scales, but does not reflect specializations to particular soil properties within a community. Community-aggregated trait distributions seem to be influenced by species composition rather than soil properties, with little influence of species turnover on trait shifts along environmental gradients. Although the underlying mechanisms of these processes still need to be quantified, we suggest that the inherent diversity in root morphologies in complex ecosystems contributes to coexistence within tree communities and therefore community stability. Although trait differences among species account for the largest source of root trait variation, the findings that competitive root trait displacement and lack of soil filtering effects are pervasive among coexisting species implies that below-ground interactions play a fundamental role in the structuring of diverse plant communities. More research into the consequences of root morphological variation in plant communities could reveal further examples of niche segregation and better explain species coexistence in diverse ecosystems.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Relationship between soil properties and root traits of the 14 most abundant tree species in Jennings Woods.

Fig. S2. Relationships between soil factors and root traits at the core level in Jennings Woods.

Fig. S3. Variance partitioning and ordinations of the effects of soil properties and the species identities of neighbouring roots on root traits of the 14 most abundant tree species in Jennings Woods.

Table S1. Summary of the variables measured for the description of soil properties in Jennings Woods.

Table S2. Average diameter (standard deviation) and proportional surface area by root order for 14 canopy tree species in a temperate deciduous forest.

Appendix S1. Description of root identification, root trait measurements and statistical analysis.