A PHYLOGENETIC PERSPECTIVE ON FINE ROOT ECOLOGY: ASSESSING THE ROLE OF ROOT EVOLUTION ON FINE ROOT FUNCTIONAL TRAITS AND ECOLOGICAL INTERACTIONS IN WOODY ANGIOSPERMS.

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by

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CHAPTER ONE: INTRODUCTION

Biological processes belowground are an important factor in ecosystem carbon and nutrient cycling, therefore influencing plant productivity and community structure (Rasse et al. 2005). Belowground net primary production is mainly associated with the dynamic of the fine root systems which may comprise as much as 50% of total net primary productivity, thus contributing significantly to the recycling of plant tissue-bound carbon and nutrients (Hanson et al. 2000). It is clear that plant species can adjust their structure and function rapidly to environmental changes. For instance, shifts in root respiration, production and turnover in forest ecosystems have been linked to increases in CO₂, temperature or N availability (Vogt et al. 1996, Atkin et al. 2000). In fact, Giardina et al. (2005), in an extensive review of literature indicated that belowground responses to environmental changes are more dramatic than those aboveground, highlighting the important role that belowground dynamics might have in overall ecosystem responses to projected climatic change. Nonetheless, our knowledge of the response of most species and the mechanisms involved in differential allocation are still not completely understood (Pregitzer 2002).

Despite the recognized importance of the dynamics of fine root systems on ecological processes, the estimation of the effects of roots on biogeochemical cycles is still poorly quantified (Litton et al. 2007). Part of the reason for the lack of knowledge
about the role of fine roots in ecosystem processes is the underestimation of the functional diversity of fine roots as acquisition organs. For instance, initial root studies classified fine root systems based on arbitrary root diameter cutoffs (i.e. < 2 mm in diameter, Vogt et al 1996), assuming that structures with similar diameter had similar physiological functions both among and within species (Jackson et al 1997). Recent investigations, however, demonstrated that fine root systems work as an integral web of structures varying in function depending on their position and interaction with symbiotic components; in addition, such variation is strictly linked to the architecture of the root system, which seems to be highly influenced by species identity (Pregitzer et al 2002, Zobel 2003).

Similarly, the intricate association of roots with soil microbial communities has been oversimplified. For example, the association between roots and fungal communities has been roughly divided depending on the affinity between plants and fungal groups. The association with arbuscular mycorryzal fungi (AMF, Phylum Glomeromycota, Schüßler et al 2001) pre-dates the invasion of plants to terrestrial ecosystems and is probably the most prevalent symbiotic association in nature (Berbree and Taylor 1992, Schüßler 2002). However, plant classifications based on mycorrhizal associations tend to classify AM plants as a single category apart from species forming associations with basidiomycetous and ascomycetous fungi (Read and Perez-Moreno 2003). As a consequence, few studies have explored the diversity of interactions between AMF and
plant species, or the evolutionary process leading to the association between plant host and fungal partners (Brundrett 2002).

Another factor limiting the understanding of root ecology is the lack of a proper theoretical framework based on an appropriate context. Most of our development in root ecology has been substantially based upon the optimality theory introduced by Grime (1977), which has been developed based on observations of aboveground structures. The theory proposes that plants balance growth rates, nutrient concentration and longevity in order to maximize resource acquisition. Then, fast-growing species tend to have relatively high metabolic rates and short life spans, whereas slow-growing species have relatively low metabolic rates and slower turnover rates (Poorter et al. 1990). The theory assumes that environmental variables are the main forces shaping plant structures; thus it is expected that species evolving under similar environmental conditions will develop similar morphological and anatomical traits. In other words roots, independent of phylogeny, should develop similar morphological and physiological arrangements if exposed to the same environmental conditions (Ackerly and Reich 1999). Traits such as link length, tissue density, and diameter will be essentially different between fast and slow growing species (Eissenstat and Yanai 1997); and it is expected that roots and leaves will co-evolve in order to adapt to environmental conditions (Tjoelker et al. 2005).

However, there are fundamental differences between above and belowground environmental conditions that limit the extension of the optimality theory approach. Roots are extremely variable and exposed to a more heterogeneous environment in
comparison to aboveground structures. Moreover, root function is affected by unique interactions such as the associations with soil symbionts (Smith and Read 1997) or neighboring roots (Gersani et al. 2003). Therefore, selective forces on roots could lead to the acquisition of traits totally independent from aboveground structures. This separation in trait selection between above and belowground compartment is particularly likely among perennial woody species. In contrast with annual species, that coordinate physiological and morphological traits at the entire plant level (Tjolker et al. 2005, Freschet et al. 2010), root systems of perennial species seem to be more independent from aboveground structures (Wells and Eissenstat 2003). For instance, Withington et al. (2007) found no relationship between leaf and root lifespan, or between leaf and root tissue density among eleven temperate tree species. Similarly, Hobbie et al. (2010) and McCormack et al. (2012) found poor correspondence between morphological root traits and root lifespan among temperate tree species growing in common gardens, which contrasted with leaf lifespan patterns observed for the same species.

In an attempt to reconcile the apparent discrepancy for the optimality theory between above and belowground tissues, I argue that root traits are better explained as the outcome of alternative evolutionary pathways more associated with functional specialization and symbiotic interactions. After land invasion, plants were possibly facing increasing selection to augment the surface area of their absorptive organs as well as to reinforce the mechanical support of larger aboveground structures. Therefore, it is possible that vascular plants gradually developed in two contrasting belowground
structures: perennial thicker systems dedicated to support, conduction and storage, and ephemeral, thinner, absorbing structures specialized in nutrient uptake and interactions with symbionts (Raven and Edwards 2001). This divergence would drive contrasting patterns between organs: thicker roots then would be highly lignified and isolated from the environment, show long lifespan and scarce branching. In contrast, short-living absorptive roots would increase permeability and interactions with other organisms by forming a specialized tissue (the cortex) around vascular vessels, reduce diameter and branch more profusely in order to increase the soil area explored, and harbor symbiotic microorganisms that might enhance nutrient foraging (Brundrett 2002).

There are relatively few hypotheses explaining further modifications in root structures after belowground organ differentiation. Plants however show continuous modification from the primeval differentiation between absorptive and structural roots. Absorptive roots exhibit large differences in their morphological appearance, which include reduction in the cortex area, higher branch frequency and the development of root hairs. Fitter (1987) introduced a dual model of classification for root architectural patterns. Root systems with sparse nodes (i.e., few branches) have low differentiation in root diameter, minimize intra-plant competition and maximize resource uptake, but decrease transportation capacity. The low differentiation in root structure requires thicker, costlier, slow growing roots, and consequently produces a herringbone branching pattern with few root branches. In contrast, root systems with high frequency of nodes undergo an concomitant decrease in root diameter with branching, which creates a dichotomous
branching pattern. These dichotomous root systems require lower construction cost and can increase total soil exploration without large resource expenditure. This kind of system is supposed to be more efficient at transportation of water and nutrients in comparison with the herringbone system, but it is also more likely to show higher susceptibility to pathogens (Newsmann et al 1995) and lower lifespan (Eissenstat et al 2002).

Baylis (1975) proposed that these contrasting patterns in root morphology reflected a deep phylogenetic divergence during the evolution of root systems. Baylis (1975) and St John (1980), analyzing temperate and tropical species respectively, proposed a division between “magnolioid” and “graminoid” roots. The former consisted of herringbone root systems with coarse fine-roots denuded of root hairs, which apparently depend largely on AMF associations for the acquisition of nutrients. In contrast “graminoid roots” were finer in diameter, highly branched and profusely covered with root hairs; making them more efficient nutrient scavengers. They proposed that

Figure 1. Examples of fine root morphological variation among tree species of North America. The branching patter can be described in terms of the length of paths that traverse the system. These paths are shorter in a dichotomous system (Acer) and longer in a herringbone system (Liriodendron); with multiple intermediate possibilities as exemplified by Quercus. Modified from Pregitzer et al (2002).
magnolioid roots” were restricted to the order Magnoliales, considered by them as the most primitive lineage among angiosperms, whereas the “graminoid roots” were predominantly found among sedges (Cyperaceae) and grasses (Poaceae) “considered as the group (of plants) furthest removed from the ancestral stock” (Baylis 1975).

Although the relationship between root morphology and mycorrhizal dependency was proposed more than three decades ago, the assessment of variation in root traits has only been evaluated recently. Pregitzer et al (2002) pioneered the comparative study of fine root systems in temperate species. Their results demonstrated large variation in root traits among trees with species maintaining similar morphological traits independently of nutrient availability, suggesting important endogenous constraints in root plasticity among woody species. More recent studies (i.e. Comas and Eissenstat 2004, 2009, Guo et al 2009, Holdaway et al 2012) have shown large variation in root traits among coexisting tree species, which seem to indicate alternative morphologies reflecting niche specialization. Moreover, Comas and Eissenstat (2009) and Comas et al (2012) indicated that the distribution of traits among trees is strongly structured phylogenetically, supporting Baylis’ hypothesis and suggesting an intrinsic relationship between mycorrhizal requirements, belowground niche segregation and root functional traits.

Despite the recent advancements in root ecology, there are still fundamental questions regarding the role of root traits in the assembly of tree communities and its implications on niche segregation in forest ecosystems. How extensive is the morphological divergence between basal and more derived angiosperm groups? To what
extent are those trait syndromes coupled with aboveground adaptations, particularly between structural and acquisition organs above and belowground? More importantly, what are the ecological implications of contrasting traits in the coexistence of species in natural ecosystems? Do these trait syndromes evolve as means of segregation over soil resources, resulting in trait associations with particular soil conditions, or do they reflect complementary approaches to the problem of nutrient uptake from soil? I begin to provide answers to these questions in this document in the following chapters:

Chapter 2: Woody angiosperm fine root morphology is phylogenetically structured but chemistry is related to the plant economics spectrum

In this chapter we explored in more detail Baylis’ phylogenetic hypothesis explaining the evolution of root morphological traits in angiosperms (Baylis 1975). We utilized root trait information from 34 woody species growing in two separate common gardens in Midwestern USA. In addition, we expanded Baylis’ approach including chemical traits as additional traits, which have been poorly evaluated from a phylogenetic perspective. We also wanted to investigate the extent of trait integration at the entire plant level. Thus we paired roots with comparable aboveground traits and tested for their possible coordination at the entire plant level, giving us an excellent opportunity to evaluate Grime’s optimality theory in woody plants (Grime 1977).

Chapter 3: Phylogenetically constrained traits in root systems influence arbuscular mycorrhizal colonization in woody angiosperms.
In this chapter we explored how root traits are associated with AMF colonization. Although contrasting root morphologies are usually associated with the level of mycorrhizal dependency of the host (Guo et al 2008, Chen et al 2013) few studies have actually quantified the relationship between root traits and mycorrhizal colonization. Using the same set of species sampled in the previous chapter, we measured mycorrhizal colonization with microscopy and molecular approaches. This study gave us a great opportunity to explore evolutionary changes in the relationship between AMF and woody plants. One important aspect of this study was the quantification of anatomical changes across phylogenetic groups. Previous studies of root anatomy have usually been restricted to commonly studied species (corn, alfalfa, Lotus, etc., Seago and Fernando 2006), and fine root remains are conspicuously sparse in the fossil record (Raven and Edwards 2001). Thus, the inclusion of less studied plant taxa provides fundamental information in the understanding of the steps involved in the reduction of root diameter throughout angiosperm evolution (Comas et al 2012). Moreover, the fact that we sampled trees growing in similar conditions help us to control for environmental conditions or differences in AMF communities, providing an opportunity to compare how species with contrasting root trait syndromes cope with mycorrhizal symbionts.

*Chapter 4: The distribution of belowground traits is explained by intrinsic species differences and intraspecific plasticity in response to root neighbors.*
Here we explored the question: how do these contrasting root patterns among trees explain the distribution of belowground traits at the ecosystem (i.e. forest) scale? As mentioned above, the optimality theory assumes adaptive evolution as the balance of inherent trade-offs between components of a system that will optimize fitness. In the case of fine root systems, contrasting root topologies should reflect the limitation of soil resources (Leuchner et al. 2004, Richter et al. 2007). Thus, it is expected that dichotomous root systems would prevail among fast-growing species adapted to proliferate in nutrient-rich patches. In contrast, herringbone systems would be dominant among slow growing species, adapted to stable conditions, and that invest in high-cost tissues as a way to maintain resources. The filtering effect of soil conditions should be a good predictor of the distribution of root types in a community. Moreover, species should respond to soil heterogeneity either through plasticity (local modification depending on environmental conditions, Valladeres et al. 2007) or specialization to allocation in specific soil patches (Taub and Goldberg 1996, de Kroon et al. 2003). Alternatively, we tested if the combination of traits were the result of the interaction among species present in close proximity or whether trait assemblages were random.

Working in Jennings Woods, an extensively studied ~70 yr old broadleaf forest owned by Kent State University, we tackle these questions sampling >130 soil cores and identifying individual root systems employing molecular techniques. This study is one of the first attempting to quantify functional trait distributions belowground at the species level in natural environments. Moreover, it involved an extensive use of molecular
techniques for root identification at the species level, making this study highly relevant in
current root ecology.

Chapter 5: Aggregated and complimentary: fine root distribution patterns in a temperate
deciduous forest.

The importance of fine root biomass in biogeochemical cycles has led to several
attempts to elucidate factors controlling the distribution and dynamics of fine roots in
forest ecosystems (Hendrick and Pregitzer 1993, Madji et al 2001., Tierney et al 2003,
Ruess et al 2003). However, few generalizations describing the factors controlling root
biomass accrual have been made (Finer et al 2011). In this final chapter we combined
both the interactions of functional traits and species identity with soil resource
heterogeneity in order to explain fine root biomass variation in a temperate broadleaf
forest. From previous studies it is clear that roots tend to cluster in nutrient-rich patches
and that species tend to proliferate roots in areas of nutrient enrichment (Hutchings and
John 2003). Moreover, it is expected that species show differences in their ability to
detect and compete for soil hotspots, suggesting spatial (niche) segregation among
coexisting species, either vertically or horizontally (Campbell et al 1991, Wijesinghe et al
2001). On the other hand, different trait syndromes may reflect alternative resource use,
making possible the complementary use of soil resources even among neighboring roots.
In this case, in addition to the expected positive relationship between soil resources and
biomass, we would expect an additive effect of root diversity on biomass (i.e.
overyielding effect, Cardinale et al 2007).
In this study, we explore the relationship between species diversity, root trait distribution and soil conditions on fine root biomass distribution. The study was based on some of the trait information examined in the previous chapter, plus a new approach integrating biomass, phylogenetic, and trait information to help elucidate the effect of species diversity on biomass accrual. Moreover, we also included the spatial distribution of ecosystems, to determine if the interactions between habitats influence fine root biomass variation at the ecosystem level.

References


Summary: Plant functional traits have revealed tradeoffs related to life-history adaptations, geographical distributions, and ecosystem processes. Although fine roots are essential in plant and ecosystem processes, little is known about their functional trait syndromes or integration at the whole-plant level.

We examined chemical and morphological traits of 34 temperate arbuscular mycorrhizal tree species, representing three main angiosperm clades (super-orders rosids, magnoliids, and asterids). We tested whether fine root traits are structured by phylogeny or by tradeoffs similar to those expected from leaves.

Root traits did not display tradeoffs similar to leaves (e.g., specific root length was not correlated with root N, whereas specific leaf area was correlated with leaf N). Moreover, 75% of belowground traits were phylogenetically structured, as opposed to 28% aboveground. Magnoliids had thicker, less branched roots than asterids or rosids, whereas rosid roots exhibited lower N and higher non acid-hydrolyzable content. Leaf traits did not differ among super-orders. At the whole-tree level, aboveground and belowground chemical traits were correlated, but morphological traits were not.

Our results reveal broad evolutionary trends in belowground root traits. Although we found partial support for the idea of whole-plant trait integration, phylogenetic
information will improve predictions about belowground traits and processes in forests.

**Introduction**

Plant functional traits can be defined as morphological and chemical attributes that significantly influence the fitness of individuals in their ecosystems (Reich *et al.* 2003). Trait-based approaches have provided important insights into the distribution of species across environmental gradients (Ackerly & Cornwell 2007, Engelbrecht *et al.* 2007, Kraft *et al.* 2008), the effect of species on biogeochemical cycles (Aerts & Chapin 2000, Eviner 2004) and the possible responses of communities to global environmental change (Diaz *et al.* 2007, Suding & Goldstein 2008). Comparisons of functional traits have also revealed important axes of variation across species that reflect fundamental biophysical tradeoffs (Reich *et al.* 2003, Westoby & Wright 2006). The best known example is the “leaf economic spectrum” (LES), which predicts a tight correlation between chemical and morphological traits in leaves, ranging from fast-growing but short-lived leaves that maximize CO₂ capture rates by increasing metabolic activity, to leaves with lower metabolic activity and growth rates but higher tissue protection and longer life span (Reich *et al.* 1997, Wright *et al.* 2005). For plant stems, tradeoffs in trait variation between construction cost, growth rate and tolerance to stress have also been described (Chave *et al.* 2009, Poorter *et al.* 2010), suggesting an association with life history strategy similar to that described for leaf tissues. The “plant economics spectrum” (PES) hypothesis broadens this line of reasoning to the whole plant, predicting that there are tradeoffs between functional traits associated with the activity, cost, and longevity of individual plant organs (Freschet *et al.* 2010). In addition, the PES hypothesis proposes
that these tradeoffs are coordinated across the whole plant to correspond with an ecological strategy of fast resource acquisition and growth, or slower resource acquisition but greater conservation and stress tolerance (Reich et al. 2003, Tjoelker et al. 2005, Freschet et al. 2010).

Fine roots (the 2-3 most distal links in a root system, Hishi 2007) exhibit wide interspecific variation in morphological and chemical traits (Guo et al. 2008, Comas & Eissenstat 2009, Holdaway et al. 2011). Such trait diversity might be driven by tradeoffs that reflect different resource uptake strategies across species (Comas & Eissenstat 2004), and possibly whole-plant life-history strategies (Grime et al. 1997, McCormack et al. 2012). Because leaves and fine roots are both short-lived organs specialized for resource capture, it is often assumed that morphological and chemical variation in fine roots follows patterns described for leaves. For example, modeling studies predict that long, thin roots with high specific root length (SRL) maximize nutrient uptake rate per unit area and are less expensive to construct (Eissenstat & Yanai 1997), which parallels the relationship between construction cost and light interception potential described for leaves (Wright & Westoby 1999, Wright et al. 2004). Concomitant increases in N content and root respiration rates may reflect a relationship between root tissue chemistry and metabolic activity similar to that described for aboveground organs (Reich et al. 1997, 2008). Nevertheless, comparative studies of woody plant root morphology and chemistry are rare (Withington et al. 2006), and it therefore remains uncertain whether root trait
tradeoffs are similar to those described by the LES or if they exhibit alternative patterns not related to the morphological and chemical coordination reported in foliar tissues.

The integration of functional traits across plant organs as a consequence of life history adaptations has been long predicted (Grime 1977, Craine 2005), and has found support in several studies, particularly among non-woody species. Freschet et al. (2010), for example, showed strong integration among tissue types (leaf, stem and roots) across 40 species of subarctic flora. Integration among leaf and root functional traits has also been found in several grass or herb-dominated communities (Craine et al. 2002, Birouste et al. 2011, Kembel & Cahill 2011, but see Tjoelker et al. 2005). Woody plants, on the other hand, seem to show less among-tissue integration. Although some degree of integration between woody organs has been previously reported (Reich et al. 1998, 2008), more recent studies indicated independence of tissue functional traits. For instance, Baroloto et al. (2010) and Fortunel et al. (2012) reported poor correlation between foliar and stem traits in tropical ecosystems, whereas Hobbie et al. (2010) reported no correspondence between leaf and root chemical traits for 11 European tree species. Similarly, Withington et al. (2006), Espeleta et al. (2009) and McCormack et al. (2012) found poor correspondence between morphological root traits, root lifespan, and leaf lifespan among temperate tree species grown in common gardens.

An alternative to the PES hypothesis is that the integration of functional traits in woody species is constrained by their evolutionary context. Contrary to the integration of foliar traits, which seem rarely affected by their phylogenetic background (Ackerly &
Reich 1999, Reich 2003, Milla & Reich 2011), evolutionary constraints are frequently cited to explain stem and root functional trait variation (Swenson & Enquist 2007, Comas & Eissenstat 2009). For example, tradeoffs among stem traits associated with mechanical strength, protection from pathogens, and growth rates seem significantly structured by phylogeny (Swenson & Enquist 2007, Chave et al. 2009, Zhang et al. 2011) and independent from leaf traits (Baroloto et al. 2010). In the case of fine roots, it has been hypothesized that their functional traits could be phylogenetically structured as the result of selection by evolutionary processes unique to roots, such as their dependency on mycorrhizal associations (Brundrett 2002). Baylis (1975) and St. John (1980) proposed that basal angiosperm groups exhibited thick roots, and therefore low SRL, as a consequence of higher mycorrhizal dependency. Although Baylis’ hypothesis is frequently mentioned to explain root trait variation across species (Pregitzer et al. 2002, Comas & Eissenstat 2009, Holdaway et al. 2011), few comparative studies have addressed this hypothesis directly.

In this study, we examined above- and belowground traits of temperate tree species representing the major phylogenetic groups of woody angiosperms. Our objectives were twofold. First, we determined the extent to which the coordination between morphological and chemical traits in fine roots are phylogenetically structured or correspond with those expected based on the LES hypothesis. If fine root functional traits follow tradeoffs similar to those observed in foliar tissues, we would expect significant correlations between root morphological and chemical traits. For instance, we
should observe a negative relationship between SRL and root C:N ratio across species (Eissenstat 1991, Freschet et al. 2010). Alternatively, if root trait syndromes are structured by phylogeny, we would expect similar trait syndromes among closely related species despite the fact that foliar traits usually lack phylogenetic structure (Milla & Reich 2011). Second, we tested the level of integration between above and belowground functional traits. If resource capture strategies are integrated at the whole plant level (PES hypothesis, Freschet et al. 2010), species should show a full integration of above- and belowground trait syndromes commonly associated with life history strategy (Reich et al. 1998). Therefore, chemical and morphological root traits should be correlated with leaf traits, with little effect of phylogeny on the relationships. However, if different plant structures are subject to divergent selective pressures, we would expect independent functional trait syndromes across organs, resulting in orthogonal axes of variation (Baroloto et al 2010).

**Methods:**

**Study site and sample collection:** We sampled 34 woody angiosperm species, plus two gymnosperms used as an external group, growing in two separate living tree collections (Table S1, Fig 2.1): The Holden Arboretum near Kirtland, Ohio (40°57’N and 82°28’W) and Boone County Arboretum in Florence, Kentucky (38°57’N and 84°43’W). The Holden Arboretum (~300 m elevation) receives an average of 116 cm precipitation per year, with an average annual temperature of 8 °C. The soils at Holden were formerly
under mixed mesophytic and beech-maple forest and are characterized by gently sloping ground (2 to 6% slope) and moderately drained silt to clay loam soils from the Platea Series (Fine-silty, mixed, active, mesic Aeric Fragiaqualfs). Boone County Arboretum (~300 m elevation) receives 105-112 cm precipitation annually, with an average annual temperature of 11°C. The site is located mostly on gently sloped fine, silty Aquic Fragiudalfs in the Rossmoyne series, which, prior to clearing and conversion to agriculture, was covered with deciduous hardwood forest species typical of the oak-hickory region.

All species sampled are known to form only arbuscular mycorrhizal associations (Brundrett et al. 1990, Wang & Qiu 2006). We classified all species into three main phylogenetic categories at the super-order level: Magnoliids (including basal families Annonaceae, Lauraceae and Magnoliaceae); Asterids (including Aquifoliaceae, Bignoniaceae, Cornaceae, Ericaceae, Oleaceae, and Styracaceae) and Rosids (including Altingiaceae, Cercidiphyllaceae, Fabaceae, Hamamelidaceae, Juglandaceae, Rosaceae, Salicaceae, Sapindaceae and Ulmaceae) (Table S1).

Above and belowground samples were collected during mid-summer (July-August) of 2010 and 2011. Samples from each species were obtained from four healthy adult trees (two individuals from each site) relatively isolated from other woody plants. Root samples were obtained by extracting two large soil cores (10 cm diameter X 15 cm deep PVC pipes) within 2 m of the main stem of each individual tree, avoiding large (>5cm diameter) roots. Samples were placed in air-tight bags and stored at 4°C.
Additionally, roots exposed and left in the soil during the core extraction were carefully excavated and a small segment (~10 cm long) that included 3-4 most distal root orders was extracted, tagged and preserved in a 45/5/50 water-acetic acid-formalin mixture as a reference sample. In the lab, roots were separated from soil by soaking in water for 12 hrs, gently washing with deionized water, and then comparing washed roots to the reference sample before further analysis.

Aboveground tissue was collected from each individual by sampling whole branches with fully expanded sunlit leaves. Leaves and twigs (long shoots of first-year growth tissue with leaves still attached to the stem) were detached from branches immediately after collection. Senesced or damaged leaves were avoided. Leaves, twigs and branches were sealed individually in air-tight polyethylene bags and maintained at 4°C until fresh weight and area could be measured (usually within two days; Wilson et al. 1999). Leaf thickness was estimated in situ measuring ten freshly collected leaves from each individual sampled at The Holden Arboretum. Each leaf was measured at three points (tip, medium and base points, three randomly chosen leaflets in the case of compound leaves) with an electronic thickness gauge (Eagle Technology, USA, precision 0.01 mm) avoiding major veins or irregular areas. Leaf vouchers of each species were collected and deposited in the Kent State University Herbarium (accession numbers KS65950-KS65980).

**Morphological and architectural traits:** For root morphological analyses, we used five randomly selected 5-10 cm long root systems comprised of the three most distal root
orders, which has been considered the portion of root systems analogous to leaf tissues (Xia et al. 2010). Roots were weighed to the nearest 0.01mg, scanned with a flat-top digital scanner (800 DPI resolution, 256-level gray-scale, TIFF format; Epson Scanner Perfection V700 Photo, USA), dried at 65 °C for 48 hr, and then reweighed. Image analysis of the scanned roots was performed using WinRhizo software (2007 Pro version, Instrument Regent, Quebec, Canada) to estimate three traits: specific root tip abundance (SRTA, tips mg⁻¹ DRM, (Meinen et al. 2009)), fractal dimension (Wang et al. 2004) and average link length (cm, Bouma et al. 2001). Additional root systems from each tree were dissected into three orders following Strahler’s stream ordering system (i.e. the most distal roots labeled as the first order (Fitter et al. 1991)) until ~2 g of tissue was obtained from each root order. Roots from each order (kept hydrated during dissection) were then scanned as described above, dried for 24-48 hr at 65 °C and weighed. WinRhizo image analysis and mass values from each root order were combined to estimate average diameter, specific root length (SRL, m g⁻¹ DRM), specific root surface area (SRA, cm² g⁻¹ DRM) and root tissue density (RTD, g DRM cm⁻³) for each root order from each sampled tree.

For leaf area estimates, a minimum of five fully expanded leaves including petioles were scanned on a flatbed scanner (Cornelissen et al. 2003). Leaves were blotted dry with absorbent tissues to remove all excess moisture, immediately weighed and scanned, then dried individually in paper envelopes at 65 °C for 48 hours and reweighed. Total leaf area and dry weight were used to estimate SLA (cm² g⁻¹). Leaf dry matter content (LDMC) was estimated as a percentage of saturated weight (Wilson et al. 1999).
Twigs and branches were also scanned independently. For each tissue type, 3-5 segments 2-5 cm long were scanned together, weighed, dried in a paper envelope at 80 °C for three days and reweighed. Density values for twigs and branches were expressed as dry matter per volume, using volume estimates from WinRhizo software. Additionally, we determined the relationship between fresh area and dry weight for twigs (specific twig area, STwigA) and branches (specific branch area, SBranchA) in order to have a common measured variable for all tissues (including SRA for roots and SLA for leaves).

**Chemical traits:** A subsample of each root set that was separated by order was ground using a GenoGrinder (Spex SamplePrep, Methuchen, NJ, USA) at 500 rpm for 1 minute. Samples with excessive fibrous material were frozen with liquid nitrogen, and ground manually with a mortar and pestle. All samples were oven-dried again at 65 °C for 24-48 hrs and stored in 2ml polypropylene tubes for further chemical analysis. Total %C and %N was determined on duplicate 0.2-0.5 mg dried tissue samples using an elemental analyzer (Costech Analytical Model 4010, Valencia, CA, USA). Proximate tissue composition was analyzed in duplicates with a series of extractions as described by Ryan *et al.* (1990). In brief, 0.2 – 0.5 mg of dry ground material from each tissue was placed in a 2ml polypropylene tube. Polar extractives and non-polar extractives were removed by consecutive extractions with methanol and dichloromethane, respectively. Polar and non-polar content was estimated gravimetrically as the difference between the initial weight and the weight after extraction. The residue from the polar/non-polar extractions was then separated into acid-hydrolyzable (AH; representing crystalline carbon polymers like
cellulose) and non acid-hydrolyzable (NAH; representing lignin-like polymers) compounds using a two-stage sequence of sulfuric acid digestions (Cusak et al. 2009). Residual material after acid digestion was collected by filtration, and the AH fraction was estimated as the weight lost during acid digestion. Finally, all fractions were corrected for ash content after incineration of the NAH fraction at 500°C for 4hr.

**Phylogenetic data:** The phylogenetic relationships for the 36 species were constructed and visualized using previously published angiosperm phylogenies provided by Phylomatic (Davies et al. 2004, Webb & Donahue 2005). The original tree from Phylomatic presented several polytomies, which were resolved *a posteriori* based on a variety of published trees for the families Magnoliaceae (Kim et al. 2001), Saxifragaceae (Wang et al. 2009) and Oleaceae (Wallander & Albert 2000) using MESQUITE software (ver 2.7, Madison & Madison 2011). We considered species belonging to the Saxifragales order as part of the rosid clade because this group has been catalogued as nested in the rosids or its sister clade (Wang et al. 2009, Soltis et al. 2013). For evolutionary analyses, all branches were transformed to equal length in order to improve contrast diagnostics (Kembel & Cahill 2011, Fortunel et al. 2012).

**Data analysis:** All statistical analyses were performed in the R 2.12 statistical platform (R development core team, 2012) using the packages picante (Kembel et al. 2010), ape (Paradis et al. 2004), phytools (Revell 2012), ade4 (Dray & Dofour 2004), and vegan (Oksanen et al. 2008). Trait variables were log transformed when necessary before
statistical analysis to account for heteroscedasticity and non-normal distribution of residual error.

Testing for phylogenetic signal in traits: As part of our experimental design we focused our analysis on differences between phylogenetic groups at the super-order level. First, we performed a mixed-model ANOVA for each measured trait. Fixed factors in this ANOVA included super-order (magnoliid, asterid, and rosid), site (Holden or Boone County Arboretum), and tissue position (root order for belowground traits, and leaf, twig or branch for aboveground traits). Species nested in clades was designated a random factor to account for the non-independence of individuals of the same species. A post-hoc Tukey HSD test was performed to determine significant differences among super-order groups.

Second, we tested the hypothesis of phylogenetic conservatism in root traits by performing a Moran’s I coefficient autocorrelation test (Paradis 2012). A high positive Moran’s I value at a particular taxonomic level indicates that a trait is more clustered than expected at that taxonomic level, whereas negative values indicate that a trait is more evenly dispersed than expected. Moran’s I values were tested for significance by permutation, randomly shuffling trait values at the tree tips and estimating the number of times the randomized Moran’s I value was larger than the observed value (Paradis 2012). The test was initially performed at the super-order level, and then repeated at several other taxonomic levels (class, order, family and genus) to visualize the consistency of our results across different taxonomic levels.
We also tested if phylogenetic structure was detected belowground more commonly than aboveground using a randomization test. We repeated the Moran’s I autocorrelation test for aboveground traits at the super-order level, and then calculated the difference between number of traits above and belowground that were significantly phylogenetically structured. Then, we simulated the distribution of significantly structured traits under the null hypothesis that above and belowground traits were equally likely to be phylogenetically structured. This simulation consisted of randomizing whether each trait was significantly phylogenetically structured, keeping the total number of significantly structured traits equal to the observed values. Finally, we ran 999 iterations of the null model simulation and calculated a $P$ value estimating the number of times the randomized data yielded equal or higher differences than that observed between compartments.

To test for phylogenetic structure in an explicitly multivariate context, we first created separate morphological and chemical trait matrices for each root order and aboveground tissue type, employing mean values of each species. Variables were standardized (mean equal to zero and standard deviation equal to one). Principal component analysis (PCA) was then performed separately for the morphological and chemical trait matrices to find major axes of trait variation in each tissue type. Evolutionary distance, inferred from the phylogenetic tree described above and assuming a Brownian motion error structure, was incorporated into the PCA as an additional constraint to account for the non-independence of species due to phylogenetic structure.
(pPCA, Revell 2009). It is important to note that this procedure shifts allocation of variation in the ordination so that principal components are uncorrelated (orthogonal) at each level in the phylogenetic tree. However, phylogenetic structure is not removed from the data and may be represented on different pPCA axes (Revell 2009). From each ordination, we then selected the informative pPCA axes (i.e., axes that explained greater amount of variation than expected by chance under a broken-stick null model, Legendre & Legendre 1998). We tested for differences among super-orders at the multivariate level by performing a redundancy analysis (RDA, ter Braak 1986). For this analysis, we used the new matrix of species scores created during the pPCA analysis as the response variable, and a categorical vector describing the classification of species at the super-order level as factor (Jombart et al. 2010).

Correlations between morphology and chemistry within plant tissues: We examined correlations among traits separately for leaves and first order root tissues using both mean species trait values and trait values that were corrected for phylogenetic effects. First, we tested the hypothesis of correlated chemical and morphological traits by calculating the Pearson correlation coefficient between species mean SRL and C:N values for first order roots and SLA and C:N ratio for leaves. We also used phylogenetically independent contrasts (PIC) to perform this bivariate analysis while accounting for phylogenetic structure (Felsenstein 1985).

We tested for multivariate correlation between morphological and chemical traits for each root order by performing a coinertia analysis (Dray et al. 2003) on the
informative morphological and chemical pPCA axes selected previously. This procedure finds a new set of ordination axes that maximizes the coinertia, or sum of squared covariances, between two data matrices, without being constrained by colinearity or numbers of variables in the data matrices (Dray et al. 2003). Coinertia analysis is not predicated on one data matrix being designated as independent and the other as dependent; therefore the coinertia value is analogous to a Pearson correlation coefficient.

We tested whether the observed coinertia value was higher than expected by chance by performing a Monte-Carlo simulation test using 999 random permutations of the rows in each matrix (Dolédec & Chassel 1994).

We also determined to what extent the coinertia between root morphology and chemistry was influenced by phylogeny. Phylogenetic pairwise distances among species were calculated based on our constructed phylogenetic tree (Paradis et al. 2004). From this distance matrix, principal coordinate analysis (PCoA, Dray et al. 2003) was used to create a matrix of orthogonal vectors that captured phylogenetic structure in the tree. We used the first two vectors (summarizing 77% of all variation of phylogenetic distances) to represent phylogenetic structure. Next, we regressed the selected trait pPCA axes against the phylogenetic PCoA vectors, and calculated residuals of this regression. The new residual trait matrix represented trait structure after phylogenetic correction. Then we performed a new coinertia analysis with the residual trait matrices obtained after correcting by phylogeny (Dray et al. 2003). Additionally, we performed the same
multivariate analysis for all foliar tissues, determining whether the expected trait coordination (LES hypotheses) was actually present at the leaf level.

Integration of functional traits above and belowground: To test the PES hypothesis, we first performed a Pearson correlation test between C:N ratio and SRL for first order roots and SLA for leaves. Correlations were also calculated using phylogenetically independent contrasts to account for phylogenetic non-independence of species. At the multivariate level, we performed an additional coinertia analysis contrasting all informative pPCA axes from each tissue selected previously. Similar to the analysis comparing chemical and morphological traits, we determined the coinertia between above and belowground pPCA axes before and after phylogenetic correction, in order to assess the effect of species phylogenetic affiliation on correlation coefficients. Finally, all vectors were projected in a global ordination using the coinertia scores obtained after the phylogenetic correction.

Results
Phylogenetic signal and trait syndromes: For the univariate mixed model ANOVAs, the factors that significantly affected traits varied among tissues (Table S2.2). Differences across tissues (root orders or twigs vs. leaves) were the most important factor for most measured traits. Super-order was a significant factor explaining most belowground but not aboveground trait variation, even after controlling for site and root order, suggesting a stronger phylogenetic effect influencing belowground trait distribution (Fig 2.1). Moreover, the significant interaction between root order and super-order suggests that,
not only root traits, but also the integration of root systems among orders is highly structured by species phylogenetic affiliation (Table S2.2). Site differences seemed to influence root chemical traits such as polar and non-polar compounds and nitrogen content, with Boone samples showing higher root N and polar compound concentration but lower non-polar content than Holden samples (Table S2.2). Aboveground, polar, non-polar and acid hydrolysable content was also affected by site. In a few cases we detected an interaction between site and super-order (Table S2.2). However, the interaction did not alter the overall trait patterns observed among super-orders.
Figure 2. Phylogenetic relationship and trait value distribution for 36 woody species grown in common gardens in the Midwestern USA. The traits displayed are A) specific root length (SRL) for first order roots; B) ratio of non acid-hydrolyzable to nitrogen contents (NAH:N) ratio for first order roots; C) aboveground branch tissue density and D) specific leaf area (SLA). Symbol size indicates relative trait values for each species, with smaller symbols closer to the mean value; black symbols represent values above the mean and white symbols are below the mean. Branch lengths were standardized to have the same length. The gymnosperms Ginkgo biloba and Thuja occidentalis were used as outgroup for the tree.
The contrasting importance of phylogeny on trait variation between above and belowground structures was confirmed by the phylogenetic signal analysis. The Moran’s I analysis was consistent with the hypothesis of larger phylogenetic conservatism belowground, with a higher proportion of root traits showing significant phylogenetic signal compared to aboveground traits (75 vs 28% at the super-order level, Fig. 2.2a). Furthermore, the randomization test indicated that the proportion of phylogenetically structured belowground traits was significantly greater than the proportion of phylogenetically structured aboveground traits (p=0.0002). In addition, this trend was maintained at other taxonomic levels (order, family and genus), suggesting a consistently higher level of phylogenetic structuring in root traits independently of the phylogenetic level contrasted (Fig. 2.2b, Table S2.3). Aboveground, those traits that were phylogenetically conserved tended to be related to structural traits, such as branch tissue density, whereas most leaf traits showed no phylogenetic signal (Table S2.3).

Similarities among phylogenetic groups varied depending on the traits compared, as shown by the RDA ordination plots (Fig. 2.3). The morphology of magnoliid roots was significantly different from asterid or rosid species (RDA analysis p< 0.005 for first order roots, Fig. 3a), which corresponds with our observations of predominantly thicker, scarcely branched root systems among magnoliids. In contrast, the chemistry of rosid species roots was significantly different from magnoliid and asterid roots (RDA analysis p<0.005 for first order roots, Fig. 3c). In general, rosid species exhibited consistently lower N content and higher NAH:N ratio than other angiosperms (Fig. 1.1, Table S2).
Figure 2. 2 Comparison of the level of phylogenetic structuring of 39 belowground and 36 aboveground tissue traits measured in 34 species of angiosperms. A) Proportion of traits showing significant phylogenetic structuring (Moran’s I index) at the super-order level in leaves, twigs, and branches and three orders of fine roots (N=12 traits per tissue), as well as the entire root system (ERS, N=3 including specific root tip abundance, fractal dimension, and link length). The higher proportion of root traits structured phylogenetically is significant (p<0.001); B) Comparison of phylogenetic signal (Moran’s I index) for specific leaf area (SLA), leaf non acid-hydrolyzable compound:nitrogen (NAH:N) ratio, first order root specific root length (SRL) and first order root NAH:N ratio across different taxonomic levels. Filled symbols represent significant phylogenetic structure at that particular taxonomic level (p<0.05).
**Figure 2.3** Ordination of first order fine root and leaf morphological and chemical functional traits. For all panels, axis values represent the first two pPCA axes, and the amount of variation explained by each axis. Labels represent the centroid values for each super-order (magnoliids, asterids and rosids). P values were obtained from redundancy analysis (RDA) testing differences in traits among super-orders. pPCA loadings for traits are shown in Table S4.
In contrast, foliar tissues showed no difference among phylogenetic groups for either morphological or chemical traits (p=0.11 and 0.09, respectively, Fig. 2.3b and d).

**Correlation between chemical and morphological traits within tissues:** The hypothesis that roots display a correlation between chemical and morphological traits similar to that expected from the LES hypothesis was not supported. Increases in SRL for instance, were not related to C:N values (r=0.24, p=0.15, Fig 2.4a), even though we found the expected negative relationship in leaves (r=-0.74, p<0.001, Fig 2.4b). At the multivariate level we found a weak correlation between root chemical and morphological pPCA trait axes (coinertia r ranging from 0.17 to 0.34, Tables 1 and S4). Moreover, coinertia values decreased after phylogenetic correction (36 % on average, Table 1) suggesting that part of the correlation was explained by coupled root morphological and chemical trait evolution. Similarly, twigs and branches showed low coinertia between morphology and chemical pPCA trait axes, which also decreased after phylogenetic correction (Table 2.1). In contrast, leaf trait matrices showed higher coinertia values (r=0.40, p=0.001) with no effects of phylogeny on the relationship (Table 2.1).

**Integration between above and belowground traits:** SRL was not related to SLA (r = -0.01, p=0.97, Fig 2.5a). However, in partial support of the PES hypothesis, the C:N ratio between leaves and roots was closely related (r = 0.67, p<0.001, Fig 2.5b). Displaying all informative pPCA axes in multivariate space revealed two orthogonal axes between morphological and chemical functional traits (Fig. 2.6). The first axis of variation seemed highly associated with chemical traits (mostly representing root and leaf N content).
variation), with leaf morphology (SLA) as the only morphological trait aligned with the chemical traits.

Table 2.1 Coinertia coefficients comparing the coordination between morphological and chemical traits within different organs of 34 woody angiosperm species grown in common gardens in the Midwestern USA. Coinertia coefficients represent the sum of squared variances between matrices representing morphological and chemical traits for each organ before (Uncorrected) and after phylogenetic correction (Phylo-corrected). Bold numbers represent cases where a significant amount of variance (p< 0.05) was explained.

<table>
<thead>
<tr>
<th></th>
<th>Uncorrected</th>
<th></th>
<th>Phylo-corrected</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Coinertia</td>
<td>P-value</td>
<td>Coinertia</td>
<td>P-value</td>
</tr>
<tr>
<td>Root 1</td>
<td>0.17</td>
<td>0.03</td>
<td>0.04</td>
<td>0.67</td>
</tr>
<tr>
<td>Root 2</td>
<td>0.32</td>
<td>0.001</td>
<td>0.23</td>
<td>0.03</td>
</tr>
<tr>
<td>Root 3</td>
<td>0.34</td>
<td>0.001</td>
<td>0.26</td>
<td>0.001</td>
</tr>
<tr>
<td>All belowground</td>
<td>0.22</td>
<td>0.009</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Leaf</td>
<td>0.40</td>
<td>0.001</td>
<td>0.40</td>
<td>0.001</td>
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<tr>
<td>Twig</td>
<td>0.18</td>
<td>0.01</td>
<td>0.05</td>
<td>0.20</td>
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<tr>
<td>Branch</td>
<td>0.04</td>
<td>0.63</td>
<td>0.02</td>
<td>0.53</td>
</tr>
<tr>
<td>All aboveground</td>
<td>0.29</td>
<td>0.005</td>
<td>0.24</td>
<td>0.003</td>
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In contrast, other morphological traits align across a second axis, depicting a weak coordination between root and stem morphological traits, but still largely independent from the chemical axis. Moreover, trait syndrome correlations were influenced by relatedness among species, reflected as a decrease in coinertia between chemical and morphological traits after phylogenetic correction, from r=0.38 (p=0.001) without phylogenetic correction to 0.14 (p=0.04) after phylogenetic correction (Fig 2.6).
Fig. 2.4. Correlation between morphological and chemical traits for leaves and first order roots. A) SLA and C:N in leaves; and B) SRL and C:N in first order roots. Coefficients correspond with uncorrected Pearson correlation ($r$) and phylogenetic independent contrast correlation values (PIC $r$).
Fig 2.5. Comparison of leaves and first order roots. A) Morphological traits specific root length (SRL) and specific leaf area (SLA); B) C:N ratio for leaves and first order roots. Correlation coefficients correspond with uncorrected Pearson correlation (r) and phylogenetic independent contrast correlation values (PIC r).
**Fig 2.6.** Coinertia ordination of aboveground and belowground chemical and morphological functional traits of leaves, twigs, branches and three orders of roots. Traits shown are informative pPCA axes, and names represent the individual trait with the highest loading value for that pPCA axis (see Table S4). For the morphological traits, SRL = specific root length for root orders 1, 2 and 3; RTD3 = root tissue density for third order roots; SLA = specific leaf area; SBranchA = specific branch area; STwigA = specific twig area; D_Branch = branch diameter; D_Twig = twig diameter. For chemical traits, N represents tissue nitrogen for each tissue; Branch_AH = branch acid hydrolyzable content.
Discussion

Trait integration and evolution in angiosperm roots: Strong correlations among leaf functional traits such as SLA and N content have been consistent across species differing in growth form, phylogenetic background and biome distribution (Reich et al. 1997, Wright et al. 2005). This striking global pattern suggests fundamental biophysical tradeoffs that limit the possible phenotypic combinations in foliar traits that can be successful under natural selection (Donovan et al. 2011). Because LES has such significant influence on the understanding of functional trait variation (Wright et al. 2004, McGill et al. 2006), the spectrum separating ‘fast’ and ‘slow-growing’ species has been repeatedly applied to functional trait variation in other organs, particularly fine roots (Tjoelker et al. 2005, Kembel & Cahill 2011). Our results, however, do not support this assumed similarity between roots and leaves. We did not find significant correlations between chemical and morphological traits of roots, suggesting that morphological and chemical traits are decoupled at the root system level. In fact, we found some species with root trait combinations that seem contradictory to patterns expected from the LES. For instance, some species with high SRL showed relatively high C:N ratio, whereas species with the lowest SRL showed consistently lower C:N (Fig 2.3). From the same individual trees, we did find the expected correlations among leaf traits, implying that the belowground results are not an artifact of biased sampling or lack of trait variation across species.

The decoupling of root chemical and morphological traits suggests that roots display a broader array of possible trait combinations than foliar tissues for maximizing
functional gains and minimizing construction and maintenance costs (Donovan et al. 2011; i.e. broader ‘phenotypic space’ sensu Pigliucci 2007). This may be possible because, in contrast to leaf function that is mostly limited to the maximization of carbon fixation while minimizing water losses (Beerling & Franks 2010), roots can operate effectively using a broader set of morphological strategies that cannot be simultaneously maximized. For instance, the uptake of limiting nutrients can be achieved either by maximizing root surface exposure to the soil or by enhancing habitat conditions for mycorrhizal fungi. However, increasing surface area requires decreasing root diameter, with a concomitant decrease in habitat for symbiotic fungi. Therefore, natural selection could lead to opposite morphological patterns to solve the same resource limitations (Brundrett 2002). Similarly, Comas & Eissenstat (2004) showed no correlations between root N content and nutrient uptake or root respiration rates of temperate trees, suggesting that the relationship between chemical traits and root physiology are more complex than predicted from the LES hypothesis.

Evolutionary forces explaining root trait syndromes: The strong phylogenetic signal exhibited by root traits indicate that functional trait syndromes emerged as the outcome of alternative evolutionary pathways, rather than the coordination of traits based on whole-plant growth strategies. Our results support Baylis’ original hypothesis of a morphological separation between basal and more derived angiosperms (Baylis 1975), which is also consistent with data reported elsewhere (Holdaway et al. 2009, Comas & Eissenstat 2009). However, to our knowledge, this is the first time that, in addition to root
morphology, root chemical makeup (particularly root lignin:N ratio) has been reported to be structured by phylogenetic history within angiosperm lineages. Moreover, the significant interaction between phylogeny and root orders suggests that the degree of trait differentiation from distal to basal roots is also phylogenetically structured. Therefore, the evolutionary process shaping functional traits in tree roots has affected not only individual root links but also the integration of root orders at the entire root system level.

Baylis’ hypothesis proposed that mycorrhizal dependency is the main driver of root morphological change among angiosperms. This view is consistent with theoretical evolutionary models and experiments indicating high stability in obligate mutualistic relationships, particularly in mycorrhizal associations (Kiers & van der Heijden 2006, Kiers et al. 2011). Moreover, microbial associations with plant roots are governed by complex genetic signaling, which is possibly highly conserved across evolutionary lineages as demonstrated in the association between angiosperms and N-fixing bacteria (Markmann & Parniske 2008). Nevertheless, it has been recently proposed that root trait diversity in angiosperms emerged as an adaptation of angiosperms to a decrease in atmospheric CO$_2$ concentrations during the Late Cretaceous (Comas et al. 2012, Fletcher et al. 2008). According to this hypothesis, new angiosperm lineages cope with lower CO$_2$ concentrations by increasing photosynthetic rates and leaf venation (Boyce et al. 2009), which increases water demand. Moreover, angiosperms expanded to more xeric and cold temperate biomes, where water limitation was higher and nutrient release from organic forms was slower than in wet tropical environments where basal angiosperms originated.
(Feild & Arens 2007, Feild et al. 2009). Thus, increases in SRL, decreases in diameter and changes in tissue lignification were possibly a consequence of selection for more efficient root systems in novel environments, independently of mycorrhizal dependency.

One important observation from our study is that typical ‘rosid-like’ roots (highly branched, short lateral roots with high concentration of non-acid hydrolyzable compounds) are present in basal families of the rosid clade (i.e. Altingiaceae, Cercidiphyllaceae and Hamamelidaceae). Because basal rosids are almost exclusively associated with arbuscular mycorrhizal fungi (Wang & Qiu 2006), we hypothesize that the acquisition of the rosid-like root trait syndrome occurred prior to the evolution of ectomycorrhizal (ECM) associations among rosid trees. Moreover, fossil records and current geographical patterns indicate that these families were historically distributed in temperate or high altitude tropical forests (Wolfe 1997, Zhou et al. 2001, Qi et al. 2012). Therefore, our data supports the hypothesis that evolution of the rosid-like root trait syndrome occurred as an adaptation to temperate environmental conditions by an ancestral rosid group, rather than due to selective pressure for new mycorrhizal symbionts. However, it is possible that the rosid-like root trait syndrome did facilitate subsequent evolution of the ECM symbiosis within rosids. This is suggested by the fact that the rosid clade includes ~80% of angiosperm ECM species (Brundrett 2002, Wang & Qiu 2006) and that AM and ECM rosid species tend to have similar root traits (Guo et al. 2008, Comas & Eissenstat 2009). Further morphological and chemical descriptions of
roots forming AM and ECM associations in Gymnosperms and additional Angiosperm families could help to elucidate the generality of the patterns we detected.

Chemical and morphological trait coordination for whole woody plants: In agreement with other studies involving woody plants (Ishida et al. 2008, Baroloto et al. 2010, Fortunel et al. 2012, Chen et al. 2013) we found limited support for the idea that all tree organs are coordinated in a whole-plant resource use strategy. Instead, our results suggest that trait syndromes have evolved independently among structures in order to cope with particular selective pressures for each tissue. As explained above, the acquisition of functional trait syndromes in fine roots was possibly obtained independently among plant lineages as adaptations to deal with soil nutrient and water limitation. Since roots are exposed to a more complex abiotic and biotic environment than leaves, and the possibilities to maximize tissue performance seem broader than those of foliar tissues, it is not surprising that these organs have evolved independently. In contrast, the low but significant correlation between morphological traits of roots and stems (i.e., branches) may indicate common selective forces (e.g., similar hydraulic stress experienced by roots and stems), which could lead to the integration of a similar set of functional traits between organs. The fact that some traits, such as tissue density, are structured phylogenetically in both roots and stems (Swenson & Enquist 2007, Martínez-Cabrera et al. 2009, Poorter et al. 2010), could also indicate that the ontological process involved in the construction of these tissues is shared. However, the fundamentally different functions of fine roots and stems, and the additional selective pressure associated with
soil biotic interactions in fine roots, may limit the extent to which these tissues can be coordinated.

Contrary to morphological traits, the strong correlation in N content across plant tissues highlights the possibility of a coordinated trait axis representing ecological strategies in plants. Nitrogen levels in plant tissues are usually associated with protein content, respiration and overall metabolic activity (Evan & Seemann 1989, Lambers et al. 2005). A previous metaanalysis by Reich et al. (2008) proposed a common relationship between N content and respiration rates for all plant organs that is independent of phylogenetic association. In grasses, the full integration of root and leaf N content is well-established (Craine et al. 2002) but relatively few studies have evaluated similar integrations in woody plants (Reich et al. 1998, Wright & Westoby 1999, Laughlin et al. 2010). Our results support the hypothesis that N content reflects inherent physiological and life-history tradeoffs across ecological guilds that are consistent across the entire plant (Wright et al. 2004), and that is independent of phylogenetic background (Reich et al. 2008).

This work highlights the importance of broad phylogenetic patterns for understanding ecological adaptations of individual species (Donoghue 2008) and entire communities (Mueller et al. 2010). However, it is important to note that we intentionally minimized the influence of environmental factors that could mask important patterns present in natural communities. For instance, soil conditions can influence the link between above and belowground functional traits at the community level (Zangaro et al.
2007, Holdaway et al. 2011) and alternative root traits have been reported among closely related species adapted to contrasting soil conditions (Comas & Eissenstat 2004, Espeleta et al. 2009). Nonetheless, large variation in root traits within communities may indicate that alternative root trait syndromes influence species coexistence, possibly reflecting belowground niche segregation mechanisms among coexisting species (Valverde-Barrantes et al. 2013). Analysis of additional taxa, including conifers and important woody tropical families (i.e. Lecythidaceae and Sapotaceae) would improve our assessment of the amount of root trait variation structured by phylogeny across biomes. If the patterns found in this study are maintained across larger biogeographical scales (as suggested by Chen et al. 2013), use of phylogenetic information could improve our ability to predict the distribution of belowground traits at global scales. Such information could be useful not only for understanding belowground ecological processes but also for explaining and predicting past, current, and future trends in forest communities (Wiens & Donaghue 2004, Donaghue 2008, Crisp et al. 2009).

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Jean Burns, and Kevin Mueller for commenting on earlier drafts of the manuscript. This study was funded by startup funds provided by Kent State University, an Art and Margaret Herrick Research Grant, a David and Susan Jarzen Scholarship, The Holden Arboretum Trust, and The Corning Institute for Education and Research.

References


**Table S1** Life history traits of 36 temperate tree species used for the description of above and belowground traits in this study.

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References
‡ Qualitative descriptions of growth rate and lifespan compiled from USDA Database (www.plants.usda.gov).
*Potential growth rate estimated from typical annual vertical increase. Slow < 30 cm; Moderate >30cm -50 cm <, Rapid > 50 cm.
Table S2. Analysis of variance comparing phylogenetic groups (Super-order) and tissue differences for all traits measured in 34 angiosperm tree species in two different common gardens. Values correspond with F-values from the mixed model ANOVA analysis. Bold values indicate significant factors for p< 0.05. Letters at the bottom indicated post-hoc significant difference between super orders (Tukey HSD test). SRL= Specific root length, SRA=Specific root area, RTD=Root tissue density, SRTA=Specific root tip abundance, AH= Acid hydrolysable compounds, NAH= Non-acid hydrolysable compounds, NAH:N=NAH:nitrogen ratio, STA=Specific tissue area comparing leaf, twig and branch specific area, DCM=dry mass content.

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<td>0.82</td>
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| Asterid                 |    | A           | A                   | A                      | A                      | A                     | A                      | A          | A           | AB    | A    | A     | AB    |
| Magnoliid               |    | A           | A                   | A                      | A                      | B                     | A                      | A          | A           | A     | A    | A     | A     |
| Rosid                   |    | A           | A                   | B                      | A                      | A                     | A                      | A          | B           | A     | A    | A     | B     |

* Leaf thickness in the case of leaves.
Table S3. Phylogenetic signal at the Super-order level for each trait. The phylogenetic signal was determined with a Moran’s I index (Moran.I) autocorrelation approach. Positive values indicate that closely related species show higher trait similarity than expected under a random distribution. Traits that showed significantly higher phylogenetic signal (p < 0.05) are indicated in bold.

<table>
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<th>Trait</th>
<th>Root 1st</th>
<th>Root 2nd</th>
<th>Root 3rd</th>
<th>Aboveground</th>
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<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>0.61</td>
<td>-0.04</td>
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<td>0.24</td>
<td>0.05</td>
<td>-0.05</td>
</tr>
<tr>
<td>SRA(cm²/g)</td>
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<td>-0.07</td>
<td>-0.02</td>
</tr>
<tr>
<td>RTD (g/cm³)</td>
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<tr>
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<td>-0.02</td>
<td>-0.06</td>
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<tr>
<td>Non Polar (mg/g)</td>
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<td>-0.04</td>
<td>-0.05</td>
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<tr>
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<td>-0.07</td>
</tr>
<tr>
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<tr>
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<tr>
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<td></td>
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</tr>
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<td>Thickness (mm)*</td>
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<td>NAH:N</td>
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</table>

SRL= Specific root length, SRA=Specific root area, RTD=root tissue density, AH= acid hydrolysable compounds, NAH=Non-acid soluble compounds, SLA=Specific leaf area, STwigA=Specific twig area, SBranchA=Specific branch area, DCM=dry mass content. * In the case of twigs and branches, thickness refers to diameter in mm.
Table S4. Loadings for phylogenetic principal component analysis (pPCA) on morphological or chemical traits for three orders of roots, leaves, twigs and branches of 34 species of woody angiosperms. Eigenvalues of informative PCA axes (i.e. explaining a non-trivial amount of variation) are denoted in bold. SRL= Specific root length (m/g), SRA=Specific root area (cm$^2$/g), RTD=root tissue density (g/cm$^3$), SRTA=Specific root tip abundance (tips/mg), AH= acid hydrolysable compounds (mg/g), NAH=Non-acid soluble compounds (mg/g), SLA=Specific leaf area (cm$^2$/g), STwigA=Specific twig area (cm$^2$/g), SBranchA=Specific branch area (cm$^2$/g), DCM=dry

<table>
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<th>PC2</th>
<th>Leaf</th>
<th>PC1</th>
<th>PC2</th>
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<td>Thickness</td>
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Continuation. Table S4.

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<td>Explained variance (%)</td>
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<td><strong>33.85</strong></td>
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| Root Polar     | 0.25| -0.93| Twig N  | 0.91| -0.07|
| Root NoPolar   | 0.15| -0.39| Twig C  | -0.13| -0.74|
| Root AH        | 0.73| 0.05| Twig CN | -0.90| 0.04|
| Root NAH       | -0.69| 0.79| Twig Polar | 0.76| -0.29|
| Root N         | 0.97| -0.17| Twig NoPolar | 0.21| -0.78|
| Root C         | -0.24| 0.22| Twig AH  | -0.15| 0.71|
| Root CN        | -0.97| 0.19| Twig NAH | -0.69| -0.11|
| Root NAH:N     | -0.99| 0.27| Twig NAH:N | -0.98| 0.01|
| Eigen value    | **40.83**| 5.09| Eigen value | **28.31**| 5.81|
| Explained variance (%) | **80.36**| 10.01| Explained variance (%) | **75.60**| 15.52|
Continuation. Table S4.

<table>
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<th>Branch</th>
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<th>PC2</th>
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CHAPTER THREE: PHYLOGENETICALLY STRUCTURED TRAITS IN ROOT SYSTEMS INFLUENCE ARBUSCULAR MYCORRHIZAL COLONIZATION IN WOODY ANGIOSPERMS.

Paper to be submitted to the journal American Journal of Botany

Summary: An emergent hypothesis in root ecology is that different plant lineages have evolved particular root trait syndromes that reflect their dependency on mycorrhizal symbiosis for nutrient acquisition. However, the lack of detailed chemical, morphological and mycorrhizal descriptions for a in a representative group of angiosperms has limited the general tests of this hypothesis. Here we examined in detail the chemical, morphological and mycorrhizal status for the three lower root orders of 34 woody species representing three main phylogenetic groups in angiosperms (i.e. asterids, magnoliids and rosids). Our main goals were 1) to quantify the relationship between stele and cortex area for the three most distal roots of woody angiosperms; 2) to contrast the rate of change in chemical and morphological traits along root orders and; 3) to examine how root trait integration influence the level of colonized length and fungal abundance across phylogenetic groups in trees. Our findings indicated that decreased cortical area has been more pronounced than the reduction in vascular area along the angiosperm phylogeny, suggesting that reduction in mycorrhizal habitat has been strongly selected over time in angiosperms. Basal angiosperms and some asterid species showed less variation in root
traits along root orders, including low tissue density, similar stele:root diameter ratio and high N content for all the orders analyzed. In contrast, most rosid and asterid species showed sharper changes in root traits, suggesting high functional specialization among distal root orders. Finally, phylogenetic groups showed different levels of mycorrhizal colonization, with magnoliids exhibiting on average up to 65% more fungal abundance per unit of root mass than rosids. We conclude that the interaction with mycorrhizal symbionts has been an important driver explaining root trait syndromes in woody angiosperms, which could have potential species and ecosystem-level effects on carbon and nutrient cycling belowground.
Introduction

Fine roots are the most distal portions of root systems. In perennial plants, these organs account for most of the nutrient and water acquisition and can comprise as much as 50% of total net primary productivity in forest ecosystems, thus contributing significantly to net ecosystem exchange (Hanson et al. 2000, Litton et al 2007). Nevertheless, the specific roles and functional significance of different fine root segments has been largely debated, particularly among woody plants (Pregitzer 2002).

Traditionally, fine roots have been separated into arbitrary diameter categories, with the assumption that all roots in the same category have similar functional and ecological roles (Vogt et al 1996, Jackson et al. 1997, Gaudinski et al. 2001). A growing body of evidence, however, indicates that root morphology, anatomy and function vary with the relative positions of each link in a root system (i.e., root order), rather than as a simple function of diameter (Pregitzer et al. 2002, King et al. 2002, Guo et al. 2008).

Detailed descriptions of individual root systems have revealed that, in most woody species, the two most distal root orders (first and second) are nutrient and water acquisition specialists. These roots are characterized by the presence of a living parenchymatous cortex, limited development of vascular tissue, and maintenance of interactive associations with microbial symbionts (Hishi 2007, Xia et al. 2010). At higher root orders the stele develops and becomes lignified, cortical cells become suberized, and cork layers accumulate in the exodermis (Hishi 2007). Stele development curtails uptake
capabilities and ability to maintain symbiotic interactions, but improves transport and stress tolerance, indicating a transition from acquisition to structural roots (Kumar et al. 2008). Thus, morphological and chemical trait differences among root orders correlate with function (Brundrett et al. 1990, Comas and Eissenstat 2009). For instance, the root length per unit mass (specific root length (SRL), m/ g dry mass) and N content usually decrease from acquisition to structural roots, whereas lignification and tissue density increase (Pregitzer et al. 1997, Ostonen et al. 2007).

Despite the generalized trait and functional trends across root orders discussed above, it is possible that not all species restrict acquisition capabilities to the first two root orders. Temperate tree species such as *Liriodendron tulipifera* and *Fraxinus mandshurica* can maintain high N concentrations, low lignification, and high mycorrhizal colonization up to the fourth root order (Pregitzer et al. 2002, Guo et al. 2008, Xia et al. 2009). These results imply contrasting acquisition strategies among woody species. However, there is little information about what evolutionary or environmental factors determine the trait integration and mycorrhizal status among root orders in angiosperm trees.

A common proposed hypothesis is that root morphology reflects the evolutionary dependency of plants on mycorrhizal associations for nutrient acquisition (Brundrett 2002, Comas and Eissenstat 2009, Comas et al. 2012). Baylis (1975) and St John (1980) were the first to propose a distinct morphological division between angiosperm roots.
They proposed that coarse, scarcely branched fine-roots are predominant among basal angiosperm groups (i.e. ‘magnoliid roots’). In contrast, finer, highly branched root systems are more common among derived angiosperm groups. The reason for this phylogenetic segregation in root morphology corresponds with the degree of mycorrhizal dependency among angiosperm lineages (Newman et al. 1995). The hypothesis states that roots with larger diameter and small SRL provide optimal habitat for AMF communities but reduce surface area and sacrifice the capacity for soil exploration (Janos 2007). On the other hand, decreased diameter increases root length per unit mass, thereby improving direct root water and nutrient uptake, but reducing the amount of suitable AMF habitat (Brudrett 2002, Seago and Fernando 2013, Chen et al. 2013). Therefore, more derived angiosperms tend to have smaller root diameter and both greater branchiness and SRL than basal angiosperms (Holdaway et al. 2009, Fig. 3.1), suggesting a selection for reduced dependency on AMF over the course of angiosperm evolution (Comas et al. 2012).

Baylis’ hypothesis, however, has three important limitations. First, although the hypothesis emphasizes that root diameter reflects mycorrhizal dependency, it is still unclear what anatomical modifications occurred during angiosperm evolution to reduce total root diameter in the most distal roots. Second, the original hypothesis did not incorporate the importance of relative root link position (root order) on root function. Therefore, it is not clear if these evolutionary changes are limited to the most distal root
orders or if the level of integration for the entire root system has also been modified over time. Third, the hypothesis assumes that morphological modification is the primary mechanism regulating mycorrhizal dependency in angiosperms. However, regulation of AMF colonization in woody plants may involve a myriad of mechanisms, such as anatomical alterations (Guo et al. 2008), tissue lignification (Wells and Eissenstat 2003, Hishi, 2007), or accumulation of phenolic compounds or labile sugars in cortical cells (Bago et al. 2000, Vierheilig 2004, Comas and Eissenstat 2009). Nonetheless, few studies have tested how the integration of chemical, morphological and anatomical root traits may affect mycorrhizal colonization rates, or how these selective pressures may affect total mycorrhizal colonization among different angiosperms lineages (Guo et al. 2008).

In this study, we tested the following hypotheses: 1) If reduction in root diameter in more derived plant groups corresponds with adaptations that limit mycorrhizal colonization, root diameter decrease should be associated with a reduction in cortical area rather than an isometric reduction of both cortical and vascular tissues. 2) We expect that trait transitions among root orders within root systems will be highly structured by phylogeny. More specifically, we predict that basal angiosperms will show less chemical and morphological differentiation along root orders than more derived angiosperms, suggesting a lesser degree of specialization among root orders. 3.1) We extended Baylis’ hypothesis to suggest that that basal angiosperm species not only have greater AMF
colonization but also less variation in AMF length colonization across root orders when compared to other angiosperm groups. In addition, we expect that AMF colonization is regulated by both morphological and chemical root traits (Hypothesis 3.2); total AMF colonization is therefore explained by interactions among root traits, even after accounting for phylogenetic relatedness among species.

**Figure 3.1** Changes in root traits along the three most distal root orders among 34 tree species representing three main angiosperm clades (superorders magnoliid, asterid and rosid). Symbols represent mean (±SE) values at each order for A) specific root length (SRL, g/m) and B) root lignin/nitrogen ratio.

**Methods**

**Site description:** In order to reduce the impact of soil conditions on both root trait variation and AMF colonization we sampled a set of 36 woody plant species growing in
two separate living tree collections; The Holden Arboretum (northeast Ohio, USA) and Boone County Arboretum (northern Kentucky, USA). Both sites were initially under mixed deciduous mesophytic forests and are characterized by gently sloping ground (2 to 6% slope) and moderately drained silt to clay loam soils (see details in Valverde-Barrantes et al. in review, Chaper two). All tree species sampled have been previously reported to form only arbuscular mycorrhizal associations (Brundrett et al. 1990, Wang and Qiu 2006) and were sampled in order to represent three main angiosperm super-orders (thereafter clades) including families Annonaceae, Lauraceae and Magnoliaceae (within the super-order magnoliid); Aquifoliaceae, Bignoniaceae, Cornaceae, Ericaceae, Oleaceae, and Styracaceae (within asterids); and Altingiaceae, Cercidiphyllaceae, Fabaceae, Hamamelidaceae, Juglandaceae, Rosaceae, Salicaceae, Sapindaceae and Ulmaceae (within rosids, for detailed information about individual species see Valverde-Barrantes et al. in review).

**Phylogenetic background:** The phylogenetic relationships for the 36 species were constructed and visualized using previously published angiosperm phylogenies provided by Phylomatic (Davies et al. 2003, Webb and Donahue 2005). The original tree from Phylomatic presented several polytomies, which were resolved *a posteriori* based on a variety of published trees for the families Magnoliaceae (Kim et al. 2001), Saxifragaceae (Wang et al. 2009a) and Oleaceae (Wallander and Albert 2000) using MESQUITE software (ver 2.7, Madison and Madison 2011). We considered species belonging to the
Saxifragales order as part of the rosid clade because this group has been catalogued as nested within rosids or its sister clade (Wang et al. 2009a, Soltis et al. 2013). For evolutionary analyses all branches were transformed to equal length in order to improve contrast diagnostics (Kembel and Cahill 2011, Fortunel et al. 2012).

**Collection of root systems:** Two healthy, relatively isolated individuals of each species were located in each sampling location during July and August 2010. From each tree, roots were obtained from two large (10 cm idiam. X 15 cm depth) soil cores excavated within two meters of the target individual. Additionally, roots from the core were traced back to the main stem of each individual and a small sample (3-4 root orders, ~10 cm long) was washed and tagged and preserved in a 45/5/50 water-acetic acid-formalin mixture as a reference sample. Soil cores were stored at 4 °C until root extraction. Samples were then placed in deionized water overnight (~12 hours) and then gently separated from soil. Using the reference samples, roots were identified and separated for further morphological and chemical analyses. An additional ~0.2 g of fresh first and second order root material was stored at -80 °C for molecular analysis.

**Chemical and morphological analyses:** Root systems encompassing 3-4 root orders were randomly selected for morphological measurements. Roots were initially dissected into orders (1st, 2nd and 3rd), scanned (800 DPI resolution flatbed scanner, 256-level gray-scale, TIFF format; Epson Scanner Perfection V700 Photo, USA), dried at 65 °C for 48 hr, and then weighed. Morphological measurements including specific root length (SRL,
m/g), root tissue density (RTD, g/cm³) and average diameter (mm) were calculated for each root order using WinRhizo image analysis (2007 Pro version, Regent Instruments, Inc., Quebec, Canada). Additional details are described in Valverde-Barrantes et al. (in review). Total C and N content was determined by dry combustion on a Costech Analytical Model 4010 elemental analyzer (Valencia, CA, USA). Polar (methanol soluble), non-polar (dichloromethane soluble), acid-hydrolysable, and non-acid hydrolysable compounds were determined gravimetrically using a series of extractions and acid digestion modified from Ryan et al (1990). Ash content was determined by incineration of non-acid hydrolysable residue for 4 hr at 500°C. Polar extract soluble polyphenolic concentration was determined by the Folin-Ciocalteu method using tannic acid (Riedel-deHaen, Belgium) as a standard (Singleton et al. 1999), while total reducing sugar concentration was determined using the phenol-H₂SO₄ procedure, utilizing glucose as a standard (Sigma-Aldrich, St Louise, MO, Rao and Pattabiraman 1989). Soluble phenolic and reducing sugar concentrations were estimated by measuring absorbance at 760 and 490 nm, respectively, on a Synergy HT microplate reader (BioTek, Winooski, VT).

**Root system anatomy and microscopic estimation of mycorrhizal colonization:**
Representative samples from each root order in each individual tree were selected for mycorrhizal analysis. Samples were placed in Simport cassettes and cleared with 10% KOH at 65 °C for 12 hours; if roots were not totally cleared they were placed sequentially
for 30 min each in alkaline-H$_2$O$_2$ solution and 10% KOH until cleared. Once cleared, samples were placed for 5 min in 5% acetic acid, stained with 0.05% trypan blue for 10 min and mounted on slides for mycorrhizal quantification (Pit et al 2009). Colonization was determined using the gridline intersect method at 200X magnification following McGonigle et al. (1990). Only areas with arbuscules or vesicles were quantified as colonized by AMF (Brundrett 2009). Trypan blue also stains lignified plant tissues (Vierheilig et al 2005, Fig 1), providing and obvious contrast between the lignified stele and the surrounding parenchymatous tissue. We used this contrast to estimate stele diameter, as well as total root diameter, measuring the cross-sectional length of each tissue at five random points using a calibrated ocular. Cortex area was determined as the difference between the average total root cross-section area and stele area, assuming perfect circles. Finally, the relative amount of vascular tissue per root was calculated as the ratio between stele and total root diameter (Hummel et al 2006, Guo et al 2008).

**Molecular estimation of mycorrhizal colonization:** Roots saved for molecular analysis were ground in a Genogrinder 2000 (SPEX CertiPrep, Metuchen, NJ). The mass of each root sample was determined before DNA extraction. Extractions were performed with the CTAB method following Wu et al. (2011). The 18S ribosomal RNA region was amplified using arbuscular mycorrhizal fungal-specific primers AMV4F (5’–AAGCTCGTAGTTGAATTTCG–3’) and AMDGR (5’–CCCAACTATCCCTATTAATCAT–3’) (Sato et al 2005, Lumini et al 2010). PCRs were
performed with a DNA Engine Dyad Peltier Thermal Cycler (Bio-Rad, Hercules, CA) following Lumini et al (2010).

We initially tested the specificity of the primers by sequencing clone libraries for three randomly chosen samples (> 90% of clones matched Glomeromycota fungi). 90 µL of PCR product were purified using the UltraClean PCR Clean-up kit (MoBio, Carlsbad, CA). Amplicons were then ligated into a pGEM-T vector with overnight incubation and used to transform competent E. coli cells following the manufacturer’s instructions (Promega, Madison, WI). Thirty-two white colonies per sample were used to inoculate wells of LB broth with 10% glycerol. Cultures were grown overnight and stored at -80ºC. Sequencing was performed at the Genome Sequencing Center at Washington University in St. Louis, MO using AMV4F- AMDGR primers. For the sequenced product, plasmid information was removed and amplicon sequences were trimmed for quality in Sequencher (Gene Codes Corporation, Ann Arbor, MI) using default settings. Clustering of sequences into operational taxonomic units (OTUs) was performed using CD-hit tool website (http://weizhong-lab.ucsd.edu/cdhit_suite/cgi-bin/index.cgi). Sequences were only used if they contained both forward and reverse primer sequences or contained one primer and were longer than the longest sequence containing both primers (i.e., longer than 250 bp). This criterion ensures that Blast score variation is not affected by length variation of partially-sequenced amplicons. A percent identity threshold of 97% was used to define OTUs. For OTUs with ≥ 5% relative abundance on
a leaf, nearest neighbors in Genbank were identified using Blast searches on representative sequences (Altschul et al. 1997).

Estimations of AMF hyphal abundance in roots were obtained using SybrGreen-based quantitative PCR (qPCR, Brankatschk et al. 2005) using a Mx3005P thermocycler (Stratagene, La Jolla, CA). Typical PCR reactions were performed using 0.02 U/µL Taq DNA polymerase, 1.5 mM MgCl2, 1X ammonium polymerase buffer (B-Bridge International, Mountain View, CA), 0.2µM each primer (Integrated DNA Technologies, Coralville, IA), 0.16 mM each dNTP, and 0.1 µg/µL bovine serum albumin (New England Biolabs, Ipswich, MA). PCR reaction conditions were: initial denaturation for 3 min at 94 ºC, 40 cycles of denaturation for 45 s at 94 ºC, primer annealing for 45 s at 60 ºC, extension for 60 s at 72 ºC, and a final extension for 7 min at 72 ºC. One of the plasmid samples from the specificity test was purified, and its DNA molecular concentration was quantified with Quant-iT PicoGreen fluorescent stain (Invitrogen, Carlsbad, CA) using a Synergy 2 microplate reader (BioTek, Winooski, VT). Then the purified plasmid was made into a dilution series, and used as a standard in every qPCR run (Brankatschk et al. 2005). We tested for inhibition of amplification by comparing qPCR standard curves for standards alone and after amendment with template DNA extracts. Samples were amplified after dilution to 1/10, 1/100 and 1/500 strength, in order to evaluate the best compromise between avoiding inhibition and maintaining amplification. After this, all samples were run using 1/100 dilutions in duplicates. All
PCR reactions were performed in a Engine Dyad Peltier Thermal Cycler (Bio-Rad, Hercules, CA) and negative controls were included in each PCR run. PCR products were run on a 1.5% agarose gel to confirm successful amplification. For the qPCR reactions we followed the same PCR procedures plus the addition of SYBR Green I and ROX dyes were added at a final concentration of 0.167X and 30nM respectively. After initial denaturation (3 min 95°C), the PCR program consisted of 40 cycles, including an 88°C step for fluorescence quantification, followed by a 7 min extension at 72°C and a melting curve analysis. Amplicon copy numbers were estimated using both Ct values and amplification efficiency over the exponential phase (Kontanis & Reed 2006, Brankatschky et al. 2012). Finally, copy numbers were corrected by the initial root weight in the sample to obtain AMF gene copy number per unit of root mass.

Statistical analysis: All statistical analyses were performed in the R 2.15 statistical platform (R development core team, 2012) using the packages nmle (Pinheiro et al. 2013), ape (Paradis et al. 2004), adephylo (Jombart and Dray 2008), phytools (Ravell 2009) and vegan (Oksanen et al. 2008). Trait variables were log transformed and mycorrhizal colonization was arcsin-transformed before statistical analysis to account for heteroscedasticity and non-normal distribution of residual error. For Hypothesis 1), we tested for a symmetrical reduction in both stele and cortex area (i.e. slope=1) across species, representing a common evolutionary selection for the symmetrical reduction of both tissues (Brakefield 2006, Frankino et al 2007). A slope < 1 will indicated a lower
reduction rate in total cortex area with respect to the vascular tissue, suggesting a preference in the reduction of mycorrhizal habitat. To test for relationships among traits we fitted two separate models to the data, one using ordinary least-squares (OLS), and one using phylogenetic least squares (PGLS) regressions to account for phylogenetic dependence (Duncan et al. 2007). The phylogenetic model was fitted using Pagel’s correlation structure to account for non-independence of residuals (Pagel 1999). We calculated the phylogenetic correlation, lambda (λ), using a residual maximum likelihood method (REML, Paradis 2012) with λ values close to 1 indicating strong phylogenetic dependence in the data. The most appropriate model was chosen using the Akaike Information Criterion (AIC). Since there is not a clear causal relationship between cortex and stele diameter, we also tested the regression using reduced major axes (RMA, Smith 2009) and, to be conservative, only report significance if the relationship was significant for both approaches.

For hypothesis 2), if there is a phylogenetic effect of species lineages on root system integration (i.e., the differentiation among root orders), we expected a significant effect of the interaction between root order and phylogeny (clade). Similar to hypothesis A), we incorporated the phylogenetic non-independence of the data in the analysis. However, because of the multiple levels in the model it was not possible to directly include the phylogenetic variance-covariance matrix in the error structure. Instead, we included clade (i.e. super-order magnoliid, asterid, and rosid), site (Holden or Boone
County Arboretum), and root order as fixed factors and species nested in clades as a random factor that would account for the non-independence of species within clades (Swenson and Enquist 2007, Roumet et al 2008). Then, we tested for the significance of the interaction between root order and phylogenetic group (super-order).

In addition we tested which traits were more influential in the overall trait structuring of fine roots using a multivariate phylogenetic approach (Jombart et al. 2010). First, we created trait matrices by root order employing standardized species mean values (mean equal 0 and standard deviation equal 1). From a previous study we knew that individual traits were highly structured phylogenetically (Valverde-Barrantes et al in review), so we performed a principal component analysis considering the phylogenetic relationship among species (assuming a Brownian motion error structure) as an additional constraint (pPCA, Revell 2009). This analysis accounted for the non-independence among samples but maintained the phylogenetic structure if present (Revell 2009). Therefore, we determined the level of root trait conservatism at the multivariate level testing for phylogenetic structuring after imposing PCA vectors along the phylogeny. Then, phylogenetic signal for the new PCA axis was measured using a K-statistic (a measure of phylogenetic signal that compares the observed signal in a trait to the signal under a Brownian motion model of trait evolution; values close to 1 represent highly conserved traits, Blomberg et al. 2003). The statistical significance of phylogenetic signal was evaluated by comparing the observed PCA K-value to a null model of shuffling taxa
labels across the tips of the phylogenetic tree (999 iterations, Kembel et al 2010). In order to visualize the importance of individual traits on the multivariate structuring of the data, we compared PCA trait loadings assuming that those with higher loadings (higher and lower quartiles) were more influential in the evolutionary patterns observed in the phylogeny (Jombart et al. 2010, Paradis 2012).

For hypothesis 3, the influence of root traits on mycorrhizal colonization was evaluated by redundancy analysis (RDA, ter Braak 1986). Briefly, we created a standardized matrix of root traits for each site as the factor matrix and compared it to the mycorrhizal length colonization following Blanchet et al. (2008). First, a global analysis was performed testing the importance of each root trait in the matrix separately. If significant, a stepwise RDA was performed, removing non-significant factors using increasing explanatory power and increasing adjusted R-squared as stopping criteria (Blanchet et al. 2008). Then, to test for possible interactions among traits, we performed a mixed-model ANCOVA using the subset of significant factors selected from the RDA analysis. Similar to hypothesis 2, we incorporated the phylogenetic non-independence of the data in the analysis using super-order (magnoliid, asterid, and rosid), site (Holden or Boone County Arboretum), and root order as fixed factors and species nested in clades as a random factor and the selected trait variables as co-variables. Then, we performed a stratified subset model, looking for the most parsimonious combination of trait metrics that explained better the distribution of mycorrhizal colonization. Models were compared
using AIC, Bayesian Information Criterion (BIC) and a Chi-square test evaluating whether reductions in the residual sum of squares were statistically significant (Chambers and Hastie 1992). We tested for Hypothesis 3.1 looking for mean colonization differences among clades (Tukey HDS test) and Hypothesis 3.2 testing for the significance of co-variables in the model. Finally, we repeated the same procedure employing the qPCR estimations of AMF abundance as a response factor. However, in this case we did not include a root order factor because we did not separate root orders during DNA extraction.

**Results**

**Hypothesis 1. Comparing changes in stele and cortex area across species:** As hypothesized, we found no evidence of symmetric reduction between cortical and vascular tissue. The relationship between cortex and stele area varied among orders. First order roots showed a positive relationship between cortex and stele area but significantly lower than 1 (phylo-RMA =0.28, p<0.001, Fig 3.2a). The regression was also strongly influenced by the phylogenetic relationship among species (λ=0.68, Table 3.1). Second order roots showed no relationship between cortex and stele area and a weak phylogenetic influence on the regression (λ=0.18, Table 3.1). Third order roots had a slope similar to first order roots (phylo-RMA =0.30, p<0.001, Fig. 3. 2c) but were not influenced by the phylogenetic relationship among species (λ=-0.10, Table 3.1). Species in the magnoliid clade had similar stele but higher cortex area than other species,
particularly in the first two root orders, which contributes to a large difference in the stele:root ratio among clades (Fig S1, Supplemental Table 3.1).

**Hypothesis 2. Integration of root traits across root orders:** From the total 14 traits measured, eight (57%) showed a significant interaction between root order and clade, even after accounting for other factors such as site, and site interactions with other factors (Supplemental Table 1). As predicted, traits showed the expected lower differences across root order within the magnoliid clade when compared to the other clades. For instance, average RTD only increased from 0.13 to 0.18 g/cm$^3$ between first and third root order in magnoliids (27% increase), whereas in asterids went from 0.12 to 0.29 g/cm$^3$ and rosids from 0.13 to 0.30 g/cm$^3$ (57 and 58% increase respectively). Similarly, average root N was not only significantly higher in magnoliids than other clades but also it decreased more slowly along orders. In magnoliids, N content decreased from 2.38 to 1.54 % in first and third order magnoliid roots respectively (35% decrease), whereas asterids and rosids showed a 50% and 40 % decrease respectively (Asterids from 1.94 to 0.96 % and Rosids from 1.63 to 0.95%). In contrast, the proportion of stele with respect to total diameter changed more drastically among rosids (66% increase) than in asterids and magnoliids (44 and 45% respectively, Fig. 3.2d), suggesting a stronger transition from acquisition to structural roots in rosids with respect to other phylogenetic groups. Other traits, particularly those related to root chemistry traits such as lignin, reduced
sugars, phenol and polar compounds, showed similar rates of change across order independent of clade (Supplemental Table 3.1).

**Table 3.1:** Relationship between stele and cortex area among 34 woody angiosperms. Values represent 95% confidence interval for slope values taken from the best-fitting ordinate least square (OLS), phylogenetic general least square (PGLS) and the slope from a Phylogenetic Reduced Major Axis (PhyloRMA) analysis. Lambda (λ) values represent the level of phylogenetic correlation among species. All values were log-transformed.

<table>
<thead>
<tr>
<th>Root Order</th>
<th>OLS</th>
<th>PGLS</th>
<th>Phylo RMA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>R²</td>
<td>Slope</td>
</tr>
<tr>
<td>1st</td>
<td>0.26 - 0.65</td>
<td>0.18</td>
<td>0.36 – 0.89</td>
</tr>
<tr>
<td>2nd</td>
<td>-0.01 - 0.35</td>
<td>0.05</td>
<td>-0.02 – 0.41</td>
</tr>
<tr>
<td>3rd</td>
<td>0.08 – 0.47</td>
<td>0.25</td>
<td>0.19 – 0.44</td>
</tr>
</tbody>
</table>

*Pseudo R-squared for the pglS model involved a comparison of the log-likelihood for the fitted model against the log-likelihood of a null/restricted model with no predictors (Long 1997).

At the multivariate level, the first PCA vector of each root order matrix (representing 42, 43 and 41% of the total variance for first, second and third order roots respectively) was strongly structured by phylogeny (K-values 0.97, 1.05 and 0.84 for first, second and third order respectively). All PCA vectors represented magnoliid species with low and rosids with high eigenvector values, whereas asterids showed intermediate values (Fig. 3.3).

Stele:root ratio, C:N, lignin:N and lignin content showed highly positive values, which correspond with the high lignin values found in rosid roots. In contrast, nitrogen content, cortex area and link length showed high negative values, suggesting that these traits were more influential characterizing magnoliid roots (Fig. 3.3).
**Figure 3.2** Relationship between cortex and stele area for the first three root orders among 34 tree angiosperm species. Panels A, B) and C) represent the relationship for 1st, 2nd and 3rd order respectively. Line represent the calculated reduced major axis slope for each regression, and p-value indicate whether the slope value is different than 1 (See Table 3.1 for more details). Panels D), E) and F) show the mean values for stele:root ratio, stele, and cortex area respectively.
Figure 3. Hypothesized phylogenetic relationships and phylogenetic distribution of PCA eigenvectors calculated for 14 root traits in 3 root orders of 34 woody angiosperms. The phylogenetic tree depicts species eigenvectors representing the PCA ordination of 14 measured variables for each root order. Symbol size represents the eigenvector departure from the mean. Black symbols indicate values above the mean and white symbols value below the mean. The right panel compares loading values for each trait. The dashed line indicates traits included in the lowest and highest quartile.
Hypothesis 3. Relationship between root traits and mycorrhizal colonization:

Mycorrhizal colonization decreased along root order (Fig 4a), ranging from 100% colonization in first order of most magnoliids to < 20 % in third order roots of some species such as *Ilex opaca*, *Liquidambar styraciflua* and *Ulmus americana*. In partial support of Baylis’ hypothesis, we found extensive AMF colonization in magnoliid species, particularly for the first two root orders. However, high length colonization (> 70%) was also detected in first order asterid and rosid roots, which explains the weak phylogenetic signal found in AMF colonization for first order roots (K=0.37, p= 0.06, Fig. 3. 4a). Second order roots showed stronger separation among clades. Rosids in particular exhibited a drastic decrease in AMF colonization (from 73% to 58%), compared to asterids that decrease from 88% to 78% and magnoliids that maintained >85% root length colonization across root order (K=0.63, p = 0.001). At third root order, AMF colonization decreased substantially for all species with averages ranging from 72% in magnoliids to 48% in rosids (K=0.36, p=0.06). Comparing the three orders for all clades, we found significant differences among clades (F=4.96, p = 0.003) but no significant interaction between root order and clade (F=1.13, p=0.76), suggesting that despite overall differences among phylogenetic groups, the decreasing trend from low to high root orders was similar among clades (Fig 3.4a). Root AMF abundance obtained from the qPCR approach was moderately correlated with first and second order AMF colonization (Pearson’s r=0.31 and 0.28, p < 0.01 for first and second order respectively) but not third order roots (Pearson’s r=0.17, p = 0.08). Nevertheless, AMF abundance
followed the same patterns observed in AMF length colonization, with higher abundance in magnoliid and asterid than rosid species (65% and 46% difference on average respectively, F=12.4, p<0.001, K= 0.48, p=0.004, Fig 3.4b).

**Table 3.2:** Summary of the most parsimonious model describing the relationship between mycorrhizal colonization and root traits for 34 woody angiosperm species.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Squares</th>
<th>Mean Square</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mycorrhizal colonization</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Order</td>
<td>2</td>
<td>1.70</td>
<td>0.85</td>
<td>27.20</td>
<td>0.000</td>
</tr>
<tr>
<td>Clade</td>
<td>2</td>
<td>0.39</td>
<td>0.20</td>
<td>6.25</td>
<td>0.002</td>
</tr>
<tr>
<td>Site</td>
<td>1</td>
<td>2.56</td>
<td>2.56</td>
<td>82.11</td>
<td>0.000</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>1</td>
<td>0.53</td>
<td>0.53</td>
<td>17.05</td>
<td>0.000</td>
</tr>
<tr>
<td>Stele:root ratio</td>
<td>1</td>
<td>1.60</td>
<td>1.60</td>
<td>51.15</td>
<td>0.000</td>
</tr>
<tr>
<td>Order:Site</td>
<td>2</td>
<td>0.22</td>
<td>0.11</td>
<td>3.48</td>
<td>0.033</td>
</tr>
<tr>
<td><strong>Mycorrhizal abundance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clade</td>
<td>2</td>
<td>4.238</td>
<td>2.119</td>
<td>4.1</td>
<td>0.022</td>
</tr>
<tr>
<td>SRL</td>
<td>1</td>
<td>2.256</td>
<td>2.256</td>
<td>4.365</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Comparing root traits and AMF colonization, we found that stele:root ratio, root N and reduced sugars were the best explained mycorrhizal length colonization across root orders (most parsimonious RDA F=7.62, p<0.005, explained 53% of variation). However, once we incorporated clade, root order and site information into our model, reduced sugars were no longer significant (F= 0.07, p= 0.93). In contrast, stele:root ratio was the best trait for predicting AMF colonization and was negatively related to the
proportion of length colonized by AMF (F=51.14, p<0.0001, Table 2, adjR²=0.55 for species mean values, Fig 3.5a). Root N was also associated, although positively, with AMF colonization (F=17.05, p<0.0001, adjR²=0.31, Fig 3.5b). The most parsimonious model did not reveal a significant interaction between stele:root ratio and root N, suggesting that these two traits influence AMF colonization independently. Mycorrhizal abundance, based on molecular estimations, was also influenced by root traits (most parsimonious RDA F=7.62, p<0.005, variation explained = 28%). However, in this case only SRL was selected as a significant factor (F=4.48, p<0.04, adjR²=0.17, Fig 3.5c).

**Figure 3.4** Mycorrhizal length colonization and mycorrhizal abundance in roots of 34 angiosperm woody species forming AMF associations. Panel A) represents the mean proportional mycorrhizal colonization (±SE) for each root order among three angiosperm clades. Panel B) indicates the mean AMF amplicon number (±SE) for the AMF-specific primers AMV4F - AMDGR (Lumini et al 2010) for each clade. Average Amplicon abundance was significantly higher in asterid and magnoliid species compared to rosids (ANOVA F=12.4, p<0.001).
Figure 3.5. Relationship between root traits and mycorrhizal colonization in woody angiosperms. Panel A) represents the relationship between stele:root ratio and proportional colonized length. Panel B) represents the relationship between root N content and proportional colonized length. Panel C) represents the relationship between specific root length (SRL) and mean AMF amplicon number per mg of root. Symbols indicate mean trait value per species.
Discussion

Root trait modifications during angiosperm evolution: Substantial differences in root diameter have been widely reported in the literature and are often associated with evolutionary trends in angiosperms (Pregitzer et al. 2002, Withington et al. 2006, Comas et al. 2012). Root development studies often assume that stele and cortex development are coordinated, and therefore total root diameter corresponds to the combined development of both tissues (Hishi 2007, Seago and Fernando 2012). However, few hypotheses have been proposed to explain how diameter reduction arose or how diameter reduction associates with the development of the cortical and vascular tissues in roots (Brundrett 2002). We proposed that the evolutionary decline in root diameter reflects a reduction in cortex area rather than a symmetrical decrease in both cortical and vascular tissues. Our results supported this hypothesis, indicating that stele and cortical cell allometry is not related to internal development constraints, but rather is shaped by different selective forces (Frankino et al 2005). We found surprisingly similar stele diameters across species, indicating that the size of fine root vascular tissue in woody plants has been relatively stable since at least the appearance of the magnoliids (~100 MYA, Crepet et al. 2004). This might reflect the superior transport capacity of angiosperm vessels (Sperry et al. 2007). More studies, including conifer AMF species in particular, would be most valuable to confirm our observations.
First order cortex area decreased approximately three times faster than stele area across species, with relatively low variation in cortex area in most derived clades (i.e. asterids and rosids, Fig 3.2). Magnoliids showed consistently larger cortex area and smaller stele:root ratios across all root orders. This result suggests that the reduction in cortex area in the most distal roots has been consistent among more derived angiosperm groups, indicating the intense selection for either an increase in surface area or reduction in mycorrhizal habitat in the most recent clades. Our results also showed a relatively weak correlation between stele and cortex area ($R^2$ ranging between 0.05-0.25, Table 3.1). Second order roots in particular exhibited no cortex and stele area relationship across species. Moreover, second and third order roots showed a decreasing importance of phylogeny explaining the relationship between cortical and vascular tissues in fine roots (Table 1). These results confirm our hypothesis that angiosperms not only have reduced cortex area more than vascular area over time, but also that the integration of the entire root system is affected by these morphological divergences.

This developmental separation between root tissues could be related to alternative resource acquisition strategies that appear independently among angiosperm lineages. The maintenance of cortical cells is strongly associated with mycorrhizal activity, to the point that the cortex is physiologically inactive in the absence of mycorrhizal fungi (Smith and Smith 1990). Moreover, the AMF association with plants can demand up to 20% of all photosyntates (Litton et al 2007), and AMF colonization is not always
beneficial to the host (Klironomos et al 2003). This energetic demand combined with low nutrient uptake benefits could translate into strong selection pressures to diminish cortical area, particularly in areas where AMF associations are not the most efficient (Fitter 2006). For instance, there are report of plants that posses the capacity to impede cortical development in fine roots. Refer to as beaded roots, these fine roots show a limited cortical development in the 1st and 2nd root orders as a consequence of suberin deposition in the cortex that impede a full development of the tissue, creating a beaded shape (Brundrett et al. 1990, here we observed beaded roots in Acer, Aesculus, Cercidiphyllum, Liquidambar and Ulmus species). In most cases, after beading, roots totally or partially slough their cortex tissue, followed by a sharp transition from acquisition to structural roots (Duhoux et al. 2000). Interestingly, this type of roots has only being reported among rosid species and few temperate conifers, and never in the tropics (Duhoux et al 2000, Brudrett 2002), suggesting environmental and genetic factors involved in the development of this particular type of roots.

In contrast, we found extensive root length still devoted to AMF colonization in most magnoliids and some asterids, even at third root order and possibly higher orders (Xia et al 2012). These observations are consistent with previous order-based observation in temperate tree root systems, including some common asterid genera like Fraxinus and Nyssa (Pregitzer et al. 2002, Guo et al. 2008, Valverde-Barrantes et al, in review). One possible explanation for this pattern is that magnoliid-like roots tend to maximize
physiological activity and acquisition capacity by extending active cortical cells to relatively high root orders. Therefore, they compensate for the relatively low exploitable soil surface area. However, our results are limited to observations and not direct physiological measurements. Further investigation comparing uptake rates and transport capacity are crucial to validate our observations (Comas and Eissesntat 2004, McCormack et al. 2012).

**Root trait integration across root orders**: As an extension of Hypothesis A), we proposed that basal angiosperms have a slower rate of differentiation across root orders, which could be interpreted as reduced specialization across root links. Consistent with this hypothesis we found that most traits, particularly those related to morphology, showed less differentiation across order in magnoliids compared to other groups. Other studies have also shown that the degree of trait differentiation related to link position varies among plants (Guo et al 2008), particularly comparing magnoliid and rosid trees (Pregitzer et al 2002, Chen et al 2012), but never examined from a phylogenetic perspective. The strength in this study is that we examined a representative number of species from far related lineages sharing the same mycorrhizal association (in contrast to the often biased representation of temperate rosid species with both AMF and ectomycorrhizal associations), providing better evidence to confirm previous observed root trait patterns among woody plants. Consistent with previous studies, we found that the stele:root diameter ratio is a key variable distinguishing phylogenetic groups,
probably reflects inherent acquisition resource strategies among plant lineages (Guo et al 2008). Nonetheless, other factors such as N content and lignin:N ratio were also influenced trait syndrome synchronization among clades (Fig 3.3). Our study both confirms initial observations and extends the Baylis’ concept of phylogenetic divergence in angiosperm root evolution, demonstrating that this trait segregation is not limited to diameter, but also chemistry and anatomy at the entire root system.

These results raise important ecological questions about the integration of root systems in woody angiosperms. For instance, how might root trait integration affect belowground C allocation and nutrient cycling? Previous research has shown that root traits such as root diameter, N content and SRL are significantly associated with root longevity in temperate (McCormak et al 2012) and tropical trees (Valverde-Barrantes et al 2007). Because all these traits seem to be relatively conserved within a given phylogenetic group (Comas et al 2012, Valverde-Barrantes et al in review), phylogenetic affiliation could be an important factor explaining root lifespan and therefore the rate and quality of C and nutrient inputs into soils.

The integration of trait syndromes among phylogenetic clades could also have an important impact on root construction cost. Previously, it was assumed that decreases in root diameter were a strategy to reduce root construction cost because smaller diameter exponentially increases root surface area, thereby decreasing root construction cost (Eissenstat 1992, Eissenstat and Yanai 1997). However, this conclusion is only true if
investment in structural tissues is proportional to diameter. Our results, in contrast, demonstrate that species with small diameter roots, such as *Ulmus americana*, invest heavily in structural tissue (i.e. lignification) whereas magnoliids root systems maintain low tissue density and large parenchymatous areas across the first three orders. Thus, investment in root size can be offset by root toughness (McCormack et al. 2012), modifying the assumed linear tradeoff between diameter and construction cost. Additionally, root N content also varies among species and is not necessarily associated with root diameter (Comas and Eissenstat 2009, Chen et al. 2013, Valverde-Barrantes et al in review). N content relates to root physiological activity and respiration (Pregitzer et la 1997), making estimations of total C cost during root construction and maintenance even more complex.

The independent variation between chemical and morphological traits can also have important implications in root decay rates. Root decomposition rates are largely influenced by tissue biochemistry (Silver and Miya 2001, Goebel et al. 2011, Lin et al. 2011) and morphology (Raich et al. 2007). From the results of this study we propose that decay rates will vary substantially within root systems depending on the set of traits exhibited by the species. More specifically, because of the lower functional differentiation, magnoliid roots should show similar decay rates along root orders, whereas differences in decay will be more prominent along rosid root orders. Moreover, different trait syndromes should also influence the quality of C compounds released in
soils. In the case of magnoliid roots, the large cortex area, high N content and low lignification would imply the release of more labile carbon compounds during decay. In contrast, rosid roots with high lignin, low N and small cortex area are more likely to decay slowly (Lin et al 2011) and possibly enter the soil carbon pool as small fragments rather than soluble compounds. If the structuring of fine root systems described here holds for most angiosperms, the representation of phylogenetic groups in tree communities would have a predominant effect on soil carbon dynamics. At global scales, magnoliid and asterid trees tend to be more abundant in tropical areas (Gentry 1988), whereas rosids are largely dominant in temperate areas (Wang et al 2009b). Chen et al (2013) already reported a substantial increase in root diameter from temperate to tropical areas in China, in part associated to the differences in taxonomic composition across forests. We predict the uneven representation of angiosperm lineages will have an important impact in the distribution of root traits at global scales, and largely determine the dominant forms of C allocated belowground.

**Root trait integration and mycorrhizal colonization:** Based on Baylis’ (1975) initial observations, our third hypothesis stated that the phylogenetic segregation in root morphology corresponded with the degree of mycorrhizal dependency among angiosperm lineages. Therefore, we expected that the root trait syndromes exhibited by magnoliid roots would correspond with higher mycorrhizal length colonization and higher fungal abundance per unit of root. Moreover, we expected that the interactions
between chemical and morphological root traits would provide a better explanation of the mycorrhizal status among samples, indicating the existence of complementary mechanisms controlling mycorrhizal proliferation in roots. As expected, our results confirmed contrasting patterns in mycorrhizal colonization between magnoliid and rosid species, with asterids showing intermediate values. More interesting, stele: root diameter ratio and root N content, both highly influential traits describing differences between angiosperm clades, were also the most important traits associated with mycorrhizal colonization.

If patterns in trait integration and mycorrhizal colonization found in this study reflect a general trend among woody angiosperms, it could have important implications in our understanding of root ecology at global scales. What is considered a “fine root” (i.e. the portion of the root system specialized in nutrient and water acquisition, Pregitzer 2002), for instance would be determined by the evolutionary context of the species or group of species in a community. In temperate areas for example, it has been assumed that the first two root orders comprised most of the roots specialized in nutrient acquisition (Comas and Eissenstat 2009, Espeleta et al 2009). However, this result could be influenced by the biased dominance of rosid families in temperate forest (i.e Fagaceae, Rosaceae, Sapindaceae, Zhang et al 2011). Increases in diversification and particularly the higher abundance of magnoliids in other ecosystems could change this perspective. In tropical areas is plausible that higher orders with thicker diameters will more commonly
show signs of high metabolic activity, shifting our current perception of mycorrhizal activity limited to root tips.

The large variation is root morphology and trait integration across root systems could have important implications for the assemblage of AMF communities. Recent molecular evidence suggests that AMF community assemblages are more specific than previously thought, challenging the preconceived idea of low specificity among plants and AMF (Rosendahl 2008). Studies in natural conditions showed that even when several AMF species can coexist in an array of hosts, the AMF community composition was not random among hosts (Vanderkoonhuyse et al. 2002, Scheublin et al. 2004). Such community specialization could be related to the apparent distinctive functional roles among AMF lineages and its complementarity with root morphology (Maherali and Klironomos 2007). In fact, Öpik et al. (2010) showed that the taxonomic identity of plant host at the super-order level has a significant effect explaining AMF communities and similar geographical distribution between plant and AMF groups. Molecular mechanisms of fungal partners and gene activation between plants and symbiotic organisms have been largely described (Markmann et al 2008). Those studies have revealed unique gene assemblages in rosids that make this group able to recognize and associate with a broader ranges of symbionts compared to other angiosperm lineages (Markmann and Paniske 2008). However, how these genes affect root development and trait syndromes as not been properly addressed. Future studies relating evolutionary paths
in symbiont recognition and root structural development, as well as the inclusion of basal angiosperms, seem to be a promising field in the understanding of root-mycorrhiza symbiosis.

We conclude that root structural and chemical evolution in woody angiosperms has been substantially constrained by their level of compatibility with AMF communities (Brundrett 2002). Even the integration of traits along root links seems to be influenced by the level of mycorrhizal dependency among species, probably as a compensatory measure for the reduction in surface area bound to the provision of large cortex areas. Evolutionary advances in root morphology and chemistry confer more derived plant groups the ability for greater control on AMF colonization, usually expressed as their confinement to the most distal tips in the root system. Our work therefore highlights the utility that phylogenetic information can provide to root ecology and its implications in the understanding of belowground processes.

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**Supplemental Table 3.1.** Analysis of variance comparing clade (Super-order) and tissue differences for all traits measured in 34 angiosperm tree species grown in two different common gardens. Values correspond with F-values from the mixed model ANOVA analysis. Bold values indicate significant factors for p< 0.05. SRL= Specific root length, RTD=Root tissue density.

<table>
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<th>Chemical traits</th>
<th>DF</th>
<th>Phenol</th>
<th>Sugar</th>
<th>Lignin</th>
<th>N</th>
<th>C:N</th>
<th>Lig:N</th>
<th>Cellulose</th>
<th>Polar</th>
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<th>Stele</th>
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<td>1.06</td>
<td>0.28</td>
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**Supplemental Table 3.2.** Loading values for the first five PC axes calculated from the ordination of 14 root trait variables measured in three root orders of 34 woody angiosperms. K-values indicate the phylogenetic signal observed in each axis. Bold values indicate that the phylogenetic signal was higher than that expected from a random phylogenetic association.

| Root trait | PC1  | PC2  | PC3  | PC4  | PC5  | PC1  | PC2  | PC3  | PC4  | PC5  | PC1  | PC2  | PC3  | PC4  | PC5  | PC1  | PC2  | PC3  | PC4  | PC5  |
|------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Polar      | -0.53| -0.33| -0.61| 0.22 | -0.09| 0.19 | -0.67| 0.49 | 0.26 | 0.30 | -0.38| -0.72| 0.06 | -0.32| 0.39 |      |      |      |      |      |
| Phenol     | 0.23 | -0.43| -0.62| 0.03 | 0.06 | -0.70| -0.39| 0.36 | -0.03| 0.12 | 0.51 | -0.56| 0.19 | -0.25| 0.19 |      |      |      |      |      |
| Sugars     | -0.22| 0.08 | -0.60| -0.40| -0.32| -0.37| -0.20| 0.70 | -0.15| 0.17 | 0.18 | -0.64| 0.26 | -0.05| 0.05 |      |      |      |      |      |
| Cellulose  | -0.43| 0.52 | 0.29 | 0.50 | 0.23 | 0.69 | 0.51 | 0.01 | 0.12 | 0.06 | -0.30| 0.14 | 0.34 | 0.82 | -0.13|      |      |      |      |      |
| Lignin     | 0.74 | -0.17| 0.22 | -0.56| -0.11| -0.77| 0.18 | -0.37| -0.34| -0.23| 0.58 | 0.53 | -0.28| -0.24| -0.35|      |      |      |      |      |
| Nitrogen   | -0.63| 0.39 | -0.02| -0.51| 0.35 | 0.78 | 0.13 | 0.18 | -0.49| 0.09 | -0.84| 0.30 | 0.18 | -0.04| 0.11 |      |      |      |      |      |
| C:N ratio  | 0.78 | -0.41| -0.07| 0.41 | -0.12| -0.91| -0.14| -0.15| 0.28 | 0.04 | 0.87 | -0.33| -0.16| 0.16 | -0.04|      |      |      |      |      |
| Lignin:N ratio | 0.89 | -0.34| 0.06 | -0.01| -0.16| -0.95| -0.09| -0.22| 0.06 | -0.12| 0.91 | -0.12| -0.26| 0.02 | -0.20|      |      |      |      |      |
| Diameter   | -0.45| -0.64| 0.45 | -0.01| -0.32| 0.54 | -0.67| -0.40| 0.21 | 0.05 | -0.38| -0.65| -0.22| 0.04 | -0.22|      |      |      |      |      |
| SRL        | 0.45 | 0.75 | 0.12 | -0.05| -0.07| -0.07| 0.89 | -0.14| 0.17 | 0.26 | 0.04 | 0.85 | -0.02| -0.23| 0.14 |      |      |      |      |      |
| RMD        | -0.36| -0.76| -0.27| 0.04 | 0.22 | -0.40| -0.67| 0.20 | -0.06| -0.39| 0.49 | -0.40| 0.40 | 0.30 | -0.17|      |      |      |      |      |
| Link       | -0.55| -0.50| 0.15 | -0.12| 0.28 | 0.47 | -0.51| -0.22| -0.49| 0.05 | -0.65| -0.17| -0.28| 0.20 | 0.01 |      |      |      |      |      |
| Cortex area| -0.55| -0.48| 0.46 | -0.03| -0.41| 0.54 | -0.59| -0.45| 0.22 | 0.03 | -0.48| -0.33| -0.68| 0.05 | -0.23|      |      |      |      |      |
| Stele area | 0.06 | -0.75| 0.33 | -0.12| 0.33 | -0.40| -0.51| -0.51| -0.21| 0.47 | 0.16 | -0.05| -0.77| 0.33 | 0.44 |      |      |      |      |      |
| Stele: root ratio | 0.76 | -0.30| 0.01 | -0.03| 0.51 | -0.82| 0.28 | -0.08| -0.14| 0.36 | 0.62 | 0.29 | 0.01 | 0.31 | 0.56 |      |      |      |      |      |
| Eigenvalue | 2.28 | 1.55 | 1.10 | 0.85 | 0.70 | 6.43 | 3.30 | 1.90 | 0.80 | 0.75 | 6.19 | 2.53 | 1.88 | 1.14 | 0.89 |      |      |      |      |      |
| Proportion Explained | 0.46 | 0.21 | 0.11 | 0.06 | 0.04 | 0.43 | 0.22 | 0.13 | 0.05 | 0.05 | 0.41 | 0.17 | 0.13 | 0.08 | 0.06 |      |      |      |      |      |
| K-value    | **0.97** | **0.66** | **0.62** | **0.38** | **0.40** | **1.05** | **0.39** | **0.38** | **0.50** | **0.32** | **0.84** | **0.31** | **0.68** | **0.43** | **0.27** |
CHAPTER FOUR: THE DISTRIBUTION OF BELOWGROUND TRAITS IS EXPLAINED BY INTRINSIC SPECIES DIFFERENCES AND INTRASPECIFIC PLASTICITY IN RESPONSE TO ROOT NEIGHBORS

Paper submitted to Journal of Ecology

Summary
1. Large variation in tree root architecture and morphology has been reported for temperate forest communities. However, it is not clear if this variation is an adaptation of species to specific soil properties or represents alternative resource acquisition strategies among co-occurring species. 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 northeast 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 among coexisting species were more important

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1 This paper is already published as Valverde-Barrantes OJ, Smemo KA, Feinstein LM, Kershner MW, Blackwood CB. 2013. Journal of Ecology 101, 933–942
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 among coexisting species is the result of inherent differences in root traits among species and plasticity-mediated responses to neighbors. Hence, the large variation in root traits seems to allow individuals to exploit distinct niches in the same area rather than reflecting adaptations to specific soil properties.
**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 aboveground tissues (Litton *et al.* 2007). Although belowground niche partitioning is regarded as a fundamental factor explaining plant species co-existence (Hutchings *et al.* 2003, Silvertown 2004), few *in situ* ecological studies have evaluated the role of belowground components structuring plant communities (Casper *et al.* 2003) and only a handful of recent studies have resolved species-specific root patterns in mixed forest communities (Comas and Eissenstat 2009, Espeleta *et al.* 2009, Meinen *et al.* 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 belowground community dynamics also precludes inferences about the role of fine root traits in plant community assembly (Westoby and Wright 2006).

A central goal in plant ecology is to understand the factors associated with morphological trait variation in plant populations and communities (Mooney and Dunn 1970, Grime 2006). At the population level, individuals vary their phenotype in order to cope with environmental heterogeneity (i.e., plasticity *sensu* Valladeres *et al.* 2007), but the magnitude of both interspecific and intraspecific trait variation in natural communities has been rarely quantified (Lake and 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 and 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 et al. 1998, Cornwell and 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, have found that deterministic (i.e., competitive and filtering mechanisms) rather than stochastic processes are involved in the landscape-scale distribution of community traits (Ackerly and Cornwell 2007, Kraft et al. 2008). Abiotic factors, such as moisture availability, were correlated with aboveground community-aggregated trait shifts along environmental gradients, and this change was correlated with species turnover (Cornwell and Ackerly 2009). These results suggest that filtering effects are more important than competitive
interactions or intraspecific trait plasticity. The distribution of belowground plant traits with respect to environmental gradients remains mostly uninvestigated (Schenk 2006). Roots have evolved as highly plastic and interactive organs (Brundrett 2002), capable of responding physiologically and morphologically to both biotic and abiotic factors (Hodge 2004, Wijesinghe et al. 2005). In fact, they often express traits, particularly among woody plants, not necessarily correlated with leaf trait syndromes (Comas and Eissenstat 2004, Withington et al. 2006), which has limited our understanding of the factors affecting root trait tradeoffs.

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 (Espeleta et al. 2009, Alvarez-Uria and Korner 2011, Holdaway et al. 2011, Comas and Eissenstat 2004, 2009). 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 neighbor species where roots were found. A few studies have shown alternative root morphological patterns between communities established in contrasting environmental conditions (Taub and Golberg 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, moisture gradients (Richter 2007, Kandza and Schmid 2009, Coleman et al. 2009) or mycorrhizal colonization (Seifert et al. 2009). Finally, the existence of highly contrasting root traits among co-occurring species has also been demonstrated (Pregitzer et al. 2002, Roumet et al. 2006, Comas and Eissenstat 2009), suggesting some degree of niche segregation within communities.

In addition to environmental effects, the distribution of belowground 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 and Casper 2000, Gersani et al. 2001, Bartelheimer et al. 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 non-random, even spacing of traits among species in direct competition (Stubbs and Wilson 2004). This pattern could be generated through competitive exclusion from a site, or trait plasticity in response to competition (Cornwell and 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 if competitive interactions lead coexisting species to exploit distinct niches, enhancing morphological differentiation among them (Fitter 1991).

To distinguish among 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 if the observed variation was related to soil properties and/or the identity of root neighbors. 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. This 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. 3) 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.

Methods
Study site and data collection: We conducted the study in Jennings Woods, a 30 ha forest in northeast Ohio, USA, which was subjected to selective timber extraction during the 1920-30’s 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 Endoaquept) more common along riverbeds. Typical soil profiles are comprised of an 11-16 cm deep A horizon, 12-16 cm B horizon and 18-27 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 understory is dominated by *Carpinus caroliana, Hamamelis virginiana* and *Lindenia benzoin*. Very fine root biomass (VFRB, roots <1mm in diameter) ranged from 290 to 526 g m\(^{-2}\) between bottomland and upland areas, with no significant differences among sites. The uppermost 15 cm accumulated 45 to 61% of the total VFRB with a shallower distribution of root biomass in upland areas. Despite differences in root distribution among sites, we did not find evidence of niche segregation by depths for the tree species studies (sampling up to 60 cm deep, n=44, details in Valverde-Barrantes et al., in prep, Chapter 5).

Samples were collected from 130 plots (2.5 x 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 x 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 hours in deionized water and sieved through # 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 (< 1mm in diameter) and fine (1-3 mm in diameter) categories. Very fine root clusters < 10 cm in length and that generally included three orders of non-lignified roots attached to a woody central segment (4th order) were selected for species identification and trait measurements. On average, 7 ± 2 root system cluster samples were extracted from each core and represented 21.6 ± 10.8% of the total very fine roots from each core.

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 analyzed seven different root traits: four morphological traits typically associated with tradeoffs 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 et al 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 et al. 2003, Leuschner et al. 2004, Meinen et al. 2009) and average link length (Pregitzer et al. 2002, Dupuy et al. 2010)]. On average, non-woody 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. Reference barcodes were generated by amplifying the plastid gene \textit{trnL} and the non-coding spacer region \textit{trnH-psbA} (Kress \textit{et al.} 2005), from leaves of 33 canopy species and some abundant understory species such as \textit{Hammamelis virginiana}, \textit{Podophyllum peltatum}, \textit{Symlocarpus foetidus}, and \textit{Thelypteris noveboracensis}. We combined RFLP profiles of both genes using GelCompar II software (version 4.5, Applied Maths BVBA, Belgium) in order to generate a library with enough resolution to distinguish most species, at least to the genus level. RFLP patterns obtained from unknown roots were compared to 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 analyzed as described above, the rest of the root biomass in each core was also carefully removed from the soil and all biomass \textgreater 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 was obtained from five 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 two 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 neighboring 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 and 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$\times$soil factor interactions. Then, the model with selected soil variables was compared to 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 among species, whereas the interaction between species and soil properties was interpreted as variation among 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. 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 et al. (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 stepwise 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 et al. 2008). Variance partitioning among soil abiotic factors and root neighbors 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 and Cornwell 2007).
Testing of filtering and competitive trait displacement effects: 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 et al. 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 and Tilman 1994). Therefore, we would expect lower nearest neighbor standard deviation (NNSD) and lower kurtosis values than those estimated from a null distribution. Since 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.

Competitive trait displacement was tested by estimating kurtosis and NNSD values for soil cores with two or more species (84 samples). NNSD was calculated for a soil core by sorting species trait values within the core and considering ‘neighbor distance’ to be the difference between two adjacent members in the sorted list. NNSD for
a soil core is the standard deviation of these neighbor distances. To determine whether
the observed kurtosis and NNSD for a given core was smaller than expected under the
null distribution of random spacing, we created 999 randomly assembled plots holding
species richness constant. The P-value for each core was calculated as the number of
times the simulated assemblage kurtosis or NNSD values were lower than the observed.
To exclude any bias in this procedure from potential habitat filtering, species selection for
a given core was limited to those species whose PCA_fertility ranges encompassed the
value found in the given core (“potential community members” sensu Cornwell and
Ackerly 2009). A significance value for assembly of root traits across the entire site was
then estimated following Stouffer’s method as above (Whitlock 2005).

*Checkerboard patterns test procedure*. Using the presence/absence matrix for all sampled
sites, we compared our observed species distribution patterns against 99 randomly
assembled communities following the null model randomization procedure recommended
by Gotelli (2000). Simulated matrices were assembled maintaining row and column sums
(SIM9 model, Gotelli (2000); see swap modification (Miklós and Podani 2004) and
matrices were compared using both the C-score (Stone and Roberts 1990) and the more
conservative CHECKER index (Gotelli 2000, Diamond 1975). P-values were estimated
as the proportional number of times that the simulated checkerboard index was higher
than the observed index.
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 among species (Tables 4.1 and 4.2). 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 and Eissenstat 2009, Meinen et al. 2009). Species exhibited a relatively broad tolerance to varying soil properties (as defined by Ackerly and Cornwell 2007), with only a few genera, such as Ulmus and Liriodendron, restricted to relatively narrow ranges of soil properties.
Table 4. Root trait mean (standard deviation) for 8 variables measured 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), root diameter and average length for each species. Diameter values were limited to first order roots. Mycorrhizal types: arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal (ECM)

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Mycorrhizal type</th>
<th>SRL (m g⁻¹)</th>
<th>SRA (cm² g⁻¹)</th>
<th>RTD (g cm⁻³)</th>
<th>Diameter (mm)</th>
<th>SRTA (tips mg⁻¹)</th>
<th>Fractal</th>
<th>Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acer rubrum</td>
<td>159</td>
<td>AMF</td>
<td>26.55 (14.18)</td>
<td>460.76 (234.23)</td>
<td>0.20 (0.11)</td>
<td>0.40 (0.08)</td>
<td>3.32 (2.04)</td>
<td>1.48 (0.06)</td>
<td>0.26 (0.11)</td>
</tr>
<tr>
<td>Acer saccharum</td>
<td>219</td>
<td>AMF</td>
<td>30.47 (16.54)</td>
<td>528.55 (272.58)</td>
<td>0.18 (0.12)</td>
<td>0.41 (0.06)</td>
<td>3.71 (2.20)</td>
<td>1.46 (0.05)</td>
<td>0.29 (0.10)</td>
</tr>
<tr>
<td>Betula alleghaniensis</td>
<td>5</td>
<td>ECM</td>
<td>26.49 (15.23)</td>
<td>432.15 (213.13)</td>
<td>0.22 (0.16)</td>
<td>0.37 (0.04)</td>
<td>2.80 (1.73)</td>
<td>1.54 (0.06)</td>
<td>0.28 (0.24)</td>
</tr>
<tr>
<td>Carpinus caroliniana</td>
<td>16</td>
<td>ECM</td>
<td>20.89 (17.32)</td>
<td>381.23 (267.17)</td>
<td>0.21 (0.07)</td>
<td>0.39 (0.05)</td>
<td>2.78 (2.50)</td>
<td>1.51 (0.05)</td>
<td>0.22 (0.05)</td>
</tr>
<tr>
<td>Carya cordifolia</td>
<td>20</td>
<td>ECM</td>
<td>23.88 (20.24)</td>
<td>441.28 (372.94)</td>
<td>0.22 (0.11)</td>
<td>0.38 (0.05)</td>
<td>4.06 (3.97)</td>
<td>1.52 (0.04)</td>
<td>0.19 (0.04)</td>
</tr>
<tr>
<td>Carya ovata</td>
<td>99</td>
<td>ECM</td>
<td>20.73 (10.79)</td>
<td>366.39 (168.16)</td>
<td>0.24 (0.12)</td>
<td>0.36 (0.06)</td>
<td>2.88 (1.74)</td>
<td>1.50 (0.07)</td>
<td>0.22 (0.08)</td>
</tr>
<tr>
<td>Fagus grandifolia</td>
<td>65</td>
<td>ECM</td>
<td>17.97 (11.73)</td>
<td>317.25 (165.78)</td>
<td>0.28 (0.16)</td>
<td>0.37 (0.07)</td>
<td>2.09 (1.35)</td>
<td>1.47 (0.05)</td>
<td>0.28 (0.09)</td>
</tr>
<tr>
<td>Fraxinus americana</td>
<td>45</td>
<td>AMF</td>
<td>19.07 (11.12)</td>
<td>394.08 (207.72)</td>
<td>0.18 (0.08)</td>
<td>0.48 (0.08)</td>
<td>1.95 (1.11)</td>
<td>1.49 (0.06)</td>
<td>0.35 (0.11)</td>
</tr>
<tr>
<td>Fraxinus nigra</td>
<td>27</td>
<td>AMF</td>
<td>26.99 (15.19)</td>
<td>478.30 (260.47)</td>
<td>0.21 (0.14)</td>
<td>0.45 (0.09)</td>
<td>1.67 (0.73)</td>
<td>1.43 (0.06)</td>
<td>0.51 (0.21)</td>
</tr>
<tr>
<td>Liriodendron tulipifera</td>
<td>7</td>
<td>AMF</td>
<td>10.46 (3.89)</td>
<td>306.31 (115.69)</td>
<td>0.16 (0.08)</td>
<td>0.73 (0.05)</td>
<td>0.68 (0.30)</td>
<td>1.50 (0.04)</td>
<td>0.74 (0.41)</td>
</tr>
<tr>
<td>Platanus occidentalis</td>
<td>5</td>
<td>AMF</td>
<td>27.91 (20.21)</td>
<td>516.87 (316.93)</td>
<td>0.17 (0.08)</td>
<td>0.40 (0.10)</td>
<td>2.75 (2.24)</td>
<td>1.45 (0.03)</td>
<td>0.34 (0.06)</td>
</tr>
<tr>
<td>Prunus serotina</td>
<td>30</td>
<td>AMF</td>
<td>19.60 (9.81)</td>
<td>381.05 (162.00)</td>
<td>0.20 (0.09)</td>
<td>0.52 (0.10)</td>
<td>1.57 (0.89)</td>
<td>1.43 (0.06)</td>
<td>0.55 (0.20)</td>
</tr>
<tr>
<td>Quercus rubra</td>
<td>23</td>
<td>ECM</td>
<td>21.17 (10.81)</td>
<td>355.45 (164.00)</td>
<td>0.26 (0.15)</td>
<td>0.36 (0.07)</td>
<td>2.50 (1.25)</td>
<td>1.46 (0.04)</td>
<td>0.24 (0.07)</td>
</tr>
<tr>
<td>Ulmus sp</td>
<td>18</td>
<td>AMF</td>
<td>49.28 (31.46)</td>
<td>868.58 (521.32)</td>
<td>0.11 (0.06)</td>
<td>0.37 (0.05)</td>
<td>6.35 (4.91)</td>
<td>1.48 (0.04)</td>
<td>0.25 (0.08)</td>
</tr>
<tr>
<td>Entire community</td>
<td>738</td>
<td></td>
<td>25.86 (15.48)</td>
<td>459.86 (257.86)</td>
<td>0.20 (0.12)</td>
<td>0.40 (0.09)</td>
<td>3.44 (2.17)</td>
<td>1.47 (0.06)</td>
<td>0.30 (0.15)</td>
</tr>
</tbody>
</table>
Table 4.2 ANCOVA results describing effects of species identity and fine root trait plasticity along soil gradients (n = 234).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Biomass</th>
<th>SRL</th>
<th>SRA</th>
<th>Diameter</th>
<th>RTD</th>
<th>SRTA</th>
<th>Fractal</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil factor</td>
<td>***</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td></td>
<td>***</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Species (intercept)</td>
<td>NS</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Soil factor *Species</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Model adjusted $R^2$</td>
<td>0.38</td>
<td>0.24</td>
<td>0.19</td>
<td>0.30</td>
<td>0.12</td>
<td>0.32</td>
<td>0.25</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*Biomass, SRL, SRA, Diameter, RTD, and SRTA were significantly related with PCA_fertility. Fractal was related with % moisture, and Length with Proportional POM Carbon. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$
Intrinsic trait differences among species (i.e. intercept differences reflecting a degree of canalization) was the most important factor explaining fine root trait variation at the individual plant scale (ANCOVA test, $P < 0.001$, Table 4.2 and Figs. 4.1a and S4.1). In addition to the species-intercept effect, PCA_fertility was also significantly related to variation in all traits except fractal dimension (related to percent moisture) and average link length (related to POM C) (AIC-based factor selection; post hoc ANCOVA test $P < 0.05$, Table 4.2 and Figs. 4.1a and S4.1). With the exceptions of SRL and average link length, the interactions between species and the relevant soil properties were not significant (Table 4.2), indicating that intraspecific plasticity in response to soil properties was similar across species, despite their broad differences in morphology, mycorrhizal association and phylogenetic background (Fig. 4.1a and S4.1).

Trait variation within each species was also analyzed separately in order to incorporate the possible effects of neighboring roots of other species due to competitive displacement. In addition to the expected influence of soil properties on trait values (affecting 6 out of 14 species), the presence of other species also affected root trait variation in 8 of the 14 species analyzed ($P < 0.05$). In fact, some species such as *Quercus rubra*, were influenced more by biotic interactions than soil factors, suggesting that co-existing species can differentially affect the level of trait plasticity among neighbors (Fig. 4.2a, Fig. S4.3). Results were species-specific, with variance explained by either neighboring roots or soil factors ranging from 0 to 24% (Fig. 4.2a, Fig. S4.3).
Figure 4. 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 4.2), 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.
Figure 4. 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.
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 Fig. 4.1a and Fig. S1, Table 4.3). Trait values for SRL, SRA and SRTA were significantly associated 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 4.4), 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 out 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 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 is a product of root plasticity in response to neighboring 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.

**Table 4.3.** Least square mean fit using species means and soil fertility scores (Fertility PCA axis) for each species in Jennings Woods (n = 14)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Intersection</th>
<th>Slope</th>
<th>R²</th>
<th>R²(adj)</th>
<th>F</th>
<th>Prob(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRL</td>
<td>23.53</td>
<td>4.05</td>
<td>0.43</td>
<td>0.38</td>
<td>8.92</td>
<td>0.01</td>
</tr>
<tr>
<td>SRA</td>
<td>430.08</td>
<td>56.26</td>
<td>0.35</td>
<td>0.30</td>
<td>6.47</td>
<td>0.03</td>
</tr>
<tr>
<td>Diameter</td>
<td>0.63</td>
<td>-0.03</td>
<td>0.17</td>
<td>0.10</td>
<td>2.41</td>
<td>0.15</td>
</tr>
<tr>
<td>RTD</td>
<td>0.21</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.02</td>
<td>0.89</td>
</tr>
<tr>
<td>Fractal</td>
<td>1.48</td>
<td>0.00</td>
<td>0.01</td>
<td>0.00</td>
<td>0.06</td>
<td>0.80</td>
</tr>
<tr>
<td>SRTA</td>
<td>2.73</td>
<td>0.71</td>
<td>0.38</td>
<td>0.33</td>
<td>7.43</td>
<td>0.02</td>
</tr>
<tr>
<td>Length</td>
<td>0.34</td>
<td>-0.05</td>
<td>0.15</td>
<td>0.07</td>
<td>2.04</td>
<td>0.18</td>
</tr>
</tbody>
</table>

**Community-aggregated traits:** Community-aggregated traits showed shifts along soil condition gradients; although in many cases the patterns were relatively weak (adjusted R² ranged from 0 to 0.11 (Fig. S4.2) compared to 0.12 to 0.50 for trait variation at the species level (Table 4.2)). The community showed a tendency to increase biomass and
decrease root tip abundance along the soil fertility gradient (Fig. S4.2), as well as decrease SRL and SRA and increase RTD with percent moisture, whereas soil factors had no effect on root diameter, fractal dimension or average link length (Fig. 4.1b and S4.2). 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. 4.2b).

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

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Soil filtering‡</th>
<th>Competitive trait displacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trait</td>
<td>Variance</td>
<td>Range</td>
</tr>
<tr>
<td>SRL</td>
<td>0.25</td>
<td>0.41</td>
</tr>
<tr>
<td>SRA</td>
<td>0.23</td>
<td>0.42</td>
</tr>
<tr>
<td>Average Diameter</td>
<td>0.18</td>
<td>0.44</td>
</tr>
<tr>
<td>RTD</td>
<td>0.24</td>
<td>0.46</td>
</tr>
<tr>
<td>Fractal</td>
<td>0.23</td>
<td>0.37</td>
</tr>
<tr>
<td>SRTA</td>
<td>0.24</td>
<td>0.42</td>
</tr>
<tr>
<td>Length</td>
<td>0.23</td>
<td>0.37</td>
</tr>
</tbody>
</table>

‡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 to 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 neighbor standard deviation. It was estimated by sorting species trait values within the core and considering ‘neighbor distance’ to be the difference between two adjacent members in the sorted list

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 4.2), 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 et al. 1989, Comas and Eissenstat 2009, Meinen et al. 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 neighbor species (Table 4.4, Fig. 4.2 and Fig. S4.3). Moreover, community-aggregated traits were primarily structured by species composition (Fig. 4.2b), because species with intrinsically different root traits
were distributed across the entire soil gradient and responded to other species within a soil core (Table 4.4). Our results also highlight the utility of using molecular methods to measure root neighborhood composition under natural conditions (Jones et al. 2011).

**Plasticity-mediated competitive trait displacement belowground:** 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, although it is unclear whether this is the direct result of competitive interactions among these species or the outcome of divergent evolutionary histories. The responses of some species to the identities of root neighbors and the even distribution of traits within cores suggest that some degree of plasticity in root systems corresponds with trait shifts, possibly avoiding trait overlap. In contrast, previous studies have linked different root morphologies as adaptations to alternative soil properties (Taub and Goldberg 1996, Neatour et al. 2007, Espeleta et al. 2009) or alternative mycorrhizal associations (Guo et al. 2008, Comas and Eissenstat 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 et al. 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 et al. 1996, Bezemer et al. 2010).

The ability of roots to recognize the identity of neighboring root systems, and in turn modifying allocation and morphology, has been reported previously under controlled conditions (Caldwell et al. 1996, Gersani et al. 2001, Callaway 2002, Dudley et al. 2008), but rarely evaluated against the effect of soil gradients and neighbor interactions in natural conditions. Our results suggest that competitive trait displacement is an important process underlying belowground species coexistence in the same location. This contradicts previous studies focused on aboveground trends, where species turnover had a strong effect on leaf trait distribution (Kraft et al. 2008, Cornwell and Ackerly 2009). This disagreement suggests either that contrasting processes shape the distributions of above and belowground community traits (such as greater symmetry of competition belowground compared to aboveground (Cahill and Casper 2000)) or that there is unmeasured variation in aboveground traits at a scale smaller than what has been considered in previous studies (as suggested by Lake and Ostling 2009). It also implies that diverse fine root morphologies could play an important role in differentiating resource use belowground, complementing the reported aboveground trait similarity among coexisting species (Kraft et al. 2008). Future studies should address the role that root-root interactions play in regulating species coexistence and structuring plant communities belowground.
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 et al. 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 and Pregitzer 2009), as well as variations in depth distribution among coexisting species (Hertel et al. 2001). Hence, alternative root morphologies may be segregated at a vertical scale not quantified in this study, implying that the observed even trait distributions could be the result of avoidance and partitioning by soil horizon. Future studies on root colonization by differing species at different soil depths could help elucidate the main mechanisms involved in root trait distributions in forest communities. 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 belowground.

**Controls on community-aggregated root traits:** We found that community-aggregated traits were better explained by species composition than soil properties, and 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 et al. 2011). Furthermore, our study highlights the need to expand the description of root traits within and between species in diverse ecosystems in order 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 belowground processes such as root mortality, carbon allocation, and soil carbon turnover (Litton et al. 2007, Klumpp and Soussana 2009), more detailed studies of interspecific interactions and intraspecific plasticity may be necessary. For example, our study suggests that more detailed descriptions of belowground species interactions could improve the understanding of diversity effects on belowground productivity (Cadotte et al. 2009, Mommer et al. 2010).

**Conclusion:** Our study indicates 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. If, within the context of large trait differences among species, competitive root
trait displacement and lack of soil filtering effects are pervasive among coexisting species, it would imply that belowground 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.

**Acknowledgments:** We thank Hafiz Maherali for his comments on this manuscript. We also thank Chris Dejelo, Eugene Ryee, and Melissa Brewster for their invaluable assistance during field work and image analysis. We are also grateful to Seth Brown and Charlotte Hewins for their assistance in the analysis of soil samples.

**References**


Kembel S. W., de Kroon H., Cahill Jr., J. F., Mommer L. (2008) Improving the scale and precision of hypotheses to explain root foraging ability. Ann Bot 101: 1295-1301


**Supplement Table S1.** Summary of the variables measured for the description of soil conditions in Jennings Woods

<table>
<thead>
<tr>
<th>Variable</th>
<th>Average</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Soil C (%)</td>
<td>4.94</td>
<td>2.31</td>
<td>0.66</td>
<td>18.02</td>
</tr>
<tr>
<td>Total Soil N (%)</td>
<td>0.32</td>
<td>0.11</td>
<td>0.06</td>
<td>0.90</td>
</tr>
<tr>
<td>C:N</td>
<td>14.95</td>
<td>2.49</td>
<td>9.17</td>
<td>22.84</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>29.49</td>
<td>8.44</td>
<td>8.20</td>
<td>52.22</td>
</tr>
<tr>
<td>pH</td>
<td>4.84</td>
<td>0.80</td>
<td>3.59</td>
<td>7.57</td>
</tr>
<tr>
<td>Particulate Organic Matter C (%)</td>
<td>2.23</td>
<td>1.30</td>
<td>0.13</td>
<td>10.09</td>
</tr>
<tr>
<td>Particulate Organic Matter N (%)</td>
<td>0.12</td>
<td>0.07</td>
<td>0.00</td>
<td>0.47</td>
</tr>
<tr>
<td>POM C: POM N</td>
<td>18.81</td>
<td>3.31</td>
<td>7.75</td>
<td>25.86</td>
</tr>
<tr>
<td>Total phosphorus (mg/kg D.M.)</td>
<td>166.64</td>
<td>92.98</td>
<td>7.78</td>
<td>456.62</td>
</tr>
<tr>
<td>Inorganic phosphorus (mg/kg D.M.)</td>
<td>137.63</td>
<td>81.49</td>
<td>7.78</td>
<td>445.57</td>
</tr>
<tr>
<td>Organic phosphorus (mg/kg D.M.)</td>
<td>29.02</td>
<td>30.50</td>
<td>0.00</td>
<td>166.44</td>
</tr>
<tr>
<td>Clay %</td>
<td>7.86</td>
<td>3.43</td>
<td>0.96</td>
<td>15.18</td>
</tr>
<tr>
<td>Silt %</td>
<td>32.85</td>
<td>12.49</td>
<td>4.81</td>
<td>58.26</td>
</tr>
<tr>
<td>Sand %</td>
<td>59.29</td>
<td>15.76</td>
<td>28.17</td>
<td>94.23</td>
</tr>
</tbody>
</table>
Table S2. Average diameter (standard deviation) and proportional surface area by root order for 14 canopy tree species in a temperate deciduous forest. Values obtained from the analysis of root clusters that included three ephemeral root orders attached to a woody central root. N indicates the number of samples per species.

<table>
<thead>
<tr>
<th>Species</th>
<th>First order roots</th>
<th>Second order roots</th>
<th>Third order roots</th>
<th>Fourth order roots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter (mm)</td>
<td>Total surface (%)</td>
<td>Diameter (mm)</td>
<td>Total surface (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acer rubrum</td>
<td>159</td>
<td>0.40 (0.08)</td>
<td>0.39 (0.08)</td>
<td>0.49 (0.11)</td>
</tr>
<tr>
<td>Acer saccharum</td>
<td>219</td>
<td>0.41 (0.06)</td>
<td>0.39 (0.09)</td>
<td>0.50 (0.10)</td>
</tr>
<tr>
<td>Betula alleghaniensis</td>
<td>5</td>
<td>0.37 (0.04)</td>
<td>0.37 (0.04)</td>
<td>0.47 (0.08)</td>
</tr>
<tr>
<td>Carpinus caroliniana</td>
<td>16</td>
<td>0.39 (0.05)</td>
<td>0.35 (0.06)</td>
<td>0.52 (0.11)</td>
</tr>
<tr>
<td>Carya cordifolia</td>
<td>20</td>
<td>0.38 (0.05)</td>
<td>0.37 (0.09)</td>
<td>0.51 (0.07)</td>
</tr>
<tr>
<td>Carya ovata</td>
<td>99</td>
<td>0.36 (0.06)</td>
<td>0.36 (0.09)</td>
<td>0.48 (0.10)</td>
</tr>
<tr>
<td>Fagus grandifolia</td>
<td>66</td>
<td>0.37 (0.07)</td>
<td>0.34 (0.11)</td>
<td>0.49 (0.14)</td>
</tr>
<tr>
<td>Fraxinus americana</td>
<td>45</td>
<td>0.48 (0.08)</td>
<td>0.43 (0.07)</td>
<td>0.63 (0.13)</td>
</tr>
<tr>
<td>Fraxinus nigra</td>
<td>27</td>
<td>0.45 (0.09)</td>
<td>0.51 (0.10)</td>
<td>0.58 (0.11)</td>
</tr>
<tr>
<td>Liriodendron tulipifera</td>
<td>7</td>
<td>0.73 (0.05)</td>
<td>0.44 (0.09)</td>
<td>0.87 (0.16)</td>
</tr>
<tr>
<td>Platanus occidentalis</td>
<td>5</td>
<td>0.40 (0.10)</td>
<td>0.36 (0.05)</td>
<td>0.51 (0.11)</td>
</tr>
<tr>
<td>Prunus serotina</td>
<td>30</td>
<td>0.52 (0.10)</td>
<td>0.43 (0.07)</td>
<td>0.61 (0.13)</td>
</tr>
<tr>
<td>Quercus rubra</td>
<td>23</td>
<td>0.36 (0.07)</td>
<td>0.38 (0.13)</td>
<td>0.49 (0.11)</td>
</tr>
<tr>
<td>Ulmus sp</td>
<td>18</td>
<td>0.37 (0.05)</td>
<td>0.34 (0.05)</td>
<td>0.47 (0.08)</td>
</tr>
</tbody>
</table>
**Supplement Figure S4.1.** Relationship between root trait plasticity and soil conditions in a temperate deciduous forest. Each panel describes the relationship between an individual root trait and the most influential soil factor explaining trait variation for the entire community. Black points represent the weighted-mean trait values for the 14 most abundant species, whereas the crosses represent trait values for individual root systems. PCA_fertility describes the first PCA component for the variation in soil C, N and P among soil samples. Perc_POM_C correspond with the percentage of carbon in soil particulate organic matter. Dotted lines (as well as adjusted $R^2$ and $P$-values) represent the least-squares fit for intraspecific trait variation, covering the full range of soil conditions where root systems of each species were recorded. The dashed line represents the overall trend for the entire community.
A) \( \text{adj}_R^2 = 0.24 \)
\[ \text{p value} = 0.0001 \]

B) \( \text{adj}_R^2 = 0.19 \)
\[ \text{p value} = 0.002 \]

C) \( \text{adj}_R^2 = 0.30 \)
\[ \text{p value} = 0.01 \]

D) \( \text{adj}_R^2 = 0.30 \)
\[ \text{p value} = 0.01 \]
E) PCA_fertility

adj $R^2 = 0.32$
p value = 0.001

F) Fractal

adj $R^2 = 0.25$
p value = 0.008

G) Proportional Soil POM C

adj $R^2 = 0.50$
p value = 0.02
**Supplement Figure S4.2.** Relationships between soil factors and root traits at the core level in a temperate deciduous forest. Panels A), B), C), D) and E) show the relationship between % soil moisture and specific root length (SRL), specific root area (SRA), average root diameter (Diameter), fractal dimension and root tissue density (RTD) respectively. Panel F) represents the relationship between specific root tip abundance (SRTA) and soil fertility (PCA_fertility; first PCA component for the variation in soil C, N and P). Panel G) shows the relationship between average root link length and percentage nitrogen in soil particulate organic matter (Prop_POM_N). Dashed lines represent least-squared fit for the regression.
A) \( \text{adj}_R^2 = 0.09 \)
\( p \text{ value} = 0.002 \)

B) \( \text{adj}_R^2 = 0.11 \)
\( p \text{ value} = 0.0004 \)

C) \( \text{adj}_R^2 = \text{NS} \)
\( p \text{ value} = 0.81 \)

D) \( \text{adj}_R^2 = 0.07 \)
\( p \text{ value} = 0.02 \)
E) adj$R^2 = 0.08$
$p$ value = 0.01

F) adj $R^2 = $ NS
$p$ value = 0.23

G) adj$R^2 = $ NS
$p$ value = 0.94
Supplement Figure S4.3. Partial contribution of root community composition and soil conditions to the plasticity of root traits at the species level for ten canopy trees in a temperate deciduous forest. All factors included in the figures were significant (p<0.05) after a stepwise RDA procedure where all the response matrix factors were tested individually (Blanchett et al 2008). Four species for which no factors explained a significant portion of root trait variation are not shown (*Carya cordifolia, Carpinus caroliniana, Liriodendron tulipifera, Platanus occidentalis*). For the remaining species acronyms were created using the three first letters of both genus and epitet. *Acer rubrum* (Ace_rub), *Acer saccharum* (Ace_sac), *Betula alleghanienssis* (Bet_all), *Carya ovata* (Car_ova), *Fagus grandifolia* (Fag_gra), *Fraxinus americana* (Fra_ame), *Fraxinus nigra* (Fra_nig), *Prunus serotina* (Pru_ser), *Quercus rubra* (Que_rub), *Ulmus sp* (Ulm_sp).

Left panels (arrow graph) summarize the amount of root trait variation explained by soil conditions (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 between the environmental factors and species root trait variation.

Right panels depict a graphical ordination showing the most influential soil conditions (blue arrows) and neighbor species (green arrows) on the variation of root traits for the focus species (red arrows).
Species (4%)
S-Sp (13%)
Soil (10%)

Acer rubrum

Species (NS)
S-Sp (0%)
Soil (24%)

Acer saccharum

Soil (10%)
S-Sp (13%)
Species (4%)
Acer rubrum

RDA1 (27%)
RDA2 (1%)
SRL
SRA
Diam
RMD
LinkLenght
Fractal
SRTA
Prop_POM_N
PCA_fert
PCA_phos
Ace_sac
Lir_tul

RDA1 (28%)
RDA2 (1%)
Prop_POM_N
Acer_rub
PCA_fert
PCA_phos
Ace_rub
Fra_ame
Fra_nig
Summary: Belowground plant interactions are assumed to have a profound impact on fine root biomass (FRB) productivity. However, the lack of studies describing root distributions at the species level has hampered the evaluation of this assumption, particularly in diverse forest ecosystems. Here we studied fine root distributions at the species level in a temperate forest in order to address the following questions: 1) Are fine root systems of canopy trees segregated vertically in the soil profile? 2) How the interaction between soil resource heterogeneity, diversity, and functional traits explain FRB spatial variation in forest ecosystems? 3) Are these relationships similar in core areas of ecosystems compared with ecotones near ecosystem edges? 4) Can the proportional species distribution of FRB be predicted from aboveground species abundance? We tackled these questions using molecular tools to identify and describe morphologically > 730 fine root samples (< 1 mm in diameter) representing 14 coexisting canopy tree species in an Acer-Fagus dominated forest in northeast Ohio, USA. We found extensive root overlapping, indicating little evidence of spatial segregation among canopy trees. Total FRB was better explained by soil heterogeneity than tree composition, particularly in core ecosystem areas. Root traits associated with uptake
capacity decrease but species diversity increase as soil resources increase, suggesting that FRB increases as an aggregative effect of overlapping species rather than the dominance of individual species. Moreover, there was a positive effect of phylogenetic diversity on FRB, even after correcting by soil fertility. These results suggest that symmetric competition and evolutionary relationships between coexisting species are important factors explaining soil resource use in tree communities. Finally, our findings indicated that FRB abundance roughly corresponds with aboveground tree abundance. However, ectomycorrhizal (ECM) species as a category were under-represented belowground, possibly reflecting tradeoffs between FRB productivity and root maintenance costs.
Introduction

In forest ecosystems, fine roots (usually non-woody roots < 1mm in diameter) represent an important sink of carbon (Jackson et al. 1997, Ruess et al. 2003), mediate most tree nutrient and water acquisition (Wardle et al. 2004, Litton et al. 2007), and maintain most of the biological interactions between plants and the associated soil community (Coomes and Grubb 2000, Lambers et al. 2009). Root interactions also play a prominent role defining the relationships between plant species diversity, ecosystem functions, and community invasibility (Shenck 2006, de Kroon et al. 2012). Despite the increasing number of studies focusing on the distribution of root traits in diverse forest ecosystems (Holdaway et al. 2009, Comas and Eissenstat 2009, Brassard et al. 2013), there is still little information about the factors driving species root productivity in forest ecosystems, or how species interactions affect the spatial variation of fine root biomass (FRB) (Schmid 2002, Meinen et al. 2009, McNickle et al. 2009).

A commonly mentioned factor driving FRB spatial variation is the heterogeneity of soil resources in natural conditions (Hutching and de Kroon 1994, Hutching et al. 2003). Root proliferation in nutrient hotspots is considered a common competition strategy, with prolific species having a competitive advantage by preemptively obtaining available resources before competitors (de Kroon et al. 2003, 2012). Thus, competition for nutrient-rich patches is expected to be intense (Wilson 2000), with a substantial effect on plant fitness (Hodge 2004) and community productivity (Wijesinghe et al. 2005).
However, although modeling studies indicate that species that do not proliferate in high 
nutrient patches are at a competitive disadvantage (O’Brien et al. 2007), some species 
show no selectivity in the distribution of their roots in patchy environments (Robinson 
1994, 1996), or respond physiologically rather than by root proliferation (Jackson et al. 
2006). Thus, in addition to spatial variation in soil nutrients, variation in foraging 
strategies could have an important effect on belowground relative abundance and spatial 
biomass variability in forest stands (Mou et al. 1997, Giardina et al. 2005, Coleman 
2007). Therefore, it is still unclear how the interaction of resource heterogeneity in soils 
and alternative foraging strategies among coexisting species determines the variation in 
FRB in natural ecosystems. Moreover, it also raises the question of whether certain 
species have consistently larger or smaller relative abundance belowground than 
avoveground because of tradeoffs in resource acquisition strategies (Meinen et al. 2009, 
Rewald and Leuschner 2009).

If some species are adapted for preemptive colonization of nutrient rich patches, 
we would expect them to exhibit traits, such as high specific root length (SRL) or low 
tissue density (RTD), which would allow them to proliferate quickly and exclude other 
species from these areas (Campbell et al. 1991, Hutchings et al. 2003). Other species 
would be expected to allocate most roots to areas not exploited by the “preemptive” 
species, and to exhibit traits more associated with resilience (de Kroon and Mommer
High soil nutrient areas would accumulate higher biomass because fast root-growing species will dominate these patches. Thus, under this asymmetric root proliferation scenario, FRB should be positively associated with nutrient availability but not with species diversity. In contrast, in a symmetric scenario, all species should have similar capacity to detect and proliferate in nutrient hotspots, and both FRB and species diversity (regardless of species traits) should be associated with high soil resource availability (Table 5.1).

In addition to the response to soil resource conditions, FRB could also be affected by biotic interactions among neighbors. Positive interactions may result in “overyielding”, in which mixtures of species obtain greater biomass than would be expected from species monocultures (i.e., biomass is correlated with diversity; Berensde 1979, Cardinale et al. 2007, Kesanakurti et al. 2011). Enhancements in biomass productivity with increasing diversity have been attributed to inherent complementary differences between species in a community, resulting in a more thorough use of available resources with increasing diversity (Cadotte et al. 2008). Although most studies demonstrating overyielding are based on aboveground measurements (Hooper et al. 2005); there is growing evidence of increased belowground productivity with species diversity (Hodge 2004, Rajaniemi 2007, Fornara and Tilman 2008, 2009). Several mechanisms have been proposed to explain the effect of diversity on belowground productivity including the increased root filling of soil space (i.e. root aggregation; von...
Felten and Schmid 2008, Mommer et al. 2010, Brassard et al. 2011), vertical niche partitioning (Dimitrakopoulos and Schmid 2004, Mueller et al. 2013), and lower pathogen constraints on FRB production (Schnitzer et al. 2011, de Kroon et al. 2012). However, most of these studies have focused on communities dominated by herbaceous species (de Kroon et al. 2012).

Evidence for belowground overyielding in forests remains controversial, ranging from negative (Bolte and Villanueva 2006) or neutral (Meinen et al. 2009) to positive effects of tree diversity on FRB (Brassard et al. 2011, 2013, Lei et al. 2012). Similarly, the vertical distributions of root tress vary from clear spatial segregation with soil depth (Leuschner et al. 2001, Bennett et al. 2002, Brassard et al. 2013) to clumping of roots in the uppermost soil layers (Meinen et al. 2009, Jones et al. 2011). These contrasting findings suggest that belowground biomass productivity could be affected by the identity of species in root neighborhoods. Previous studies have demonstrated that combinations of complementary functional traits or alternative phylogenetic groups tend to better explain ecosystem overyielding than total species richness, particularly in natural ecosystems (Cardinale et al. 2007, Flombaum and Sala 2008). Moreover, plants are able to recognize and respond to the identities of neighbor roots (Semchencko et al. 2007, de Kroon 2007). In the case of forest ecosystems, root assemblages are not random and functional traits tend to be evenly distributed in root neighborhoods, suggesting strong belowground biotic interactions (Leuschner et al. 2001, Valverde-Barrantes et al. 2013).
Nonetheless, the lack of studies describing root distributions at the species level has hampered the evaluation of this assumption, particularly in diverse forest ecosystems.

Finally, the influence of tree diversity and soil conditions on FRB accrual may vary among ecosystems, as well as due to other features of natural landscapes that can have an effect on mechanisms of community assembly. In particular, transitional areas between ecosystems, called ecotones (Cadenasso et al. 2003), can influence the coupling between biotic and abiotic factors (Blackwood et al. 2013) that may ultimately affect the spatial distribution of FRB. In a comparison of ecosystems, the “ecotone decoupling” hypothesis indicates that the influence of soil properties would diminish near ecotones due to the increased importance of dispersal and establishment of individuals in non-optimal soils (Blackwood et al. 2013). This weakened selection by soil filtering provides an opportunity for the effects of niche partitioning and over-yielding to become more apparent.

Our objective in this study was to use a natural landscape with three adjacent forest ecosystems to examine: 1) vertical segregation by roots of different tree species; 2) the effects of soil nutrients and root diversity (species, phylogenetic, and trait diversity) on total FRB; 3) the influence of ecosystem transitions on the controls over FRB spatial variation; and 4) the belowground relative abundance of species compared to their aboveground relative abundance. For our first objective, we examined biomass distribution by depth among ecosystems and tree species, and tested the hypothesis that
species partition soil resources by allocating root biomass in different soil horizons. For our second objective, we looked for FRB patterns associated with vertical and horizontal segregation of species, and associations between root biomass, root traits, and root diversity. More specifically, we tested for patterns consistent with the asymmetric or symmetric root proliferation hypotheses (Hypotheses 2.1 and 2.2), as well as overyielding (Hypothesis 2.3, see Table 1 for details). For our third objective we hypothesized that soil resource availability will predict FRB variation better when comparing core areas of ecosystems, whereas the inclusion of areas near ecotones will decrease the explanatory ability of soil conditions but will increase the influence of community composition on FRB spatial variation (Hypothesis 3, Blackwood et al. 2013). Finally, for our fourth objective we tested the hypothesis that belowground relative abundance should mirror aboveground relative abundance regardless of typical root traits of different species (Hypothesis 4.1; Kesarnakurti et al. 2011, Finér et al. 2011). Alternatively, considering that ectomycorrhizal fungi can be more C demanding (Marschner 1995) and better nutrient scavengers than AM fungi (Phillips et al. 2013), we proposed that ECM tree root biomass may be under-represented compared to AM tree species (Hypothesis 4.2).
Table 5.1 Hypothetical effects of spatial soil resource heterogeneity on fine root biomass accrual and root trait distribution in a temperate forest.

<table>
<thead>
<tr>
<th>Root proliferation pattern</th>
<th>Asymmetric root proliferation (H2.1)</th>
<th>Symmetric root proliferation (H2.2)</th>
<th>Overyielding (H2.3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertical segregation</td>
<td>Yes</td>
<td>No</td>
<td>Possible</td>
</tr>
<tr>
<td>Horizontal segregation</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Particular species associated with root biomass</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Trait association with root biomass</td>
<td>Positive, associated with species abundance</td>
<td>No, random association of traits</td>
<td>No, but non random association of traits</td>
</tr>
<tr>
<td>Root biomass association with soil resources</td>
<td>Yes, prolific species dominating hotspots</td>
<td>Yes, aggregative</td>
<td>Possibly</td>
</tr>
<tr>
<td>Species diversity association with root biomass</td>
<td>Neutral</td>
<td>Possibly positive, but not additive with soil resources</td>
<td>Positive and additive to soil resources</td>
</tr>
</tbody>
</table>

Methods

Site description: We conducted the study in Jennings Woods, a 30 ha forest in northeast Ohio, USA. Current vegetation is dominated by *Acer saccharum*, *Fagus grandifolia*, and *Acer rubrum*, encompassing nearly 50% of the forest tree cover (Blackwood et al. 2013).

The understory is dominated by *Hammamelis virginiana*, *Carpinus caroliniana* and *Lindenia benzoin*. The property includes three main ecosystems (Blackwood et al. 2013).
A riparian forest with fine-loamy Entisols dominates areas within 80 m from the river. Soils in the riverbed can vary from flood-deposited sand dunes to poorly drained oxbows. An upland forest thrives in a plateau 15-20 m higher in elevation than the riparian forest. The upland forest soils include fine-loamy Alfisols, which are interspersed with mesic spots that can have standing water during most of the year, depending on weather conditions. Finally, a bottomland forest is located at the southwest of the property. This area includes a lower elevation plateau which is very poorly drained, with mesic Alfisols similar to those found in the upland forest but saturated with water throughout the year. The forest was selectively logged early during the nineteenth century, and has remained undisturbed for at least 60 years.

**Vertical root distribution samples:** Vertical root biomass distribution was studied by collecting soil samples (hereafter referred to as “deep cores”) with a 5-cm diameter core from 44 locations. Samples were located in a layout design to target each component of the landscape (i.e. upland, bottomland, and riparian forest) and transition points between ecosystems (see Blackwood et al. 2013). Mean core depth was 52±8.75SD cm, reaching in most cases the C horizon. Deep cores were carefully dissected by horizon, and roots were manually retrieved from each horizon and sorted separately between dead and live roots based on friability, color and cortex conditions (Bouma et al. 2000). Live roots were separated into three diameter categories: Fine root biomass (<1mm, FRB), coarse root biomass (1-3mm, CRB), and woody root biomass (3-5mm, WRB). A subsample of FRB
from each horizon (0.01-0.2 g depending on total horizon biomass) was stored at -80°C for subsequent DNA extraction. The rest of the roots from each category were dried at 60 °C for 24 h and then weighed.

**Sampling roots across ecosystems and ecotones:** Based on the results from the deep cores, ≥50% of the total biomass was concentrated in the first 15 cm of the soil profile, independent of ecosystem type (Fig. 3. 1a). Consequently, we collected additional “surface samples” from 130 plots (2.5 x 5 m) arranged to maximize the detection of transitional areas between main ecosystems (i.e. ecotones) and ensure good spatial coverage of the different environments. The plot arrangement is described in detail in Blackwood et al. (2013), and root sampling is described in detail in Valverde-Barrantes et al. (2013). In brief, samples were obtained with a 10 cm diameter × 15 cm deep PVC corer. Once obtained, each soil sample was placed in ice, transported to the lab and refrigerated at 4 °C until further processing. Each soil core was soaked in water for at least 12 hours and all intact fine root clusters (i.e. root clusters consisting of 3-4 root orders ~ 10 cm in length and < 1 mm in diameter, Xia et al. 2010) were carefully removed and stored in deionized water. Randomly, 5-10 intact fine root clusters (comprising on average 20.01% ±0.95 SE of the total FRB per core) were separated for subsequent analysis. Each of these root clusters was divided into two sections: a small sample (~0.05 g), consisting of 1st and 2nd order roots was frozen at -80 °C for DNA extraction; the rest of the sample was scanned and then weighed after oven-drying. The
remaining soil sample was washed through a 105 \( \mu \text{m} \) sieve and all root fragments were classified as described previously for the deep cores.

**Soil conditions and species distribution above and belowground:** Measurement of aboveground species abundance and soil properties are described in detail elsewhere (Blackwood et al. 2013, Valverde-Barrantes et al. 2013). Briefly, five soil cores (1.5 cm diameter, 10 cm depth) were collected from each plot and subsequently pooled. We measured 14 soil variables including % clay, % silt, % sand, % moisture, pH, total soil carbon and nitrogen (%), C:N ratio, particulate organic matter C (POM C, %), particulate organic matter N (POM N, %), POM C:N ratio, and organic, inorganic and total bicarbonate-extractable phosphorus. We performed a PCA analysis on the original data to summarize the information collected from soils (Valverde-Barrantes et al. 2013). To summarize nutrient variation, we used the first two PCA axes (explaining 79% and 14% of variance in C, N, P, and POM variables), hereafter referred to as Fertility PCA axes 1 and 2. To summarize soil texture, we selected the first PCA axis (explaining 98% of variance in % clay, silt, and sand), hereafter referred to as the Texture PCA axis. In total, five soil variables were utilized for subsequent statistical analysis, including Fertility PCA axes 1 and 2, Texture PCA axis, % moisture and pH. Trees were identified within 15 m radius circular plots centered on the soil core plots described above. The distance of each tree to the center of the plot was recorded, as well as species identity and diameter at breast height (dbh).
Molecular root identification: Extraction of DNA from root samples was performed using standard procedures (Murray and Thompson 1980, Valverde-Barrantes et al. 2013). Briefly, we froze and ground root tissue extracted from each soil sample using liquid nitrogen, then suspended the powder in CTAB buffer containing 50 mM EDTA, 10 mM Tris-HCl (pH 8.0), 1% CTAB, and 0.2% β-mercaptoethanol. Proteins were then removed by washing with chloroform, followed by sequential precipitation in isopropanol and 70% ethanol. Finally, the isolated DNA was dissolved in 50 µl of sterile distilled water.

For the deep cores, the presence of tree species in different soil horizons was assessed using terminal restriction fragment length polymorphism (T-RFLP, see details for molecular procedures in Supplementary text 1). The non-coding spacer region trnH-psbA was PCR-amplified (Kress et al. 2005), followed by restriction digestion and capillary electrophoresis (Blackwood et al. 2003, Taggart et al. 2011). We also created a reference library running a similar T-RFLP procedure from leaf DNA samples.

The composition of the root community in the surface samples was determined using restriction fragment length polymorphism (RFLP) of the selected intact root cluster samples (Valverde-Barrantes et al. 2013). In this case, we identified root cluster samples by comparing DNA barcodes to a reference library based on leaf collections from 33 tree canopy species (Valverde-Barrantes et al. 2013). Barcoding patterns were generated by combining HinfI RFLP patterns of the plastid gene trnL and non-coding spacer region trnH-psbA (Brunner et al. 2001, see Supplementary text 1). Barcoding patterns from both genes were analyzed using GelCompar II software (version 4.5, Applied Maths BVBA, 175
Belgium), allowing us to identify most samples to genus or species level. At the end, 738 root systems belonging to 14 canopy tree species was used for all calculations of proportional biomass and trait diversity effects (Valverde-Barrantes et al. 2013).

**Root trait measurements:** Root clusters from the surface cores were scanned and the following root traits were measured on each root cluster, as described in Valverde-Barrantes et al. (2013): specific root length (SRL (m/g)), specific root surface area (SRA (cm$^2$/g)), root tissue density (RTD (g/cm$^3$)), average diameter for first order roots (mm), fractal dimension (D, unitless), specific root tip abundance (SRTA (tips/mg)) and average link length (L (cm)). These traits have all been employed in the characterization of root systems in previous studies (Eshel 1998, Hertel et al. 2003, Meinen et al. 2009, Wang et al. 2009, Dupuy et al. 2010).

**Statistical analysis:** All analyses were conducted using the software R 2.15.1, packages picante 1.2 (Kembel et al. 2010) and vegan (Oksanen 2010), and R scripts modified from Ackerley and Cornwell (2007) and Cadotte et al. (2009).

**Hypothesis 1. Vertical FRB distribution:** We created two presence-absence matrices from the deep core T-RFLP data, and performed the following analyses on each. The first matrix included all T-RFLP bands, assuming that different bands represented different species. The second matrix included only bands confirmed to represent tree species, based on comparison with T-RFLP patterns derived from leaves. To account for the fact
that all deep core samples were nested within plots and ecosystems with differing species compositions, we initially performed a redundancy analysis (RDA, Legendre and Gallagher 2001) using plot identity as an explanatory factor. Then, we used the residual values from the first RDA to test the hypothesis of vertical niche segregation among tree species in a second RDA, with maximum depth of the soil horizon used as a predictor in this analysis. Additionally we directly tested the mean depth distribution values for the species identified during the TRFL-P analysis, using a univariate ANOVA with depth at which roots were detected as a response variable, species identity as predictor, and plot identity as a random effect (Fig. 3.1b).

Hypothesis 2.1 and 2.2 Comparing belowground root proliferation hypotheses: Under the root asymmetric-proliferation scenario we predicted an association between FRB accrual and the dominance of species bearing traits associated with fast growth rates (i.e. long SRL, high SRTA). In contrast, for the symmetric proliferation scenario we expected no relationship between dominance of particular species or root traits and FRB. To test if belowground species abundance or trait distribution was associated with FRB accrual, we performed a multi-step procedure using RDA following Valverde-Barrantes et al. (2013). A matrix of log-transformed FRB values from each core was used as the response variable. To test for an affiliation of particular species with high or low FRB, we created a predictor variable matrix of Hellinger-transformed values of proportional root abundance of each species in each soil core for the 14 most abundance species. A second
predictor variable matrix contained the average core-level (community-aggregated) root traits for all traits measured. The analysis procedure included an initial global analysis for each predictor variable matrix. If the global analysis was significant, posterior forward selection of variables in the predictor variable matrix was performed to obtain a parsimonious model (Blanchet et al. 2008). Then, variance partitioning was performed including only selected root traits and relative species abundances, and the significance of each predictor type was determined in a partial test after correcting for variance explained by the other predictor type (Peres-Neto et al. 2006). Finally, we tested the effect of soil conditions on both root traits and belowground species abundance, to determine whether these factors could explain associations between FRB and particular root traits or species root relative abundance detected in the analysis described above. Since preliminary analysis showed a non-linear relationship between soil conditions and FRB (Valverde-Barrantes et al. 2013), the soil matrix included the normalized linear and squared values for all soil variables included in the analysis. The analysis followed the same steps described for the previous multi-step RDA. We also performed partial tests on selected root traits and species relative root abundances to determine whether they explained significant variation after accounting for the effects of selected soil variables or species aboveground relative abundance.

**Hypothesis 2.3 Testing overyielding effect on FRB variation:** Here our goal was to separate between FRB accrual due to soil conditions and biotic interactions among
species. Soil factors associated with FRB were previously selected during the RDA in objective 2.2. For the diversity effects, we calculated a series of diversity metrics summarizing the interactions among species in each core based on their functional traits (Cadotte et al. 2009). First, we scaled all root traits to have a mean of zero and variance of one. We then calculated a Euclidean distance matrix and performed hierarchical clustering on this matrix (Ward’s method, Legendre and Legendre 1998). Functional attribute distance (FAD) was calculated as the sum of pairwise Euclidean distances among all species present in a sample, and functional distance (FD) was calculated as the sum of the branch lengths required to connect all species present in a sample (Petchey and Gaston 2002). In order to account for intraspecific trait variation, calculations for FAD and FD were based on core-specific measurements rather than species averages. Additionally, we calculated a functional diversity index that accounts for possible multi-collinearity among traits by performing a non-metric multidimensional scaling (NMDS) ordination on the trait matrix to obtain two summary axes (Cadotte et al. 2009). We then performed a cluster analysis with the resulting fitted values and calculated a NMDS distance similar to FD. Phylogenetic diversity (PD) was calculated after constructing a phylogenetic tree using the Phylocom tool to assemble species lists into phylogenies based on APG3 angiosperm supertree (Webb and Donahue 2005, Fig. S5.1). From the phylogeny, we calculated PD as the sum of the branch lengths required to connect all species present in a core (Faith 1992). We used Pearson correlation to examine
consistency of the diversity indices (see more details about diversity indexes in Table S5.1).

We tested whether diversity (species richness, functional trait diversity, and phylogenetic diversity indices) significantly explained variance in residual variation in FRB after correcting for soil conditions (Cadotte et al. 2009). Only cores with at least one of the evaluated species were included in the analysis (n=86, Loreau and Hector 2001, Dybzynki et al. 2008). Similar to the previous test, we included the normalized linear and squared values for all soil and diversity variables. Then, we performed the same RDA multi-step procedure described above (Blanchet et al. 2008). In this case however, we obtained the residual values from the best fit soil-biomass RDA analysis and tested whether diversity indexes explained any additional information. A positive significant relationship between the residual FRB values and diversity metrics was interpreted as evidence for the overyielding hypothesis.

**Objective 3. Ecotone effects on controls over FRB distribution:** To test the importance of the decoupling between biotic and abiotic factors across ecosystem transitions we followed randomization test described in Blackwood et al. (2013). We classified core samples as “Core” if they were located more than 30 m from a topographically-defined ecosystems boundary, and “Edge” if they were located within 30 m of the boundary (see Blackwood et al. 2013 for details about the classification of core and transitional areas at Jennings Woods). Tests described above to determine controls on the spatial variation of
FRB were initially performed on core plots only. Then, using factors obtained in the previous analysis, the variance partitioning was performed again on the core and edge plots together. Comparison between models was based on adjusted-R² to correct for the effect of differing numbers of samples on variance explained, and the significance of the change in variance explained was tested using a restricted randomization test (Fagan et al. 2003, Peres-Neto et al. 2006). To calculate a P value, the empirical proportional increase in adjusted-R² due to exclusion of edge plots was compared to a null distribution of values derived from randomization under the null hypothesis that there was no effect of including ecotone areas on the controls over FRB.

**Objective 4. Predicting FRB abundance based on aboveground information:**

Aboveground species abundance was estimated based on basal area measurements from each species in each plot. Species abundance belowground was based on the proportional amount of roots per species identified in each core. To test the idea of equal representation of species above and belowground, we performed a linear regression between mean basal area relative abundance and FRB relative abundance of each species, and tested if the slope differed from 1.0. For each species individually, we also tested whether the average difference in above and belowground relative abundance was different from zero using a randomization procedure. First, we calculated the difference between above and belowground relative abundance for each plot, and then calculated the observed mean difference across all plots where the species was present. Then, we
performed 999 random permutations under the null hypothesis of no difference in representation above and belowground, in which values of relative abundance were randomly assigned as above or belowground, and the mean difference between compartments was calculated for each species. To calculate a $P$ value, we determined the proportion of times that the difference between above and belowground relative abundance simulated under the null hypothesis was larger than the observed value. Finally, we determined whether the ratio of above to belowground relative abundance was different between AM and ECM tree species. In this case we compared the above and belowground relative abundance of AM and ECM species in each plot. Then, we randomize the proportional representation of mycorrhizal types at each plot both above and belowground and compared the relative abundance of each mycorrhizal type after randomization. We performed 9999 iterations for the random communities and calculated a $p$-value based on the proportion of times the ratio between proportions in the iterations was larger than the observed.

**Results**

**Objective 1. Vertical FRB segregation:** All ecosystems showed a relatively shallow fine root distribution, with 50, 56 and 71% of total FRB in the upper 15 cm of the soil profile for riparian, upland, and bottomland forest, respectively (Fig. 1a). Despite differences in root distribution and root accrual between ecosystems, we found no evidence of species segregation by depth. In the T-RFLP matrix representing only tree species, RDA
indicated that soil horizon depth did not explain the presence of species (F=0.95, p=0.4, adj R² =0.01). Moreover, species showed large overlap in the depths at which they were detected, with no significant difference in their mean depth value (ANOVA, F=0.2, p=0.7, Fig. 5.1b). However, when including all detected T-RFLP’s, there was a limited but significant effect of depth on species distribution (F=1.37, p=0.02, adj R² =0.02), indicating limited vertical sorting of species, probably discriminating between herbaceous and woody plants.

**Objective 2.1 Comparing belowground root proliferation hypotheses:** Comparing FRB in surface cores among ecosystems, only the finest root class showed significant differences among ecosystems, with riparian forest having 24% lower FRB than upland forest and 26% lower FRB than bottomland forest (Table 5.1). After factor selection (RDA stepwise selection test), we found that both trait distribution (Traits selected: SRL and SRTA, F=5.5, p=0.01) and species relative root abundance (Species selected: *Acer saccharum* and *Ulmus sp* F=3.86, p=0.04) were significantly correlated with spatial biomass variation, explaining 14 and 17% of the total variation respectively. However, the effect of species relative root abundance was not significant after correcting by root traits (F=1.52, p=0.13). On the other hand, root traits were still explaining a significant proportion of FRB variation even after correcting for belowground species abundance (F=2.5, p=0.01, total variance explained 15%, Fig 5.2a). Soil conditions had an overriding effect on FRB distribution (Variables selected Fertility PCA1 and pH, F=8.98, p=0.01).
p<0.001). Once corrected by soil conditions, neither species relative root abundance nor trait distribution were significantly associated with FRB (F=1.59, p=0.13 and F=0.96, p=0.62 for root traits and species relative root abundance, respectively). Once included, soil conditions were the dominant factor matrix explaining FRB distribution (F=8.08, p<0.0001, 44% of variation explained, with Fertility PCA1 and pH, including quadratic factors, as the most important variables explaining FRB variation, Fig 5.2b).

Table 5.2. Root biomass distribution at three different ecosystems in a deciduous temperate forest in northeastern Ohio. Categories correspond with fine root (FRB, roots < 1 mm in diameter), coarse root, (CRB, roots 1-3 mm) and woody root biomass (WRB, 3-5 mm) in the uppermost 15 cm of the soil profile.

<table>
<thead>
<tr>
<th></th>
<th>FRB (g/m²)</th>
<th>CRB (g/m²)</th>
<th>WRB (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>95% CI</td>
<td>Mean</td>
</tr>
<tr>
<td>Bottomland</td>
<td>357.96</td>
<td>293.07 - 423.11</td>
<td>111.43</td>
</tr>
<tr>
<td>Riparian</td>
<td>189.48</td>
<td>160.65 - 219.72</td>
<td>95.19</td>
</tr>
<tr>
<td>Upland</td>
<td>289.02</td>
<td>245.85 - 335.57</td>
<td>120.37</td>
</tr>
</tbody>
</table>
Figure 5.1. Vertical distribution of fine root biomass in Jennings Woods. a) Vertical distribution of fine root biomass (< 1mm in diameter) at Jennings Woods. Bars indicate mean (±SD) root biomass values at different depths. Letters indicate significant differences in FRB crop between ecosystems. b) Vertical distribution of roots by species based on T-RFLP detection from root samples collected at different depths. Species represented are: *Acer rubrum* (Acerub), *Acer saccharum* (Acesac), *Fagus grandifolia* (Faggra), *Carya ovata* (Carova), *Carya cordifolia* (Carcor), *Populus sp* (Populus), *Ulmus sp* (Ulmus), *Prunus serotina* (Pruser), *Carpinus caroliniana* (Carcar), *Ostria virginiana* (Ostvir), *Fraxinus sp* (Fraxinus), and *Quercus sp* (Quercus).

Contrary to the expected relationship between particular traits and FRB, we found a negative trend between FRB and SRL (F=9.45, p=0.002) or SRTA (F=15.99, p<0.001, Fig. 5.3a, Table S5.2). Similarly, the two species selected based on their proportional abundance and core FRB showed a significant negative relationship (F=5.67, p=0.02, and F=19.17, p<0.001, for *Acer* and *Ulmus* respectively, Fig 5.3b). Moreover, this trend was highly influenced by samples in which the species were absent. If the regression was
limited to only samples where species were detected, only *Ulmus* root abundance showed a significant relationship with FRB (F=8.79, p=0.02).

**Figure 5.2.** Variance explained (Adjusted R²) in FRB by functional root traits, proportional root abundance by species and soil conditions after variable selection. The graph summarizes the amount of variation explained a) in FRB by functional root traits and species relative root abundance; and b) the effect of including soil conditions in the analysis. For panel a) the amount of variation explained by proportional root biomass is not significant after correction by functional root traits. In panel b) neither functional traits nor proportional species abundance are significant after incorporating soil variables.

**Objective 2.2. Testing overyielding effect on FRB variation:** The clustering of species based on root traits essentially followed the species phylogenetic relationships, confirming the high conservatism of functional root traits among trees in this community (Figure S1). In general, clustering by traits separated AM and ECM species, with the exception of *P. occidentalis* and *Ulmus sp*, which were clustered within the ECM clade. Similarly, the NMDS-based clustering separated ECM and AM species, with the
exception of *Q. rubra* that classified within the AM clade and *P. occidentalis* that classified within the ECM clade. Moreover, *Ulmus sp* and *L. tulipifera* were located at the root of the tree, confirming the large, but contrasting, differences in morphology between these species and the rest of the community (Valverde-Barrantes et al. 2013). Species richness (S) averaged approximately three species per core, with a maximum of seven in a single core, out of the 14 species studied. Phylogenetic diversity, species richness, and functional diversity indices were highly correlated, with Pearson correlation coefficients ranging from 0.72 to 0.89 (Table S5.3). Despite the close relationship between trait diversity and phylogenetic distances, PD was the only variable significantly related to residuals of FRB after correcting for soil fertility. Similar to soil conditions, the better fit model included a quadratic PD factor (*F*=6.13, *p*=0.003) but did not any of the other diversity indexes.
Objective 3. Ecotone effects on controls over FRB distribution: For this objective we compared the best fitted model obtained describing soil conditions and FRB, but limiting the samples to core ecosystem areas and compared with the previous model including all samples. The relationship between FRB and soil conditions was significantly stronger in core areas of ecosystems compared to when plots close to ecotones were also included (core adj $R^2 = 0.61$, $F=15.8$, $p<0.0001$; edge + core adj $R^2 = 0.37$, $F=13.81$, $p<0.0001$; $p=0.014$ testing the difference between model adj $R^2$, Fig 5.4a), suggesting a decoupling between soil conditions and FRB in ecotone areas. In contrast, we found no significant difference in the relationship between PD and FRB biomass when edge areas were
included in the analysis (adj $R^2 = 0.10$, $F=3.14$, $p=0.05$, edge + core  adj $R^2 = 0.11$, $F=6.13$, $p=0.003$, $p=0.9$ for difference between adj$R^2$, Fig 5.4b).

**Objective 4. Predicting FRB abundance based on aboveground information:**

Belowground relative species abundance was related to aboveground basal area relative abundance, with no significant difference from the expected 1:1 ratio between both compartments (Fig 1a, slope 95% confidence interval = 0.5 – 1.25). However, when species were tested individually, the representation of roots from some species was significantly lower than expected from aboveground abundance. Both ECM species (*Fagus grandifolia* and *Quercus rubra*) and AMF species (*Fraxinus americana* and *Prunus serotina*) showed lower abundance of roots compared to aboveground basal area ($P < 0.05$, Fig 5.5a). However, when mycorrhizal types were compared, ECM species had significantly lower proportional abundance of roots compared with the proportional abundance of tree aboveground ($p=0.02$, Fig 5.5b).
Figure 5.4. Relationship of FRB with soil nutrient availability and phylogenetic diversity. A) Relationship between FRB and soil fertility (Fertility PCA axis 1); B) Relationship between the residual FRB values after correcting for soil fertility and phylogenetic distance (PD) of each core. Statistical values were estimated considering only core ecosystem samples (filled points) and core plus ecotone samples (open symbols). Parameters for A) were adj R² = 0.61, F=15.8, p<0.0001 and adj R² = 0.37, F=13.81, p<0.0001 for models including only core samples and cores + ecotone samples respectively. For B) parameters were adj R² = 0.10, F=3.14, p=0.05 for a model with only core samples and adj R² = 0.11, F=6.13, p=0.003 for a model with core + ecotone samples. Differences between models was significant only for the effect of soil conditions on FRB. Lines illustrate the best polynomial fit for both regressions.
Figure 5. Relationship between estimated relative basal area abundance and relative fine root biomass abundance at Jenning Woods. A) Regression relationship across species. Gray-filled symbols represent ECM species, open symbols AMF species. B) Mean ratio of species aboveground to belowground relative abundance for different mycorrhizal groups. The asterisk indicates a significant departure from the expected 1:1 ratio. Error bars indicate ±SD.

Discussion

Diversity, complementarity and productivity belowground in natural ecosystems:

Root systems are traditionally depicted as highly territorial, usually in response to intense competition for belowground limiting resources (Yeaton et al. 1977, Brisson and Reynolds 1994, Pecháčková et al. 1999). For instance, vertical and horizontal segregation of roots has been frequently mentioned as a common mechanism of soil resource partitioning (Fitter et al. 1991, Caldwell et al. 1996) and is expected to be common across ecosystems (Tilman et al. 1997, Schenk et al. 1999). Also, it is commonly assumed that root traits correspond with specialized foraging patterns (Campbell et al. 1991, Coleman 2007). For instance, it was assumed that fast-growing roots are adapted to forage in and dominate areas with higher nutrient availability, and therefore would show functional
traits associated with their ability to proliferate in nutrient hostspots (Taub and Goldberg 1996, Aanderud et al. 2003). Our results, however, do not support these assumptions for root systems of canopy trees. First, similar to other studies using molecular techniques for root identification (von Felten and Schmid 2008, Mommer et al. 2010) and studies in a forest with similar floristic composition (Meinen et al. 2009), we found no evidence of vertical segregation among root systems of canopy trees. Moreover, the root mass depth profile was similar for all ecosystems, and indicates that species in this forest tend to accumulate roots in the uppermost soil horizon, rather than distribute FRB evenly along the soil profile (Fig 1a).

Second, we did not find a strong effect of species identity on FRB spatial distribution. Combined with the overlapping species ranges previously documented to occur over the soil fertility gradient at Jennings Woods (Valverde-Barrantes et al. 2013), these results show an extensive overlap in foraging areas among neighboring trees, rejecting the idea of high territoriality or horizontal niche segregation based on soil resources among species (H2.1). Instead, all species roots tend to proliferate in nutrient-rich spots, and higher FRB is associated with the aggregation of species, supporting H2.2. The fact that FRB was better explained by community-aggregated trait values than above and belowground relative abundance supports the idea of symmetric root proliferation belowground (Cardinale et al. 2007). In other words, our study suggests that all species showed similar opportunities to access fertile soil patches and proliferate to a similar
extent (Schenk 2006, Lang et al. 2010) and soil heterogeneity does not promote asymmetry in belowground resource uptake (Blair 2001).

Interestingly, we also found that, at the community level, there is a trend to increase root tissue density and decrease the number of root tips and root length with FRB. This is consistent with our previously documented trends between soil resources and community-aggregated and individual species traits (Valverde-Barrantes et al. 2013). These results suggest that tree species generally proliferated root biomass but also decreased uptake capacity when resources were readily available, contradicting our initial hypothesis of increasing traits typically associated with rapid proliferation in fertile, high FRB patches (H2.1). Decreases in FRB are commonly observed in tree plantations after fertilization (see Litton et al. 2007 for a review), which is usually attributed to a shift in carbon allocation to aboveground tissues as soil resource availability increases. Similarly, Ostonen et al. (2011) and Montagnoli et al. (2012) reported higher root length and an increase in tip abundance in low fertility areas, suggesting that morphological root traits often associated with rapid growth are actually related to nutrient scarcity, rather than pre-emptive colonization of areas with high nutrient abundance. Additionally, competitive interactions among neighbor roots can limit the level of root branchiness among individuals (de Kroon 2003, 2007), suggesting strong biotic interactions preventing a single species to take over nutrient hotspots (Gersani et al. 2001). Similar results to those found in Jennings Woods were proposed in game-theory models (O’Brien
et al. 2007) where higher biomass in nutrient-rich patches was explained in terms of co-limitation among neighbors sharing common high quality foraging areas, but more species investing in those hotspots.

We also found a relationship between species diversity and FRB even after correcting for nutrient availability, supporting the overyielding hypothesis (H2.3). The identity of neighboring species also plays a role in the overall community response, with higher biomass in cores occupied by distantly related species (Cadotte et al. 2009). Similar relationships between phylogenetic diversity and ecosystem overyielding have been described previously (Cadotte et al. 2008, Burns and Strauss 2011), particularly in late successional stages in natural ecosystems (Cardinale et al. 2007, Flombaum and Sala 2008). In this study, root traits were highly structured by phylogeny (Table S3), and we previously found that some species root traits shift in response to the identity of root neighbors, resulting in a more even distribution of traits than expected (Valverde-Barrantes et al. 2013). These results indicate that the enhanced root productivity among distantly related species is a result of complementary tradeoffs to avoid competition among overlapping roots. A related mechanism by which phylogenetic diversity may result in root overyielding is through the relationships between roots and associated microorganisms (Newman et al. 1995, Maherali and Klironomos 2007). Theoretical studies have predicted stable mutualistic associations between microorganisms and plants (Kier et al. 2003, Kiers and van der Heijden 2006) and molecular studies have revealed
that observed symbiotic associations are not random (Lambers et al. 2009, Öpik et al. 2010). Moreover, the release from species-specific soil pathogens in species mixtures has been hypothesized to contribute to species coexistence, root overyielding (Maron et al. 2011, de Kroon et al. 2012), and the maintenance of higher patch fertility (Fornara et al. 2009). Further analyses are necessary to evaluate the role of plant phylogenetic diversity and the associated rhizosphere community on ecosystem productivity, but our results suggest that it probably plays an important role in soil patch and ecosystem productivity.

**Species abundance above and belowground:** A main objective in our study was to test the hypothesis that species are equally represented below and aboveground. Root:shoot ratios have been used as a surrogate of allocation strategies and a linear relationship among species is expected, assuming a direct correspondence between canopy demand and belowground root proliferation (Farrar and Jones 2003, Finér et al. 2007, Jones et al. 2011). However, belowground under and over representations are commonly reported. For instance, Meinen et al. (2009) found a 30-40% belowground under-representation of *Fagus sylvatica* in mixed temperate forests. Rewald and Leuchner (2009) reported > 35% difference between leaf area index and root biomass for *Quercus petraea* in mature stands, which is similar to the ~33% difference between basal area and root biomass difference for ECM species in Jennings Woods.

Differences in rhizodeposition and mycorrhizal maintenance could be a possible reason for the contrasting differences in belowground biomass between species.
(Marshner 1995, Matamala et al. 2003). For instance, ECM roots may be stronger sinks of C when compared with AM roots, leading to contrasting patterns in fine root productivity and rhizosphere activity (Phillips and Fahey 2005, 2006). Within AM species, root productivity could be also strongly affected by mycorrhizal dependency, with species with larger root diameter and scarcely branched systems depending more on physiological adaptations (Poorter et al. 1990) or mycorrhizal interactions for nutrient uptake (Brundrett 2002, Johnson and Gehring 2007), instead of high root biomass and surface area. Studies in tree plantations, for example, have shown that stands dominated by different species can have contrasting FRB accrual but similar belowground carbon allocation and total soil respiration, indicating similar amounts of photosynthates allocated belowground (Giardina and Ryan 2002, Russell et al. 2007). Moreover, species with thick, slow growing root systems showed lifespans almost twice as long as species with finer root systems (Valverde-Barrantes 2007, McCormark et al. 2012), suggesting a tradeoff between root maintenance cost, longevity and proliferation rates among trees. These results are consistent with previous findings on differences in root longevity and maintenance cost between colonized and non-colonized mycorrhizal roots (Eissenstat et al. 2000) and between non-woody species with distinct mycorrhizal dependency (Manjunath and Habte 1990, Newman et al. 1995).

Conclusions and implications: We explicitly tested the importance of soil resource heterogeneity, species identity, root neighborhood diversity, and root trait variation in
determining the distribution of fine roots in a relatively diverse temperate forest. We conclude that both nutrient availability and root neighborhood diversity have a significant effect on FRB distribution, with little evidence for spatial segregation among individuals or specialization of particular root traits for different soil conditions. Moreover, the effect of soil conditions on FRB accrual seems to vary when moving from central ecosystem areas toward ecotones.

These results have important implications for future belowground studies. For instance, our conclusions suggest that quantification of FRB must consider the level of resource heterogeneity within both central ecosystem areas and ecotones to accurately represent the natural variation of root biomass accrual at the stand level. In the case of habitat restoration and soil carbon sequestration, if one of the goals is to maximize belowground productivity, species assemblages should incorporate a wide representation of evolutionary lineages. Similarly, our data suggest that commercial tree plantations should consider phylogenetically mixed communities, rather than monocultures, in order to maximize soil resource use and reduce pathogen pressure belowground. Finally, our results highlight the important role that root interactions may have in ecosystem functionality. The idea of segregated foraging areas among coexisting individuals is possibly limited to strongly nutrient or water restricted communities (Schenk 2006), with direct interactions among roots in forests being more pervasive than can be inferred simply by examining the overlap of plant canopies (Jones et al. 2011). In addition, we
found belowground foraging behavior in trees to be influenced by nutrient heterogeneity in soils independently of species identity, in contrast to the hypothesized scale-precision tradeoff mechanism commonly mentioned as a driver of plant coexistence in natural communities (Kembel and Cahill 2005, Kembel et al. 2008). Instead, our results suggest an optimality foraging framework where most species will invest in foraging from areas with higher resource availability, and the level of biomass productivity in the patch will depend on carbon allocation patterns and phylogenetic relatedness among neighbors (McNikle and Cahill 2009).

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Table S5.1 Diversity indexes calculated in this study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Acronym</th>
<th>Definition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Richness</td>
<td>S</td>
<td>Number of species.</td>
<td></td>
</tr>
<tr>
<td><strong>Functional attribute diversity</strong></td>
<td>FAD</td>
<td>Sum of Euclidean distances estimated from a scaled trait matrix including all traits for each species present in a sample.</td>
<td>Walker et al. 1999</td>
</tr>
<tr>
<td>Functional diversity</td>
<td>FD</td>
<td>Total branch length from a hierarchical clustering tree calculated from Euclidean distances of a scaled trait matrix in each sample.</td>
<td>Petchey and Gaston (2002)</td>
</tr>
<tr>
<td><strong>Functional diversity NMDS</strong></td>
<td>FD-NMDS</td>
<td>Similar to FD but clustering based on dimensions generated from a NMDS analysis, thus accounting for correlations between traits.</td>
<td>Cadotte et al (2009)</td>
</tr>
<tr>
<td>Phylogenetic diversity</td>
<td>PD</td>
<td>Total branch lengths from a phylogenetic tree constructed with all species in the community. PD for each sample includes only members of the community present in each sample.</td>
<td>Cadotte et al (2008).</td>
</tr>
</tbody>
</table>
Table S5.2. Least square mean fit using average core root trait values and fine root biomass in each core (n=95)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Intersection</th>
<th>Slope</th>
<th>R^2</th>
<th>R^2(adj)</th>
<th>F</th>
<th>Prob(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRL</td>
<td>355.88</td>
<td>-2.94</td>
<td>0.09</td>
<td>0.08</td>
<td>9.45</td>
<td>0.002</td>
</tr>
<tr>
<td>SRA</td>
<td>372.80</td>
<td>-0.20</td>
<td>0.11</td>
<td>0.10</td>
<td>11.36</td>
<td>0.001</td>
</tr>
<tr>
<td>Diameter</td>
<td>327.80</td>
<td>-81.10</td>
<td>0.10</td>
<td>-0.01</td>
<td>0.11</td>
<td>0.65</td>
</tr>
<tr>
<td>RTD</td>
<td>171.63</td>
<td>527.37</td>
<td>0.10</td>
<td>0.09</td>
<td>10.45</td>
<td>0.002</td>
</tr>
<tr>
<td>Fractal</td>
<td>122.30</td>
<td>106.50</td>
<td>0.01</td>
<td>-0.01</td>
<td>0.36</td>
<td>0.54</td>
</tr>
<tr>
<td>SRTA</td>
<td>374.21</td>
<td>-30.07</td>
<td>0.14</td>
<td>0.13</td>
<td>15.99</td>
<td>0.0001</td>
</tr>
<tr>
<td>Length</td>
<td>226.87</td>
<td>178.85</td>
<td>0.01</td>
<td>-0.01</td>
<td>0.01</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Table S5.3 Correlation matrix for all diversity distance parameters considered in this study. Diversity index acronyms explained in Table S5.1

<table>
<thead>
<tr>
<th></th>
<th>PD</th>
<th>S</th>
<th>FD</th>
<th>FAD</th>
<th>FAD-NMDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>1.00</td>
<td>0.89</td>
<td>0.79</td>
<td>0.72</td>
<td>0.86</td>
</tr>
<tr>
<td>S</td>
<td>0.89</td>
<td>1.00</td>
<td>0.83</td>
<td>0.86</td>
<td>0.75</td>
</tr>
<tr>
<td>FD</td>
<td>0.79</td>
<td>0.83</td>
<td>1.00</td>
<td>0.92</td>
<td>0.68</td>
</tr>
<tr>
<td>FAD</td>
<td>0.72</td>
<td>0.86</td>
<td>0.92</td>
<td>1.00</td>
<td>0.60</td>
</tr>
<tr>
<td>FAD-NMDS</td>
<td>0.86</td>
<td>0.75</td>
<td>0.68</td>
<td>0.60</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Figure S5.1. Alternative clustering arrangement based on phylogenetic and functional root traits for 14 dominant canopy tree species in a deciduous temperate forest. A) The phylogenetic tree was obtained as a subset of an angiosperm super-tree previously published (Webb and Donahue 2005). B and C represent the clustering of species based on functional trait similarities for eight root functional traits (See Table S1). Black squares at tree tips represent ectomycorrhizal species and gray squares represent arbuscular-mycorrhizal species.
CHAPTER SIX: SYNTHESIS

The importance of root system architecture in determining the degree of benefit available to plants from forming AMF associations is now a commonplace idea (Fitter 2004, Comas et al 2012). Nonetheless, there are still important gaps in the understanding of the process leading to the actual patterns in root morphology and their ecological implications that have not been properly addressed. Throughout this dissertation I tried to expand our knowledge of root ecology by focusing on two important topics: 1) The exploration of the possible steps involved in the evolution of root traits during woody angiosperm evolution, and 2) the ecological implications that these adaptations may play in the belowground interactions of tree communities. For the first topic I decided to focus on woody plants because this possibly was the first habit showed by ancestral angiosperms (Feild et al 2009), giving us the opportunity to control for additional adaptations leading to non-woody root systems. Moreover, the studies were conducted in common gardens where site conditions and plant interaction factors were minimized (Comas et al 2012). For the second topic, our efforts were focused on the description of root functional traits in natural conditions, quantifying the importance of biotic and abiotic factors in the assemblage of root neighborhoods. We conducted this study in an extensively studied forest ecosystem with relatively high diversity (Blackwood et al 2013). We were able to integrate a large set of information including ecosystem spatial distribution, floristic composition and detailed soil descriptions in our analysis of root trait distribution.
Topic 1: Angiosperm root evolution and its implications for AMF communities.

Thoughts on root function in ecology had a major shift in the last decade. Initial studies quantifying fine roots had to deal with the problem of identifying the structures within root systems that perform the acquiring function belowground. Unlike aboveground tissues that present a clear division between photosynthetic and structural functions, roots are a continuum of organs that vary mostly in diameter but without a clear division between functional types. Moreover, there was a lack of information about basic processes such as tissue lifespan and chemical composition that could help elucidate how the entire root system is integrated (Pregitzer 2002). As a consequence, most researchers adopted the ‘pick and weigh’ approach (Gaudinski et al 2001), where arbitrary diameter categories were selected in order to separate root functions (e.g., fine roots were usually defined as roots < 2mm in diameter, Vogt et al 1996, Jackson et al 1997). Further advances demonstrated that root traits vary relative to the position of a link in the entire system, changing the previous simplified categorical perspective (Pregitzer et al 2002, Guo et al 2004, 2008). Now it is largely accepted that the first three root orders are the main contributors to nutrient uptake among woody species (Comas and Eissenstat 2009).

In this document we explored two relevant questions related to this new view of root ecology. First, if the first three most distal root orders in woody plants are the ones specialized for nutrient acquisition, how are functional traits in these organs related to the rest of the plant? Previous approaches, based on optimality theory (Grime 1977) and the ‘plant economics spectrum’ (Wright et al 2004, Freschet et al 2010), predicted that traits associated with resource acquisition should be positively correlated. If this is the case,
there should be an integration of similar resource acquisition strategies at the entire plant level (Mommer and Weemstra 2012). As an alternative hypothesis, we evaluated the possibility that root traits could be better explained as an acquisition strategy adopted independently of aboveground traits by different plant lineages over time.

Our results did not support the hypothesis of a single axis of variation explaining trait variation across plant organs. In fact, we found low correlation not only between above and belowground tissue traits but between leaf and branch traits as well. Only N concentration showed consistent variation across all plant organs. More interestingly, we found the typical negative relationship between N concentration and tissue density only in leaves, whereas roots showed no correlation between morphology and chemistry. In support of the phylogenetic hypothesis, we found a significantly higher phylogenetically conserved trait distribution in belowground tissues. As groups, magnoliids had a consistently different morphology than the other angiosperms, but rosid roots were chemically different than other major angiosperm clades.

The second question we examined during the study of angiosperm root evolution was about the importance that those traits may have on variation in mycorrhizal colonization rates among species. The consistent effects of plant phylogeny on mycorrhizal responsiveness has been commonplace in the literature, with several studies referring to plant family as a good factor to infer mycorrhizal dependency (Pringle and Beaver 2008, Hoeksema et al 2010). However, these studies have lacked a proper representation of the angiosperm phylogeny, mainly due to the biased dominance of rosid trees in temperate areas where most studies have been conducted (Chen et al 2013).
Studies have also been limited to examination of the relationships between anatomical or morphological traits and mycorrhizal colonization, overlooking the importance of chemical mechanisms of control on AMF (Bago et al. 2000, Vierheilig 2004, Comas and Eissenstat 2009).

Our results in this section provide two important contributions to the study of the coevolution between angiosperms and AMF. First, it provides for the first time a quantitative description of the interaction between cortical and vascular tissue and its relationship with mycorrhizal colonization for a phylogenetically diverse set of angiosperms. This approach helped to elucidate the remarkable similarities in vascular area showed for most trees, and therefore the large differences in cortex area provided to AMF. It also helped to illustrate how variation in cortical and vascular tissue may reflect adaptations for AMF control (Smith and Read 2008). Second, the study provides compelling information supporting Baylis’ hypothesis of morphological divergence among angiosperm lineages. Furthermore, the phylogenetic clustering of traits is not limited to morphology but extends to chemical and anatomical traits too, and encompasses the entire root system rather than just first order roots. The idea that resource acquisition is limited only to very fine distal roots may be biased by the overrepresentation of rosid species in the literature (Comas et al 2012). It is possible that this impression may vary as studies in root ecology move to tropical and subtropical areas, where there is much greater representation of basal phylogenetic clades, there may be more species with thicker roots, and acquisition capacities may be maintained at higher root orders (see Chen et al 2013). Our results suggest that future studies regarding
the phylogenetic background of plants will be increasingly informative, especially if less studied plant taxa get incorporated (Brundrett 2009).

**Topic 2: The integration of root functional traits in community ecology:**
Variation in fine root biomass can be understood as a function of the interaction between availability of resources and species response to nutrient patches. Nutrients and roots are unevenly distributed in natural environments and roots tend to proliferate in high-nutrient patches (Hutchings et al 2003). A prevalent vision in root ecology is that root trait differences among species reflect alternative acquisition strategies, implying some degree of belowground niche partitioning (Campbell et al 1991, Kembel et al. 2008). Species with traits associated with high root growth capacity (i.e., high specific root length, small diameter, low tissue density) should be better adapted to proliferate in nutrient-rich patches whereas traits associated with nutrient conservation should be prevalent in less fertile areas (Hutchings et al 2003). According to this hypothesis, variations in root morphology could either reflect an adaptation for specific soil conditions, or an adaptation to partition soil resources among roots of different species in relatively close proximity. Moreover, it predicts that species should be strongly segregated belowground and differences in root distribution should correspond with differences in biomass productivity among coexisting species (Casper and Jackson 1997).

In order to understand competition belowground in a natural community and the root characteristics that drive competitive ability, we studied the distribution of root traits and fine root biomass at the species level in a relatively diverse temperate deciduous forest. We found that intraspecific variation in root traits was more influenced by the
identity of other species within samples than by soil conditions, suggesting that biotic interactions play an important role shaping root trait distributions. We also found an extensive overlap among species with respect to soil conditions, indicating that species are able to proliferate roots in diverse environmental conditions, and possibly extend far from their area of direct influence (Jones et al 2011). Using molecular techniques to identify roots to the species level, we found no evidence of spatial segregation, either vertically or horizontally, among the most dominant species in the forest. Thus, our results contradict the idea of high specialization in root distribution based on functional trait or competitive ability.

When fine root biomass spatial variation was included in the analysis, we found that fine root biomass was positively related to resource availability. We further explore the idea that other dimensions of biodiversity like functional or phylogenetic diversity could be related to biomass accumulation. Here, we found a positive relationship between root biomass and phylogenetic diversity, even after correcting by nutrient availability. These results suggest that the identity of species, rather than particular traits, was important explaining the relationship between resource availability and root productivity (Cardinale et al 2008, Cadotte et al 2009). Our results highlight the importance of the belowground component in the understanding of ecological process such as biomass accrual, and the need to incorporate other dimensions of biodiversity to better understand ecosystem productivity belowground.
Conclusion and future research: Throughout this dissertation I provided evidence that fine root systems of woody angiosperms can vary widely. In chapter two, we provided compelling evidence that that variation is in large measure due to alternative trait syndromes evolved independently among angiosperm lineages. In chapter three, I tested the idea that the described separation in root traits among phylogenetic angiosperm groups reflects their dependency from mycorrhizal associations (Baylis 1975, Comas et al 2012). I extended this hypothesis, providing evidence that the observed root trait syndromes in woody angiosperms reflect different evolutionary pathways for nutrient acquisition and includes the entire root system rather than only the root tips.

In chapters four and five, I explored the role that root trait diversity and species identity may play in the interactions of species belowground in a natural ecosystem. In chapter four, results showed that the interspecific variation in root morphological traits is not related to specific soil conditions. Rather, it seems to enhance the ability of species to coexist in relatively aggregated conditions. Accordingly, in chapter five, we found no evidence for spatial segregation among species. In fact, root biomass variation was explained by both resource availability and phylogenetic diversity, highlighting the importance that evolutionary process may have in ecosystem stability (Cadotte et al 2009).

Future work should cover some aspects not included in this study. One important limitation of this study was the relatively restricted set of species studied in my comparison of phylogenetic groups. Important angiosperm groups, including basal eudicots and some basal families such as Amborellaceae and Chloranthaceae, could add
important information and extend the conclusions derived here. Similarly, a parallel study contrasting root evolution in angiosperms and conifers could provide important insights, not only on the relationship between woody plants and AMF, but also on root modifications involved in the transition to other mycorrhizal associations (Brundrett 2002).

Another important avenue of research derived from this dissertation involves the importance of biotic interactions defining the distribution of traits belowground. For instance, higher root biomass associated with phylogenetic relatedness among neighbors suggests that strong interactions between roots and rhizosphere microbes may determine efficiency in plant resource uptake (Whitbeck and Cardon 2007). Understanding these mechanisms could have profound implications in forest ecology and conservation. First, this approach could help to predict the ultimate impact of human alterations in forest ecosystems (Matamala et al 2005). Investigations at the root system level may also predict how species will be affected by changes in resource availability and eventually which species will be displaced from the system. More detailed studies looking at the processes of root growth and proliferation in natural conditions could shed light on the effect of root dynamics on forest carbon cycling. Such information could enhance our capacity to increase soil carbon accrual in plantations or managed forests (Giardina et al 2005).
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