Aggregated and complementary: symmetric proliferation, overyielding, and mass effects explain fine-root biomass in soil patches in a diverse temperate deciduous forest landscape

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Summary

• Few studies describe root distributions at the species level in diverse forests, although belowground species interactions and traits are often assumed to affect fine-root biomass (FRB).
• We used molecular barcoding to study how FRB of trees relates to soil characteristics, species identity, root diversity, and root traits, and how these relationships are affected by proximity to ecotones in a temperate forest landscape.
• We found that soil patch root biomass increased in response to soil resources across all species, and there was little belowground vertical or horizontal spatial segregation among species. Root traits and species relative abundance did not explain significant variation in FRB after correcting for soil fertility. A positive relationship between phylogenetic diversity and FRB indicated significant belowground overyielding attributable to local root diversity. Finally, variation in FRB explained by soil fertility and diversity was reduced near ecotones, but only because of a reduction in biomass in periodically anoxic areas.
• These results suggest that symmetric responses to soil properties are coupled with complementary species traits and interactions to explain variation in FRB among soil patches. In addition, landscape-level dispersal among habitats and across ecotones helps explain variation in the strength of these relationships in complex landscapes.

Introduction

In forest ecosystems, fine roots (<1 mm in diameter) represent an important belowground carbon (C) sink (Jackson et al., 1997; Ruess et al., 2003). Root interactions also play a prominent role in defining the relationships between plant species diversity and ecosystem function (Schenk, 2006; de Kroon et al., 2012). Nevertheless, there is still little information about factors driving species-level root productivity in natural, diverse forest ecosystems or how interactions among species affect the spatial variation of fine-root biomass (FRB) (McNickle et al., 2009; Meinen et al., 2009). Spatial variation in FRB is often attributed to soil resource heterogeneity (Hutchings & de Kroon, 1994; Hutchings et al., 2003). Root proliferation in nutrient hotspots is considered a common competitive strategy, with prolific species gaining an advantage by pre-emptively obtaining available resources (de Kroon et al., 2003, 2012). Thus, competition for nutrient-rich patches is expected to be intense (Wilson, 2000; Blair, 2001), with a substantial effect on plant fitness (Hodge, 2004) and community productivity (Wijesinghe et al., 2005). Modeling studies also suggest that species that do not proliferate in high-nutrient patches are at a competitive disadvantage (O’Brien et al., 2007). However, some species show no selectivity in the placement of their roots in patchy environments (Robinson, 1994, 1996) or respond through altered metabolic rates rather than by root proliferation (Jackson et al., 1990; Lambers & Poorter, 1992; Schimel & Bennett, 2004; Phillips & Fahey, 2005, 2006). Thus, in addition to spatial variation in soil nutrients, variation in foraging strategies could have important effects on belowground relative abundance and spatial biomass variability in forest stands (Mou et al., 1997; Giardina et al., 2005; Coleman, 2007).

If some species are adapted for pre-emptive colonization of nutrient-rich patches, they are expected to exhibit traits that would allow them to proliferate quickly and exclude other species, including high specific root length (SRL) and/or low root tissue density (RTD) (Campbell et al., 1991; Hutchings et al., 2003). Other species would be expected to allocate most roots to areas not exploited by the ‘pre-emptive’ species, and to exhibit traits more associated with resilience (de Kroon & Mommer, 2006). Thus, under this ‘asymmetric root proliferation’ scenario, FRB should be positively associated with nutrient availability in a soil patch, as well as the abundance of roots of the pre-emptive,
aggressively proliferating species, but FRB would not be correlated with species diversity of roots. By contrast, if all species have a similar capacity to detect and proliferate in nutrient hot-spots (symmetric root proliferation), then both FRB and species diversity (regardless of species traits) should be associated with high soil resource availability (Table 1).

In addition to responses to soil resource heterogeneity, FRB could also be affected by biotic interactions among neighbors. ‘Overyielding’ occurs where mixtures of species obtain greater biomass than would be expected from species monocultures (i.e. biomass is positively correlated with diversity; Berendse, 1983; Cardinale et al., 2007; Kesnakurti et al., 2011). Several mechanisms have been proposed to explain strong belowground overyielding in herbaceous plant communities. Mixtures of species exploiting different belowground niches (e.g. different soil depths) may result in overall greater resource uptake (Dimitrakopoulos & Schmid, 2004). Plastic root allocation in response to heterospecific root neighbors can result in increased root biomass due to greater soil depth exploration (Mueller et al., 2013) or common responses to growth-stimulating root exudates or rhizosphere microorganisms (Mommer et al., 2010). Species mixtures may also result in lower pathogen pressure, allowing for increased FRB production (Maron et al., 2011; Schnitzer et al., 2011; de Kroon et al., 2012). However, evidence for belowground overyielding in forests remains controversial, with reports of negative (Bolte & Villanueva, 2006), neutral (Meinen et al., 2009), and positive effects of tree diversity on FRB (Brassard et al., 2011, 2013; Lei et al., 2012). Similarly, the vertical distributions of tree roots vary from spatial segregation by 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 diversity effects on belowground biomass could be affected by the particular species in root neighborhoods. The importance of species-specific interactions is also consistent with identity of root neighbors driving morphological trait plasticity to achieve an even distribution of traits, which reduces trait overlap and may help avoid negative competitive interactions (Leuschner et al., 2001; Valverde-Barrantes et al., 2013). Thus, functional or phylogenetic diversity may be the strongest driver of the overyielding response of FRB, rather than total species richness (Cardinale et al., 2007; Flombaum & Sala, 2008).

Finally, the influence of tree diversity and soil conditions on FRB 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), have been found to decouple community composition from the environment as a result of independent transport of propagules and abiotic material (Blackwood et al., 2013). Dispersal of propagules from an optimal environment across an ecotone can result in ‘mass effects’, in which propagules establish under nonoptimal conditions, but reduced reproductive output limits further spread in the nonoptimal environment. This weakened filtering of community composition by the environment may provide an opportunity for the effects of niche partitioning and overyielding on root biomass to become more apparent. However, ecosystem-level phenomena such as biomass accumulation are mediated by traits that are often shared by a variety of species (Grime, 2006). Thus, root biomass may be more tightly linked to environmental conditions, and therefore more resistant to ecotone decoupling, than community composition.

Our objective was to use a natural landscape with three adjacent, mixed-species forest ecosystems to examine soil and community controls on FRB. First, we sought patterns consistent with three hypotheses: (H1.1) asymmetric root proliferation, in which certain species are able to dominate root biomass in high-resource soil patches; (H1.2) symmetric root proliferation, in which there is no inherent advantage of a particular species in colonizing high-resource soil patches; (H1.3) overyielding, in which local root diversity explains additional variation in FRB that was not explained by soil fertility and symmetric or asymmetric root proliferation. Previously at our study site, we showed that both community-aggregated and species-level root traits were associated more closely with species identity of interacting roots than with soil conditions (Valverde-Barrantes et al., 2013), which seems inconsistent with trait-mediated asymmetric root proliferation (H1.1). Here we tested for a variety of other patterns in root

Table 1 Predicted effects of spatial soil resource heterogeneity and root species diversity on fine-root biomass (FRB) accrual and root trait distribution in soil patches under three hypothesized mechanisms (H1.1–1.3)

<table>
<thead>
<tr>
<th>Root proliferation pattern</th>
<th>Asymmetric root proliferation (H1.1)</th>
<th>Symmetric root proliferation (H1.2)</th>
<th>Overyielding (H1.3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root biomass increases with soil resources</td>
<td>Yes</td>
<td>Yes</td>
<td>Possibly</td>
</tr>
<tr>
<td>Patterns in species distributions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertical species segregation</td>
<td>Possibly</td>
<td>No</td>
<td>Possibly</td>
</tr>
<tr>
<td>Horizontal species segregation(^1)</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Particular species associated with high root biomass</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Patterns in trait distributions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trait association with root biomass</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Distribution of root traits within cores(^1)</td>
<td>Clumped</td>
<td>Random</td>
<td>Even</td>
</tr>
<tr>
<td>Effects of root diversity on FRB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species diversity increases with root biomass</td>
<td>No</td>
<td>Possibly</td>
<td>Yes</td>
</tr>
<tr>
<td>Effect of species diversity additive to soil resource effect</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

\(^1\)These tests were originally reported in Valverde-Barrantes et al. (2013). Results are used to inform our interpretation of other tests shown in this table.
biomass, species composition, soil resources, root traits, and root diversity (Table 1) that would be consistent with the hypotheses described above. Secondly, we hypothesized that landscape position with respect to ecotones has an effect on controls over FRB; in particular, we tested the hypothesis that (H2) areas near ecotones decrease the explanatory ability of soil conditions but increase the influence of community composition on FRB spatial variation. Again, at our study site we previously showed that tree community composition is decoupled from soil properties near ecotones (Blackwood et al., 2013), but the effects of ecotones on controls over biomass accumulation have not been explored.

Materials and Methods

Site description

The study was conducted in Jennings Woods, a 30-ha forest in northeast Ohio, USA. *Acer saccharum* Marsh., *Acer rubrum* L., *Fagus grandifolia* Ehrh., and *Quercus rubra* L. encompass nearly 60% of the forest tree cover, with 11 other species accounting for most of the remaining basal area (Blackwood et al., 2013). The property includes three main forest ecosystems and additional smaller scale variation in soil characteristics and tree communities (Blackwood et al., 2013). A riparian forest with fine-loamy Inceptisols dominates areas within 80 m of the West Branch of the Mahoning River. A plateau 15–20 m above the riparian forest is dominated by upland forest with fine-loamy Alfisols. A third ecosystem is represented by bottomland forest on a lower elevation plateau with Alfisol soils that are very poorly drained. The forest was selectively logged during the early 20th Century, and has remained undisturbed for at least 60 yr.

Vertical root biomass and species distributions

Vertical root biomass distributions were determined by collecting soil samples with a 5-cm-diameter core from 44 locations. Core depth was 52 ± 8.75 cm (mean ± SD), reaching the C horizon in most cases, and this set of samples is hereafter referred to as ‘deep cores’. Samples were located on a grid to ensure coverage of the entire area and to include each distinct ecosystem within the landscape (i.e. upland, bottomland, and riparian forest) and transition points between ecosystems. Deep cores were dissected by horizon and gently sieved to <2 mm. During this process, fine roots (<1 mm diameter) were manually retrieved from each horizon and sorted into dead and live roots based on friability, color and cortex conditions (Bouma et al., 2000). 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. Remaining roots from each category were dried at 60°C for 24 h, and then weighed.

Tree species presence in different soil horizons was assessed using DNA extraction from pooled (mixed-species) root samples from each horizon, followed by terminal restriction fragment length polymorphism (T-RFLP; see details in Supporting Information Methods S1). The noncoding spacer region *trnH-psbA* was PCR-amplified (Kress et al., 2005) using fluorescently tagged primers, followed by restriction digestion and capillary electrophoresis (Blackwood et al., 2003; Taggart et al., 2011). We also created a reference library by running a similar T-RFLP procedure from leaf DNA samples.

Sampling root biomass, traits, and species across ecosystems and ecotones

Based on 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. 1a). Consequently, we collected additional ‘surface cores’ from 130 plots (2.5 × 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. Each soil core was soaked in water for at least 12 h and all intact fine-root clusters (i.e. roots consisting of three to four root orders c. 10 cm in length and <1 mm in diameter; Xia et al., 2010) were carefully removed and stored in deionized water. Five to 10 intact root systems were separated from each core for trait analysis and identification. All remaining root fragments from each soil sample were collected by washing soil through a 500-μm sieve, after which they were dried and weighed for calculation of total FRB in the core (see Valverde-Barrantes et al., 2013).

We identified individual root samples from the surface cores to species by comparing their restriction fragment length polymorphism (RFLP) patterns to a reference library based on leaf collections from 33 tree canopy species (see Methods S1 and Valverde-Barrantes et al., 2013). We generated barcodes by combining *Hinfl* RFLP patterns of the plastid gene *trnL* and the non-coding spacer region *trnH-psbA* (Brunner et al., 2001). Seven hundred and thirty-eight root systems belonging to 14 canopy tree species were identified. Relative abundance of a species in a core was calculated as the sum of individual root system biomasses identified for that species in that core divided by the total biomass of all roots identified in that core. Calculation of diversity in each core is described in the Statistical Analysis section below.

Individual root samples from the surface cores were also 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⁻¹), specific root surface area (SRA; cm² g⁻¹), root tissue density (RTD; g cm⁻³), average diameter for first-order roots (mm), fractal dimension (*D*, unitless), specific root tip abundance (SRTA; tips per mg) and average link length (*L*; cm). These traits have all been employed in the characterization of root systems in previous studies (e.g. Eshel, 1998; Hertel et al., 2003; Meinen et al., 2009; Wang et al., 2009; Dupuy et al., 2010).

Soil conditions and aboveground species distribution

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 and pooled from each of the 93 plots. We measured 14 soil variables including per cent clay, per cent silt, per cent sand, per cent moisture, pH, per cent soil carbon (C) and nitrogen (N), 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 (P). To summarize overall nutrient variation, we used the first two principal components analysis (PCA) axes from an analysis of C, N, and P (explaining 79% and 14% of variance), hereafter referred to as Fertility PCA axes 1 and 2 (Valverde-Barrantes et al., 2013). POM C and N were also used separately as measures of active organic matter that may be mineralized on a shorter time-scale (Zeller & Dambrine, 2011). To summarize soil texture, we used the first PCA axis from an analysis of per cent clay, silt, and sand (explaining 98% of variance), hereafter referred to as the Texture PCA axis. In total, 11 soil variables were utilized for subsequent statistical analyses, including Fertility PCA axes 1 and 2, Texture PCA axis, per cent moisture, pH, C : N ratio, per cent POM C, per cent POM N, and POM C : N ratio.

Statistical analysis

All analyses were conducted using the software R 2.15.1, packages PICANTE 1.2 (Kembel et al., 2010) and VEGAN (Oksanen et al., 2008), and R scripts modified from Ackerly & Cornwell (2007) and Cadotte et al. (2009).

Testing for vertical species segregation We used depth profiles of roots identified by T-RFLP in the deep cores to look for vertical segregation of species, which would be inconsistent with symmetrical species proliferation (H1.2). First, we tested for different mean depths among species using a mixed model ANOVA where depth at which roots were detected was the response variable, species identity was the predictor, and plot identity was the random effect. Secondly, we tested for different maximum depths among species using a univariate ANOVA with species identity as the predictor, but using only the maximum depth for each species in each core. Thirdly, we tested for shifts in depth of a target species as a result of the presence of other species using the variable selection framework provided by redundancy analysis (RDA; Blanchet et al., 2008). For each target tree species, we used presence/absence vectors of other species as predictors of either maximum or average root depth of the target species in each core. In this analysis, no significant relationships were found, so we did not proceed further with variable selection. Fourthly, for each target tree species, we examined Pearson correlation coefficients between maximum or average depth and richness of either tree species or all T-RFLP bands detected in a core.

Fine-root biomass correlation with soil fertility, root traits, and species identity To test if FRB accrual was associated with soil variables, we performed a multi-step variable selection procedure using RDA following Blanchet et al. (2008). Log-transformed FRB from each core was used as the response variable. We conducted a preliminary analysis including all soil variables as predictors. We included linear and squared values of soil variables in this analysis because examination of bivariate plots showed an expected nonlinear (saturating) relationship between soil conditions and FRB. If the preliminary analysis was significant,
forward selection of predictor variables was performed to obtain a parsimonious model (Blanchet et al., 2008).

Under the asymmetric root proliferation hypothesis (H1.1, and in contrast to H1.2), we predicted an association between FRB accrual and the dominance of species bearing traits associated with fast growth rates (i.e. long SRL and high SRTA). To test for an affiliation between root traits and FRB accrual, we repeated the RDA variable selection procedure described above using average core-level (community-aggregated) root traits as predictors. To test for an affiliation of particular species with FRB accrual, we performed regressions between each species relative abundance and FRB. However, we performed the regression for each species using a restricted set of samples to avoid spurious results related to root absences in soil conditions outside the normal range of the species. For each species, we only used samples that fell within the ranges of soil pH, moisture, or Fertility PCA1 where that species was detected. Species were selected for further analysis if their relative abundance was significantly related to FRB within one or more of these sample sets.

After selecting soil properties, root traits, and species abundances related to FRB, variance partitioning and partial tests were performed to determine the significance of each factor after correcting for effects of other factors (Peres-Neto et al., 2006).

**Overyielding effects on FRB variation** Here, our goal was to test for overyielding (H1.3) by determining the amount of variation in FRB that could be explained by local species diversity, after accounting for soil factors selected during RDA as described in the previous section. Detailed descriptions of diversity indices are given in Methods S1 and Cadotte et al. (2009). We calculated a series of diversity metrics summarizing root diversity in terms of species richness (S), phylogenetic dispersion (mean phylogenetic distance (MPD) and mean nearest taxon distance (MNTD)), phylogenetic diversity (PD, which aggregates species richness and phylogenetic dispersion), and functional traits (functional attribute distance (FAD), functional distance (FD), and nonmetric dimensional scaling-based functional distance (NMDS)). We then tested whether these diversity indices significantly explained variance in residual FRB variation after correcting for soil conditions (Cadotte et al., 2009). Diversity indices were evaluated using the same RDA variable selection procedure described in the previous section. Only cores with at least one of the 14 most abundant species were included in the analysis (n = 86; Loreau & Hector, 2001; Dybzinski et al., 2008). We included the normalized linear and squared values of diversity variables. A significant positive relationship between the residual FRB values and diversity metrics was interpreted as evidence for overyielding.

**Ecotone effects on FRB distribution** To test for decoupling between biotic and abiotic factors across ecosystem transitions (H2), we used the randomization test described in Blackwood et al. (2013). In brief, we classified plots and soil samples as within the ecosystem ‘core’ area if they were located > 30 m from a topographically defined ecosystem boundary, and within an ‘ecotone’ area if they were located within 30 m of the boundary.

Tests for determining controls on FRB spatial variation (described in the previous two sections) were initially performed on all plots, including core and ecotone areas. Here, the variance in FRB explained in the core plots only was also calculated. For this calculation, we reused the explanatory variables retained during variable selection on the entire data set, resulting in a conservative estimate of the amount of variance that can be explained in the core areas only. Fits of the regression models to each data set were compared based on adjusted- $R^2$ values 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- $R^2$ attributable 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 predictability of FRB.

**Results**

**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 FRB between ecosystems, we found no evidence of species segregation by depth, in support of symmetric root proliferation (H1.2), as opposed to asymmetric proliferation (H1.1). Species showed large overlap in the depths at which they were detected, with no significant differences in their mean or maximum depths ($P > 0.1$; Fig. 1b).

To test for shifts in root depth of a particular target species, we only used other species as predictors if they co-occurred in two or more soil cores. In agreement with H1.2, there were no target species where RDA indicated that average or maximum root depth was predicted by the presence of any other species ($P > 0.1$). We also did not detect any species with significant correlations between average or maximum root depth and terminal restriction fragment richness of trees or all plants in soil cores ($P > 0.1$).

**Fine-root biomass correlation with soil fertility, root traits, and species identity**

Comparing FRB in surface cores, only the finest root class showed significant differences among ecosystems, with riparian forest having 34% lower FRB than upland forest and 47% lower FRB than bottomland forest (Table 2). The preliminary tests of root traits and soil fertility variables explaining FRB were both significant ($P < 0.05$). The selected soil factors explained 39% of the variation in FRB and included Fertility PCA1 and its squared term, and C : N, POM C : N, POM C and POM N ($P = 0.001$). The root traits retained during variable selection included average diameter, SRTA, and RTD, together explaining 22% of the variation in FRB ($P = 0.001$). In contrast to our expectations for
traits based on H1.1, SRTA and SRL showed negative associations with FRB, whereas RTD was positively associated with FRB (Fig. 2a–c). The species with relative abundance significantly predicting FRB included *Acer saccharum*, *A. rubrum* and *Prunus serotina* Ehrh. Together, these species explained 11% of variation in FRB (Fig. 2d–f). See Tables S1 and S2 for details about relationships between FRB and other root traits and species.

Soil properties were still significantly related to FRB even after partitioning out the effect of root traits and species relative abundance (*P*=0.005; Fig. 3). Consistent with symmetric root proliferation (H1.2), species relative abundance was not significantly associated with FRB residuals after correcting for soil conditions (*P*=0.62), and the effect of root functional traits was only marginally significant (*P*=0.052). Although the effect of species relative abundance was mostly confounded by soil properties, 67% of the variance explained by soil properties was unrelated to species relative abundance (Fig. 3).

**Overyielding effect on FRB variation**

For surface cores, species richness (*S*) averaged approximately three species per core, with a maximum of seven. Phylogenetic diversity (PD), species richness, and functional diversity indices were highly correlated, with Pearson correlation coefficients ranging from 0.72 to 0.89, whereas indices of phylogenetic dispersion were poorly correlated with other measures of diversity (Fig. S1; Table S3). The preliminary RDA test including all diversity indices indicated that residual FRB (after correcting for soil fertility) was significantly related to root diversity in soil cores with roots of at least one of the 14 most abundant trees. Despite the close relationship between trait diversity and phylogenetic distances, PD and PD² were the only variables retained during variable selection (*P*=0.003). Inclusion of PD² created a unimodal relationship between phylogenetic distance and residual FRB, with residual FRB clearly increasing at low levels of PD (Fig. 4d). Residual FRB begins to decline at the highest levels of PD, although this may be an artifact of reduced sampling at the highest levels of phylogenetic diversity.

**Ecotope effects on strength of soil and diversity controls on FRB**

Analyses described in the previous two sections included soil samples from all landscape positions including ecotone and core ecosystem areas. To test for an effect of proximity to ecotones (H2), we calculated variance explained in samples limited to core ecosystem areas only. The relationship between FRB and soil conditions was almost twice as large in ecosystem core areas (adjusted $R^2 = 59\%$) compared with when plots near ecotones were included (adjusted $R^2 = 32\%$) (Fig. 4a,b). Similarly, variance explained by PD more than doubled in ecosystem core areas (19%), compared with when ecotone areas were included (8%) (Fig. 4c,d). The restricted randomization test showed that this dilution of variance explained was statistically significant for both soil conditions ($P=0.0001$) and phylogenetic diversity ($P=0.014$), suggesting that the relationships between these factors and FRB are decoupled at ecotones.

Examination of individual sample points in Fig. 4 indicates that the reduction in variance explained by inclusion of ecotone areas was associated with particular ecotone types. Samples from upland-bottomland and upland-riparian ecotones were farthest from the best fit curves describing the effects of soil fertility and PD on root biomass (Fig. 4b,d). However, the unexplained variation introduced by these samples was not random; these samples had less root biomass than expected based on their levels of both soil fertility and PD. These samples came from the base of topographic slopes in the bottomland or riparian forests, areas that are normally saturated with water as a result of runoff and seepage from the slopes, and are probably anoxic multiple times each year.

**Discussion**

**Dominance of symmetric root proliferation among species controlling FRB**

Vertical and horizontal root segregation resulting from asymmetric root proliferation by certain plant species is often mentioned as a mechanism of soil resource partitioning (Fitter et al., 1991; Caldwell et al., 1996) and is a pattern expected to be common across ecosystems (Tilman et al., 1997; Schenk et al., 1999). Although we found that root biomass did increase in areas of high resource availability, this is consistent with both asymmetric and symmetric root proliferation responses by different species. However, comparison of the remainder of our results to predictions in Table 1 provides more support for the hypothesis of symmetric species root proliferation responses for canopy tree root systems (H1.2). First, we found no evidence for vertical segregation among canopy tree root systems, similar to other studies.

### Table 2 Root biomass distribution for three different ecosystems in a deciduous temperate forest in northeastern Ohio (USA)

<table>
<thead>
<tr>
<th></th>
<th>FRB (g m⁻²)</th>
<th>CRB (g m⁻²)</th>
<th>WRB (g m⁻²)</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>

Categories correspond to fine root biomass (FRB; roots < 1 mm in diameter), coarse root biomass (CRB; roots 1–3 mm) and woody root biomass (WRB; 3–5 mm) in the uppermost 15 cm of the soil profile. CI, confidence interval.
using molecular techniques for root identification (von Felten & Schmid, 2008; Frank et al., 2010; Mommer et al., 2010; Ravenek et al., 2014) and in forests with similar floristic composition (Meinen et al., 2009). Moreover, the root mass depth profile indicated that species in this diverse forest tend to accumulate roots in the uppermost soil horizon, rather than distribute FRB evenly across the soil profile (Fig. 1a). Secondly, we found only a weak effect of species identity on horizontal FRB spatial distribution. Out of 14 common tree species, only the relative abundance of *P. serotina* and *A. rubrum* was strongly positively associated with FRB. At the scale of the entire community, this accounted for a small portion of the overall effect of soil properties on FRB. Thirdly, we previously showed lack of horizontal species segregation in tree roots at Jennings Woods (Valverde-Barrantes et al., 2013), similar to the unexpectedly high diversity and overlap in root systems found in other systems (Lang et al., 2010; Jones et al., 2011; Hiiesalu et al., 2012). These results indicate that there is extensive overlap in foraging areas among neighboring trees, in contrast to the idea of high among-species territoriality or niche differentiation based on asymmetric species responses to soil resources (i.e. in contrast to H1.1). Instead, roots of all species tended to proliferate in nutrient-rich areas, and higher FRB was associated with aggregation of species in nutrient-rich soils, supporting symmetric ability of species to proliferate roots in response to soil nutrients (H1.2). Thus, our study suggests that all species had relatively similar opportunities to access patchily distributed soil resources, implying that soil heterogeneity may not substantially alter belowground competitive interactions (Schenk, 2006).

**Fig. 2** Relationships between fine-root biomass (FRB), representative root traits and relative species root biomass abundance in 93 surface soil cores (15 cm deep). Upper panels: relationship between FRB and (a) average specific root tip abundance (SRTA; slope = -30.07; *F* = 15.99; *P* < 0.0001), (b) specific root length (SRL; slope = -2.94; *F* = 9.45; *P* < 0.002) and (c) root tissue density (RTD; slope = 527.37; *F* = 10.45; *P* < 0.002) (see Supporting Information Table S2 for other root traits). Lower panels: relationship between FRB and proportional abundance in root biomass of (d) *Acer saccharum* (*F* = 9.85; *P* < 0.002), (e) *Acer rubrum* (*F* = 4.45; *P* < 0.03) and (f) *Prunus serotina* (*F* = 7.69; *P* < 0.007). In each species relative abundance panel, only samples that fall within the range of soil fertility principal components analysis (PCA) axis 1 inhabited by the species are shown. The dotted line represents the linear regression fit (see Table S3 for details about other species).
Fig. 3 Variance in fine-root biomass (FRB) (adjusted $R^2$) partitioned among groups of variables after selection of a parsimonious set of significant variables ($P < 0.05$) as described in the Results section. The analysis was performed on 93 surface soil cores (15 cm depth). After accounting for variation explained by other factors (i.e. subtracting the overlap among factors), only the amount of variance explained by soil conditions remained significant ($P = 0.05$, although the effect of root traits was marginally significant). Functional root traits in this analysis include average diameter, specific root tip abundance (SRTA), and root tissue density (RTD). Proportional root abundances used included the species Acer saccharum, Acer rubrum, and Prunus serotina. Soil conditions included Fertility PCA1 and its squared term, and carbon (C) : nitrogen (N), particulate organic matter (POM) C : N, POM C, and POM N. See Fig. 2 and Tables S1 and S2 for information about individual variable relationships with FRB.

biomass in nutrient-rich areas was not the result of rapid proliferation of roots with maximum potential for resource uptake, but was instead attributable to an accumulation of roots with presumably reduced uptake capacity and longer lifespan. Similarly, Osten et al. (2011) and Montagnoli et al. (2012) reported increased root density and reduced tip abundance in high-fertility areas. These results suggest that morphological root traits often associated with rapid growth and pre-emptive colonization are actually related to nutrient scarcity. The increased root tissue density and reduced tip abundance we found associated with high biomass are thought to increase root lifespan and provide better protection from pathogens and herbivores (Poot & Lambers, 2003; Tjoelker et al., 2005). Long-term occupation and survival of roots in high-resource locations may be particularly beneficial if resource conditions persist for long periods.

Despite large trait differences among species, the variance in FRB explained by root traits did not correspond with the variance in FRB explained by species composition, suggesting that intraspecific root trait plasticity drives the variation in community-aggregated traits that helps explain FRB. This is consistent with our previous finding that individual species traits shift to avoid trait overlap with other species in the same soil core, creating a within-core trait distribution that is more even than expected by chance (Valverde-Barrantes et al., 2013). Avoiding trait overlap may be particularly important to avoid competitive interactions, pathogens, and herbivores in higher resource soil where there is consistently greater root biomass and species diversity (de Kroon et al., 2012). Interspecific interactions among neighboring roots can limit root branching (de Kroon et al., 2003; de Kroon, 2007), suggesting strong biotic interactions that prevent a single species from dominating nutrient hotspots (Gersani et al., 2001).

Overyielding of root biomass

We also found a relationship between diversity and FRB, even after correcting for soil resources, supporting the overyielding hypothesis (H1.3). Although O’Brien et al. (2007) showed that higher biomass in areas of root overlap (i.e. increased diversity) could arise from competitive foraging in a simple game-theory framework, additional patterns in our data suggest that the mechanisms behind overyielding may be more complex. The identity of neighboring species 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, but only with respect to aboveground biomass productivity (Flombaum & Sala, 2008; Burns & Strauss, 2011).

The diversity index that predicted residual FRB the best was PD, an integrated index that represents species richness, phylogenetic dispersion, and any trait diversity that is phylogenetically structured. In this study, root traits were highly structured by phylogeny (Fig. S1; Table S3). Coupled with the shift in some species’ root traits to achieve a more even distribution of traits than expected (Valverde-Barrantes et al., 2013), this indicates that the enhanced root productivity among distantly related species is a result of complementary tradeoffs to avoid competition among overlapping roots. Although all species seemed to access the resources in high-nutrient soil patches, overyielding indicates that they may not overlap completely in their access to all types of resources within the patches. In addition, phylogenetic diversity may also result in root overyielding through phylogenetic conservatism in relationships with mutualistic or pathogenic microorganisms (Schweitzer et al., 2008; Brandt et al., 2009; Lambers et al., 2009; Opik et al., 2010). Moreover, release from species-specific soil pathogens in species mixtures has been hypothesized to contribute to species coexistence and root overyielding (Maron et al., 2011; de Kroon et al., 2012), as well as the maintenance of higher patch fertility (Fornara & Tilman, 2009). Further analyses are necessary to evaluate the role of plant phylogenetic diversity and the associated rhizosphere community in ecosystem productivity, but our results suggest that these factors play an important role in root distributions and productivity.

Reduced explanatory power of soil fertility and phylogenetic diversity near ecotones

We had previously observed that relationships between tree community composition and soil properties were decoupled near the ecotones at Jennings Woods (Blackwood et al., 2013). Here, we have again found that landscape positions near ecotones
contributed substantial noise to otherwise strong patterns. In our analysis of root biomass, the explanatory power of both soil properties and PD was dramatically increased by excluding ecotone areas, despite the fact that our variable selection procedure resulted in a conservative estimate of the amount of variance explained when ecotones were excluded. However, unlike the effects of ecotones on community composition–soil property relationships, the reduction in explanatory power for root biomass was driven by specific types of ecotones and landscape positions. Ecosystem processes such as biomass accumulation may be less sensitive to phenomena (in this case, mass effects) that cause variation in community assembly (Kardol et al., 2013). The fact that species can establish in nonoptimal environments in ecotones (despite lower reproductive output) implies that there is a degree of physiological plasticity that increases the functional redundancy of species, even from different ecosystems (Violle et al., 2011; Comte et al., 2013).

Ecotone plots that did vary from expectations based on soil fertility or PD were at the base of slopes transitioning from upland to bottomland or riparian forest. These plots are poorly drained, periodically contain standing water, and may frequently become anoxic. Soil anoxia limits respiration and induces fermentation, resulting in root energy limitation and possibly root mortality and reduced photosynthetic rates unless the plant has metabolic, physiological, or anatomical adaptations to these conditions (Parent et al., 2008). Because root production by trees is limited under these conditions if they do not have specialized traits (Schmull & Thomas, 2000; Neatrour et al., 2007; Ferner et al., 2012), it is possible that poorly adapted upland species that established through mass effects caused the low root biomass in these ecotone areas. However, the species with roots in our low-biomass ecotone samples that were affiliated with upland forest are actually generalists (A. rubrum, Carya ovata (Mill.) K. Koch and F. grandifolia), and their roots were also sometimes found in samples from the core areas of bottomland and riparian forests, which did not have reduced biomass. Because of their broad distributions, they might be expected to physiologically adapt to prevalent soil anoxia in the upland-bottomland and upland-riparian ecotones (Neatrour et al., 2007). However, root growth during periods of waterlogging and soil anoxia has been shown to vary among genotypes within species, and this variation is associated with adaptation to local conditions (Jaeger et al., 2009; Silva et al., 2010). Thus, our results suggest that there may be populations of generalist species that are locally adapted to increased moisture levels in the core areas of our bottomland and riparian ecosystems. Reduced root biomass in ecotone areas that are often anoxic could be the result of dispersal of poorly adapted genotypes into bottomland or riparian areas from the upland forest.

Fig. 4 Relationship of fine-root biomass (FRB) with soil nutrient availability and phylogenetic diversity in 86 surface soil cores (15 cm depth) where one or more of the most abundant 14 species were detected. (a) Relationship between FRB and soil fertility (Fertility principal components analysis (PCA) axis 1) for core ecosystem areas (adjusted $R^2 = 59\%$). (b) Relationship between FRB and soil fertility (Fertility PCA axis 1) for core plus ecotone areas (adjusted $R^2 = 32\%$). (c) Relationship between residual FRB values, after correcting for soil fertility, and phylogenetic distance (PD) for core ecosystem areas (adjusted $R^2 = 19\%$). (d) Relationship between residual FRB values, after correcting for soil fertility, and phylogenetic distance (PD) for core plus ecotone areas (adjusted $R^2 = 8\%$). Symbols represent ecosystem core areas (black symbols), ecosystem areas under the influence of ecotones (gray symbols), and transitional ecotone points at the interface between two ecosystems (open diamonds). Curves show polynomial regressions including squared explanatory variables. Variance explained was significantly increased when ecotone samples were excluded from the analysis; see text for details.

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The population-level mass effects of locally adapted genotypes provides another potential mechanism by which ecological relationships may be decoupled at ecotones, in addition to the community-level mass effects described in Blackwood et al. (2013). This mechanism may be particularly important for ecosystem-level phenomena, such as biomass accrual, under harsh abiotic conditions.

Conclusions

Our results highlight the important role that root interactions may play in forest communities, as well as landscape-scale species distribution and dispersal patterns. 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, contradicting the hypothesized scale-precision tradeoff mechanism commonly mentioned as a driver of plant coexistence in natural communities (Kembel & Cahill, 2005; Kembel et al., 2008). Instead, we suggest an optimality foraging framework where most species preferentially forage in areas with higher resource availability and the level of biomass productivity in the patch will depend on phylogenetic relatedness among neighbors and the persistence of nutrient patches (McNickle & Cahill, 2009). However, in contrast to the relative lack of differences among species in FRB accrual in response to soil resources, reduced FRB persistence of nutrient patches (McNickle & Cahill, 2009).

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