Tree species diversity increases fine root productivity through increased soil volume filling

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Summary

1. Although fine roots (< 2 mm in diameter) account for a major share of the production of terrestrial ecosystems, diversity effects on fine root productivity and their mechanisms remain unclear.

2. We hypothesized that: (i) fine root productivity increases with tree species diversity, (ii) higher fine root productivity is a result of greater soil volume filling due to species-specific patterns of root placement and proliferation, and (iii) differences in fine root productivity and soil volume filling associated with tree species diversity are more pronounced in summer when plants are physiologically active and demand for water and nutrients is at its greatest.

3. We investigated the effects of tree species diversity on fine root productivity and soil volume filling of boreal forest stands that have grown naturally for 85 years on similar sites.

4. Annual fine root production was 19–83% higher in evenly mixed- than single-species-dominated stands, and increased with tree species evenness, but not tree species richness. Fine root biomass was higher in evenly mixed- than single-species-dominated stands in summer months, but not in spring or fall. Higher fine root productivity in evenly mixed- than single-species-dominated stands was realized by filling more soil volume horizontally and vertically in the forest floor in the mixtures of deep- and shallow-rooted species vs. the deeper mineral soil in the mixtures of deep-rooted species.

5. *Synthesis*. Our results provide some of the first direct evidence for below-ground species complementarity in heterogeneous natural forests, by demonstrating that tree species evenness increases fine root productivity by filling/exploiting the soil environment more completely in space and time, driven by differences in the inherent rooting traits of the component species and variations of root growth within species.

Key-words: below-ground production, boreal forest, fine root biomass and necromass, mixed- and single-species stands, over-yielding, plant–plant interactions, spatial rooting heterogeneity, species complementarity

Introduction

Over the past two decades, diversity–ecosystem function experiments have shown that biodiversity is critical for maintaining ecosystem productivity, stability and other services

(Isbell *et al.* 2011). Experiments using short-lived species such as bacteria, fungi, annual grasses, and algae and larger and longer-lived perennial grasses can allow for measurable effects of diversity on productivity to be determined within a few months or years of the establishment of an experiment. Research on diversity–productivity relationships, however, lags behind in forest ecosystems due to the size and longevity of trees. Diversity–productivity experiments in forest

ecosystems would take decades in tropical forests (e.g. Powers *et al.* 2009) to centuries in temperate coniferous rain forests (e.g. Franklin *et al.* 2002) to allow for trees to reach maturation so that site resources can be optimally used and the expression of species complementarity may be found (Cavard *et al.* 2011b). As a result, diversity–productivity studies in forest ecosystems are based almost exclusively on sampling naturally established stands of different levels of species diversity that grow on similar site conditions, with a few focused on young plantations, for examining diversity–productivity relationships (Zhang, Chen & Reich 2012). Furthermore, although fine root production accounts for 33% of the total net primary production of terrestrial ecosystems globally (Jackson, Mooney & Schulze 1997) and up to 76% in some ecosystems (Gower, Pongracic & Landsberg 1996), few studies have investigated tree diversity effects on the biomass and production of fine roots relative to above-ground woody structures and foliage. This is probably because sampling plant roots at the stand level in terrestrial ecosystems is destructive, laborious and technically challenging.

Observed positive diversity effects in plant communities are commonly attributed to increased total resource use through niche differentiation or facilitation (Hooper *et al.* 2005) and/or decreased host-specific disease susceptibility (Schnitzer *et al.* 2011), that is, species complementarity. Furthermore, the magnitude of diversity effects has been linked to phylogenetic and functional trait differences among component species that result in a higher degree of niche utilization in space or time (Cadotte, Cardinale & Oakley 2008). However, possible mechanisms for diversity effects have rarely been demonstrated directly in heterogeneous natural environments (Naeem, Duffy & Zavaleta 2012), especially for below-ground functions.

Positive diversity effects on below-ground productivity (over-yielding) may be stimulated by increased filling of soil volume, reduced competition and/or altered morphological and physiological plasticity in species-rich stands (Goldberg *et al.* 1999; Casper, Cahill & Jackson 2000; Hodge 2004; Rajaniemi 2007) that facilitate higher relative root growth in a non-self neighbourhood in mixtures (Schenk 2006; de Kroon 2007). Alternatively, pathogen-constrained root growth in monocultures can also be responsible for a lower below-ground productivity compared to species mixtures (de Kroon *et al.* 2012). However, empirical evidence for below-ground over-yielding remains controversial. For example, fine root biomass has been reported to be higher in species mixtures than corresponding monocultures (Schmid & Kazda 2002; Meinen, Hertel & Leuschner 2009b; Brassard *et al.* 2011), whereas other studies have reported no effect (Bauhus, Khanna & Menden 2000; Leuschner *et al.* 2001; Meinen, Hertel & Leuschner 2009a) or even a negative effect of species diversity on fine root biomass (Bolte & Villanueva 2006). But three recent studies have all reported positive diversity effects on annual fine root production (Meinen, Hertel & Leuschner 2009b; Brassard *et al.* 2011; Lei, Scherer-Lorenzen & Bauhus 2012). The contrasting findings of these studies may reflect variations in diversity indices used, functional traits and

phylogenies among component species, stand development and stand origin (Zhang, Chen & Reich 2012) or a lack of adequate replications. No difference in fine root biomass but greater production could also be attributable to greater production matched by faster turnover (Nadelhoffer 2000) and/or morphological and physiological plasticity including spatially and temporally explicit root proliferation (Casper, Cahill & Jackson 2000; Hodge 2004).

In this study, we examined spatial and temporal patterns of fine root productivity, measured as annual fine root production and fine root biomass (live roots), in four types of stands of varying tree species richness and evenness in boreal forest, and tested the hypotheses that: (i) more diverse stands with component species differing functionally (deep rooted vs. shallow rooted) and phylogenetically (coniferous vs. broad-leaved) have higher stand-level fine root productivity than less diverse stands and (ii) higher fine root productivity is a result of greater soil volume filling of fine roots due to species-specific patterns of root placement and proliferation. We also expect that (iii) differences in fine root productivity and soil volume filling associated with tree species diversity are more pronounced in summer when plants are physiologically active in the boreal forest and demand for soil resources is at its greatest.

Materials and methods

STUDY AREA

The study was conducted in the boreal forest north of Lake Superior and west of Lake Nipigon in the Upper English River (B. 11) Forest Region (Rowe 1972) approximately 150 km north of Thunder Bay, Ontario, Canada, between 49º27 N to 49º38 N and 89º29 W to 89º54 W. The closest meteorological station is located in Thunder Bay, Ontario (48º22 N, 89º19 W, 199 m elevation). The study area has a moderately dry, cool climate with short summers. The average annual precipitation for Thunder Bay (1971–2000) is 712 mm and the average annual temperature is 2.5°C (Environment Canada 2011). Topographic features were shaped by the retreat of the Laurentide Ice Sheet approximately ten millennia ago. Soils on upland sites are relatively deep glacial tills of the Brunisolic order (Soil Classification Working Group 1998). The natural stand-initiating disturbance of the area is predominantly stand-replacing crown fire (Johnson 1992) with an average fire return interval of approximately 100 years for the past century (Senici *et al.* 2010).

SAMPLING DESIGN

Similar to other studies that investigate tree species diversity effects in naturally established mature stands (MacPherson, Lieffers & Blenis 2001; Brassard *et al.* 2011), and in following the definitions for single- and mixed-species stands in the forest resource inventory, criteria for stand selection were that single-species-dominated stands would contain ≥ 80% stand basal area of a single species, while in evenly mixed-species stands, none of the component species would have ≥ 80% stand basal area. On mesic sites in central Canada, which support a wide range of forest compositions due to the variation of local propagule availability (Ilisson & Chen 2009), we sampled mature stands from four stand types: single-species-dominated early

successional coniferous *Pinus banksiana* Lamb. (Pb), single-species-dominated early successional broadleaved *Populus tremuloides* Michx. (Pt), *P. banksiana* mixed with the late-successional coniferous species *Picea mariana* (Mill.) BSP, *Picea glauca* (Moench) Voss and *Abies balsamea* L. (Pb + LSC), and *P. banksiana* mixed with *P. tremuloides* (Pb + Pt), each with three replications (Table 1). This provides two kinds of useful test contrasts: first, of Pb with Pb + LSC stands, and second, of Pb and Pt with Pb + Pt stands. The Pb + LSC stands had higher tree species evenness but not richness than Pb stands, whereas Pb + Pt stands had higher evenness than Pb and Pt, and higher richness than Pt but not Pb stands (Table 1). We did not sample stands dominated by late-successional *Picea* spp. and *A. balsamea* because their dominance does not occur in the study area until later stages of stand development (Brassard *et al.* 2008; Taylor & Chen 2011), and they would be less productive both above- and below-ground than stands at earlier stages of stand development (Paré & Bergeron 1995; Finér, Messier & DeGrandpré 1997; Yuan & Chen 2010). Both *P. banksiana* and *P. tremuloides* are habitually deep rooted, whereas the other species are shallow rooted with a slower rate of growth (Wendel & Smith 1990).

All selected stands established naturally 85 years prior to sampling after stand-replacing crown fire and developed without silvicultural treatments. Sample stands were allocated several kilometres apart from each other, by selecting them from different road accesses using forest resource inventory maps and stratified random sampling, to minimize neighbourhood influences and unknown environmental influences that may be spatially correlated among sample stands. To help ensure that tree species composition was the only significant source of variation among stands, all stands were selected on mesic sites using an ecological classification approach (Taylor *et al.* 2000), by allocating all sites on mid-slope positions of well-drained glacial moraines > 50 cm in thickness. Soil moisture regime class was confirmed by examination of a soil profile, dug to the parent material, within each selected stand. Similarity of sites was further validated by soil physical and chemical properties, that is, concentrations of total nitrogen and total carbon, cation exchange capacity and soil texture composition of the mineral soil at a depth of 30–55 cm (see Table S1 in Supporting Information). Mineral soil at this depth is generally unaffected by current vegetation (Augusto, Dupouey & Ranger 2003).

DATA COLLECTION

In each sample stand, a 400 m^2 circular plot was randomly established to represent the stand. The d.b.h., taken 1.3 m above the root collar, height and species of all live trees d.b.h. $\geq$ 2 cm were measured and recorded. Stand basal area by species was summed to the plot level and used for assigning stand-type classification (Table 1).

Within each plot, seven soil cores (6.6 cm in diameter) were randomly extracted from the forest floor surface to a mineral soil depth of 30 cm using a power auger each month during the 2007 growing season (early May to late October). Fine root production during the non-growing season was assumed to be negligible (Burke & Raynal 1994; Haynes & Gower 1995). To facilitate extraction by layers and to minimize compaction during coring, we extracted the forest floor layer (FF, F and H layers or O_1 and O_2 horizons, respectively) and two mineral soil sections: MS1 (0–15 cm) and MS2 (15–30 cm), subsequently after removing the upper layer. In total, we took 504 cores, each separated into three layers, resulting in 1512 samples for laboratory analysis. Soil core sections were transported in an ice-filled cooler from the field to the laboratory and stored for up to 6 months at –18°C until processing.

Thawed samples were soaked in water overnight, poured into trays and rubbed gently to separate roots from soil and coarse fragments (roots would float to the top of the water column while soil and coarse fragments would stay on the bottom). Roots > 2 mm, determined using callipers, were discarded. Fine roots (< 2 mm in diameter) were then sorted according to vitality class, that is, live vs. dead. Roots were classified as 'live' if they were pale coloured on the exterior,

Table 1. Characteristics (mean and 1 SEM) of the study stands in Northwestern Ontario, Canada. Stand types are *Pinus banksiana* mixed with the late-successional conifers *Picea mariana*, *Picea glauca* and *Abies balsamea* (Pb + LSC), single-species *P. banksiana* dominated (Pb), single-species *Populus tremuloides* dominated (Pt), and mixed *P. banksiana* and *P. tremuloides* (Pb + Pt). Each stand type was replicated three times

Characteristic	Pb + LSC	Pb	Pt	Pb + Pt
Stand basal area (m^2 ha^{-1})	44.60 ± 3.10[a]	38.95 ± 5.35[a,A]	33.58 ± 6.21[A]	34.24 ± 1.09[A]
Stand density (trees ha^{-1})	2208 ± 271[a]	2016 ± 281[a,A]	733 ± 30[B]	1167 ± 254[AB]
Tree species richness (number of species per plot)	4.0 ± 1.00[a]	4.33 ± 0.33[a,A]	2.0 ± 0.0[B]	4.0 ± 0.0[A]
Tree species evenness	0.70 ± 0.08 [a]	0.41 ± 0.03[b,B]	0.21 ± 0.07[C]	0.74 ± 0.02[A]
Tree species composition (% of stand basal area)				
Pinus banksiana	63.26 ± 4.50	84.03 ± 2.20	0	42.04 ± 11.53
Populus tremuloides	1.00 ± 1.00	0	96.54 ± 1.28	43.56 ± 10.75
Picea mariana	30.41 ± 3.32	6.19 ± 1.90	0	6.65 ± 2.99
Betula papyrifera	1.96 ± 1.21	5.72 ± 0.39	3.46 ± 1.28	7.75 ± 2.80
Abies balsamea	2.69 ± 1.65	2.28 ± 1.32	0	0
Picea glauca	0.70 ± 0.70	1.78 ± 1.78	0	0
Stand volume (m^3 ha^{-1})*	345.0 ± 45.0[a]	312.1 ± 53.8[a,A]	378.2 ± 79.0[a,A]	309.4 ± 32.1[a,A]
Tree layer biomass (Mg ha^{-1})†	170.7 ± 6.1 [a]	178.5 ± 30.2[a,A]	191.4 ± 39.8[A]	170.1 ± 14.8[A]
Understorey vegetation biomass (Mg ha^{-1})‡	1.1 ± 0.6[a]	4.4 ± 1.9[a,A]	25.7 ± 11.9[A]	4.8 ± 1.6[A]

Differences in characteristics among stand types were tested using a one-way ANOVA for the two contrasts: first, of Pb with Pb + LSC stands (lowercase letters), and second, of Pb and Pt with Pb + Pt stands (uppercase letters). Different letters indicate a significant difference ($\alpha = 0.05$).
*Stand volume was the sum of individual tree volumes estimated from allometric equations developed for tree species of central and eastern Canada (Honer, Ker & Alemdag 1983).
†Tree layer biomass was the sum of individual tree biomass estimated from allometric equations developed for Canadian tree species (Lambert, Ung & Raulier 2005).
‡Understorey vegetation biomass was estimated from allometric equations developed for the study sites (Cavard *et al.* 2011a).

elastic and flexible, and free of decay with a whitish cortex, while roots were classified as 'dead' if they were brown or black in colour, rigid and inflexible, in various stages of decay, and had a dark coloured cortex (Persson 1983; Bennett, Andrew & Prescott 2002).

Live fine roots were further divided into the following species classes: (i) *P. banksiana*, (ii) *P. tremuloides* and incidental *Betula papyrifera* Marsh. (a small number of young *B. papyrifera* trees were present in some of the plots), (iii) *Picea* spp. and *A. balsamea*, and (iv) non-tree (shrubs and herbs) using a combination of morphological characteristics. These included: (i) colour (*P. tremuloides*, *B. papyrifera* and non-tree roots were more white or yellow in colour, while coniferous roots were more red or brown in colour), (ii) size (*Picea* spp. and *A. balsamea* and non-tree roots were generally finer structured than those of *P. banksiana*), (iii) branching pattern (sections of *Picea* spp. and *A. balsamea* and non-tree roots were more branched than those of *P. tremuloides*, *B. papyrifera* and *P. banksiana*) and (iv) presence or absence of root hairs (non-tree roots contained small hairs that were not present on tree roots). These criteria were developed prior to root sorting using samples of known origin from the study stands. When needed, a stereomicroscope was used to assist with root sorting. Sorted fine roots were then oven-dried to a constant mass at 65°C and weighed. However, a small fraction (< 2% of dry weight for all samples) of very small root fragments could not be sorted by species class. The species distribution of this fraction was extrapolated using the known species class proportions of the particular sample.

In each plot, we also installed 10 ingrowth cores that were made of fibreglass mesh (2.5 × 2.5 mm) (QuickCount plastic canvas; Uniek Inc., Waunakee, WI, USA) and formed into 6.6-cm-diameter cylinders with a length of 30 cm. A power auger was used to drill holes to a total depth of 30 cm. After a soil column was removed, a cylinder was placed into the excavated hole, filled with root-free soil (medium-textured sand of local origin), covered with leaf litter and marked by a steel rod. All ingrowth cores were installed in October 2007 and removed after one calendar year by carefully digging the soil away from the cylinder and cutting roots that had grown into the cylinder with a knife. In the laboratory, roots were separated from the soil, dried and weighed as described above, with the exception that roots were not separated by soil layer, vitality class or species class. No roots $\geq$ 2 mm in diameter were present in any of the ingrowth cores.

DATA ANALYSIS

Fine root biomass and necromass (dead roots) (Mg ha^{-1}) were calculated for each sampling date at each site by summing the dry weight of live and dead fine roots, respectively, in each soil core and scaling up to per ha. Because all current methods for estimating fine root production at the stand level (Mg ha^{-1} year^{-1}) have inherent strengths and weaknesses, it is recommended that multiple methods be used when possible (Vogt, Vogt & Bloomfield 1998; Hendricks *et al.* 2006). As a result, we estimated fine root production by the following four methods: (i) Ingrowth method by summing the dry weight of all live and dead roots grown into the cores, (ii) MaxMin (ΔL) method by calculating the difference in biomass between sampling dates with the highest and lowest measurements, (iii) MaxMin ($\Delta L + \Delta D$) method by summing the difference between maximum and minimum biomass and necromass, respectively and (iv) a decision matrix method that was based on summing all changes in biomass and necromass between sampling dates. The Matrix method was modified from Mcclaugherty, Aber & Melillo (1982) as follows:

if: $\Delta_l > 0$ and $\Delta_d > 0$, then production = $\Delta_l + \Delta_d$

if: $\Delta_l < 0$ and $\Delta_d < 0$, then production = 0

if: $\Delta_l > 0$ and $\Delta_d < 0$, then production = Δ_l

if: $\Delta_l < 0$ and $\Delta_d > 0$ and $\left| \Delta L \right| > \left| \Delta D \right|$, then production = 0

if: $\Delta_l < 0$ and $\Delta_d > 0$ and $\left| \Delta L \right| < \left| \Delta D \right|$, then production = $\left| \Delta L + \Delta D \right|$

where Δ_l is the biomass in a given month minus the biomass in the preceding month, and Δ_d is the same calculation for necromass.

We tested for diversity effects on fine root productivity by examining differences in annual production and monthly biomass among stand types and in relation to tree species richness and evenness, and by using a heterogeneity index to quantify horizontal and vertical spatial biomass variability. Horizontal heterogeneity of fine root biomass was calculated as the standard deviation of the biomass values of all soil layers combined among the seven soil cores within each sample plot by sampling date. Vertical heterogeneity of fine root biomass was calculated as the standard deviation of the biomass values among the three soil layers averaged from all soil cores. A lower horizontal and vertical heterogeneity value would imply a more homogenous distribution of biomass among the soil cores and soil layers, respectively. Examination of spatial configurations of fine root biomass by species is important for understanding diversity effects on fine root productivity, as patterns of below-ground niche utilization and species complementarity should be reflected in fine root distribution patterns (Casper & Jackson 1997; Brassard, Chen & Bergeron 2009; de Kroon *et al.* 2012).

All statistical analyses were performed using the R for Windows Version 2.13.1 statistical software (R Development Core Team 2011). The effect of tree species diversity on annual fine root production was separately tested for the two stand-type contrasts using one-way analysis of variance (ANOVA) and linear regression. We also used general linear models to examine the effects of both tree species richness and evenness on fine root production by pooling data from the two stand-type contrasts. The effects of stand type and sampling date on fine root biomass and necromass and horizontal and vertical heterogeneity were determined using a repeated measures ANOVA since sampling date reflected a repeated measure of each sample stand. To describe the joint variation of biomass and heterogeneity, we used standardized major axis regression, a Model II regression method recommended when variables cannot be clearly distinguished as independent or dependent, and when error is associated with the measures of both. Differences in the relationships between biomass and heterogeneity among stand types were tested using SMATR package (Warton 2007). Assumptions of normality and homogeneous variances were examined by Shapiro–Wilk's test and Levene's test, respectively. While the assumption of normality was met for all analyses, the assumption of homogeneous variances was not for some analyses, so that the respective dependent variable was transformed by base 10 logarithm to better meet the assumption.

Results

For both test cases, annual fine root production in the top 30 cm of soil was higher in evenly mixed- than single-species-dominated stands, although differences between stand types were not always significant (Fig. 1). Pb + LSC had 32–72% higher annual production than Pb stands, depending on the method of estimation. Pb + Pt had 57–80% and 19–83% higher annual production than Pb and Pt stands, respectively (Fig. 1a). When examined by tree species richness and evenness, annual fine root production increased with evenness, but not with richness (Fig. 1b–i, Table 2).
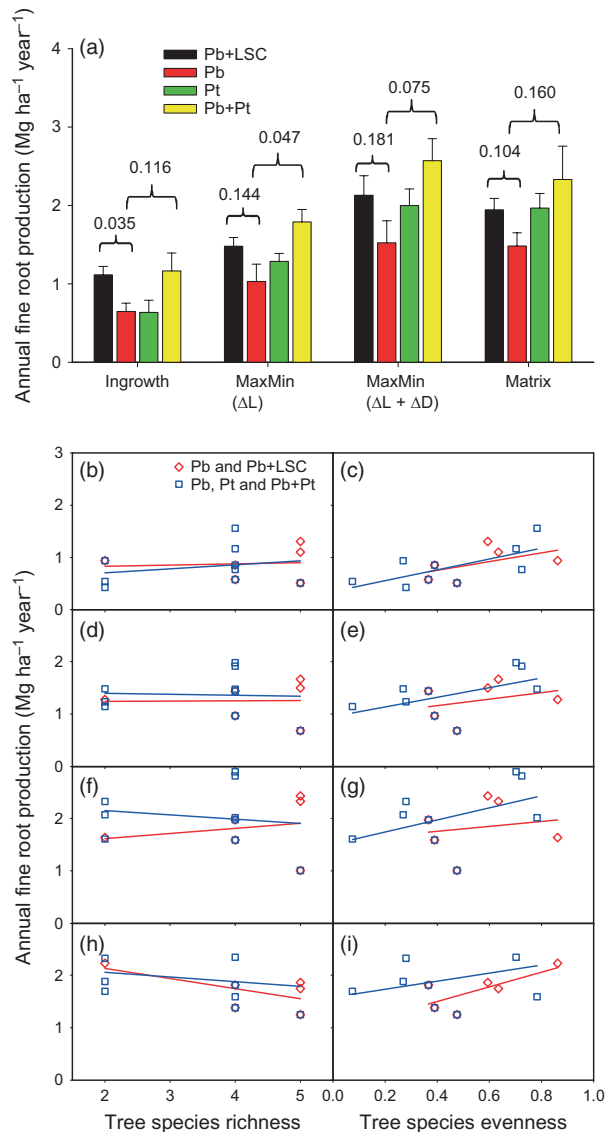
Fig. 1. Effects of tree species diversity on annual fine root production in the forest floor and top 30 cm of mineral soil. (a) Fine root production by stand type and method [Ingrowth, MaxMin (ΔL), MaxMin (ΔL + ΔD) and Matrix]. Stand types are evenly mixed *Pinus banksiana* and late-successional conifers (Pb + LSC), single-species *P. banksiana* dominated (Pb), single-species *P. tremuloides* dominated (Pt), and evenly mixed *P. banksiana* and *P. tremuloides* (Pb + Pt). Error bars represent 1 SEM (*n* = 3). Values above the error bars are the *P*-values for the stand-type contrasts. (b), (d), (f) and (h) Fine root production in relation to tree species richness by Ingrowth, MaxMin (ΔL), MaxMin (ΔL + ΔD) and Matrix method, respectively. (c), (e), (g) and (i) Fine root production in relation to tree species evenness by Ingrowth, MaxMin (ΔL), MaxMin (ΔL + ΔD) and Matrix method, respectively.

The effect of stand type on fine root biomass differed with sampling date (Table 3). For all stand types, biomass peaked in the summer months (July to September) (Fig. 2a). Biomass was greater in Pb + LSC than Pb stands, and in Pb + Pt than Pb and Pt stands in summer (July to September), whereas differences in biomass between stand types were less apparent in spring and fall. Fine root necromass had a reversed seasonal pattern compared to biomass, that is, lower in summer and

higher in spring and fall for all stands types, but did not differ or only differed marginally among stand types (Table 3, Fig. 2b).

The horizontal and vertical indices of fine root biomass differed with stand type and sampling date (Table 3). In summer, horizontal heterogeneity was higher in single-species-dominated stands than their respective mixtures (Fig. 2c). Vertical heterogeneity was higher in Pb + LSC than Pb stands, whereas Pb + Pt stands had lower vertical heterogeneity than Pb and Pt stands in summer (Fig. 2d). The differences in horizontal and vertical heterogeneity among stand types were much less apparent in spring and fall (Fig. 2c and d). The higher summer vertical heterogeneity in Pb + LSC stands compared with that in Pb stands was the result of a larger difference between biomass in the FF vs. the MS1 and MS2 layers due largely to a greater biomass of *Picea* spp. and *A. balsamea* roots in the FF layer of the Pb + LSC stands (Fig. 3). The lower summer vertical heterogeneity in Pb + Pt stands resulted from fine roots being more evenly distributed among the soil layers due to a greater biomass of roots in the MS1 and MS2 layers in the Pb + Pt stands than Pb or Pt stands (Fig. 3). As expected, both biomass and necromass decreased with increasing soil depth in all stand types (Fig. 3).

Fine root biomass was negatively related to horizontal heterogeneity in Pb + LSC and Pb + Pt, but not in Pb or Pt stands (Fig. 4a). By contrast, biomass was positively related to vertical heterogeneity in Pb + LSC stands, but negatively related in Pb + Pt stands (Fig. 4b). In both Pb and Pt stands, however, biomass was not significantly related to vertical heterogeneity.
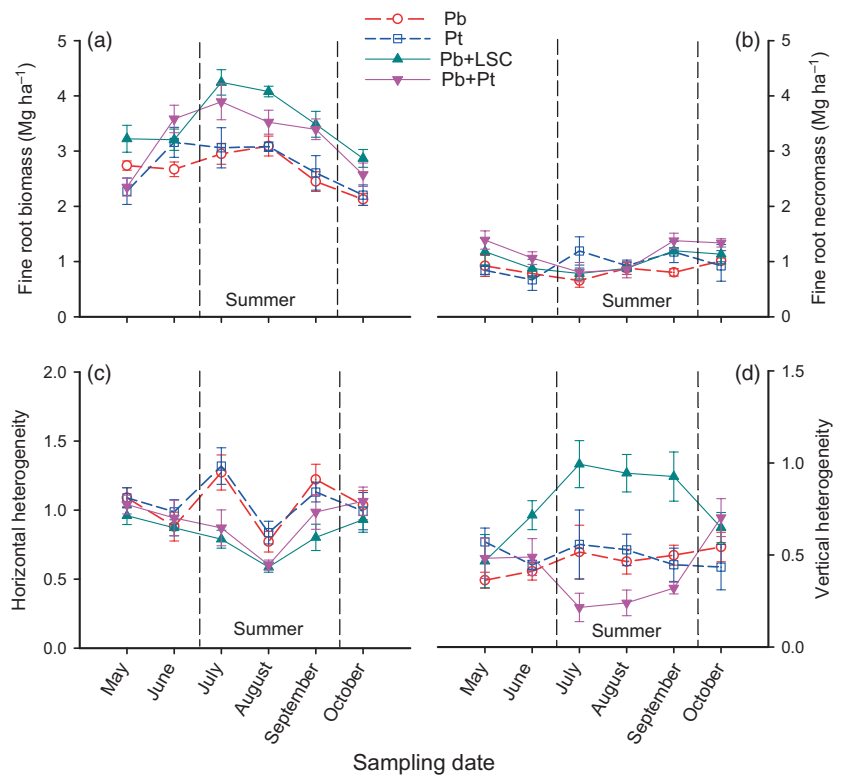
Discussion

Our results support the prediction of greater fine root productivity in both types of species mixtures than their respective single-species-dominated stands. The species mixture effect on fine root productivity in both our contrasts was attributable to higher tree species evenness but not tree species richness. This finding extends the understanding of the important role tree species evenness plays in above-ground productivity to below-ground productivity in forest ecosystems (Zhang, Chen & Reich 2012). Our results and those from previous studies (Schmid & Kazda 2002; Meinen, Hertel & Leuschner 2009b;

Table 2. The effects (*P*-values) of tree species richness, evenness and contrast type on annual fine root production, analysed by general linear models

Source	d.f.	Ingrowth	MaxMin (ΔL)	MaxMin (ΔL + ΔD)	Matrix
Richness	1	0.957	0.377	0.399	0.111
Evenness	1	0.022	0.055	0.059	0.080
Contrast	1	0.315	0.369	0.245	0.608
Contrast × Richness	1	0.315	0.464	0.402	0.557
Contrast × Evenness	1	0.162	0.132	0.064	0.344
Error	9				

Table 3. The effects of stand type and sampling date on fine root biomass, necromass, and horizontal and vertical heterogeneity for the two contrasts (Pb vs. Pb + LSC; Pb and Pt vs. Pb + Pt)

Source	d.f.	Biomass		Necromass		Horizontal heterogeneity		Vertical heterogeneity	
		MS	P	MS	P	MS	P	MS	P
Contrast: Pb vs. Pb + LSC									
Between subject									
Stand type	1	6.452	0.002	0.248	0.090	0.441	0.009	0.902	0.001
Error	4	0.129		0.050		0.019		0.014	
Within subject									
Sampling date	5	1.086	< 0.001	0.117	0.082	0.113	0.005	0.095	0.038
Stand type × Sampling date	5	0.148	0.181	0.029	0.724	0.053	0.092	0.047	0.255
Sampling error	20	0.087		0.050		0.024		0.032	
Contrast: Pb and Pt vs. Pb + Pt									
Between subject									
Stand type	2	1.630	0.055	0.404	0.164	0.109	0.050	0.042	0.298
Error	6	0.334		0.163		0.021		0.028	
Within subject									
Sampling date	5	1.601	< 0.001	0.134	0.047	0.208	< 0.001	0.601	< 0.001
Stand type × Sampling date	10	0.223	0.055	0.052	0.034	0.035	0.432	0.044	0.111
Sampling error	30	0.105		0.050		0.033		0.025	

Fig. 2. Biomass and necromass of fine roots and horizontal and vertical heterogeneity of fine root biomass in relation to sampling date by stand type. (a) Fine root biomass, (b) fine root necromass, (c) horizontal heterogeneity and (d) vertical heterogeneity. Stand types are described in Fig. 1. Error bars represent 1 SEM ($n = 3$).

Brassard *et al.* 2011; Lei, Scherer-Lorenzen & Bauhus 2012) indicate that positive diversity effects on fine root productivity in forest ecosystems are common. It is important to note that our results and the results of these other studies all show greater fine root productivity in mixed than the most productive single-species-dominated stand type, precluding attribution of the below-ground over-yielding to a selection/sampling effect, that is, that higher productivity of mixtures than the average of monocultures is due to mixtures having a greater probability of being dominated by the most productive species (Wardle 1999).

The lower horizontal heterogeneity in the mixtures when compared with their respective single-species-dominated stands in summer, but not in spring or fall, appears to indicate more complete below-ground soil volume filling in the evenly mixed- than single-species-dominated stands during the summer, when demand for fine roots to uptake water and nutrients is at its highest, via a greater occupancy of the soil
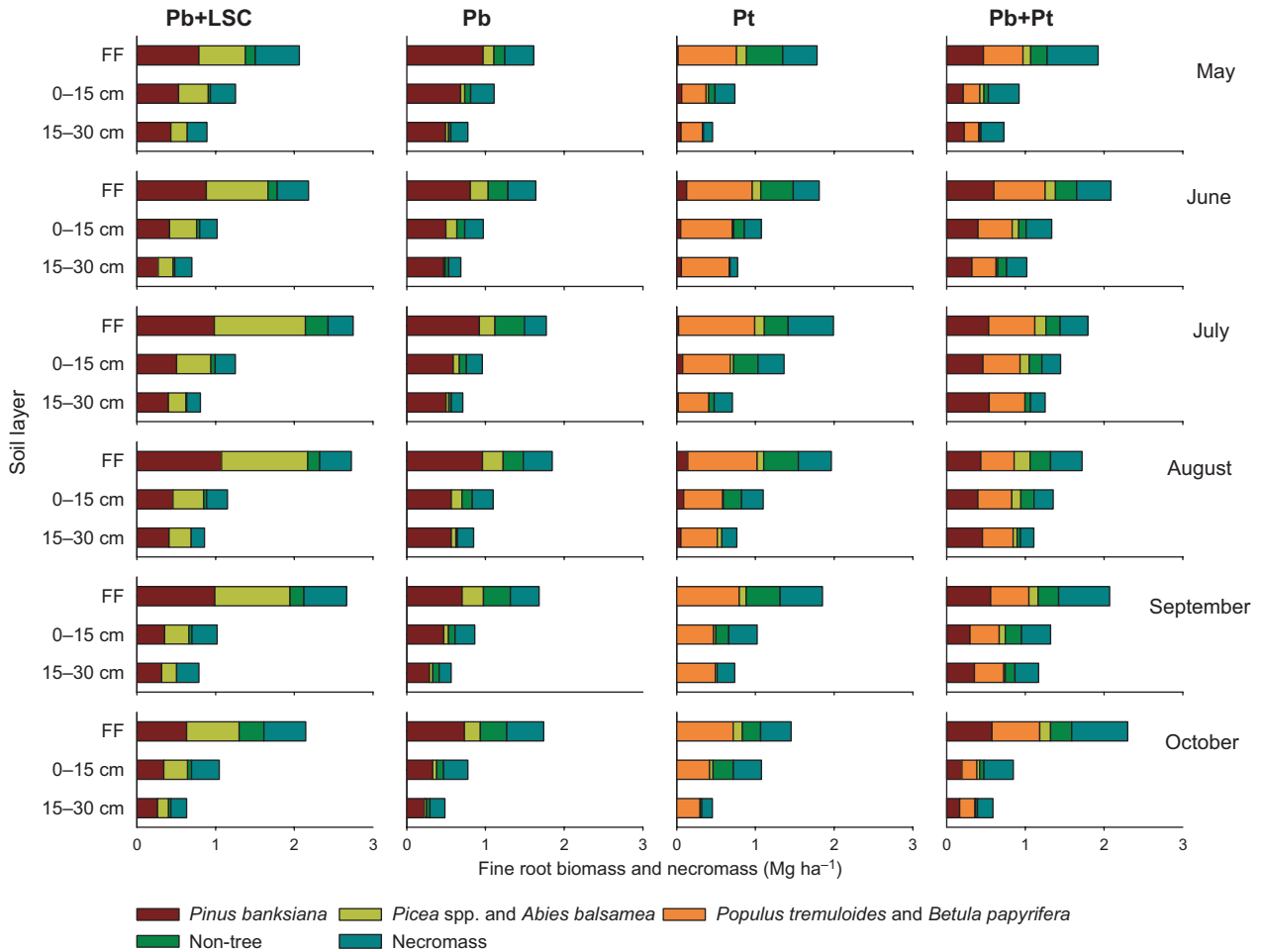
Fig. 3. Vertical distribution by soil layer of fine root biomass by species class (*Pinus banksiana*, *Picea* spp. and *Abies balsamea*, *Populus tremuloides* and *Betula papyrifera*, and non-tree) and necromass in relation to stand type and sampling date. Columns are Pb + LSC stands (first column), Pb stands (second column), Pt stands (third column) and Pb + Pt stands (fourth column); rows are May (first row), June (second row), July (third row), August (fourth row), September (fifth row) and October (sixth row). Stand types are described in Fig. 1 and species classes in the text. Soil layers are forest floor (FF), mineral layer 1 (MS1, 0–15 cm) and mineral layer 2 (MS2, 15–30 cm).
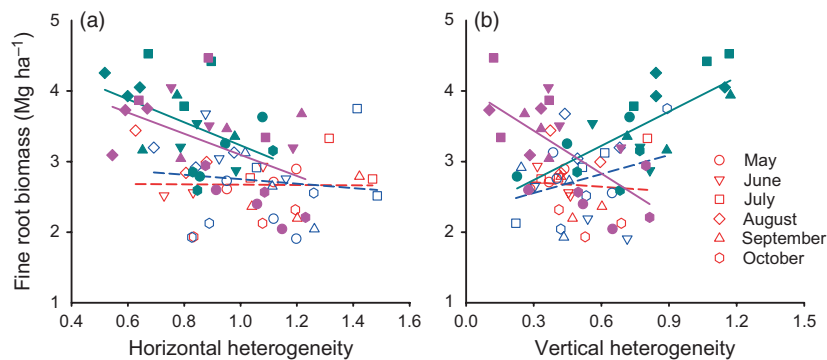

Fig. 4. Relationships between fine root biomass and heterogeneity of fine root biomass. (a) Fine root biomass vs. horizontal heterogeneity with fitted type II regressions (solid cyan line for Pb + LSC stand types, $y = 6.45 - 3.57x$, $R^2 = 0.21$, $P = 0.056$; solid pink line for Pb + Pt stand types, $y = 6.01 - 3.04x$, $R^2 = 0.24$, $P = 0.038$; dash red and blue lines for Pb and Pt stands, respectively, with $P > 0.1$ in both cases) and (b) Fine root biomass vs. vertical heterogeneity with regressions (dash cyan line for Pb + LSC stand types, $y = 1.73 + 2.29x$, $R^2 = 0.50$, $P = 0.001$; dash pink line for Pb + Pt stand types, $y = 4.49 - 3.11x$, $R^2 = 0.42$, $P = 0.004$; dash red and blue lines for Pb and Pt stands, respectively, with $P > 0.5$ in both cases). Incremental symbols represent sampling dates. Colours are red for Pb, blue for Pt, Cyan for Pb + LSC and pink for Pb + Pt stand types, respectively. Stand types are described in Fig. 1.

space horizontally by fine roots. Similarly, Brassard *et al.* (2011) showed that greater horizontal soil space filling of fine roots resulting in higher evenness was linked to higher fine root productivity in evenly mixed stands of *P. tremuloides*, *P. mariana*, *P. glauca* and *A. balsamea* than single-species-dominated stands of *P. tremuloides*. These results collectively provide some of the first direct evidence that increased horizontal soil volume filling by fine roots is associated with increased fine root productivity of more diverse stands in heterogeneous natural environments.

Based on our examination of the differences in fine root biomass stratification between soil layers among the stand types, the two mixture types show different depth-related root foraging strategies from each other and the single-species-dominated stands. While the positive association between biomass and vertical heterogeneity in Pb + LSC stands resulted from increased summer fine root biomass of shallow-rooted *Picea* spp. and *A. balsamea* in the nutrient-rich forest floor, the negative association in Pb + Pt stands between biomass and vertical heterogeneity was attributable to increased summer fine root biomass of deep-rooted *P. banksiana* and *P. tremuloides* to the deeper mineral soil. A thicker FF layer in Pb + LSC than Pb stands (Table S1), probably due to slower decomposition of litter from the foliage and roots of *Picea* spp. and *A. balsamea*, may have also contributed to the higher fine root biomass in the FF layer of the Pb + LSC than Pb stands. While it is not surprising that the different inherent rooting characteristics of the *Picea* spp. and *A. balsamea* vs. *P. banksiana* could have facilitated greater root growth in Pb + LSC mixtures relative to Pb stands, it is intriguing that *P. banksiana* and *P. tremuloides*, despite having similar rooting characteristics, still produced higher fine root biomass in mixture than their respective single-species-dominated stands by growing comparably more roots in deeper soil layers, suggesting that root growth was stimulated in a non-self neighbourhood (Schenk 2006; de Kroon 2007). These results provide some of the first empirical evidence for root plasticity in patterns of placement and proliferation in natural forests. In conjunction with the results on the fine root biomass–horizontal heterogeneity relationships, these findings support the ideas that plants can adapt their foraging behaviours in heterogeneous environments (Cahill *et al.* 2010) and that plastic responses of individuals within species are important for species coexistence (Clark 2010). They also support the idea that monocultures are 'under-rooted' (de Kroon *et al.* 2012) and that increased soil volume filling is an important mechanism supporting increased root productivity in mixtures (Rajaniemi 2007).

The different horizontal and vertical fine root spatial biomass patterns observed during the growing season in the evenly mixed stands, that is, Pb + LSC and Pb + Pt stands, had more fine root biomass in the upper and lower soil layers, respectively, and both occupied more soil space horizontally with fine root biomass, in summer compared to the other times of year appears to indicate that soil space was more completely utilized in summer only. At the other times of the year, the evenly mixed stands used the soil space to approximately the same extent as the single-species-dominated stands. These findings advance upon those of Brassard *et al.* (2011) by demonstrating that mechanisms promoting below-ground over-yielding can have a unique temporal component that is expressed only when demand for nutrients and water is at its greatest, suggesting that root foraging behaviours between and within species can vary not just spatially in plant communities, but also temporally and that both mechanisms are key to maintaining diversity and productivity in natural forests.

Our sampling strategy, that is, sequential cores taken over an entire growing season by six sampling dates, each by seven cores of three soil layers, and 10 ingrowth cores installed for an entire calendar year, in each 400 m^2 plot, allowed us to quantify annual fine root production, seasonal variation of fine root biomass, and horizontal and vertical biomass heterogeneity. Increasing the number of cores may increase the accuracy of the heterogeneity indices. Additionally, while the majority of fine roots are distributed in the forest floor and top mineral soil layers in the boreal forest (Jackson *et al.* 1996; Yuan & Chen 2010), fine roots grow deeper in other ecosystem types. Therefore, sampling deeper soil layers would be needed to adequately quantify fine root biomass, production and spatial rooting variability in those ecosystems. Furthermore, while using an arbitrary fine root size class such as < 2 mm in diameter to study fine root demographics is reasonable for understanding fine root biomass and production at the ecosystem scale, individual species-focused studies need to consider interspecific differences in branching orders and functional heterogeneity within the fine root guild (Pregitzer *et al.* 2002).

Conclusions

In summary, this study provides some of the first direct evidence for below-ground species complementarity in heterogeneous natural forests. We demonstrate that species with phylogenetic and/or functional trait differences growing in mixtures can achieve higher fine root productivity than their respective single-species-dominated stands by filling/exploiting the soil environment horizontally and vertically more completely in space and time, driven by differences in the inherent rooting traits of the component species and variations of root growth within species. These results add a new and important context of understanding on the effects of plant diversity on ecosystem services and functioning in terrestrial ecosystems.

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Supporting Information

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

Table S1. Soil characteristics of the study stands in Northwestern Ontario, Canada.