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Research paper

Specific spatio-temporal dynamics of absorptive fine roots in response to neighbor species identity in a mixed beech–spruce forest

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Received March 25, 2019; accepted July 18, 2019; handling Editor Daniel Epron

Absorptive fine roots are an important driver of soil biogeochemical cycles. Yet, the spatio-temporal dynamics of those roots in the presence of neighboring species remain poorly understood. The aim of this study was to analyze shifts in absorptive fine-root traits in monoculture or mixtures of *Fagus sylvatica* [L.] and *Picea abies* [L.] Karst. We hypothesized that root competition would be higher under single-species than mixed-species interactions, leading to changes in (i) root survivorship, diameter and respiration and (ii) spatio-temporal patterns of root growth and death. Using minirhizotron methods, we monitored the timing and location of absorptive fine-root growth and death at an experimental forest in southern Germany from 2011 to 2013. We also measured root respiration in the spring and fall seasons of 2012 and 2013. Our findings show that the absorptive fine roots of *F. sylvatica* had a 50% higher risk of root mortality and higher respiration rates in the single-species compared to mixed-species zones. These results support our hypothesis that root competition is less intense for *F. sylvatica* in mixture versus monoculture. We were unable to find confirmation for the same hypothesis for *P. abies*. To analyze spatio-temporal patterns of absorptive fine-root production and mortality, we used a mixed-effects model considering root depth (space) and seasons (time) simultaneously. This analysis showed that *F. sylvatica* shifts root production towards shallower soil layers in mixed-species stands, besides significant seasonal fluctuations in root production depths for both species. Ultimately, the impact of neighbor species identity on root traits observed in this study has important implications for where, when and how fast root-facilitated carbon cycling takes place in single-species versus mixed-species forests. In addition, our study highlights the need for inclusion of absorptive fine-root spatio-temporal dynamics when examining belowground plant interactions and biogeochemical cycles.

Keywords: absorptive fine roots, belowground species interactions, forest ecology, root respiration, root survivorship, spatio-temporal dynamics.

Introduction

The spatio-temporal dynamics of fine-root growth are crucial for understanding biogeochemical cycles and belowground plant–plant interactions in a changing climate (Bardgett et al. 2014, Eissenstat et al. 2000, Gill and Jackson 2000, Nadelhoffer and

Raich 1992). Fine roots and root-associated organisms are the primary sources of soil carbon (C) in forest ecosystems (Clemmensen et al. 2013, Rasse et al. 2005). Hence, the timing and location of fine-root production and mortality within the soil profile largely determine the distribution and cycling of

belowground C resources. Even though root competition is a ubiquitous feature of plant communities and can significantly affect plant performance (Cahill and Casper 2000, Casper and Jackson 1997, Novoplansky 2009), it is unclear to what extent the species identity of neighboring plants influences fine-root traits and thereby soil C inputs in forests.

Recently, fine roots (<2 mm in diameter) have been redefined into two distinct functional categories: (i) absorptive fine roots, the most distal, lowest-order roots with high resource uptake capacity and higher incidence of mycorrhizal colonization, and (ii) higher-order, transport roots with increased conductance rates based on their ontogeny and secondary structural development (McCormack et al. 2015). Absorptive, lower-order fine roots can include ephemeral modules, which are shorter-living than transport, higher-root orders (Xia et al. 2010, Valenzuela-Estrada et al. 2008, Espeleta et al. 2009), and therefore constitute the faster turnover component of the fine-root system. The more dynamic character of absorptive fine roots with smaller diameters also allows them to be more responsive to changes in environmental factors (McCormack and Guo 2014) and interactions with neighboring tree species. Hence, their potentially greater role in belowground plant–plant interactions and impact on soil biogeochemical cycles made them the primary focus of this study.

The heterogeneous distribution of soil resources increases the risk of being outcompeted by neighboring plants and requires root foraging strategies over space and time (Casper and Jackson 1997, Ettema and Wardele 2002, Farley and Fitter 1999, Grams and Andersen 2007). Foraging strategies including timing of fine-root production, shifts in lifespan, morphology and physiology can all contribute to the intensity of root interactions (Hodge 2006, Hutchings and de Kroon 1994, Schenk et al. 1999, Tilman 1988, Volder et al. 2005). Root proliferation often coincides with periods of increased resource availability (Eissenstat and Caldwell 1988, Pregitzer et al. 1993). Studies have shown that plant species intrinsically vary the timing of root production to preempt a resource or spatially partition its uptake (Chesson 2000, McKane et al. 2002, Stratton et al. 2000). In addition, fine-root morphology can respond to resource availability. Because fine roots are responsible for resource acquisition and associations with beneficial soil microorganisms (Pregitzer et al. 1997, Pregitzer et al. 2002), the modification of fine-root diameter or length has critical consequences for resource uptake and plant growth (Eissenstat 1992). A reduction in diameter coupled with an increase in length results in a greater absorptive fine-root surface area without augmenting C and nitrogen costs for root production (Eissenstat 1991, Eissenstat et al. 2002, Ostonen et al. 2007). Even though these previous studies have thoroughly explored how root foraging strategies respond to shifts in resource availability and distribution, it is unknown whether fine-root traits show spatial and temporal adjustments to neighbor species identity.

The root foraging strategy that may have received the least attention in the context of species interactions is fine-root longevity or lifespan. Ultimately, resource exploitation depends not only on the level of fine-root production in a resource path, but also on the lifespan of the roots occupying that space (Adams et al. 2013). Fine-root lifespan can be plastic in some species and change depending on resource availability (Aber et al. 1985, Adams et al. 2013, Burton et al. 2000). In a scenario where trees are competing for soil resources, individuals that maintain fine roots within resource patches for longer periods of time can effectively preempt soil space and ensure a greater return on investment (Eissenstat et al. 2000, Eissenstat and Yanai 1997). Yet, the benefits of space occupation may be outweighed by the C costs of producing and maintaining fine roots. For example, fine-root respiration as a measure of maintenance costs is associated with soil temperature, fine-root activity and nitrogen content (Volder et al. 2005, Zogg et al. 1996) and, therefore, likely to be affected by seasonal variations in temperature and precipitation. Yet, it is unclear whether belowground neighbor species identity affects root lifespan across seasons.

The few experiments focusing on belowground plant–plant interactions in forest ecosystems have shown that root foraging strategies differ between monotypic and mixed-species forest stands. Schmid and Kazda (2002) found that tree species shift their average rooting depth when grown in mixed-species arrangements compared to monocultures, essentially changing their niche within the soil profile. When discrete resource patches are unavailable due to a high proportion of root system overlap, roots of distinct tree species may also alter their timing of water uptake and level of root activity, allowing for the coexistence of neighbors with very similar niche requirements (Meinzer et al. 1999, Stratton et al. 2000). In addition, fine roots of *Fagus sylvatica* [L.] (European beech) showed greater surface area and specific root length when grown intermixed with *Picea abies* [L.] Karst (Norway spruce) than in monoculture (Bolte and Villanueva 2006). Yet, other studies have reported no admixing effects on root morphology (Lei et al. 2012). In terms of fine-root lifespan, one study reported that fine-root longevity decreased for *F. sylvatica* and increased for *Fraxinus excelsior* (European ash) when grown together in the same area (Beyer et al. 2013). The authors attributed these opposite trends to asymmetric competition in favor of ash trees. However, because this is one of the few studies testing the effect of neighbor identity on fine-root lifespan, further experimentation is needed to test these trends.

At the ecosystem scale, previous studies have shown a positive relationship between species diversity and productivity (Hector 1999, Hooper and Vitousek 1997, Liang et al. 2016, Pretzsch et al. 2010, Tilman 2001). So far, most biodiversity–productivity research has focused on grassland ecosystems and the majority of these studies considered solely aboveground

parameters (Meinen et al. 2009). Species differences in root traits facilitating niche complementary could explain increases in belowground root productivity observed in forests (Brassard et al. 2011). In the case where significant belowground niche overlap exists among different tree species, the pattern of increased belowground root productivity or 'over-yielding' of species involved may not be evident (Meinen et al. 2009). From these previous findings on root productivity, the question arises to what extent the outcome of belowground species interactions can be explained by static species differences in root system traits or plastic root responses to neighboring trees.

We designed an experiment aimed at testing the effects of single-species versus mixed-species root interactions on the timing and location of absorptive fine-root growth, root survivorship and respiration of *F. sylvatica* and *P. abies* forest stands in southern Germany over 3 years. We hypothesized that trees growing intermixed with a different species (mixed-species) would experience less belowground competition in comparison to trees growing in monoculture (single-species). We therefore predicted, for both species, that absorptive fine roots produced within the single-species zones of *F. sylvatica* or *P. abies* would have smaller average diameters (morphological adjustment), lower cumulative root production, higher risk of mortality (lower survivorship) and higher respiration rates (physiological adjustment) when compared to roots growing in the mixed-species zone. Moreover, we expected to find evidence for niche complementarity by identifying a significant difference in the timing and average depth of absorptive fine-root production, and mortality between the mixed-species and single-species zones of the forest.

Materials and methods

Experimental setup and field site conditions

The study was performed across 10 randomized plots containing 70–75-year-old, mature *Picea abies* [L.] Karst (Norway spruce) and *Fagus sylvatica* [L.] (European beech) trees located in the 'Kranzberger Forst', a research site near Freising, Germany (48°25'N, 11°39'E, 490 m a.s.l.). The total stand basal area was 52.0 m² ha⁻¹ with the stand area of *F. sylvatica* amounting to 22.7 m² ha⁻¹ and that of *P. abies* to 29.3 m² ha⁻¹. The total stand density was 659 trees ha⁻¹, of which 353 were *F. sylvatica* and 305 were *P. abies* (Rötzer et al. 2017). Rectangular plots (~8 × 18 m) contained monotypic groupings of spruce trees at one end and a monotypic grouping of beech trees at the opposite end (Pretzsch et al. 2014). The central portion of each plot contained a 'mixed' zone with a high degree of interspecific root mixing (Häberle et al. 2012, Mainiero et al. 2010). For a map of the research plots and tree distribution, please see Rötzer et al. (2017). To prevent cross-plot root interactions and water flow between each of the 10 experimental plots, plots were trenched in May 2010 until a known claypan was

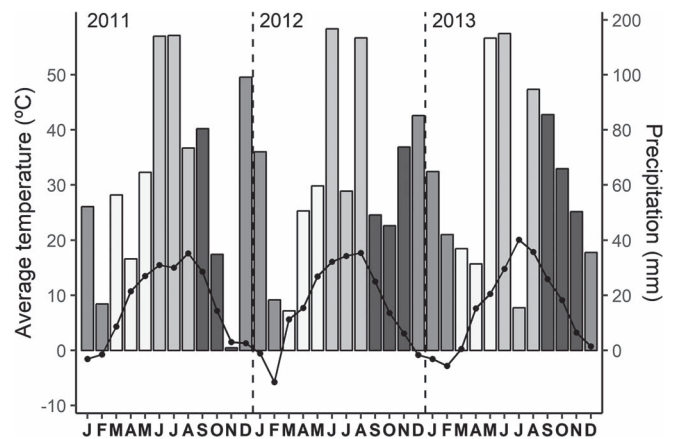


Figure 1. Average air temperature (point-line) and monthly total precipitation (bars) during 2011–2013 are in fixed proportion, 10 °C corresponding to a precipitation of 20 mm, allowing the characterization of seasonal environmental conditions, i.e., drought or freeze periods (Gausson and de Phillip 1958, Walter and Lieth 1960). During 2011–2013, mean air temperature and annual precipitation were 8.0 °C and 810 mm, respectively. Seasons are indicated by grayscale and defined as winter (Dec, Jan, Feb), spring (Mar, Apr, May), summer (June, July, Aug) and fall (Sept, Oct, Nov).

reached (~1 m), lined with low-density polyvinyl plastic and then backfilled (Pretzsch et al. 2016).

Thirty-year averages (1970–2000) of mean precipitation and air temperature were 786 mm and 7.6 °C, respectively (Matyssek et al. 2007). During the 2011–2013 period, however, mean air temperature and annual precipitation were 8.0 °C and 810 mm, respectively. Air temperature, relative humidity and precipitation were collected continuously from the 'Ecosystem Monitoring Plot Freising' (Bavarian State Institute of Forestry, LWF, Freising, Germany), 1 km south of the Kranzberger forest research site. Following Gausson and de Phillip (1958) and Walter and Lieth (1960), temporal occurrences of drought conditions as well as freeze periods can be observed at the site, i.e., the month of July in 2013 and the winter seasons of 2012 and 2013 (Figure 1), respectively.

Soils at the site are haplic luvisols and provide sufficient nutrients for tree growth (Göttlein et al. 2012). Time domain reflectometers (TDRs) used to monitor volumetric soil water content were installed in 2010 across all 10 plots at two soil depths: 0–10 and 10–30 cm (Cole 1977). Soil volumetric water content was measured weekly from April through November and monthly from December through March (TDR100, Campbell Scientific, North Logan, Utah). Averaged over 3 years, our measurements showed that average soil water content in mixed (22.6 ± 0.1%) and beech-dominated soils (21.9 ± 0.1%) in the 0–10-cm depth was higher compared to spruce-dominated soils (20.3% ± 0.1; see Figure S1 available as Supplementary Data at *Tree Physiology* online). Deeper soil layers (10–30 cm) also had higher average soil water content in mixed (28.5 ± 0.1%) and beech-dominated stands (28.1 ± 0.1%)

compared to spruce-dominated stands ($26.2 \pm 0.1\%$). During the month of August (2011–2013), the monotypic beech and spruce as well as the mixed-species zones reached equally low average moisture levels of $18.9 \pm 0.5\%$ at 0–10 cm and $23.6 \pm 0.5\%$ at 10–30 cm of soil depth.

Root observation

In 2010, clear acrylic minirhizotron tubes (70 cm in length, 6 cm in outside diameter) were installed at an angle of 60° from the horizontal reaching a vertical depth of 50 cm. The vertical depth was chosen based on previous research results at Kranzberger forest, which reported that $>90\%$ of beech and spruce roots grew between 0 and 50 cm (Häberle et al. 2012). Each plot contained six minirhizotron tubes: two in each single-species zone, from hereon called '*F. sylvatica*' and '*P. abies*' zone, and two within the beech–spruce zone, from hereon called 'mixed-species' zone. Each tube was located a minimum distance of 1 m from the plot boundaries, and in the case of the mixed-species zones, tubes were installed equidistant from both species (see Figure S2 available as Supplementary Data at *Tree Physiology* online). Average distances from the minirhizotron tubes to the trees were significantly longer in the mixed-species zone (5.4 m for *F. sylvatica* and 5.5 m for *P. abies*) than in the single-species zone (2.8 m for *F. sylvatica* and 2.9 m for *P. abies*, $P < 0.001$). However, minirhizotron tube distance to trees did not have a significant effect on cumulative fine root production within these tubes ($P = 0.83$; see Figure S3 available as Supplementary Data at *Tree Physiology* online). Moreover, we did not find a significant difference in tube distances to trees between the two species ($P = 0.67$). Before installation, minirhizotron tubes were capped at the base with plastic plugs and lined with silicon caulk to reduce water infiltration. Tubes, when not in use, were covered with large plastic caps to prevent aboveground water infiltration and light penetration.

From 18 May 2011 until 29 August 2013, 44 continuous images (width = 18 mm, height = 15 mm) were taken across the length of each tube using a minirhizotron camera system (BTC-100X Camera, Bartz Technology, Carpinteria, California), bi-weekly during the growing season and monthly during the winter months. All images were analyzed for absorptive fine-root production, death, survivorship and diameter using WinRHIZO Tron MF (Regent Inc., Quebec, Canada). To be certain of the absorptive fine-root characteristic, we solely focused on first-order roots (root tips). If a first-order root grew into a second-order root, this was marked as such and excluded from data analysis. The date of root birth was calculated as the mid-point between the appearance of a new root tip and the preceding image session. Similarly, the death date of a root was calculated as the mid-point between the disappearance or death of a root (black, shriveled) and the preceding image session (Comas et al. 2005). Roots that transected more than

one observation window were only counted once. The number of neighboring roots was defined as the number of root tips present in the proximity (within the 18 mm-by-15 mm image) of the root at the time of death. Through visual inspection of images in the single-species zones of *F. sylvatica* and *P. abies*, we determined three root-specific differences by appearance: (i) the epidermal coloration (spruce: dark brown, beech: red-dish), (ii) root-tip branching patterns (spruce: alternate branching, beech: herringbone and often opposite branching), and (iii) root-tip ending (spruce: round, beech: sharp). These three indicators were used to identify species identity of roots in the mixed-species zones.

Root respiration

To view, trace and sample roots of known species and age (Bouma et al. 2001), three in-ground plywood boxes (root box: $40 \times 60 \times 30$ cm, H $\times$ W $\times$ D) containing two acetate windows were installed in 8 out of the 10 research plots: one in each single-species zone and one in the mixed-species zone. Viewing windows of the root boxes, when not in use, were insulated year-round with polystyrene shutters, and a lid was screwed into place to prevent micro-climatic effects and exclude light penetration.

Root respiration was measured in the spring and fall of 2012 (22 May and 6 October), and 2013 (18 May and 7 October). Using the root boxes, absorptive fine-root branches containing first- and second-order roots were traced along acetate windows using colored markers for a period of 3 weeks to ensure that fine-root material was of a known species and age ($5 < x < 22$ days old). Pioneer roots, defined as first-order roots exhibiting little or no branching, lower incidence of mycorrhizal colonization and larger average diameter (Zadworny and Eissenstat 2011), were excluded in this experiment. Highlighted roots were then excised from behind the acetate windows using a razor blade, transferred to an onsite climate-controlled hut and placed into a cuvette containing oxygenated buffer (10 mM MES, 1 mM CaSO_4 and $5.5 \mu\text{M}$ K_2HPO_4 , pH = 5.8; Volder et al. 2005) within an oxygen electrode system maintained at $21 \pm 0.5^\circ\text{C}$ (Oxygraph, Hansatech, King's Lynn, UK). Oxygen consumption was measured over a period of 15 min. After 15 min, roots were removed from the cuvette, placed in a paper envelope and dried at 60°C .

Data analysis

All statistical analyses were performed in R (R Core Team 2018). We used the emmeans (Lenth 2019), survival and coxme (Therneau and Grambsch 2000) and lme4 (Bates et al. 2015) packages for Tukey honest significance difference (HSD) tests, survival analyses and mixed-effects linear models.

To determine the effect of species interaction zones (single-species *F. sylvatica*, single-species *P. abies* and mixed-species) on belowground root productivity measured as cumulative

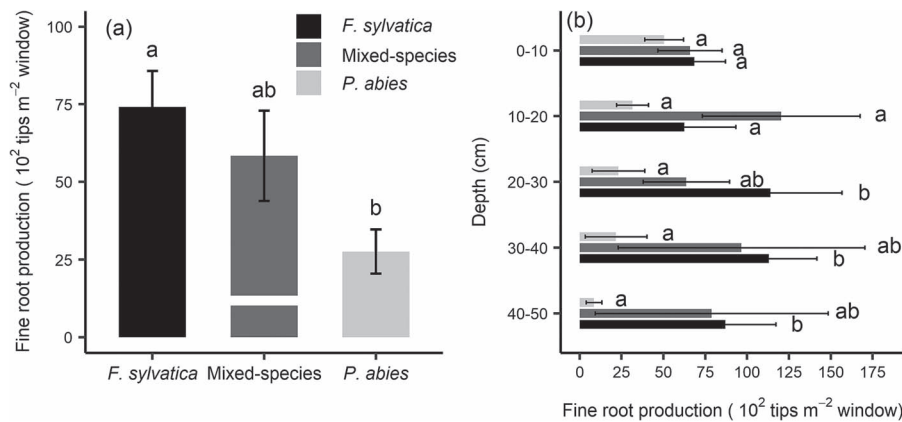


Figure 2. Cumulative root-tip production by (a) zones and (b) depth levels and zones of *F. sylvatica*, *P. abies* and mixed-species zone (roots of both species combined together). Means and standard errors are shown ($N = 10$). Contrasting letters indicate significant differences among zones within depth level ($P < 0.05$) as determined with the Tukey honest significant difference test. The area below the white line indicates the amount of roots belonging to *P. abies*, and the area above the white line indicates the amount of roots belonging to *F. sylvatica* in the mixed-species zone.

root-tip production, we used a linear model and Tukey HSD tests. Root-tip production of each species interaction zone was calculated by averaging the production of first-order roots over the two tubes in the mixed-species and single-species zones of each replicate plot and expressed as the total number of tips produced per square meter minirhizotron viewing window. To analyze root-tip production of the species interaction zones by 10-cm-soil-depth increments and seasons, we used the same approach but log-transformed seasonal root-tip production to satisfy the normality and equal variance of the residual assumptions. Seasons were defined as winter (December 01–February 28), spring (March 01–May 31), summer (June 01–August 31) and fall (September 01–November 30). Root-tip mortality patterns were analyzed using the same methods.

To analyze shifts in absorptive fine-root production and mortality patterns of *F. sylvatica* and *P. abies* over space and time, we again analyzed first-order roots only and took two different modeling approaches. First, we used two separate linear models to test the effect of neighbor identity on the proportion of fine-root tips by 10-cm-depth increments or season separately. The proportion was calculated by dividing the number of root tips in a specific temporal or spatial cohort of each year by the cumulative annual root-tip production or death over the 50-cm soil profile. Second, we developed a spatio-temporal mixed-effects model where both depth (responding variable) and seasons (independent variable) were included simultaneously. In addition, we included species neighbor identity, year and an interaction term for neighbor species identity and season as independent variables in the model. Plot was included as a random effect.

For the survivorship analysis of absorptive fine roots, we made use of Cox proportional hazards regressions (Cox 1972). Neighbor species identity, root diameter, number of neighboring root tips and root depth were included as covariates and plot as a

random effect. To satisfy Cox's proportional hazard assumptions, (i) we stratified the fine root data in the model by season and production year, and (ii) we categorized the independent variables root diameter (0–0.2 mm, 0.2–0.4 mm, >0.4 mm) and root depth (0–10 cm, 10–30 cm, 30–50 cm) into cohorts.

Lastly, we used a linear model and Tukey HSD tests to determine the effect of species, neighbor species identity and season on fine-root respiration. This was the only exception where second-order roots were also assessed. To analyze trends in first-order root diameter, we utilized a mixed-effects model with plot as random effect.

Results

Spatio-temporal patterns of absorptive fine-root production and mortality

Cumulative root-tip production was greater in the single-species zone of *F. sylvatica* than in the single-species zone of *P. abies* during 2011–2013 (Figure 2a, $P < 0.05$). Yet, cumulative root-tip production in the mixed-species zone was not significantly different from the single-species zones for either species, implying no evidence for over-yielding under mixed-species interactions. In mixture, *F. sylvatica* produced a greater number of root tips (46.6×10^2 tips m^{-2} window) than *P. abies* (11.8×10^2 tips m^{-2} window). These trends in root-tip production under single- and mixed-species growing conditions persisted at different spatial and temporal scales: at the 20–30-, 30–40- and 40–50-cm soil depth (Figure 2b) and across seasons (see Figure S4 available as Supplementary Data at *Tree Physiology* online). The ratio of *F. sylvatica* to *P. abies* root tips increased with soil depth.

Cumulative root-tip deaths in the single- and mixed-species zones during 2011–2013 showed the same pattern as production (see Figure S5 available as Supplementary Data at *Tree*

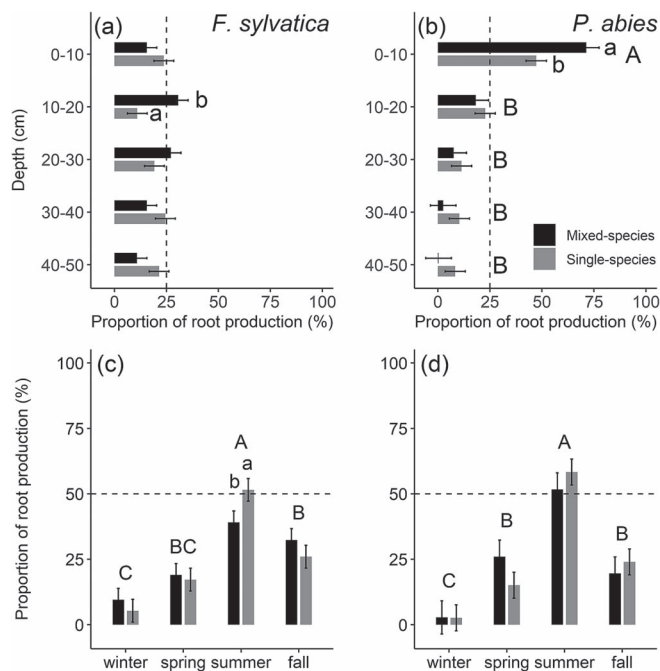


Figure 3. Relative abundance of root-tip production by 10-cm soil depth levels for (a) *F. sylvatica* and (b) *P. abies* and by season for (c) *F. sylvatica* and (d) *P. abies* averaged over the years 2011–2013 under mixed-species (black) and single-species (gray) growing conditions. Means and standard errors are shown ($N = 10$). Contrasting uppercase letters indicate significant differences between depth levels or season averaged over single- and mixed-species interaction zones ($P < 0.05$), and contrasting lowercase letters indicate significant differences between mixed-species and single-species growing conditions ($P < 0.05$) as determined with the Tukey honest significant difference test. Dashed line indicates 25% of cumulative root production in panels (a) and (b), and 50% of cumulative root production in panels (c) and (d).

Physiology online). At a greater soil depth, the single-species zone of *F. sylvatica* had a greater number of root tip deaths than the single-species zone of *P. abies* (see Figure S6a available as Supplementary Data at Tree Physiology online). Root-tip death was greater in the single-species zone of *F. sylvatica* than *P. abies* in the summer and fall, yet such differences were not observed in winter and spring (see Figure S6b available as Supplementary Data at Tree Physiology online). Whether we analyzed the total amount of dead tips during 2011–2013 or partitioned the data by season or depth, we did not find a difference in root-tip death between the single- and mixed-species zones except for the single *P. abies* zone and mixture in the fall.

Using the proportion of root tips produced over 10-cm soil depth levels or seasons by year as a responding variable, we found differences in the spatial root production patterns of *F. sylvatica* and *P. abies* under single- and mixed-species growing conditions (Figure 3). Namely, while *P. abies* had a greater proportion of absorptive fine roots growing in the upper 10 cm of the soil when grown in mixture with *F. sylvatica* than by itself, *F. sylvatica* had a greater proportion of fine roots growing at the 10–20-cm soil depth in the mixed-species arrangement

($P < 0.05$). In general, *F. sylvatica* showed an even distribution of absorptive fine roots across depth levels, while *P. abies* produced a large proportion of root tips in the upper 10 cm of the soil that decreased with soil depth. Temporal adjustments in the form of increased absorptive fine-root production were only observed in the summer for the single-species zone of *F. sylvatica* relative to mixed-species growing conditions. Overall, root-tip production varied seasonally across years for both *F. sylvatica* and *P. abies*, with most roots produced in the summer followed by spring and fall. Fine-root production was lowest in the winter for both species ($P < 0.05$). Also, for both species, root-tip production was the highest in 2011 ($P < 0.05$). Root-tip production of *P. abies* was similar in 2012 and 2013, while *F. sylvatica* produced more fine roots in 2012 than in 2013 ($P < 0.05$). It is important to note that we stopped measuring root-tip production in September 2013, and more absorptive fine roots may have been produced in the fall and winter of that year.

When including soil depth (space as dependent variable) and season (time as one of the independent variables) in the same mixed-effects model, we found that absorptive fine roots of *F. sylvatica* were produced deeper in the soil (25.5 ± 3.1 cm) when grown with itself than in mixed-species stands (21.1 ± 3.1 cm, $P < 0.001$) (Figure 4a), while *P. abies* root-tip production depth was not affected by the identity of neighboring species ($P = 0.85$). Likewise, we did not detect a significant interaction between single- versus mixed-species zones and production season, meaning that *F. sylvatica* and *P. abies* did not show temporal adjustments in root-tip growth dynamics in response to neighbor species identity ($P = 0.13$ and $P = 0.17$, respectively).

The depth of root-tip production differed by season for both species, but was more pronounced for *F. sylvatica* than *P. abies*. Of the total population of *F. sylvatica* roots produced, fall roots were produced significantly deeper in the soil compared to other seasons ($P < 0.05$). Root-tip production in spring took place at the shallowest depth but was not significantly different from the average production depth, in winter. As the seasons progressed from spring to summer to fall, root tips were produced at continuously deeper depths ($P < 0.05$). For *P. abies*, absorptive fine roots in summer and fall were produced deeper than spring roots ($P < 0.05$), but the production depth of winter roots was not significantly different from the rooting depth of the other seasons. For both species, root-tip production in 2011 was shallower than in the other years. For *F. sylvatica*, rooting depth was also shallower in 2012 than in 2013. Overall, the average root production depth of *P. abies* (15.2 ± 2.6 cm) was shallower than that of *F. sylvatica* (23.6 ± 2.6 cm).

The average depth of root-tip mortality in the single-species and mixed-species stands followed the same trend as fine-root production (Figure 4b) with two exceptions. First, we did not detect a significant effect of species interaction zones

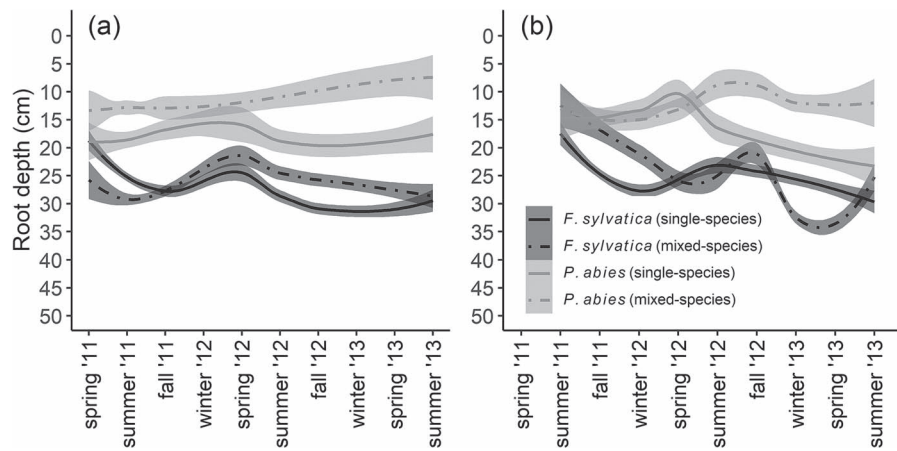


Figure 4. Average depth $\pm$ standard error of (a) root-tip production ($N = 4855$) and (b) mortality ($N = 3694$) across seasons and year for root tips in the single-species zone of *F. sylvatica* and *P. abies* as well as both species in the mixed-species zone. Means are indicated by the line graph and standard errors by a gray-tinted area on each side of the line. Seasons are defined as winter (Dec, Jan, Feb), spring (Mar, Apr, May), summer (June, July, Aug) and fall (Sept, Oct, Nov).

Table 1. Cox-proportional hazard model output for the mortality risk of root tips of *F. sylvatica*. Coefficient estimates, standard error (SE), Wald chi-square statistic (χ^2), hazard risk ratio and *P* values are shown. For the categorical variables included in the model, their reference level is in parentheses. Significant *P* values ($P < 0.05$) are in bold. A hazard ratio < 1 means a decrease in mortality risk, while a hazard ratio > 1 signifies an increase in mortality risk. Neighbors are defined as number of neighboring root tips of *F. sylvatica* and *P. abies* in the 18 mm-by-15 mm image at time of death.

Variable	Coefficient estimate	SE	χ^2	<i>P</i>	Hazard ratio
Interaction (mixed-species)	0.43	0.05	8.62	< 0.0001	1.54
Single-species					
Diameter (0–0.2 mm)					
0.2–0.4 mm	−0.58	0.05	−12.46	< 0.0001	0.56
>0.4 mm	−1.40	0.17	−8.06	< 0.0001	0.25
Depth (0–10 cm)					
10–30 cm	−0.31	0.06	−4.87	< 0.0001	0.73
30–50 cm	−0.91	0.07	−12.34	< 0.0001	0.40
Neighbors	−0.001	0.001	−1.02	0.31	1.00

for *F. sylvatica* in the spring. Second, we found a significant interaction between year and species interaction zones for *P. abies* ($P < 0.001$), which showed a difference in the depth of root-tip mortality between the single-species (17.8 ± 2.6 cm) and mixed-species (7.1 ± 3.2 cm) arrangements in 2012, but not in 2011 or 2013. However, the lack of significant difference in 2013 may be due to the absence of fall data. Interestingly, in the case of *F. sylvatica*, seasonal patterns of root-tip mortality were the inverse of production, with root-tip death taking place in the shallowest soil layers in the summer and fall and at greater depth in spring followed by winter ($P < 0.05$). In addition, root-tip death of *F. sylvatica* took place at a progressively deeper depth each year ($P < 0.05$). For *P. abies*, season did not influence the depth of fine-root mortality ($P = 0.25$).

Survivorship of absorptive fine roots

The root tips of *F. sylvatica* lived longer in mixed-species growing conditions (Table 1; see Figure S7a available as Supplementary Data at *Tree Physiology* online). The Cox proportional hazard

model estimated that beech absorptive fine roots in the single-species zone have a 54% higher risk of mortality compared to roots in mixed-species zones ($P < 0.0001$), with a median lifespan of 174 days versus 440 days, respectively. In contrast to *F. sylvatica*, *P. abies* did not show a significant difference in absorptive fine-root survivorship under mixed- or single-species conditions (Table 2; see Figure S7b available as Supplementary Data at *Tree Physiology* online). On the other hand, soil depth significantly influenced the survivorship of both *F. sylvatica* and *P. abies* root tips. The depth effect was stronger for *F. sylvatica* ($P < 0.0001$) than for *P. abies* ($P = 0.025$) at 10–30 cm in comparison to the reference 0–10-cm surface soil layer (Table 1, 2). Absorptive fine-root lifespan increased for both species in deeper soil layers (see Figure S8 available as Supplementary Data at *Tree Physiology* online). The median root lifespan of *F. sylvatica* was 147 (0–10 cm), 220 (10–30 cm) and 400 (30–50 cm) days. Yet, for *P. abies*, only the deeper roots at 30–50 cm had a significantly higher lifespan (318 days) than the upper layers which showed similar lifespans (234 days,

Table 2. Cox-proportional hazard model output for the mortality risk of root tips of *P. abies*. Coefficient estimates, standard error (SE), Wald chi-square statistic (χ^2), hazard risk ratio and *P* values are shown. For the categorical variables included in the model, their reference level is in parentheses. Significant *P* values ($P < 0.05$) are in bold. A hazard ratio < 1 means a decrease in mortality risk, while a hazard ratio > 1 signifies an increase in mortality risk. Neighbors are defined as number of neighboring root tips of *F. sylvatica* and *P. abies* in the 18 mm-by-15 mm image at time of death.

Variable	Coefficient estimate	SE	χ^2	<i>P</i>	Hazard ratio
Interaction (mixed-species)	−0.02	0.11	−0.13	0.89	0.99
Single-species					
Diameter (0–0.2 mm)	−0.20	0.29	−0.66	0.51	0.82
>0.4 mm	−0.65	0.31	−2.11	0.04	0.52
Depth (0–10 cm)					
10–30 cm	−0.23	0.10	−2.24	0.03	0.79
30–50 cm	−1.06	0.19	−5.52	<0.0001	0.34
Neighbors	0.001	0.001	0.46	0.65	1.00

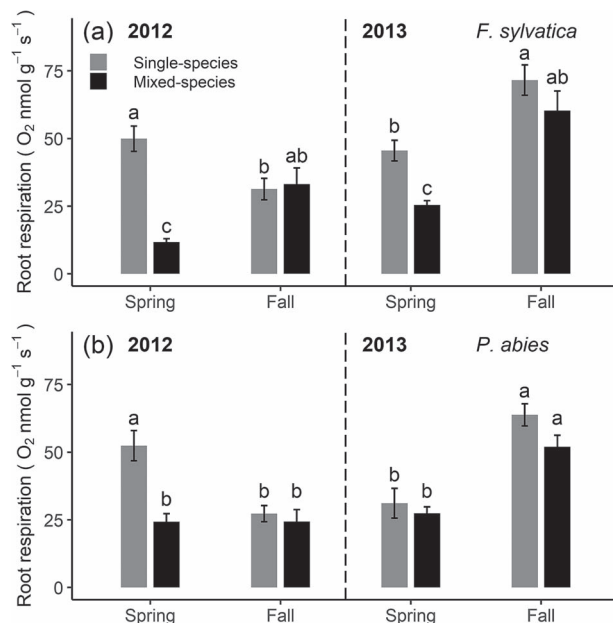


Figure 5. Respiration of absorptive fine roots (first- and second-order roots, $5 < x < 22$ days old) of (a) *F. sylvatica* and (b) *P. abies* growing under single-species (gray) and mixed-species (black) conditions in the spring (22 May 2012, 18 May 2013) and fall (6 October, 7 October) season. Means and standard errors are shown ($N = 56$ for *F. sylvatica* and $N = 56$ for *P. abies* in 2012, $N = 48$ for *F. sylvatica* and $N = 46$ for *P. abies* in 2013). Contrasting letters indicate significant differences between seasons and single- and mixed-species interaction zones within each year as determined by Tukey honest significant difference tests ($P < 0.05$).

0–10 cm and 190 days, 10–30 cm). A smaller resolution of 10-cm-depth increments also resulted in no depth effect differences. *F. sylvatica* absorptive fine roots with a diameter > 0.2 mm lived longer than the roots with a diameter < 0.2 mm (Table 1, $P < 0.0001$). In the case of *P. abies*, however, only root tips with a diameter > 0.4 mm had longer lifespans than the 0–0.2-mm-diameter cohort (Table 2, $P = 0.035$). The number of neighboring root tips present at the time of death did not have a significant effect on root survivorship for either species (Tables 1 and 2).

Morphological and physiological absorptive fine-root plasticity to neighbor species identity

Neighbor identity significantly influenced absorptive fine-root respiration for *F. sylvatica* in spring of 2012 and 2013 and, for *P. abies* in the spring of 2012 (Figure 5) with a significant third-order interaction among year, season and species interaction zones. In all these cases, absorptive fine-root respiration rates were higher in the single-species zone than in the mixed-species zone. For both species, season significantly influenced root respiration ($P < 0.0001$). In 2013, respiration was higher in the fall than in the spring for both species under single-species and mixed-species conditions ($P < 0.05$). In 2012, *F. sylvatica* roots in the mixed-species zone respired more in the fall than in the spring ($P < 0.05$), while the trend was reversed for single-species roots of *F. sylvatica* ($P < 0.05$). For *P. abies*, we did not find a significant seasonal difference in root respiration under mixed-species conditions in 2012 ($P = 0.99$). Yet, the single-species absorptive fine roots of *P. abies* showed higher respiration in the spring than in the fall of 2012 ($P < 0.05$). Overall, absorptive fine roots of *P. abies* had higher respiration rates in 2013 than in 2012 ($P < 0.05$), but root respiration of *F. sylvatica* did not vary between years ($P = 0.51$). Absorptive fine-root respiration did not differ between species ($P = 0.28$).

Root tips of *F. sylvatica* were smaller in diameter than those of *P. abies* ($P < 0.05$). Absorptive fine roots of *F. sylvatica* in the mixed-species zone were also slightly larger than roots in the single-species zone (0.28 vs. 0.27 mm, $P < 0.05$), while the opposite was true for spruce (0.36 vs. 0.40 mm, $P < 0.05$). Also, the season in which root tips were produced had a significant effect on the absorptive fine-root diameter of both species. Absorptive fine roots of *F. sylvatica* had a smaller diameter in the summer and fall than in the spring and winter ($P < 0.05$). The diameter of *P. abies* absorptive fine roots was the largest in the spring, followed by summer and lastly by fall and winter ($P < 0.05$).

Discussion

Plastic responses in absorptive fine-root traits to neighbor species identity

This study analyzed absorptive fine-root traits and spatio-temporal dynamics of root production and mortality of *F. sylvatica* and *P. abies* in single-species and mixed-species zones in an experimental forest in southern Germany. As we predicted, the species identity of surrounding trees significantly affected the absorptive fine-root lifespan of *F. sylvatica* (Table 1; see Figure S7 available as Supplementary Data at *Tree Physiology* online), respiration in spring (Figure 5) and diameter of both *F. sylvatica* and *P. abies*. We used these changes in absorptive fine-root traits as an indicator of the intensity of root competition in the single-species and mixed-species interaction zones. Contrary to our hypothesis that root competition is higher under single-species compared to mixed-species interactions for both *F. sylvatica* and *P. abies*, the directional shifts in fine-root traits observed in this study suggest that this is only the case for *F. sylvatica*. Our prediction on niche differentiation over space (soil depth) and time (seasons) was partly met and more dynamic than expected (Figures 3 and 4).

To our knowledge, this study provides the first evaluation of absorptive fine-root survivorship in mature single-species and mixed-species forest stands at the field scale. We found that the risk of root-tip mortality for *F. sylvatica* was 54% higher when grown by itself than when intermixed with *P. abies*. The higher rates of absorptive fine-root respiration of *F. sylvatica* in the single-species zone in comparison to the mixed-species zone in the spring of 2012 and 2013 line up well with the survivorship results. Namely, cost–benefit models predict that roots with higher respiration rates—indicating greater C costs—should have shorter lifespans (Eissenstat 1992, Eissenstat et al. 2000, Eissenstat and Yanai 1997). These trends in root longevity and respiration may also be connected to changes in fine-root diameter. While previous research has demonstrated that fine roots with larger diameters tend to have lower respiration rates (Pregitzer et al. 1998) and longer root lifespans (Wells and Eissenstat 2001), these studies assessed different root orders with a wider range of diameters, soil depths and functions than covered in our analysis. The absorptive fine roots of *F. sylvatica* indeed had larger diameters in the mixed-species than single-species zone of the forest. Yet, this difference only amounted to 0.01 mm. Because few studies have analyzed the impact of changes in fine-root diameter at this scale, it is difficult to conclude whether such a minor change in diameter contributes to explaining the respiration and survivorship patterns observed in this study.

The absorptive fine roots of *P. abies* showed a less plastic response in survivorship and respiration (Table 2, Figure 5), but a more plastic response in diameter to neighbor species identity than *F. sylvatica*. Absorptive fine-root diameters of *P. abies* were

0.04 mm smaller under mixed-species than single-species interactions, the opposite response to that of *F. sylvatica*. A smaller fine-root diameter associated with higher specific root length is a common morphological adjustment to low soil resources (Farley and Fitter 1999, Hodge 2004). Hence, the species differences in root diameter, together with the observed lifespan patterns of *F. sylvatica*, suggest that the soil environment under mixed-species interactions provided less competition and/or more resources to the absorptive fine roots of *F. sylvatica*, but more competition and/or fewer resources to the fine roots of *P. abies* in comparison to their single-species environments.

Our conclusion that the mixed-species zone is more favorable for *F. sylvatica*, but less favorable for *P. abies* than their respective single-species zones, is supported by the observed trends in root space occupation. Namely, the total number of root tips produced substantially decreased from single *F. sylvatica* (beech roots), to mixed-species (beech + spruce roots), to single *P. abies* (spruce roots) (Figure 2). Though not significant, this trend suggests that mixed-species interactions provide an environment with fewer roots (reduced competition) for *F. sylvatica* yet increase root competition for *P. abies*. Another potential factor contributing to shifting absorptive fine-root traits between the single-species and mixed-species zone is soil resource availability. Yet, the lower soil moisture content in the single *P. abies* zone in comparison to the mixture (see Figure S1 available as Supplementary Data at *Tree Physiology* online) goes against our conclusion that the mixed-species zone is a less favorable environment for *P. abies* root growth, or suggests that competition for soil water resources was not driving shifts in absorptive fine-root traits. Previous studies at the 'Kranzberger Forst' site identified higher nutrient concentrations in the topsoil in single *P. abies* than single *F. sylvatica* stands, which were primarily attributed to species-specific litter chemistry (Göttlein et al. 2012). This study, however, did not address soil nutrient content in mixed stands, making it difficult to confirm the role of soil nutrient shifts. Lastly, while our study focused on fine-root growth dynamics at the forest stand or plot scale, it would be relevant to consider neighborhood conditions and specifically surrounding tree density effects on fine-root traits when shifting to the individual tree scale. Overall, our findings stress the complexity of belowground plant–plant interactions and that the outcome of root competition may be asymmetric (Montgomery et al. 2010, Rewald and Leuschner 2009). Future studies are needed to confirm the role of root space occupation, soil resource availability and tree density as drivers of shifts in fine-root morphology, and physiology during belowground species interactions.

Spatio-temporal niche differentiation and fine-root production patterns under single-species and mixed-species root interactions

To assess patterns of spatial and temporal niche differentiation between the single- and mixed-species interaction zones of

F. sylvatica and *P. abies*, we utilized two different analysis methods: (i) we evaluated the spatial or temporal proportion of root tips as individual response variables in two models, and (ii) we developed one spatio-temporal model to analyze root depth as a response variable with season as the independent variable. Both modeling approaches gave similar results for spatial and temporal adjustments in absorptive fine-root production to neighboring tree species. First, we found that the single-species and mixed-species forest stands influence the spatial root distribution of *F. sylvatica*. Namely, *F. sylvatica* produced a greater proportion of root tips at the 10–20-cm soil depth overall (Figure 3a and b) and had a shallower root production depth (Figure 4a) in the mixed-species zone in comparison to the single-species zone. The insignificant effect of neighbor species identity on the root production depth of *P. abies* in the spatio-temporal model (Figure 4a) may be explained by its overall shallow root depth, which makes it more difficult to identify the significantly higher proportion of absorptive fine roots produced in the 0–10-cm soil layer in the mixed-species zone (Figure 3a and b). Schmid and Kazda (2002) also reported a shift in root production towards shallower soil layers for *P. abies* when grown in mixture. *F. sylvatica*'s root production on the other hand took place at deeper locations in the mixed zone. The fact that our study only focused on the absorptive fine roots—and not the entire root system—may explain these opposing trends. Secondly, neither model provided evidence for seasonal shifts in absorptive fine-root production between the single-species and mixed-species interaction zones apart from *F. sylvatica* producing a higher proportion of root tips in summer under single-species than mixed-species composition in the one-dimensional seasonal model (Figure 3c and d).

The spatial root adjustments of *F. sylvatica* and *P. abies* to neighbor identity may go beyond mechanisms of resource availability (de Kroon 2007, Schenk 2006). Recently, root exudates and rhizosphere microbes have been recognized as important drivers of belowground plant–plant interactions (Mommer et al. 2016). Previous studies have shown distinct root exudate profiles (Zwetsloot et al. 2018) and fine-root fungal compositions (Nickel et al. 2018) of spruce and beech tree species. These relations between species-specific non-resource cues and spatial niche differentiation within the soil profile motivate further investigation on this topic.

We recognize that our second spatio-temporal model is a less common approach to analyzing absorptive fine-root dynamics. Yet, in addition to confirming the patterns observed by the first models that evaluated root dynamics separately over space and time, the second model highlights a spatio-temporal root-tip production pattern that cannot be identified otherwise. Specifically, we found a significant effect of season on *F. sylvatica* and *P. abies* rooting depth (Figure 4a). This seasonal trend in root production depth has been observed in other studies (Lyr and Hoffman 1967; Hendrick and Pregitzer

1992) and could be explained by seasonal fluctuations in soil temperature and resource availability (Reich 1980), which are strongly associated to soil temperature (Kuhns et al. 1985, Teskey and Hinckley 1981, Tierney et al. 2003). For example, after bare soil exposure to low temperatures, shallow soil depths are the first to reach adequate temperatures for root growth in spring. In addition, studies have observed that deep root production is related to soil water availability (Hendrick and Pregitzer 1996). In our study, absorptive fine-root production in deeper soil layers may be a result of the lower soil moisture levels near the soil surface during the summer and fall months (see Figure S1 available as Supplementary Data at *Tree Physiology* online). Lastly, the location of absorptive fine-root mortality in the soil profile for *F. sylvatica* was the inverse of its production patterns through time (Figure 3b). This makes sense when we consider seasonal resource availability, where root tips are produced in areas of high nutrient or water content (shallow layers in spring, deep layers in fall) and senesce under unfavorable resource conditions (deep layers in spring, shallow layers in fall).

Belowground root productivity and implications for root-facilitated C cycling

Surprisingly, absorptive fine-root production in the mixed-species zone was not different from the single-species zone of *F. sylvatica* or *P. abies* (Figure 2a). This goes against the hypotheses and results of previous publications (Tilman et al. 1997, Brassard et al. 2011, Mommer et al. 2010) where species mixtures led to greater productivity than monocultures. The fact that we, and other studies (Meinen et al. 2008), did not find evidence of belowground over-yielding under mixed-species composition suggests, that transitioning from a single-species setting to a mixture of species is not a guarantee for increased belowground root productivity. These contrasting outcomes of belowground plant–plant interactions could be explained by the extent of niche differentiation of the species involved in these different experiments. In our study, the absorptive fine roots of both species showed substantial niche overlap in the shallow soil layers. In light of competition and accessibility to soil resources, the overlap of absorptive fine roots may explain why belowground root productivity was not higher under mixed-species zones than in the single-species zones. However, Mommer et al. (2010) showed that mixtures of grass species had shallower rooting depths, but also greater belowground root productivity than monocultures. An alternative hypothesis by Callaway and Walker (1997) states that competition is more prevalent under high resource availability, while facilitation is more common when resources are low. As in other similar studies, our conclusions are inherently limited to select species combinations under specific environmental conditions. Therefore, additional studies are needed to explore which mechanisms are driving the

outcomes of root–root interactions across ecosystems under fluctuating soil resources.

Cumulative absorptive fine-root production (Figure 2a) and mortality (see Figure S5 available as Supplementary Data at *Tree Physiology* online) in the species interaction zones showed similar trends. Yet, when we analyzed root-tip mortality and production dynamics over soil depth and seasons (Figure 4), we found distinct patterns. With absorptive fine roots functioning as a primary source of soil C in forest ecosystems (Clemmensen et al. 2013, Rasse et al. 2005) and driving seasonal trends in microbial community composition and soil extracellular enzyme activity (Kaiser et al. 2010), the spatio-temporal patterns of absorptive fine-root mortality have important implications for where and when absorptive fine roots prime soil microbial processes and provide C inputs to soil organic matter pools. In contrast with the single-species zones of *P. abies*, where the depth of absorptive fine-root mortality did not fluctuate, or remained shallow in mixed zones, the mortality of absorptive fine roots of *F. sylvatica* shifted C fluxes from deeper soil layers in the spring to shallower depths in the summer and fall. When we consider total absorptive fine-root deaths in each season (see Figure S6b available as Supplementary Data at *Tree Physiology* online), our results imply that root C supply in the single- and mixed-species zone of *F. sylvatica* fluctuates across soil depths throughout the year with stronger pulses in the summer and fall. Few studies have attempted to analyze these seasonal and depth-related fine-root patterns, or they show contrasting results (Hendrick and Pregitzer 1996), making it challenging to extract general trends across species and experimental sites. We encourage future studies to make use of the spatio-temporal analyses presented in this paper to obtain a more complete understanding of root-facilitated C cycling in forest ecosystems.

Conclusion

In summary, this study analyzed the absorptive fine-root responses of *F. sylvatica* and *P. abies* in single- and mixed-species forest stands over space and time. Our findings have important implications for plant–plant interactions and biogeochemical cycles at the ecosystem scale. Namely, the observation that the absorptive fine roots of *F. sylvatica* show differences in longevity between the mixture and monoculture, and those of *P. abies* do not, demonstrates that the plasticity of root lifespan in response to neighboring plants is species-specific. Moreover, these results suggest slower turnover of *F. sylvatica* absorptive fine roots and therefore root-facilitated C cycling in mixed-species forests. In addition, we did not detect an increase in belowground root productivity in mixed- relative to single-species stands. Our results confirm the findings from previous studies that increasing tree species diversity is not a guarantee for higher root productivity. Lastly, our spatio-temporal analyses of root growth dynamics indicate that root

carbon fluxes to the soil fluctuate within the soil depth profile throughout the year.

On a mechanistic level, future studies are necessary to disentangle what factors drive shifts in absorptive fine-root dynamics in response to neighbor species identity. We propose that soil resource availability, rhizodeposition, litter chemistry and species-specific soil microbial communities likely contribute to spatial root niche differentiation, and changes in absorptive fine-root lifespan, respiration and diameter between single-species and mixed-species forest stands.

Supplementary Data

Supplementary data for this article are available at *Tree Physiology* Online.

Conflict of interest

None declared.

Acknowledgements

We are thankful for the invaluable technical support provided by Christof Seidler, Thomas Feuerbach, Peter Kuba, Josef Heckmair, Annika Huber and Roman Meier. We also thank Karl-Heinz Häberle for his help during the entirety of this project and Stephen Parry from Cornell Statistical Consulting Unit for his advice on analyzing spatio-temporal patterns of root production and mortality.

Funding

Andrew W. Mellon Student research grant and Einaudi funding to A.P.; Grants to T.P. by the German Research Foundation (DFG, GR1881/5-1); Bavarian State Ministries of the Environment and Consumer Protection as well as Food, Agriculture and Forestry.

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