Yeste A., Blanco J.A., Imbert J.B., Zozaya-Vela H., Elizalde-Arbilla M. 2020. Pinus sylvestri L. and Fagus sylvatica L. effects on soil and root properties and their interactions in a mixed forest on the Southwestern Pyrenees. *Forest Ecology and Management* 481, 118726.

https://doi.org/10.1016/j.foreco.2020.118726

Pinus sylvestris L. and *Fagus sylvatica* L. effects on soil and root properties and their interactions in a mixed forest on the Southwestern Pyrenees

Antonio Yeste[1], Juan A. Blanco[2,*], J. Bosco Imbert[3], Helena Zozaya-Vela[4], Martín Elizalde-Arbilla[5]

[1] Universidad Pública de Navarra, Departamento de Ciencias, Institute for Multidisciplinary Research in Applied Biology – IMAB, Pamplona, Spain

E-mails:

[1] Antonio Yeste: antonio.yeste@unavarra.es

[2] Juan A. Blanco: juan.blanco@unavarra.es

[3] J. Bosco Imbert: bosco.imbert@unavarra.es

[4] Helena Zozaya-Vela: helenazozaya97@gmail.com

[5] Martín Elizalde-Arbilla: martin.elizalde.orgi@gmail.com

[*]Corresponding author:

Juan A. Blanco

Departamento de Ciencias, Institute for Multidisciplinary Research in Applied Biology - IMAB

Universidad Pública de Navarra, Campus de Arrosadía s/n, 31006, Pamplona, Spain.

E-mail address: juan.blanco@unavarra.es

Phone: (+34) 948 16 9869

Fax: (+34) 948 16 8930

Abstract

Tree species alter soil properties, potentially modifying forest nutrients cycling. In the current management context in which mixed species forests are favoured over monocultures due to their biodiversity and productivity-related advantages, the assessment of species effects on soils, as well as their interactions with other species, gains increasing relevance. In this study, the effects of Scots pine (*Pinus sylvestris* L.) and European beech (*Fagus sylvatica* L.) on soil properties were evaluated. Fine roots were paid special attention, measuring their biomass, functional traits (specific root length, root tissue density) and vertical distribution in order to discern the direction of these species interaction, either complementary or competitive. The research was carried out in the Southwestern Pyrenees (northern Spain), in an originally Scots pine stand transformed nowadays into a mixed forest by European beech natural regeneration. Soil and root samples were taken close to pine trees surrounded by other pines in areas that remain similar to pine monospecific stands, and close to pine and beech trees surrounded by both species in mixed areas. A lower C/N ratio was found in the soil close to beech stems. This suggests better quality in mixed litter in comparison to pine litter, leading to higher decomposition rates. Higher fine root biomass was found in the mixed areas mainly due to beech fine roots great abundance, which correlated positively with microbial biomass. Fine roots functional traits such as specific root length and diameter did not vary depending on their proximity to different tree species, though Scots pine fine root biomass decreased sharply when close to beech trees. This reduction, together with the already more abundant fine root biomass of beech, with higher specific root length and root tissue density than pine, lead to a competitive interaction in which European beech tends to dominate the soil at all depths. In this case, no complementarity effect at belowground level, strong enough to allow Scots pines to cope with beech soil colonization, was found under natural conditions.

Key words: mixedwoods, belowground competition, fine root biomass, specific root length, root tissue density, complementarity.

1. Introduction

Forest soils have been long recognized as a key element in sustainable forest management, but only recently the complex relationships between aboveground biodiversity and belowground processes are being study in depth. Over the last decade, mixed forests have been identified as generally more productive than pure stands (Griess and Knoke, 2013; He et al., 2013; Liang et al., 2016), and more resilient against biotic and abiotic threats (Jactel et al., 2009; Loreau and de Mazancourt, 2013). However, such advantages have been reported mostly by observing aboveground ecosystem features, and have been frequently attributed to belowground differences but without solid empirical proofs. In parallel, the focus on soil research has also grown, seeing soil increasingly as a fundamental part of the ecosystem that must be taken into account when developing sustainable policies (Doran and Zeiss, 2000; Dumanski, 2015). In fact, many of the ecosystem changes driven by biodiversity are mediated by processes that occur at belowground (Lange et al., 2015; Scheu, 2005).Hence, there is a clear need to better understand soil processes and how they affect aboveground forest structure and function, particularly in mixed forests.

In forests, soil properties can be affected by tree species in a variety of ways, through resource uptake and the deposition of organic matter that may differ in quality and quantity. Thus, changes in species dominance may lead to changes in forest biogeochemical cycles (Mueller et al., 2012). For example, litter and the exudates released into the rhizosphere have an effect on the microbial community leading to changes in decomposition rates, altering availability of nutrients and forests capacity to store carbon (Prescott and Grayston, 2013; Zhang et al., 2019). Faster decomposition is usually found in broadleaf and mixed forests along with a lower soil carbon-to-nitrogen (C/N) ratio, while conifer litter, richer in lignin and poorer in nitrogen, is linked to the opposite (Cools et al., 2014; Prescott and Grayston, 2013).

At the same time, species develop interactions when coexisting that shape the different aspects of forest functioning. Complementarity effects can facilitate coexistence between different species or, on the other hand, competition may lead to the dominance of some species over others (Cavin et al., 2013; Forrester and Bauhus, 2016; Madrigal-González et al., 2016). Although belowground has been less studied than aboveground, complementarity has been observed through changes in the root system, such as those that lead species to change vertical distribution of roots when growing next to other species, hence facilitating coexistence through niche segregation (Brum et al., 2019). Root morphology can also vary reflecting adaptation to local conditions both between different species and between individuals of the same species. In this regard, the study of functional traits, defined as morphological, physiological or phenological traits which impact fitness (Violle et al., 2007), has proven to be useful to assess the ecological role of fine root morphological variability. Plants can optimize resource uptake and carbon investment through changes in traits such as specific root length (SRL, root length per root dry mass), specific root area (SRA, root area per root dry mass) or root tissue density (RTD, root dry mass per root volume) (Addo-Danso et al., 2018; Borden et al., 2019; Freschet et al., 2017). As functional traits are linked to trade-offs between performance and competition, which in turn drive species coexistence processes, they can also help to understand species interactions (Kunstler et al., 2016). Species with high SRL and RTD tend to be strong competitors under non-limiting water and nutrient conditions (Fort et al., 2014; Mommer et al., 2011). However, when considering intraspecific fine root plasticity driven by competition, the conclusion is not clear yet. For example, Salahuddin et al., (2018) found morphological differences in the first three root orders of *Juglans mandshurica* Maxim. and *Larix gmelinii* (Rupr.) Rupr. when growing in monocultures or in mixed stands, while that was not the case for *Fagus sylvatica* L. and *Fraxinus excelsior* L. under greenhouse conditions (Beyer et al., 2013).

In this context, the establishment of mixed forests creates a more complex framework, not only due to the increase in number of species but also because the response to that increase is not linear and depends on local abiotic conditions and on the identity of the species in question (González de Andrés, 2019; Schuler et al., 2017). Particularly, Scots pine (*Pinus sylvestris* L.) and European beech (*Fagus sylvatica* L.) are among the main tree species in Europe given their natural distribution ranges. Scots pine covers about 12 x 10^6 ha and European beech 49 x 10^6 ha, including an extensive potential overlapping area in the continent (Pretzsch et al., 2015, Figure 1). With such relevance, mixed forests of Scots pine and European beech have been on the spotlight over the last years, emphasizing the need of understanding the underlying processes that drive these species mixing effects (Condés et al., 2017; Pretzsch et al., 2016, 2015).

Interactions among populations at the rear edge of their distribution, as the tree species targeted in this study, are of particular interest as they may be the first ones reacting to global change caused by land use and climate changes. Therefore, the objective of this study was to evaluate the effect of Scots pine on soil properties and how they are affected when growing together with naturally regenerated European beech. We paid special attention to the distribution and functional traits of fine roots aiming to identify the direction of these species interaction, either positive or negative. The following hypothesis were tested: 1) Soil properties in Scots pine stands will benefit from beech recruitment presenting a reduction in their carbon-to-nitrogen ratio, 2) Scots pine and European beech fine root systems traits (fine root biomass, specific root length and dry biomass per volume) will be affected by species interaction, and 3) the changes caused by this interaction will facilitate species coexistence e.g. via niche segregation.

2. Material and methods

2.1. Study site

The study site is located in the Southwestern Pyrenees, near the town of Aspurz (province of Navarre, northern Spain, Figure 1). It is a cold and wet Mediterranean site at low elevation, with frequent frosts from winter to early spring and water deficit usually in July and August. The forest was initially classified by foresters in 1999 as an even-aged Scots pine stand, but nowadays it has transformed into a mixed stand by European beech natural regeneration and natural succession processes (González de Andrés et al., 2019). Scots pine trees naturally regenerated after strip-like clear-cutting carried out in the mid-1960s (current mean age ~55 years) followed years later by spatially-heterogeneous recruitment of European beech (mean age 40-45 years). At this stand, beech is the second most abundant tree species and, while its stem density is far below pine density (Table 1), many beech trees are codominant or dominant, reaching the highest canopy layer, and sustaining a mean crown cover of 38% (Cardil et al., 2018). The result is a forest stand composed of a mixture of patches that still resemble monospecific stands dominated by Scots pines (hereafter pine patches) and patches codominated by both tree species (hereafter mixed patches). Further information on site characteristics is presented in Table 1.

The study site corresponds to the location of six 40 x 30 m plots at the long-term monitoring research site established in 1999 by the Ecology and Environment Group of the Public University of Navarre, as described in Blanco (2004) and González de Andrés et al. (2019). These plots originally belonged to a thinning trial. However, the time passed since the last thinning (11 years) has caused a lack of differences among plots for aboveground features (canopy cover and global radiation, García Sancet, 2017), as well as for stand attributes (stem density, basal area, dominant tree height, Arozarena González 2018) and soil properties (Yeste et al., 2019). For this reason, in this study rather than considering whole stand characteristics as the experimental unit (i.e., plot) to explain soil and root properties, we focused on the immediate surrounding area of individual trees within pure pine patches and pine-beech mixed patches. Data from the area surrounding each sampling point (4-m radius) are presented in Table 1.

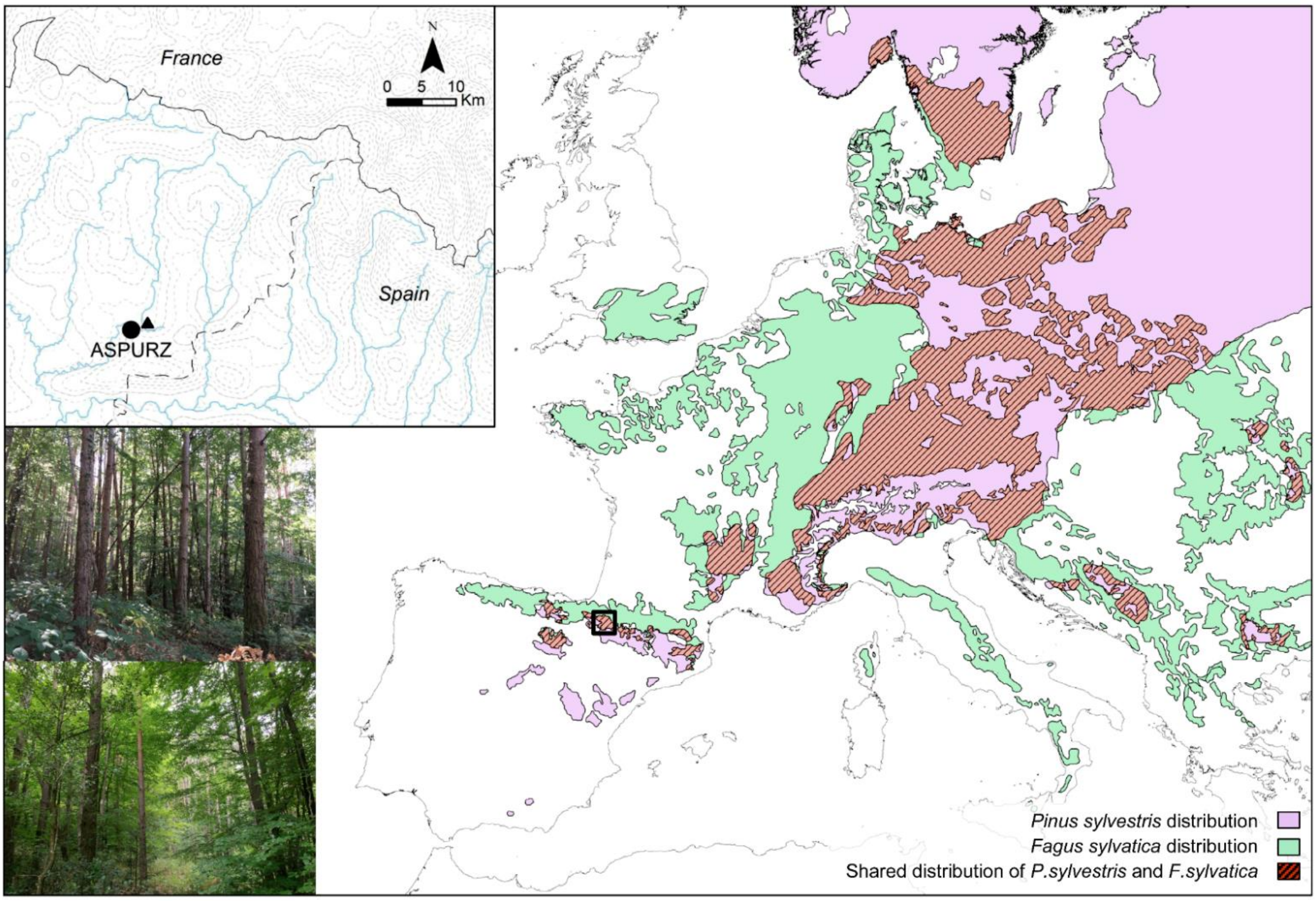

Figure 1. *Pinus sylvestris* and *Fagus sylvatica* natural distributions in Europe (EUFORGEN, 2009), and the common area between both species. The study site is indicated with a circle in the left upper inset and the weather station providing historical climate with a triangle. Top photograph shows a Scots pine-dominated canopy patch. Bottom photograph shows a mixed canopy patch.

Table 1. Main site characteristics (mean ± standard error).

Latitude	42°42'31"N	**Longitude**	1°08'40"W
Altitude (m)	642	**Slope** (%)	7
Orientation	North	**Climate** (Papadakis, 1970)	Cold-wet Mediterranean
Mean temperature (°C) [a]	12.0	**Mean precipitation** (mm) [a]	945
Soil type (FAO)	Haplic alisol		

Soil physical attributes (Blanco, 2004)

Soil [b] Horizon	**Depth** (cm)	**Clay** (%)	**Silt** (%)	**Sand** (%)	**Bulk density** (g cm^3)	**CEC** (meq 100 g^{-1})
A	0 - 10	7.2	50.5	42.3	0.96	14.2
B	10 - 45	14.2	33.3	55.5	1.31	7.2

Stand attributes	Stem density [c] (stems ha^{-1})	Basal area [c] (m^2 ha^{-1})	Quadratic mean diameter (cm)	DBH [c] (cm)	Average height [d] (m)	Top height [d] (m)
Pine-dominated patches						
Pinus sylvestris	1509 ± 91	55.81 ± 5.05	21.9 ± 1.1	21.2 ± 1.1	18.45 ± 0.20	20.87 ± 0.25
Fagus sylvatica	50 ± 29	0.03 ± 0.06	2.6 ± 1.6	2.6 ± 1.6	16.68 ± 0.99	22.05 ± 1.57
Mixed canopy patches						
Pinus sylvestris	1365 ± 112	47.89 ± 3.59	21.4 ± 0.6	21.1 ± 0.6	18.45 ± 0.20	20.87 ± 0.25
Fagus sylvatica	477 ± 74	24.00 ± 5.82	25.6 ± 2.9	23.1 ± 2.9	16.68 ± 0.99	22.05 ± 1.57

[a] Referred to the period 1988-2018 (data from Navascués weather station, located at 2.7 km from the study site 42°43'06"N, 1°06'55"W, 615 m).

[b] Soil physicochemical properties are homogeneous in the study site, and equal for all the canopy types.

[c] Measured within a 4 m radius around the sampling point, for all stems with DBH > 1.0 cm.

[d] Average heights estimated at plot level only.

2.2. Sample collection

Thirty-six dominant or co-dominant trees (24 pines, 12 beech) of similar diameter at breast height (20-25 cm) were selected as focal trees (the closest ones to the soil sampling points). Soil samples were taken at 50 cm from each of these trees. Focal trees were characterised depending on tree species and forest patch type: twelve pine trees were located on the pine patches (PP), twelve pine trees on the mixed patches (PM) and twelve beech trees on the mixed patches (BM). Soil samples were collected in May 2018 using a 5-cm diameter soil auger, at two depths (0-13 cm, corresponding roughly with mean A horizon depth, and 13-26 cm, corresponding with the top of the B horizon). A second sample was taken at 5-10 cm from the first. One soil sample was used for soil chemical analysis, whereas the other soil sample was used for fine root collection. Being thirty-six trees and sampling two soil depths, 72 samples were collected for each, chemical and fine root analyses. Deeper soil samples for fine root collection were taken in 13 cm increments until reaching 65 cm depth. However, data severely departed from a balanced statistical analysis for depths below 26 cm due to increasing stoniness, which prevented sample collection. Hence, samples from these depths were not included in further analyses.

2.3. Chemical analysis

Samples for chemical measurements were kept at 4 °C until they were processed before 24 hours passed since collection. Microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) were determined by using the chloroform fumigation method (Brookes et al., 1985, 1982; Vance et al., 1987), assuming a fumigation efficiency of 0.45 for carbon and nitrogen, and 0.40 for phosphorus (Jenkinson et al., 2004; Joergensen et al., 2011). Nitrate (NO_3^-) and ammonium (NH_4^+) were determined in 2 M KCl extracts by segmented flow colorimetry (AA3, Braun+Luebbe, SEAL Analytical, Norderstedt, Germany). Soil pH was measured in 1:2.5 soil to distilled water ratio. Total nitrogen (TN), soil organic carbon (SOC) and available phosphorus (AP) were determined by the Kjeldahl, Walkley-Black and Olsen methods respectively.

2.4. Root sorting and measurements

Fine roots (diameter < 2 mm) were handpicked, washed and sorted in order to separate live Scots pine and European beech fine roots. These were additionally classified following a functional approach identifying the first three orders as “absorptive” fine roots and the rest up to 2 mm as “transport” fine roots (Freschet and Roumet, 2017; McCormack et al., 2015). Absorptive roots functional traits were measured as this portion is expected to be the more plastic and responsive to external variability (Makita et al., 2011, Salahuddin et al. 2018). Therefore, absorptive roots were scanned at 1200 dpi and analysed using WinRHIZO 2019a (Régent Instruments Inc., 2019) to measure their specific root length (SRL, root length per root dry mass) and root tissue density (RTD, root dry mass per root volume). All fine roots (adding up both absorptive and transport portions) were oven dried (40 °C, 48 h) and weighed. The soil was oven dried (100 °C, 48 h) after excluding stones > 2 mm to determine fine root biomass (FRB, fine root dry mass per fine soil dry mass).

2.5. Statistical analysis

Spearman correlation coefficients were used to describe the relationships between the different soil chemical variables, and between soil features and FRB of both tree species. Linear mixed models were used to assess the effects of two fixed effects factors, the joined effect of focal tree species and patch type (PP, PM and BM) and depth (0-13 cm and 13-26 cm) on soil chemical variables and FRB (n = 72) and on SRL and RTD (n = 48 for pine, n = 48 for beech. In the case of functional traits data, samples containing none or low amounts of fine roots were not included in the analyses, which was the case for pine roots in BM and beech roots in PP. As different depth samples were taken at the same point, focal tree identity (the code given to each tree to identify them and, consequently, identifying each sampling point) was included nested in depth as a random effect, and a correlation structure was included in the model to account for samples taken at the same point.

Homoscedasticity and normality were checked using Bartlett and Shapiro-Wilk tests and square and cube roots variable transformations were applied when necessary to meet linear mixed models assumptions (Quinn and Keough, 2002). Differences between the means of factors levels were assessed by Tukey's HSD. All analysis were carried out using R version 4.0.1 (R Core Team, 2020), linear mixed models were fitted using *nlme* package (Pinheiro et al., 2020).

3. Results

3.1. Soil chemical variables

Five out of nine chemical variables (TN, SOC, MBC, MBN and MBP) showed significantly higher levels in the upper soil layer, pH showed lower levels, and NO_3^-, NH_4^+ and AP were unaffected by soil depth (Table 2). Patch type and focal tree species affected TN and SOC, with lower values found close to beech stems (BM). For both TN and SOC, focal tree species had a greater effect than patch type (monospecific or mixed): when comparing PP and PM, no significant differences on soil chemical composition were found near Scots pine stems whether it was a pine patch or a mixed patch. On the other hand, when comparing PM and BM, samples taken close to a beech stem contained less TN and SOC than those close to a pine stem (14.5% and 17.5% less

TN at 0-13 cm and 13-26 cm; 23% and 42% less SOC at 0-13 cm and 13-26 cm). As the decrease in SOC was more pronounced than that of TN, soil C/N was significantly lower close to beech stems (Figure 2).

Table 2. Mean values and standard deviation of the soil variables analysed. Significant differences are indicated on the right side by the p-values from the linear mixed models for both fixed effects factors (Patch type + Focal tree species and Depth). No interaction effects were found for any of the variables.

Soil characteristic	**Soil depth**	**Pine on pine patch (PP)**	**Pine on mixed patch (PM)**	**Beech on mixed patch (BM)**	**Patch + Focal tree species**	**Depth**
NH_4^+ (mg kg^{-1})	0-13 cm	2.3 ± 1.3	2.5 ± 1.7	2.9 ± 2.2	*ns*	*ns*
	13-26 cm	2.6 ± 1.8	2.0 ± 1.0	2.5 ± 1.8		
NO_3^- (mg kg^{-1})	0-13 cm	0.24 ± 0.09	0.20 ± 0.13	0.39 ± 0.39	*ns*	*ns*
	13-26 cm	0.29 ± 0.22	0.22 ± 0.11	0.33 ± 0.24		
Available P (mg kg^{-1})	0-13 cm	1.2 ± 0.8	1.8 ± 1.2	1.2 ± 0.9	*ns*	*ns*
	13-26 cm	0.8 ± 0.6	1.2 ± 1.2	1.6 ± 1.3		
pH	0-13 cm	5.0 ± 0.3	4.8 ± 0.5	4.9 ± 0.3	*ns*	< 0.0001
	13-26 cm	5.2 ± 0.3	4.9 ± 0.4	5.1 ± 0.4		
Total N (%)	0-13 cm	0.22 ± 0.05	0.23 ± 0.07	0.2 ± 0.03	0.018	< 0.0001
	13-26 cm	0.14 ± 0.02	0.14 ± 0.02	0.11 ± 0.02		
Soil organic C (%)	0-13 cm	4.0 ± 1.6	4.4 ± 2.0	3.0 ± 0.8	0.013	< 0.0001
	13-26 cm	2.8 ± 1.2	2.7 ± 1.6	1.6 ± 0.3		
C/N ratio	0-13 cm	20.7 ± 4.9	20.1 ± 5.4	14.8 ± 2.8	0.006	*ns*
	13-26 cm	20.8 ± 8.6	21.6 ± 9.1	14.2 ± 3.9		
Microbial biomass C (mg kg^{-1})	0-13 cm	478 ± 187	513 ± 193	539 ± 205	*ns*	< 0.0001
	13-26 cm	279 ± 113	317 ± 166	326 ± 138		
Microbial biomass N (mg kg^{-1})	0-13 cm	120 ± 23	120 ± 42	126 ± 45	*ns*	< 0.0001
	13-26 cm	80 ± 30	74 ± 21	78 ± 37		
Microbial biomass P (mg kg^{-1})	0-13 cm	15.4 ± 7.2	15.8 ± 8.3	16.7 ± 4.6	*ns*	0.0001
	13-26 cm	10.7 ± 3.6	10.3 ± 3.7	8.1 ± 3.2		

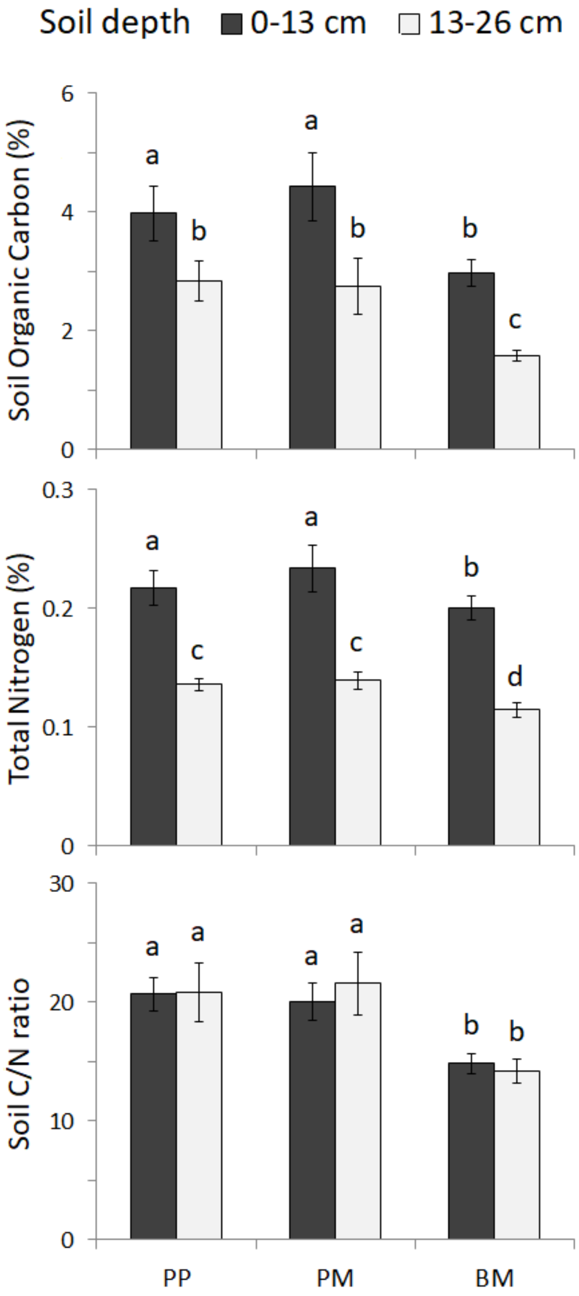

Figure 2. Mean values and standard error for soil organic carbon (SOC), total nitrogen (TN) and soil C/N ratio. The horizontal axis indicate the sampling point, near a pine tree in a pine monospecific patch (PP), a pine tree in a mixed patch (PM) or a beech tree in a mixed patch (PM). Different letters indicate significant differences (Tukey's HSD, $p < 0.05$).

3.2. Fine root biomass and functional traits

Fine root biomass (FRB) and functional traits varied greatly between Scots pine and European beech (Table 3, Figure 3). Pine absorptive fine roots had on average five times lower SRL than beech absorptive fine roots and lower RTD (45% lower at 0-13 cm, 33% at 13-26 cm). FRB varied between both species but also depended strongly on the patch type and the focal tree species. On average, beech FRB was seven times higher than pine FRB in the mixed patches, even though there were three times less beech stems than pine stems (Table 1). Pine FRB was reduced vastly on the mixed areas, independently of whether the sample was taken close to a pine stem or to a beech stem: pine FRB was six times higher on the pine patches than on the mixed patches at 0-13 cm, and twice at 13-26 cm. Stratification of pine roots between soil depths appeared only on PP. Beech FRB was higher in BM than in PP and PM, with the particularity that the difference between BM and PM was much more marked at the lower soil layer. Patch type and focal tree species did not influence root functional traits. However, the higher value in beech SRL in PM than in BM was marginally significant ($p = 0.08$). Average SRL was higher at the upper soil layer but the difference was only significant for beech. Both species had higher RTD at the deeper soil layer (18% for pine, 11% for beech). Spearman correlations showed a significant positive correlation between beech FRB and microbial biomass C, N and P, while pine FRB did not show a correlation with any of the analysed soil variables (Figure 4).

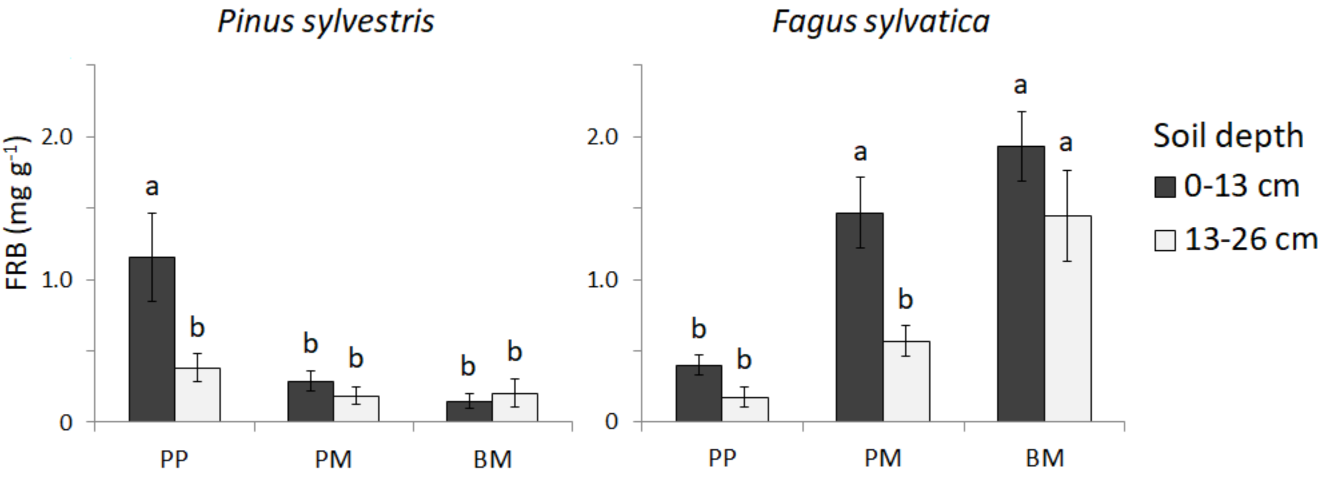

Figure 3. *Pinus sylvestris* and *Fagus sylvatica* fine root biomass (FRB, diameter below 2 mm). The horizontal axis indicates the sampling point, near a pine in a pine monospecific patch (PP), a pine in a mixed patch (PM) or a beech in a mixed patch (PM). Mean values and standard error are presented. Different letters indicate significant differences (Tukey’s HSD, $p < 0.05$).

Table 3. Mean values and standard deviation of the fine root variables analysed. Significant differences are indicated on the right side by the p-values from the linear mixed models for both fixed effects factors (Patch type + Focal tree species and Depth). No interaction effect was found for any of the variables. Fine roots were those with a diameter below 2 mm and absorptive roots the first three root orders (McCormack et al., 2015).

Tree species	**Root type and variable**	**Soil depth**	**Pine on pine patch (PP)**	**Pine on mixed patch (PM)**	**Beech on mixed patch (BM)**	**Patch + Focal tree species**	**Depth**
Pinus sylvestris	Fine root biomass FRB (mg g^{-1})	0-13 cm	1.16 ± 1.06	0.29 ± 0.24	0.15 ± 0.18	0.0015	*ns*
		13-26 cm	0.38 ± 0.35	0.19 ± 0.20	0.20 ± 0.34		
	Absorptive roots SRL (cm g^{-1})	0-13 cm	536 ± 117	569 ± 201	-	*ns*	*ns*
		13-26 cm	480 ± 129	490 ± 205	-		
	Absorptive roots RTD (g cm^{-3})	0-13 cm	0.41 ± 0.03	0.40 ± 0.03	-	*ns*	0.0036
		13-26 cm	0.47 ± 0.09	0.48 ± 0.08	-		
Fagus sylvatica	Fine root biomass FRB (mg g^{-1})	0-13 cm	0.40 ± 0.24	1.47 ± 0.86	1.93 ± 0.82	< 0.0001	0.0001
		13-26 cm	0.17 ± 0.24	0.57 ± 0.37	1.45 ± 1.11		
	Absorptive roots SRL (cm g^{-1})	0-13 cm	-	2997 ± 358	2669 ± 414	0.084	0.013
		13-26 cm	-	2787 ± 558	2522 ± 507		
	Absorptive roots RTD (g cm^{-3})	0-13 cm	-	0.57 ± 0.05	0.59 ± 0.06	*ns*	0.0002
		13-26 cm	-	0.63 ± 0.06	0.66 ± 0.04		

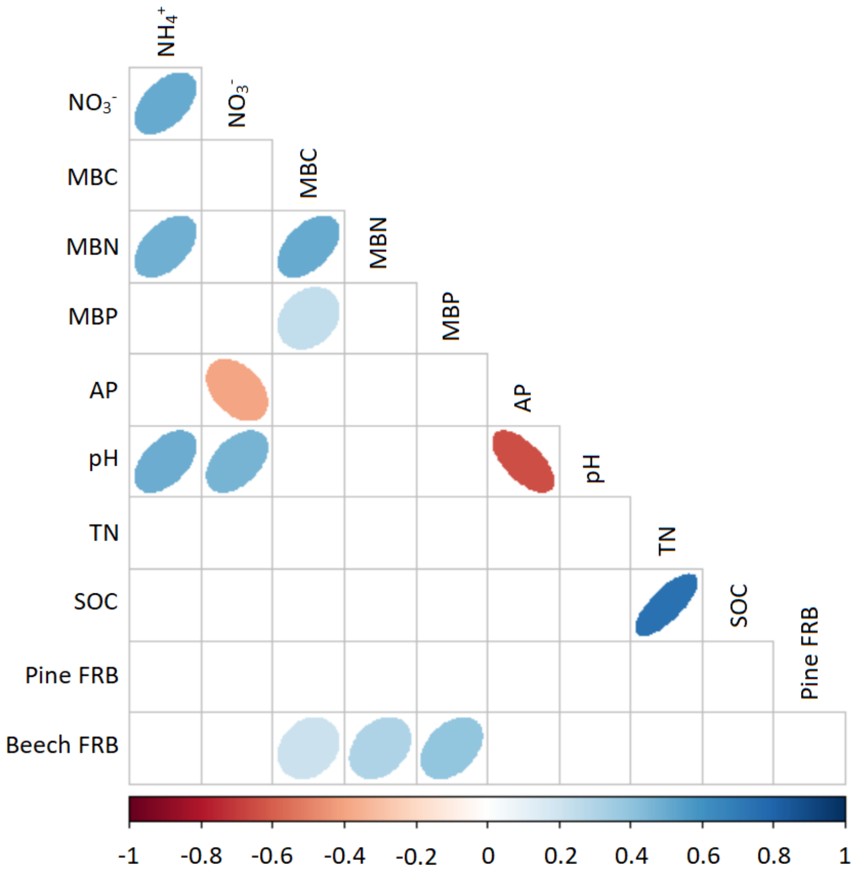

Figure 4. Spearman correlation coefficients between analysed variables. Only significant correlations are shown ($p < 0.05$) either in blue (positive) or in red (negative). Colour intensity and size of the ellipse are proportional to the correlation coefficients.

4. Discussion

4.1. Tree species effect on soil C/N ratio

Soil depth was the main driver of soil chemical variability while patch type did not alter any of the measured parameters. Focal tree species was a more important factor, with a reduction in TN and SOC close to beech stems. Although microbial biomass was unaffected by patch type and tree species, it is interesting that it remained at the same level, whereas SOC and TN decreased. In addition, the difference in SOC between the samples taken close to beech stems and those taken close to pine stems was more pronounced than the difference of TN, leading to a lower C/N ratio close to beech stems. Here, better quality organic matter may be sustaining microbial biomass levels. This result indicates that the first hypothesis could be accepted with some reservations. The soil C/N ratio in Scots pine stands may benefit from beech recruitment and growth, but the effect was only found near beech trees, rather than being caused by admixture interactions. This improvement of soil features when beech is present was hypothesized for the same study site using a modeling approach by González de Andrés et al. (2017), and it can be confirmed now empirically.

Tree species is a key factor explaining soil C/N variability (Cools et al., 2014). Previous studies have also found lower C/N ratio in broadleaves forests (e.g. Menyailo, 2009) and in particular in beech forests when compared to Scots pine forests (Błońska et al., 2018). It has been suggested that higher quality litter, with lower contents of lignin and higher nitrogen, accelerate decomposition rate, which has an impact in soil organic mass accumulation and C/N ratio (Vesterdal et al., 2012). In addition, leaf litter inputs to the forest soil are clearly different under each canopy type. In autumn 2017 (the fall season previous to the sampling date) 1131.0 kg ha^{-1} felled under pine canopy (59% pine needles, 41% beech leaves) whereas 2050.5 kg ha^{-1} were collected under mixed canopy (29% pine needles, 71% beech leaves) (Gonzalez de Andrés et al., 2019). Hence, higher organic matter inputs combined with a higher decomposition rate under beech trees driven by its better quality litter in comparison with pines could also explain the higher SOC accumulated under pine trees.

However, the effect of focal tree species on C/N was not found close to the pine trees located in the mixed patches, where beech litter also accumulates, suggesting that an additional factor related with tree species proximity could be affecting soil C/N. Here, roots and root secretions could be playing an even more important role than litter. When roots die and decompose, root exudates and root tissues are an additional input of organic matter known to drive soil microbial community composition and functioning (Broeckling et al., 2008; Eisenhauer et al., 2017; Whipps, 2001). In this study, we found higher fine root abundance in the mixed patches mainly caused by beech FRB, which in turn correlated positively with microbial biomass.

4.2. Fine root biomass and functional traits: tree species interaction at belowground level

Scots pine and beech fine roots differed greatly in both biomass and functional traits with the maximum pine FRB on PP (1.5 mg per soil g at the first 26 cm) and the maximum beech FRB on BM (3.4 mg per soil g). Although proximity to the stem and stem size are key variables to determine FRB (Sochacki et al., 2017), in this study the influence was more clear for beech than for pine. Beech FRB diminished as expected when distance to beech stems increased (BM > PM > PP), but that was not the case for pine, in which FRB suffered a sharp reduction on the mixed forest area, even close to pine stems (PP > PM = BM). Thus, in the mixed patches interaction with beech trees seemed to be a more important factor. Although pine stem density was only 10% lower in the mixed patches (Table 1), pine FRB was reduced to a third at the first 26 cm.

The low SRL found in Scots pine absorptive fine roots in comparison to beech roots could be attributed in part to its thicker root tips but also to the observed lower ramification of the first three root orders in pine and, consequently, a smaller number of first and second order roots. Even when beech trees invested in larger soil occupation, they also invested in higher RTD, which is typical of slow growth and conservative strategies with longer organ life span (Reich, 2014; Ryser, 1996). Both FRB and absorptive fine roots SRL were much higher for beech, giving the species a soil exploration and exploitation capacity much more pronounced than that of Scots pine. Given the intensive soil occupation strategy of beech, a competitive interaction could be behind the pine FRB reduction close to beech trees, in which Scots pine fails to compete with beech root systems.

This competitive exclusion took place mainly at the upper soil layer, and therefore our results also suggest a certain degree of niche segregation. Beech roots more effectively colonized the shallower portion of the soil, and the proportion of roots at greater depth was reduced when growing close to Scots pine trees. In BM, an average of 57% of beech FRB was found in the first 13 cm of the soil while this proportion increased to 72% in PM. Pine FRB followed the opposite distribution, 75% was at 0-13 cm in PP, diminishing to 61% in PM. In the case of Scots pine, the distribution pattern was driven mainly by the drastic reduction of biomass at 0-13 cm, implying that, in this situation, pine root system cannot cope with beech competition by colonizing deeper soil portions. Although due to soil stoniness only around one third of sites could be sampled down to a depth of 65 cm, a pattern emerged. Thus, on average, 69% of Scots pine FRB and 63% of European beech FRB were on the first 26 cm. The average results for deeper soil layers were similar to those found in the upper part of the soil, with a dominance of beech roots in mixed patches.

These differences lead to accept the second hypothesis in the case of fine root biomass, as both species FRB and vertical distribution were altered by the other species proximity. However, the results support that this is a competitive interaction, rejecting the third hypothesis in which complementary effects were expected. Previous research at this site had observed a continuing increase of beech foliage production over the past twenty years while pine foliage production has steadily being reduced, reaching similar production levels in 2016 (González de Andrés et al., 2019). A similar trend could be expected to have occurred belowground, as belowground niche partitioning did not explain the coexistence of these species. In fact, European beech is regarded as a highly competitive and adaptive species, currently expanding its distribution range (Bolte et al., 2007; Packham et al., 2012). Above- and belowground evidences observed so far suggest a growing dominance of beech even in the early middle stages of natural succession (~50 years since stand establishment).

Contrary to biomass, absorptive fine roots functional traits were not modified by patch type or focal tree species. Those first root orders (the finest portion of the root system) may be more susceptible to modification depending on external factors (Makita et al., 2011). However, chemical variables were mostly unaffected by patch type or focal tree species. Only TN and SOC changed significantly depending on focal tree species, with lower values close to beech trees. Depth was a more important driver of variability in root functional traits, coinciding with the way soil chemical variables were altered. Higher FRB and SRL in the upper layer benefit resource uptake at this more fertile area of the soil, which was the case for beech. Scots pine SRL showed a similar but not significant trend and pine FRB was altered in the same way but only on the pine patches. The consistent higher RTD on deeper soil may be attributed to the common finding of increasing tissue density on poorer nutrient conditions (Ryser, 1996; Ryser and Lambers, 1995) and to the better soil penetration capacity of harder roots (Hutchings and John, 2003).

5. Conclusions

Soil C/N improvement was associated with the presence of *F. sylvatica*, being significantly higher only close to beech stems. Here, the lower C/N ratio could be associated with higher quality of organic matter, enhancing decomposition rate and accelerating carbon cycling. Though there was no other significant change in soil chemical properties affected by tree species, Scots pine and beech fine root systems responded to the presence of the other species through biomass changes. The change was dramatic in the case of Scots pine, indicating a strong competitive interaction in which mature beech trees tend to dominate the whole soil profile. With no evidence of complementarity effects sufficient to overcome competition. higher production of fine roots by European beech, with higher SRL and RTD, may accelerate the eventual substitution of a pioneer species such as Scots pine on a fertile soil under natural conditions. These belowground interactions should be taken into account when planning the transition from pure to mixed stands currently advocated through Europe´s forest management community.

Acknowledgements

This research was funded by the Spanish Ministry of Economy and Competitiveness (project AGL2016-76463-P). A.Y. was funded through a Spanish Predoctoral Research Grant (BES-2017-080326). Special thanks are due to the members of the Ecology and Environmental Group at the Universidad Pública de Navarra (David Candel-Pérez, Yueh-Hsin Lo, Raquel Echeverría, Susana García and Federico J. Castillo) who provided assistance in different field and lab tasks. The authors declare not having any conflict of interests.

Author contributions

Antonio Yeste: Methodology, Formal analysis, Investigation, data curation, Writing – Original draft. Juan A. Blanco & J. Bosco Imbert: Conceptualization, Investigation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition. Helena Zozaya-Vela & Martín Elizalde-Arbilla: Methodology.

References

Addo-Danso, S. D., Prescott, C. E., Adu-Bredu, S., Duah-Gyamfi, A., Moore, S., Guy, R. D., Forrester, D. I., Owusu-Afriyie, K., Marshall, P. L., & Malhi, Y. (2018). Fine-root exploitation strategies differ in tropical old growth and logged-over forests in Ghana. Biotropica, 50(4), 606–615. https://doi.org/10.1111/btp.12556

Arozarena González, I. (2018). Influencia de las claras forestales en el crecimiento y estructua de un rodal de pino silvestre y haya en el Pre-Pirineo navarro. BSc. Thesis. Public University of Navarre. Pamplona.

Beyer, F., Hertel, D., & Leuschner, C. (2013). Fine root morphological and functional traits in Fagus sylvatica and Fraxinus excelsior saplings as dependent on species, root order and competition. Plant and Soil, 373(1–2), 143–156. https://doi.org/10.1007/s11104-013-1752-7

Blanco, J.A., 2004. La práctica del aclareo y su influencia en la estructura y función de dos bosques de pino silvestre del Pirineo Navarro. PhD Thesis, Public University of Navarre. Pamplona.

Błońska, E., Klamerus-Iwan, A., Lasota, J., Gruba, P., Pach, M., Pretzsch, H., 2018. What Characteristics of Soil Fertility Can Improve in Mixed Stands of Scots Pine and European Beech Compared with Monospecific Stands? Commun. Soil Sci. Plant Anal. 49, 237–247. https://doi.org/10.1080/00103624.2017.1421658

Bolte, A., Czajkowski, T., Kompa, T., 2007. The north-eastern distribution range of European beech - A review. Forestry 80, 413–429. https://doi.org/10.1093/forestry/cpm028

Borden, K. A., Thomas, S. C., & Isaac, M. E. (2019). Variation in fine root traits reveals nutrient-specific acquisition strategies in agroforestry systems. Plant and Soil. https://doi.org/10.1007/s11104-019-04003-2

Broeckling, C.D., Broz, A.K., Bergelson, J., Manter, D.K., Vivanco, J.M., 2008. Root Exudates Regulate Soil Fungal Community Composition and Diversity. Appl. Environ. Microbiol. 74, 738 LP – 744. https://doi.org/10.1128/AEM.02188-07

Brookes, P.C., Kragt, J.F., Powlson, D.S., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: The effects of temperature. Soil Biol. Biochem. 17, 831–835.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biol. Biochem. 14, 319–329. https://doi.org/10.1016/0038-0717(82)90001-3

Brum, M., Vadeboncoeur, M.A., Ivanov, V., Asbjornsen, H., Saleska, S., Alves, L.F., Penha, D., Dias, J.D., Aragão, L.E.O.C., Barros, F., Bittencourt, P., Pereira, L., Oliveira, R.S., 2019. Hydrological niche segregation defines forest structure and drought tolerance strategies in a seasonal Amazon forest. J. Ecol. 107, 318–333. https://doi.org/10.1111/1365-2745.13022

Cardil, A., Imbert, J.B., Camarero, J.J., Primicia, I., Castillo, F., 2018. Temporal interactions among throughfall, type of canopy and thinning drive radial growth in an Iberian mixed pine-beech forest. Agric. For. Meteorol. 252, 62–74. https://doi.org/10.1016/j.agrformet.2018.01.004

Cavin, L., Mountford, E.P., Peterken, G.F., Jump, A.S., 2013. Extreme drought alters competitive dominance within and between tree species in a mixed forest stand. Funct. Ecol. 27, 1424–1435. https://doi.org/10.1111/1365-2435.12126

Condés, S., Vallet, P., Bielak, K., Bravo-Oviedo, A., Coll, L., Ducey, M.J., Pach, M., Pretzsch, H., Sterba, H., Vayreda, J., del Río, M., 2017. Climate influences on the maximum size-density relationship in Scots pine (Pinus sylvestris L.) and European beech (Fagus sylvatica L.) stands. For. Ecol. Manage. 385, 295–307. https://doi.org/10.1016/j.foreco.2016.10.059

Cools, N., Vesterdal, L., De Vos, B., Vanguelova, E., Hansen, K., 2014. Tree species is the major factor explaining C: N ratios in European forest soils. For. Ecol. Manage. 311, 3–16. https://doi.org/10.1016/j.foreco.2013.06.047

Doran, J.W., Zeiss, M.R., 2000. Parent material and soil physical properties. Appl. Soil Ecol. 15, 3–11.

Dumanski, J., 2015. Evolving concepts and opportunities in soil conservation. Int. Soil Water Conserv. Res. 3, 1–14. https://doi.org/10.1016/j.iswcr.2015.04.002

Eisenhauer, N., Lanoue, A., Strecker, T., Scheu, S., Steinauer, K., Thakur, M.P., Mommer, L., 2017. Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. Sci. Rep. 7, 44641. https://doi.org/10.1038/srep44641

Fort, F., Cruz, P., & Jouany, C. (2014). Hierarchy of root functional trait values and plasticity drive early-stage competition for water and phosphorus among grasses. Functional Ecology, 28(4), 1030–1040. https://doi.org/10.1111/1365-2435.12217

Forrester, D.I., Bauhus, J., 2016. A Review of Processes Behind Diversity—Productivity Relationships in Forests. Curr. For. Reports 2, 45–61. https://doi.org/10.1007/s40725-016-0031-2

Freschet, G.T., Roumet, C., 2017. Sampling roots to capture plant and soil functions. Funct. Ecol. 31, 1506–1518. https://doi.org/10.1111/1365-2435.12883

Freschet, T., Valverde-barrantes, O. J., Tucker, C. M., Joseph, M., Mccormack, M. L., Violle, C., Fort, F., Blackwood, C. B., Urban-mead, K. R., Iversen, C. M., Bonis, A., Comas, L. H., Cornelissen, H. C., Dong, M., Guo, D., Hobbie, S. E., Holdaway, R. J., Kembel, S. W., Makita, N., … Roumet, C. (2017). Climate , soil and plant functional types as drivers of global fine-root trait variation. 1182–1196. https://doi.org/10.1111/1365-2745.12769

García Sancet, M.A. 2017. Influencia de los parámetros de dosel y lumínicos sobre la regeneración de *Pinus sylvestris* L. en un bosque mixto con tres intensidades de clara. MSc. thesis. Public Unviersity of Navarre, Pamplona.

González de Andrés, E., 2019. Interactions between climate and nutrient cycles on forest response to global change: The role of mixed forests. Forests 10, 609. https://doi.org/10.3390/f10080609

González de Andrés, E., Blanco, J.A., Imbert, J.B., Guan, B.T., Lo, Y.-H., Castillo, F.J., 2019. ENSO and NAO affect long-term leaf litter dynamics and stoichiometry of Scots pine and European beech mixedwoods. Glob. Chang. Biol. 25, 3070–3090. https://doi.org/10.1111/gcb.14672

González de Andrés, E., Seely, B., Blanco, J.A., Imbert, J.B., Lo, Y.H., Castillo, F.J., 2017. Increased complementarity in water-limited environments in Scots pine and European beech mixtures under climate change. Ecohydrology 10, 1–14. https://doi.org/10.1002/eco.1810

Griess, V.C., Knoke, T., 2013. Bioeconomic modeling of mixed Norway spruce-European beech stands: Economic consequences of considering ecological effects. Eur. J. For. Res. 132, 511–522. https://doi.org/10.1007/s10342-013-0692-3

He, Y., Qin, L., Li, Z., Liang, X., Shao, M., Tan, L., 2013. Carbon storage capacity of monoculture and mixed-species plantations in subtropical China. For. Ecol. Manage. 295, 193–198. https://doi.org/10.1016/j.foreco.2013.01.020

Hutchings, M.J., John, E.A., 2003. Distribution of Roots in Soil, and Root Foraging Activity., in: de Kroon, H., Viser, E.J.W. (Eds.), Root Ecology. Springer Berlin Heidelberg, pp. 33–60. https://doi.org/DOI 10.1007/978-3-662-09784-7

Jactel, H., Nicoll, B.C., Branco, M., Gonzalez-Olabarria, J.R., Grodzki, W., Långström, B., Moreira, F., Netherer, S., Christophe Orazio, C., Piou, D., Santos, H., Schelhaas, M.J., Tojic, K., Vodde, F., 2009. The influences of forest stand management on biotic and abiotic risks of damage. Ann. For. Sci. 66, 1–18. https://doi.org/10.1051/forest/2009054

Jenkinson, D.S., Brookes, P.C., Powlson, D.S., 2004. Measuring soil microbial biomass. Soil Biol. Biochem. 36, 5–7. https://doi.org/10.1016/j.soilbio.2003.10.002

Joergensen, R.G., Wu, J., Brookes, P.C., 2011. Measuring soil microbial biomass using an automated procedure. Soil Biol. Biochem. 43, 873–876. https://doi.org/10.1016/j.soilbio.2010.09.024

Kunstler, G., Falster, D., Coomes, D. A., Hui, F., Kooyman, R. M., Laughlin, D. C., Poorter, L., Vanderwel, M., Vieilledent, G., Wright, S. J., Aiba, M., Baraloto, C., Caspersen, J., Cornelissen, J. H. C., Gourlet-Fleury, S., Hanewinkel, M., Herault, B., Kattge, J., Kurokawa, H., … Westoby, M. (2016). Plant functional traits have globally consistent effects on competition. Nature, 529(7585), 204–207. https://doi.org/10.1038/nature16476

Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Mellado-Vázquez, P.G., Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B.C., Trumbore, S.E., Gleixner, G., 2015. Plant diversity increases soil microbial activity and soil carbon storage. Nat. Commun. 6. https://doi.org/10.1038/ncomms7707

Liang, J., Crowther, T.W., Picard, N., Wiser, S., Zhou, M., Alberti, G., Schulze, E.D., McGuire, A.D., Bozzato, F., Pretzsch, H., De-Miguel, S., Paquette, A., Hérault, B., Scherer-Lorenzen, M., Barrett, C.B., Glick, H.B., Hengeveld, G.M., Nabuurs, G.J., Pfautsch, S., Viana, H., Vibrans, A.C., Ammer, C., Schall, P., Verbyla, D., Tchebakova, N., Fischer, M., Watson, J. V., Chen, H.Y.H., Lei, X., Schelhaas, M.J., Lu, H., Gianelle, D., Parfenova, E.I., Salas, C., Lee, E., Lee, B., Kim, H.S., Bruelheide, H., Coomes, D.A., Piotto, D., Sunderland, T., Schmid, B., Gourlet-Fleury, S., Sonké, B., Tavani, R., Zhu, J., Brandl, S., Vayreda, J., Kitahara, F., Searle, E.B., Neldner, V.J., Ngugi, M.R., Baraloto, C., Frizzera, L., Bałazy, R., Oleksyn, J., Zawiła-Niedźwiecki, T., Bouriaud, O., Bussotti, F., Finér, L., Jaroszewicz, B., Jucker, T., Valladares, F., Jagodzinski, A.M., Peri, P.L., Gonmadje, C., Marthy, W., O'Brien, T., Martin, E.H., Marshall, A.R., Rovero, F., Bitariho, R., Niklaus, P.A., Alvarez-Loayza, P., Chamuya, N., Valencia, R., Mortier, F., Wortel, V., Engone-Obiang, N.L., Ferreira, L. V., Odeke, D.E., Vasquez, R.M., Lewis, S.L., Reich, P.B., 2016. Positive biodiversity-productivity relationship predominant in global forests. Science (80-.). 354. https://doi.org/10.1126/science.aaf8957

Loreau, M., de Mazancourt, C., 2013. Biodiversity and ecosystem stability: A synthesis of underlying mechanisms. Ecol. Lett. 16, 106–115. https://doi.org/10.1111/ele.12073

Madrigal-González, J., Ruiz-Benito, P., Ratcliffe, S., Calatayud, J., Kändler, G., Lehtonen, A., Dahlgren, J., Wirth, C., Zavala, M.A., 2016. Complementarity effects on tree growth are contingent on tree size and climatic conditions across Europe. Sci. Rep. 6, 32233. https://doi.org/10.1038/srep32233

Makita, N., Hirano, Y., Mizoguchi, T., Kominami, Y., Dannoura, M., Ishii, H., Finér, L., Kanazawa, Y., 2011. Very fine roots respond to soil depth: Biomass allocation, morphology, and physiology in a broad-leaved temperate forest. Ecol. Res. 26, 95–104. https://doi.org/10.1007/s11284-010-0764-5

McCormack, M.L., Dickie, I.A., Eissenstat, D.M., Fahey, T.J., Fernandez, C.W., Guo, D., Erik, A., Iversen, C.M., Jackson, R.B., 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. New Phytol. 207, 505–518.

Menyailo, O. V., 2009. The effect of Siberian tree species on the mineralization rate of soil organic matter. Eurasian Soil Sci. 42, 1156–1162. https://doi.org/10.1134/S106422930910010X

Mommer, L., Visser, E. J. W., van Ruijven, J., de Caluwe, H., Pierik, R., & de Kroon, H. (2011). Contrasting root behaviour in two grass species: a test of functionality in dynamic heterogeneous conditions. Plant and Soil, 344(1), 347. https://doi.org/10.1007/s11104-011-0752-8

Mueller, K.E., Eissenstat, D.M., Hobbie, S.E., Oleksyn, J., Jagodzinski, A.M., Reich, P.B., Chadwick, O.A., Chorover, J., 2012. Tree species effects on coupled cycles of carbon, nitrogen, and acidity in mineral soils at a common garden experiment. Biogeochemistry 111, 601–614. https://doi.org/10.1007/s10533-011-9695-7

Packham, J.R., Thomas, P.A., Atkinson, M.D., Degen, T., 2012. Biological Flora of the British Isles: Fagus sylvatica. J. Ecol. 100, 1557–1608. https://doi.org/10.1111/j.1365-2745.2012.02017.x

Papadakis, J., 1970. Climates of the world, their classification, similitudes, differences, and geographic distribution. Self-edited, Buenos Aires.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2020. nlme: Linear and nonlinear mixed effects models. https://CRAN.R-project.org/package=nlme.

Prescott, C.E., Grayston, S.J., 2013. Tree species influence on microbial communities in litter and soil: Current knowledge and research needs. For. Ecol. Manage. 309, 19–27. https://doi.org/10.1016/j.foreco.2013.02.034

Pretzsch, H., del Río, M., Ammer, C., Avdagic, A., Barbeito, I., Bielak, K., Brazaitis, G., Coll, L., Dirnberger, G., Drössler, L., Fabrika, M., Forrester, D.I., Godvod, K., Heym, M., Hurt, V., Kurylyak, V., Löf, M., Lombardi, F., Matović, B., Mohren, F., Motta, R., den Ouden, J., Pach, M., Ponette, Q., Schütze, G., Schweig, J., Skrzyszewski, J., Sramek, V., Sterba, H., Stojanović, D., Svoboda, M., Vanhellemont, M., Verheyen, K., Wellhausen, K., Zlatanov, T., Bravo-Oviedo, A., 2015. Growth and yield of mixed versus pure stands of Scots pine (Pinus sylvestris L.) and European beech (Fagus sylvatica L.) analysed along a productivity gradient through Europe. Eur. J. For. Res. 134, 927–947. https://doi.org/10.1007/s10342-015-0900-4

Pretzsch, H., del Río, M., Schütze, G., Ammer, C., Annighöfer, P., Avdagic, A., Barbeito, I., Bielak, K., Brazaitis, G., Coll, L., Drössler, L., Fabrika, M., Forrester, D.I., Kurylyak, V., Löf, M., Lombardi, F., Matović, B., Mohren, F., Motta, R., den Ouden, J., Pach, M., Ponette, Q., Skrzyszewski, J., Sramek, V., Sterba, H., Svoboda, M., Verheyen, K., Zlatanov, T., Bravo-Oviedo, A., 2016. Mixing of Scots pine (Pinus sylvestris L.) and European beech (Fagus sylvatica L.) enhances structural heterogeneity, And the effect increases with water availability. For. Ecol. Manage. 373, 149–166.

https://doi.org/10.1016/j.foreco.2016.04.043

Quinn, G., Keough, M., 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Viena, Austria. https://www.R-project.org/.

Reich, P.B., 2014. The world-wide “fast-slow” plant economics spectrum: A traits manifesto. J. Ecol. 102, 275–301. https://doi.org/10.1111/1365-2745.12211

Ryser, P., 1996. The importance of tissue density for growth and life span of leaves and roots: a comparison of five ecologically contrasting grasses. Funct. Ecol. 10, 717. https://doi.org/10.2307/2390506

Ryser, P., Lambers, H., 1995. Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. Plant Soil 170, 251–265.

Salahuddin, Rewald, B., Razaq, M., Lixue, Y., Li, J., Khan, F., & Jie, Z. (2018). Root order-based traits of Manchurian walnut & larch and their plasticity under interspecific competition. Scientific Reports, 8(1), 9815. https://doi.org/10.1038/s41598-018-27832-0

Scheu, S., 2005. Linkages Between Tree Diversity, Soil Fauna and Ecosystem Processes, in: Scherer-Lorenzen, M., Körner, C., Schulze, E.-D. (Eds.), Forest Diversity and Function: Temperate and Boreal Systems. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 211–233. https://doi.org/10.1007/3-540-26599-6_11

Schuler, L.J., Bugmann, H., Snell, R.S., 2017. From monocultures to mixed-species forests: is tree diversity key for providing ecosystem services at the landscape scale? Landsc. Ecol. 32, 1499–1516. https://doi.org/10.1007/s10980-016-0422-6

Sochacki, S.J., Ritson, P., Brand, B., Harper, R.J., Dell, B., 2017. Accuracy of tree root biomass sampling methodologies for carbon mitigation projects. Ecol. Eng. 98, 264–274. https://doi.org/10.1016/j.ecoleng.2016.11.004

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Vesterdal, L., Elberling, B., Christiansen, J.R., Callesen, I., Schmidt, I.K., 2012. Soil respiration and rates of soil carbon turnover differ among six common European tree species. For. Ecol. Manage. 264, 185–196. https://doi.org/10.1016/j.foreco.2011.10.009

Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional! Oikos, 116(5), 882–892. https://doi.org/10.1111/j.0030-1299.2007.15559.x

Whipps, J.M., 2001. Microbial interactions and biocontrol in the rhizosphere. J. Exp. Bot. 52, 487–511. https://doi.org/10.1093/jxb/52.suppl_1.487

Yeste, A., Blanco J.A., Imbert, J.B., González de Andrés, E., Lo, Y.H., Candel-Pérez, D., Castillo, F.J. 2019. Influence of thinning and canopy type on soil chemical composition on mixed forests on the western Pyrenees. Pp. 75 in Abstract book of the 1st Meeting of The Iberian Ecological Society (SIBECOL) & XIV AEET Meeting, Barcelona, 4-7 February. Asociación Española de Ecología Terrestre, Madrid. Doi.: 10.7818/SIBECOLandAEETmeeting.2019

Zhang, W., Yang, K., Lyu, Z., Zhu, J., 2019. Microbial groups and their functions control the decomposition of coniferous litter: A comparison with broadleaved tree litters. Soil Biol. Biochem. 133, 196–207. https://doi.org/10.1016/j.soilbio.2019.03.009