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Functional Ecology

Title page

Tree species mixing causes a shift in fine-root soil exploitation strategies across European forests

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The authors declare no conflict of interest.

Authors Contributions

All authors contributed to the design of the study. JW and LPS carried out data collection and performed the analysis. JW drafted the manuscript. All authors discussed and interpreted the results and contributed to writing of the manuscript.

Data Accessibility Statement

The datasets used for this study are archived on a data portal associated with the FunDivEUROPE and SoilForEUROPE projects and available after a 1- year embargo (root biomass data: <https://data.botanik.uni-halle.de/fundiveurope/datasets/518>; root functional trait data: <https://data.botanik.uni-halle.de/fundiveurope/datasets/521>; plot-level C, N data: <https://data.botanik.uni-halle.de/fundiveurope/datasets/520>; Soil fungal biomass and diversity data: <https://data.botanik.uni-halle.de/fundiveurope/datasets/522>).

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10

11 **Abstract**

- 12 1. Mixed-species forests have often been shown to enhance above-ground ecosystem properties
13 and processes. Despite the significance of fine roots for tree and ecosystem functioning, the
14 role of tree species diversity for below-ground processes driven by fine roots remains largely
15 unknown. Previously, an underyielding of fine-root biomass (FRB) in tree mixtures across
16 four major European forest types has been reported. To explain this phenomenon, we tested
17 here the effect of tree species mixing on fine-root traits related to soil exploitation efficiency,
18 including biotic feedbacks from ectomycorrhizal fungi (EcM), and assessed the role of root
19 trait dissimilarity.
- 20 2. We analysed morphological and chemical traits as well as ectomycorrhizal colonisation
21 intensity of absorptive fine roots (i.e. first three most distal orders) in soil samples from 315
22 mixed and mono-specific tree neighbourhoods in mainly mature, semi-natural forest stands
23 across Europe. Additionally, we quantified mycorrhizal abundance and diversity in soil
24 samples from the same stands.
- 25 3. At the community level, fine roots in tree mixtures were characterised by higher specific root
26 lengths and root nitrogen concentrations, lower diameters, and root tissue densities indicating
27 a *faster* resource acquisition strategy compared to mono-specific stands. The higher root EcM
28 colonisation intensity and soil EcM diversity in mixtures compared to mono-specific stands

29 may further provide evidence for positive biotic feedbacks. Moreover, the diversity of fine-
30 root traits influenced FRB, as mixtures characterised by a higher trait dissimilarity were
31 linked to a lower reduction in FRB. At the level of phylogenetic groups, thin-rooted
32 angiosperm species showed stronger responses to mixing than thick-rooted gymnosperms,
33 especially in terms of root morphology and EcM colonisation, indicating different strategies
34 of response to tree mixing.

- 35 4. Our results indicate that a lower FRB can reflect a shift in soil resource acquisition strategies,
36 rather than a lower performance of trees in mixtures. They show that several non-exclusive
37 mechanisms can simultaneously explain negative net effects of mixing on FRB. This study
38 sheds new light on the importance of using integrative approaches including both above- and
39 below-ground biomass and traits to study diversity effects on plant productivity.

40 **Key words:** absorptive fine roots, biodiversity-ecosystem functioning, fungal diversity,
41 functional dispersion, SoilForEUROPE, species identity effects, trait dissimilarity, tree species
42 richness

43

44 **Introduction**

45 Mixed-species forests can positively affect above-ground ecosystem properties and processes
46 (reviewed by Scherer-Lorenzen, 2014). For example, tree species mixtures can be more
47 productive (Ammer, 2019) and more resistant and resilient towards environmental changes
48 compared to their mono-specific counterparts (Jactel et al., 2017). Therefore, and especially in
49 view of global change, many current forest management strategies focus on the promotion of
50 mixed-species forests (Bauhus et al., 2017).

51 While above-ground overyielding of biomass in mixtures has frequently been observed (e.g.
52 Zhang et al., 2012), evidence for below-ground mixing effects on fine-root biomass (FRB) is
53 inconclusive (Finér et al., 2017; Ma & Chen, 2016). Yet, the commonly observed above-ground
54 overyielding suggests that below-ground resource capture is likely sustained or even enhanced in
55 mixtures (e.g. Archambault et al., 2019). Forrester et al. (2006) observed that mixing *Eucalyptus*
56 *globulus* with *Acacia mearnsii* did not change the total below-ground carbon (C) allocation
57 compared to monocultures, but strongly increased above-ground productivity, suggesting that in
58 mixtures plant C was invested more efficiently below-ground to provide trees with soil resources.
59 Similarly, in a young tree diversity experiment, simultaneous underyielding of FRB and above-
60 ground overyielding in mixtures suggested a more efficient below-ground resource acquisition
61 (Archambault et al., 2019). While FRB may be indicative of the potential to capture soil
62 resources, it cannot be used as indicator of below-ground productivity or the carbon allocation to
63 below-ground organs and processes.

64 Three types of mechanisms, i.e. resource partitioning, abiotic facilitation and positive biotic
65 feedbacks may enhance soil resource uptake by fine roots in mixtures (Barry et al., 2019). A
66 well-studied example of plant association that involves all three mechanisms relates to the
67 symbiotic nitrogen (N) fixation in trees, which may result in a) resource partitioning by leaving
68 more soil N available for non-fixing species (Forrester et al., 2006), b) abiotic facilitation of non-
69 N-fixing plants owing to increase of soil N availability through N-fixation, and c) biotic
70 feedbacks through transfer via mycorrhizal networks (Munroe & Isaac, 2014). As this example
71 shows, these underlying mechanisms often occur simultaneously, contribute to the same net-
72 effect, and are thereby difficult to separate from each other (Forrester & Bauhus, 2016).

73 A greater overall resource capture in mixtures may be achieved by spatial, temporal and/or
74 chemical resource partitioning or a combination thereof (Barry et al., 2019). A higher trait
75 dissimilarity can conceptually be linked to a greater niche differentiation (De Bello et al., 2010).
76 Hence the quantification of trait diversity using trait-based diversity indices (Laliberté &
77 Legendre, 2010) may be useful for assessing underlying mechanisms of diversity effects. Positive
78 relationships of such trait-based indices with standing biomass or productivity of fine roots were
79 reported (Mahaut et al., 2020; Sun et al., 2017), while other studies did not observe such effects
80 (Bakker et al., 2019; Zeng et al., 2020).

81 Abiotic facilitation can increase soil resource availability when plant-plant interactions increase
82 the plant-available resource pool (Barry et al., 2019). Examples include N-fixation, positive litter
83 interaction effects accelerating nutrient cycling (Hättenschwiler, 2005), or hydraulic
84 redistribution (Prieto, Armas, & Pugnaire, 2012).

85 Positive biotic feedbacks can increase fine-root resource acquisition in mixtures (Barry et al.,
86 2019). This may be the case when interactions with symbiotic fungi in mixtures enhance soil
87 nutrient exploitation of plants (Boddy, 1993; Read & Perez-Moreno, 2003). An example for such
88 a biotic feedback is the nutrient and water transfer between tree species in mixtures through a
89 common mycorrhizal network (Simard et al., 2015). Not only the abundance (e.g. fungal hyphae
90 mass), but also the diversity of mycorrhizae may enhance nutrient uptake of plants through
91 complementary fungal nutrient exploitation strategies (Agerer, 2001; Wagg et al., 2011). Yet,
92 evidence on the response of root-EcM interactions to changing tree diversity levels is still scarce
93 and inconsistent (Salahuddin et al., 2018; Weißbecker et al., 2018).

94 Tree species interactions may also enhance below-ground resource-uptake efficiency, i.e.
95 resource capture per C invested. In this case, species' interactions above-ground may cause a
96 greater below-ground resource-uptake efficiency as a result of shifts in C allocation pattern from
97 below- to above-ground biomass (Poorter et al., 2012). A reduction in herbivory and pathogen
98 pressure in mixtures could also result in less C requirement for defence structures such as tougher
99 tissue (de Kroon et al., 2012; Jactel & Brockerhoff, 2007).

100 These examples show that instead of only measuring FRB, the quantification of other fine-root
101 traits related to soil resource exploitation efficiency (i.e. C invested in roots per unit of resource
102 acquired) and capacity (i.e. potential resource uptake by roots, independent of C cost) could

103 improve our understanding of overall diversity-productivity relationships. In addition,
104 mycorrhizal symbionts, which often crucially support fine roots in soil resource capture
105 (Brundrett, 2009), are also rarely considered (Laliberté, 2017).

106 Overall, there is growing evidence for a global trade-off in root strategies along a *resource-*
107 *acquisition and resource-conservation* gradient, hereafter referred to as *acquisition-conservation*
108 gradient, ranging from roots with high root tissue density (RTD) that show a *slow* resource return
109 on investment but are long-lived and well-protected, to *fast* roots with a high N content and high
110 metabolic rate for fast resource return on investment but a short life span (Bergmann et al., 2020).
111 A high root N concentration is generally indicative of high root metabolic activity (Reich et al.,
112 2008) and may positively relate to specific root uptake activities (e.g. Legay et al., 2020). Also, a
113 low RTD, reflecting low construction cost (Chen et al., 2018), may indicate a higher efficiency in
114 conditions where the risk of root loss from herbivory and/or pathogens is not high. The strategy
115 of plants for efficient resource uptake and conservation is further defined by the *fungus-*
116 *collaboration* gradient – a trade-off between roots that efficiently acquire soil resources by
117 themselves (high specific root length, SRL - *do-it-yourself* strategy), and those with typically
118 higher fine-root diameter that rely on mycorrhizal colonisation (*outsourcing* strategy) (Bergmann
119 et al., 2020). Indeed, a higher SRL of fine roots generally translates into a larger volume of soil
120 under the influence of roots and therefore a higher soil resource uptake per biomass invested
121 (Freschet et al., 2020). Nonetheless, mycorrhizal hyphae may be as efficient in resource
122 acquisition than fine roots (Chen et al., 2018), or even more efficient under conditions of low
123 resource availability (Lambers et al., 2008). Therefore, both a higher mycorrhizal colonisation
124 intensity and a high SRL may be linked to a high resource-acquisition efficiency. Overall,
125 evidence for such changes in resource uptake strategies of fine-root systems in response to tree
126 species mixing is strongly limited. To our knowledge, only morphological root trait adaptations
127 including an increased SRL in mixtures have been observed (e.g. Bolte & Villanueva, 2006; Bu
128 et al., 2017; Salahuddin et al., 2018).

129 Phylogenetics may determine variations in fine-root traits and thereby resource acquisition
130 strategies more than environmental conditions (Comas & Eissenstat, 2009; Valverde-Barrantes et
131 al., 2017). Hence, it has been suggested to group species based on their root diameter and
132 mycorrhizal type, because these two traits may strongly reflect the species' resource acquisition
133 strategies (Chen et al., 2018). For example, we know that gymnosperm and angiosperm species

134 differ considerably in their fine-root traits and represent contrasting soil exploitation strategies
135 (Bauhus & Messier, 1999; Guo et al., 2008). Owing to their evolutionary background, thin-rooted
136 angiosperms are thought to follow a more *acquisitive* strategy characterised by faster root
137 proliferation in contrast to thick-rooted gymnosperms that generally harbour a more *conservative*
138 acquisition strategy by forming roots that are longer-lived and rely more on mycorrhizal fungi for
139 soil exploitation and nutrient acquisition (Liu et al., 2015; Ma et al., 2018). Evidence has also
140 been gathered for contrasting fine-root trait plasticity of thick vs. thin-rooted species in response
141 to changes in environmental conditions, with thin-, but not thick-rooted species enhancing root
142 proliferation and thereby showing a higher plasticity (Bauhus & Messier, 1999; Chen et al., 2016,
143 2018; Wang et al., 2019). This suggests that gymnosperm and angiosperm tree fine roots may
144 also respond differently to tree species mixing. Only a few studies have dealt with this question,
145 as species-specific fine-root data in mixtures are difficult to obtain (Bolte & Villanueva, 2006;
146 Salahuddin et al., 2018). More generally, it has also been suggested that plastic responses in
147 mixtures may be more pronounced for absorptive roots (the three most distal root orders) than for
148 higher-order roots (Salahuddin et al., 2018) suggesting that separating fine roots by root orders or
149 functions is an important approach to identify species interactions (McCormack et al., 2015).

150 Previously, underyielding of standing FRB was observed in four European forest types
151 (Wambsganss, Beyer, Freschet, Scherer-Lorenzen, & Bauhus, 2021) despite evidence for
152 predominantly positive above-ground diversity-productivity effects in these ecosystems (Jucker
153 et al., 2016; Ratcliffe et al., 2017). An increased occupation of soil volume by fine roots in the
154 most nutrient-rich soil depth in mixtures, as indicated by an increased root length density (RLD),
155 suggested an enhanced below-ground resource-uptake efficiency and complementary use of
156 space and resources in these mixed forest stands (Wambsganss et al., 2021). Here, our objective
157 was to further investigate these below-ground adaptations in mixtures by analysing fine-root
158 traits related to soil exploitation efficiency. We further aimed at assessing the effect of tree
159 species mixing on the intensity of the relationship between trees and their mycorrhizal symbionts.
160 We also intended to investigate the role of diversity of root trait values, as a proxy for resource
161 partitioning mechanisms. Moreover, we wanted to shed light on differences in tree mixing effects
162 driven by two phylogenetic groups with contrasting traits, namely thin-rooted angiosperm vs.
163 thick-rooted gymnosperm species.

164 Our hypotheses were:

- 165 1) In tree mixtures, reductions in standing FRB are generally associated with changes in root
166 traits linked to:
- 167 a) a higher fine-root resource acquisition efficiency (i.e. higher root N concentrations and
168 lower RTD) and higher length of roots deployed in soil (i.e. higher RLD), resulting in a
169 faster/greater soil volume exploitation per unit of C invested in roots, and/or
 - 170 b) facilitation in resource acquisition by mycorrhizal fungi (positive biotic feedbacks),
171 particularly higher root mycorrhizal colonisation and changes in traits related to the
172 hosting of mycorrhizae (i.e. lower SRL and higher root diameter) and/or
 - 173 c) a higher functional fine-root trait dissimilarity and hence complementary resource
174 acquisition strategies among tree species.
- 175 2) Fine-root morphology (SRL, RTD, diameter) and total fine-root length investment (RLD)
176 respond more strongly to interspecific interactions in thin-rooted angiosperms than in thick-
177 rooted gymnosperms, which rely more on adaptations through mycorrhizal associations.

178 **Material and Methods**

179 *Study Design*

180 The study sites were selected from the pan-European FunDivEurope forest-plot network,
181 representing a tree species richness gradient from mono-specific to high-diversity stands in each
182 forest type (Baeten et al., 2013). The 30×30 m plots in mostly mature uneven-aged forests were
183 chosen according to predefined criteria comprising evenness, tree age, density, species
184 composition and environmental factors (e.g. soil type). The comparative study design aimed at
185 keeping abiotic and biotic variables as constant as possible, while allowing for gradients in tree
186 species diversity. Here, we used 63 plots (30 mono-specific, 33 mixtures) across four sites
187 representing major European forest types including boreal (Finland), hemiboreal (Poland),
188 mountainous beech (Romania) and thermophilous deciduous forest (Italy) (Table S1). The plots
189 consisted of one or three tree species from a pool of three to five indigenous species that were
190 representative of the local tree community. At each plot, we chose five subplots, i.e. tree
191 neighbourhoods (*triplets*) for soil sampling following Vivanco & Austin (2008). These *triplets*
192 consisted of three healthy, dominant or co-dominant tree individuals of a single species and three
193 different species in the mono-specific stands and mixtures, respectively. Further criteria for the
194 selection of the *triplets* were approximately equal diameters at breast height (DBH) and crown
195 sizes of the three tree individuals and a homogeneous distribution of the *triplets* across the plot.
196 We then selected soil sampling locations based on the visually estimated dimensions of *triplet*
197 trees (DBH and crown dimensions) to capture a point of approximately equal influence of all
198 three trees. Sampling points were hence moved closer towards trees of smaller dimensions and
199 further away from trees with larger dimensions to ensure equal influences.

200 *Root sampling and processing*

201 In spring 2017, one soil core per subplot was extracted to a depth of 30 cm with a split-tube
202 sampler (Eijkelkamp, inner diameter 5.3 cm). Soil cores were divided into three depth layers, i.e.
203 0-10, 10-20 and 20-30 cm. Samples were frozen (-20°C) until processing. Soil samples were
204 washed under tap water and live tree fine roots (≤ 2 mm in diameter) were separated from
205 understory (herbaceous) and dead tree fine roots. Rocks and coarse roots (both > 2 mm in
206 diameter) were kept for fine-earth volume estimations. Live tree fine roots were further sorted by
207 species using reference samples and divided into absorptive and transport fine roots (McCormack

208 et al., 2015). This was done based on root orders, i.e. absorptive roots consisting of the first three
209 most distal root orders (beginning from tips) and transport roots consisting of higher root orders.
210 The functional classification approach was preferred over the traditional approach, i.e. classifying
211 fine roots as roots with a diameter ≤ 2 mm, because recent studies have shown that root functions
212 and plasticity significantly differ with root orders (Freschet & Roumet, 2017; Laliberté, 2017;
213 McCormack et al., 2015).

214 *Root traits*

215 For each subplot sample, root traits (Table S2) were measured on live absorptive fine roots for
216 each species and soil depth separately (except for the three *Quercus* species in Italy, which could
217 not be reliably distinguished and were therefore pooled, hereafter referred to as *Quercus spec.*).
218 Root tips colonized by EcM were visually identified and counted on representative subsamples
219 based on the presence or absence of a fungal sheath for the 12 species known to be associated
220 with EcM (*Acer pseudoplatanus* was associated with arbuscular mycorrhizae). Thereafter, roots
221 were scanned in water with a flat-bed scanner (resolution 800 dpi) and scans analysed with the
222 software WinRhizo (Regents Instruments, Québec, Canada, 2009) to obtain root length, area,
223 volume and diameter. Root volume and (average) diameter values were corrected by
224 recalculating them from the sum of all diameter classes' averages (Freschet et al., 2020).
225 Subsequently, all samples were dried (72 h, 40°C) and weighed. For C and N analysis, root
226 samples of the first depth layer (0-10 cm) were pooled at the plot level. Different root species and
227 functional root types (absorptive vs. transport roots) were kept separately. Then, samples were
228 milled to determine total organic C and N concentrations by dry combustion (Elementar Vario El
229 Cube, Langensfeld, Germany). Overall trait values of absorptive fine roots were calculated as
230 cumulative values, i.e. pooled across the three depth layers (with the exception of chemical traits,
231 which were only available for the first depth layer) and species, as we focussed on overall mixing
232 effects rather than species-specific effects.

233 *Diversity and biomass of fungi*

234 Data on overall mycorrhizae and EcM subgroups are based on soil samples taken adjacent to the
235 root sampling spots (0-10 cm soil depth). Molecular data of fungal diversity were obtained by
236 standard total genomic DNA extraction. Sample processing, laboratory analysis and
237 bioinformatics procedures were done according to Prada- Salcedo et al. (2021). We amplified

238 fungal ITS2, using the primers P5-5 N-ITS4 and P5-6 N-ITS4 together with P7-3 N-fITS7 and
239 P7-4 N-fITS7 (Gardes & Bruns, 1993; Ihrmark et al., 2012; Leonhardt et al., 2019) and produced
240 libraries using the Nextera XT Illumina index Kit (Illumina), based on the manufacturer's
241 instructions. Subsequently, samples were sequenced with the MiSeq Reagent kit v3 on Illumina
242 MiSeq system (Illumina Inc., San Diego, CA, United States). After bioinformatic analysis, the
243 FUNGuild V1.0 tool was used to analyse fungal taxonomy and ecological guilds (Nguyen et al.,
244 2016). Subsequently, we identified and classified fungal groups and determined fungal richness
245 and diversity using the package *phyloseq* (McMurdie & Holmes, 2013) in R (R Core Team,
246 2018). Soil total fungal biomass and specifically mycorrhizal biomass were determined by
247 phospholipid fatty acid analysis method described by Prada-Salcedo, Wambsganss, Bauhus,
248 Buscot, & Goldmann (2021) and Pei et al. (2017). After lipids extraction, GC-MS analysis and
249 peak areas conversion to nmol g soil^{-1} , the biomass was calculated accordantly to fungal
250 biomarkers 18:2 ω 6,9c, 18:1 ω 9 and 16:1 ω 5c.

251 *Plasticity index*

252 To assess the intraspecific trait plasticity of angiosperms and gymnosperms in mixed relative to
253 mono-specific stands, a plasticity index (PI) (Freschet et al., 2018) was calculated as the
254 percentage of mean deviation from the mean mono-specific trait value:

$$PI (\%) = \frac{Trait_{mix} - Trait_{mono}}{Trait_{mono}} * 100 \quad (1)$$

255
256 where $Trait_{mono}$ is the mean trait value of a species in its mono-specific stand; $Trait_{mix}$ is the mean
257 trait value of a species in the mixture.

258 *Diversity effects*

259 Diversity effects were calculated for the cumulative biomass of absorptive roots following the
260 additive partitioning method (Loreau & Hector, 2001).

261 The complementarity effect (CE) was calculated as:

$$CE = N \times \text{mean}(\Delta RY_i) \times \text{mean}(M_i) \quad (2)$$

262 where N is the number of species (here $N=3$),

263 ΔRY_i is the difference between the observed and expected relative (R) yield, i.e. FRB, of species i
264 in mixture,

265 M_i is the FRB in the mono-specific stand of species i .

266 The net diversity effect (NE) was calculated as:

$$\Delta Y = Y_O - Y_E \quad (3)$$

267 where ΔY , i.e. the net effect, is the deviation from total expected FRB in the mixture.

268 Y_O is the total observed FRB in mixture and Y_E is the expected FRB based on the observed
269 average FRB values of the component species in mono-specific stands (i.e. the sum of all three
270 species' FRB in mono-specific stands divided by 3).

271 If ΔY equals 0, effects of the component species are additive. If ΔY deviates from 0, effects are
272 non-additive (i.e. negative if <0 , positive if >0).

273 *Functional trait diversity*

274 We quantified functional trait diversity as functional dispersion (FDis), an index considering
275 multidimensional trait space and independent of species richness (Laliberté & Legendre, 2010),
276 using the function *dbFD* of the *FD* package in R (Laliberté et al., 2014) and considering the
277 following five traits measured on absorptive roots: SRL, RTD, RLD, EcM colonisation intensity
278 and root N concentration. Since we used only quantitative traits, the FDis calculations were based
279 on the Euclidean distance (Laliberté & Legendre, 2010). We used plot-specific species trait
280 values for these calculations to reflect intra-specific trait plasticity. We excluded samples from
281 *triplets* composed of the three different *Quercus* species (Italy), owing to the lack of species-
282 specific data. The considered traits were selected due to their important role for the plant
283 economics spectrum (Freschet et al., 2010; Reich, 2014) and to avoid redundancy. Species
284 abundance data were derived from the biomass proportions of the individual species' absorptive
285 fine roots (Zeng et al., 2020).

286 *Statistical Analyses*

287 All statistical analyses were performed using R version 3.5.1. (R Core Team, 2018), with
288 significance levels set at $P=0.05$.

289 To avoid model overfitting, a principal component analysis (PCA) was computed summarising
290 absorptive root traits (SRL, RTD, root diameter, RLD and EcM colonisation intensity) that were
291 available at the *triplet* level. As root chemical traits could only be measured at the plot level, we
292 computed a second PCA for the plot-level data including chemical traits.

293 The first two axes of the *triplet*-level PCA were considered most important as they retained
294 eigenvalues of >1 and described 64 % of the total variation (Table S3). Hence, they were used as
295 predictor variables in some of the models (see below). We also tested the third and fourth PC axis
296 in some of the models, however, as these models did not yield significant results (not reported),
297 we focus only on the first two axes. Moreover, bivariate correlations between absorptive root trait
298 pairs that were expected to be significant based on the PCA biplots were checked using simple
299 linear regression analyses to corroborate the interpretation of the PC axes.

300 Linear mixed-effects models (LMMs) were used for testing the hypotheses and accounting for the
301 nested study design (package *lme4*, Bates *et al.*, 2015). Random slopes and intercepts and plot
302 nested within site were used as random effect structure for all models, except for site-level
303 models and models based on plot-level data, where only site was used as random effect. Model
304 assumptions were checked using the package *DHARMA* (Hartig, 2019). In case of violations of
305 model assumptions, response variables were transformed (package *bestNormalize*, Peterson,
306 2017).

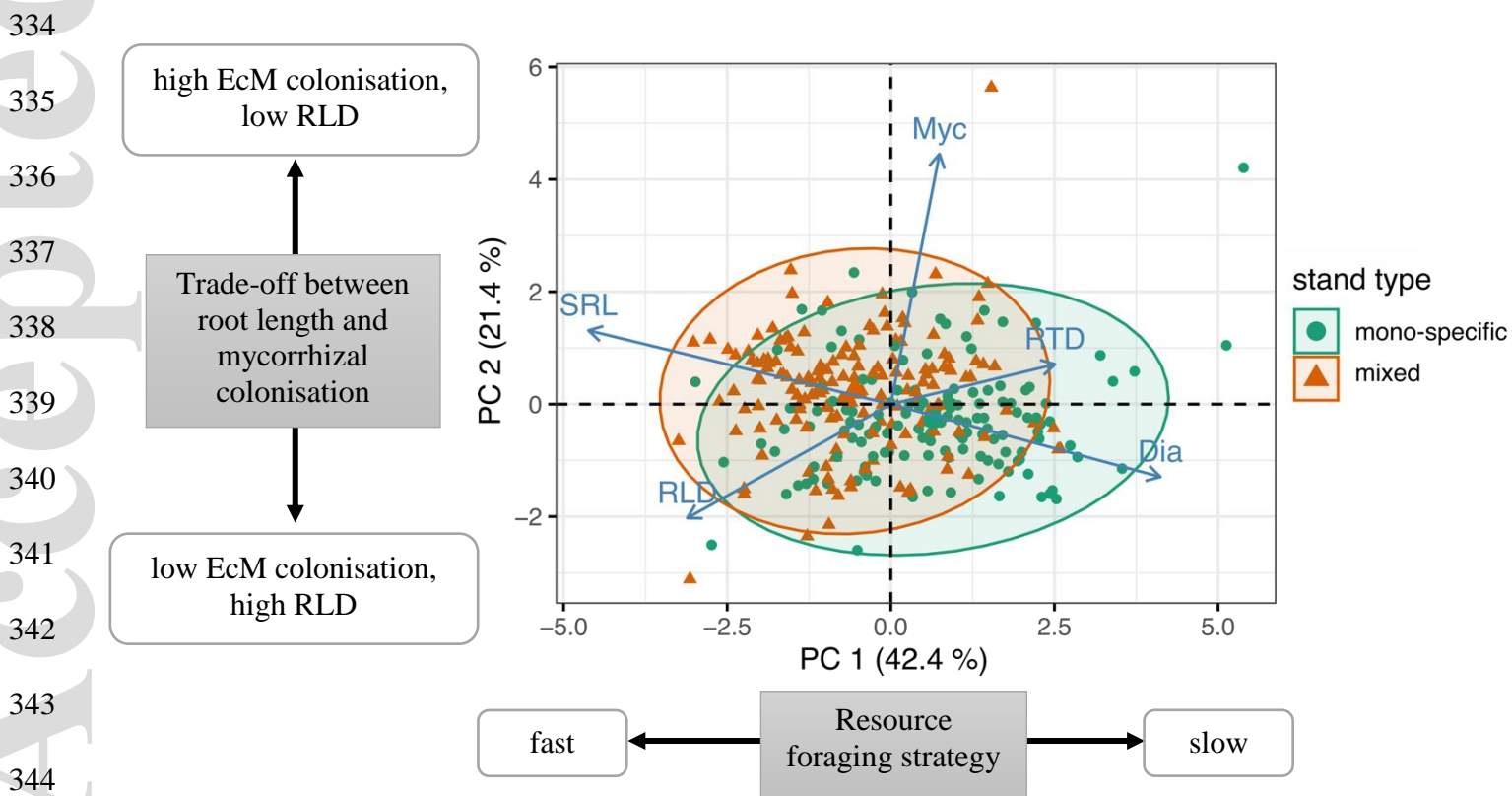
307 To assess the effect of tree mixing on the individual root traits (hypothesis 1), we tested each trait
308 (SRL, RTD, RLD, diameter, mycorrhizal colonisation, root N) separately in response to tree
309 species richness across sites. We further modelled each of the first two *triplet* root PCs separately
310 in response to tree species richness. In addition, a redundancy analysis (RDA) including site as
311 conditional factor (package *vegan*, Oksanen *et al.*, 2013) was performed to affirm our focus on
312 the first two PC axes (Fig. S1). To check whether the traits were related to standing FRB and
313 diversity effects on FRB models testing FRB as well as each diversity effect (NE, CE) separately
314 were computed in response to the *triplet* root PCs. To specifically test hypothesis 1b (biotic
315 feedbacks), we checked whether diversity effects and overall FRB were related to mycorrhizal
316 biomass and diversity. To test hypothesis 1c (trait dissimilarity), NE and CE were modelled in
317 response to functional dispersion of absorptive fine-root traits (representing functional trait
318 diversity), respectively. To test how trait values differed between mixtures and mono-specific
319 stands for angiosperms and gymnosperms, respectively, (hypothesis 2), we separated the data for

320 the two phylogenetic groups and then modelled each trait for each group separately across sites
321 and for individual sites. We further modelled FRB of both gymnosperms and angiosperms
322 separately in response to total mycorrhizal biomass and diversity as well as the diversity of EcM.
323 In addition, to specifically test for differences in responses of gymnosperms and angiosperms to
324 tree species mixing, we computed models testing each trait in response to the interaction between
325 phylogenetic type and tree species richness using the whole dataset.

326 Results

327 Fine-root trait coordination

328 The *triplet*-level PCA on absorptive root traits showed that variation of the fine-root trait data
329 was coordinated along two main axes (Fig. 1): PC1 described 42.2% of variation in root traits and
330 indicated a gradient from high-SRL, low tissue density, and low-diameter roots (negative values)
331 to low-SRL and high-diameter roots (positive values). PC2 explained 21.4% of the variation and
332 described a separate dimension indicating the reliance of trees on EcM colonisation intensity,
333 which appeared to some extent be opposed to developing high RLD.



345 **Figure 1.** Biplot of the principal component analysis (PCA) using traits of absorptive roots at the
346 *triplet* level (for the plot-level PCA including root N, see Fig. S2). PC1 represents a resource
347 foraging strategy gradient independent of mycorrhizal colonisation intensity (thus clearly distinct
348 from a collaboration gradient), from high-SRL, low tissue density, low-diameter roots, that are
349 characterised by fast foraging to low-SRL, high-diameter roots, which can be related to slow
350 foraging. PC2 represents the reliance of trees on EcM colonisation intensity, which was
351 negatively related with RLD (Fig. S3), i.e. negative values indicating a high RLD and low EcM
352 colonisation intensity, and positive values representing a high EcM colonisation intensity and low
353 RLD.

354 The *plot*-level PCA on absorptive root traits, which additionally included chemical root traits
355 only available at the plot level, further showed two axes along which the fine-root trait data were
356 coordinated (Fig. S2): PC1, which described 48.3% of the variation, represented a trade-off
357 between RTD and N concentrations, from high-RTD roots low in N concentrations (positive
358 values) to low-RTD roots that are high in N concentrations (negative values). PC2 described
359 19.7% of the variation and indicated a trade-off between SRL and root diameter, similar to *triplet*
360 PC1.

361 Bivariate trait pair analyses showed negative relationships between SRL and root diameter, SRL
362 and RTD, EcM colonisation intensity and RLD as well as root N concentrations and RTD (Fig.
363 S3).

364 **Tree mixing effects on fine-root traits**

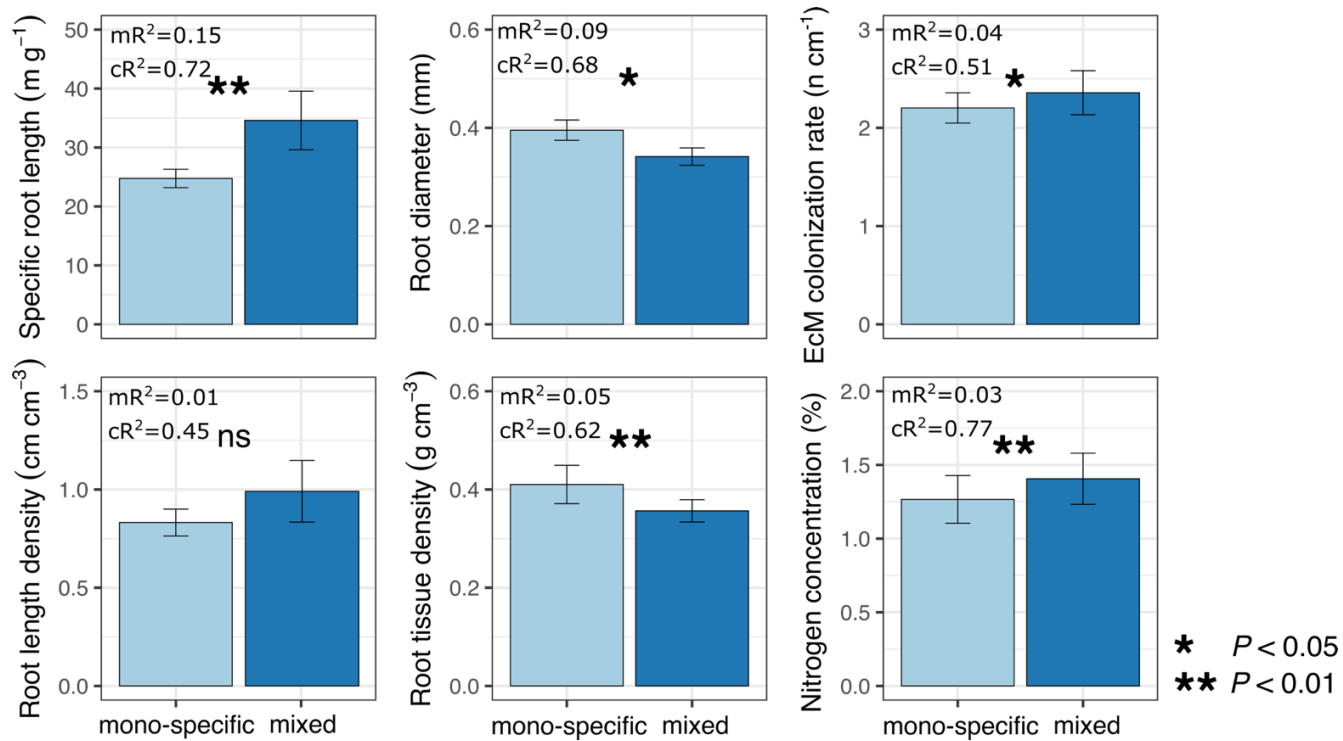
365 Across all four forest types, tree mixing had a negative effect on *triplet* PC1 (Fig. 1 & Table 1).
366 Moreover, tree mixing was positively related to *triplet* PC2, indicating a higher EcM colonisation
367 and lower RLD in mixtures compared to mono-specific stands (Fig. 1 & Table 1). Root PC1 was
368 further positively related to gymnosperm proportion in the tree *triplet*, whereas root PC2 was not,
369 suggesting that the higher the gymnosperm proportion in *triplets*, the greater the community root
370 diameter and RTD.

371 **Table 1.** Relationships between tree species richness, *triplet* gymnosperm proportion (% basal
372 area) with *triplet*-level root principal component (PC) 1 and 2, respectively tested using linear
373 mixed-effect models (from which marginal and conditional R^2 values were derived). Red and
374 green shade indicate positive and negative slopes, respectively.

Predictors (fixed effects)	Absorptive root PC1			Absorptive root PC2		
	<i>Estimate</i>	<i>t-value</i>	<i>P</i>	<i>Estimate</i>	<i>t-value</i>	<i>P</i>
Tree species richness	-1.04	-4.76	<0.001	0.45	2.88	<0.01
Triplet gymnosperm %	0.62	5.30	<0.001	-0.04	-0.42	0.68
mR ²		0.31			0.05	
cR ²		0.68			0.39	

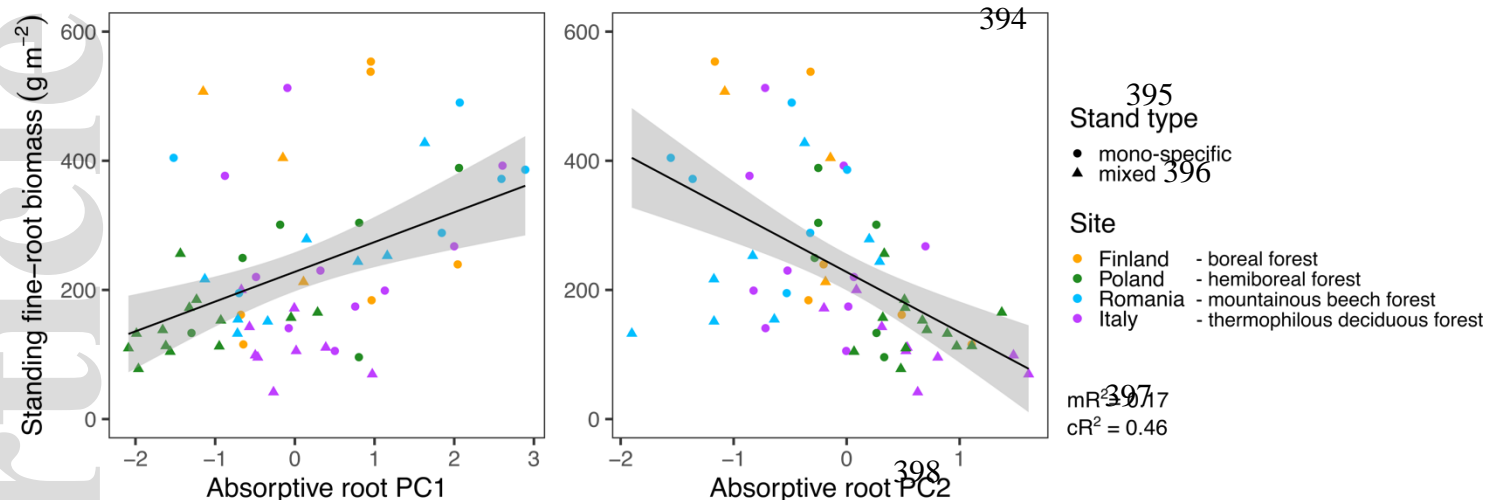
375 When analysing the traits individually, across all sites, community SRL, EcM colonisation
 376 intensity as well as root N concentrations were significantly higher, while root diameter and RTD
 377 were significantly lower in mixtures compared to mono-specific stands (LMM, Fig. 2). RLD did
 378 not significantly differ between mixtures and mono-specific stands.

379 Across sites, biomass of absorptive fine roots pooled across soil depths was positively related to
 380 *triplet* root PC1 and negatively related to *triplet* root PC2 (Fig. 3).



Figure

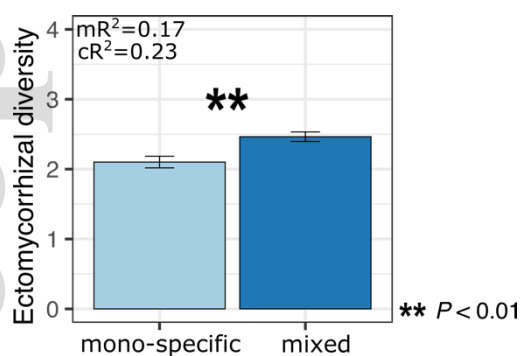
Figure 2. Mean cumulative traits of absorptive fine roots (\pm SE) by tree species richness across sites. Asterisks indicate significant differences between mixtures and pure stands tested with linear mixed-effect models (from which marginal and conditional R^2 values were derived).



399 **Figure 3.** Relationship of biomass of absorptive fine roots with *triplet*-level root principal
 400 components (PC) 1 and 2. Significance was tested using linear mixed-effect models including
 401 both PCs as fixed effects in one model (from which marginal and conditional R² values were
 402 derived). The solid lines represent linear regressions for the two variables of interest, including a
 403 95 % confidence interval (shaded grey area).

404 Role of mycorrhizae

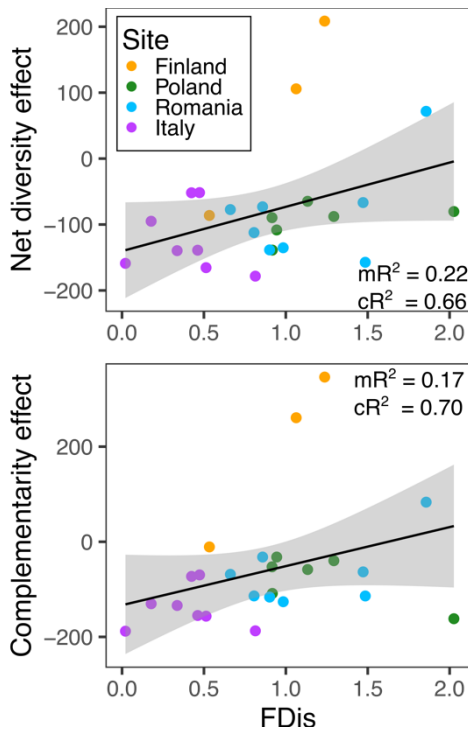
405 Across sites, total mycorrhizal as well as EcM Shannon diversity were significantly higher in
 406 mixtures than in mono-specific stands, whereas total mycorrhizal biomass did not significantly
 407 differ between the two stand types (Fig. 4 & Table S4).



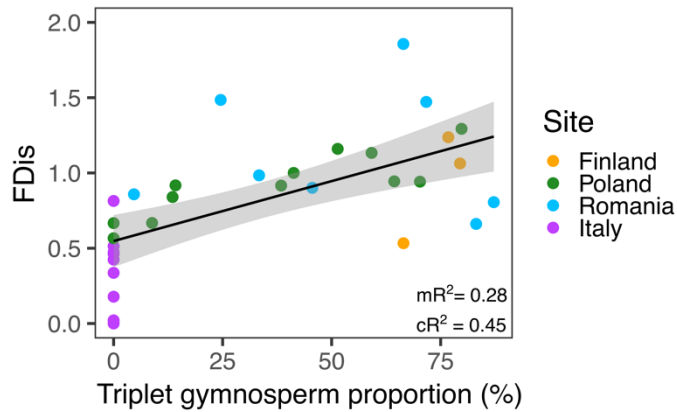
409 **Figure 4.** Mean ectomycorrhizal Shannon diversity (\pm SE) in soil samples (0-10 cm soil depth) in
 410 mixtures and pure stands across all four sites. Asterisks indicate significant differences between
 411 the two stand types tested with linear mixed-effect models (from which marginal and conditional
 412 R² values were derived).

413 Role of root trait dissimilarity

414 Across all four sites, functional trait diversity of absorptive roots in mixtures, reflected by the
415 functional dispersion index, FDis, was positively related to both NE and CE in terms of biomass
416 of absorptive fine roots (Fig. 5). Furthermore, FDis was positively related to gymnosperm tree
417 proportion in mixtures (Fig. 6) and also to total mycorrhizal as well as EcM diversity (LMM,
418 $P < 0.01$, Table S5).



419
420 **Figure 5.** Relationship between functional dispersion of traits of absorptive roots (FDis) with
421 mean net diversity and complementarity effects in terms of absorptive standing fine-root biomass
422 (g m^{-2}). R^2 (marginal and conditional) values were derived from linear mixed-effect models. The
423 solid lines represent linear regressions for the two variables of interest, including a 95 %
424 confidence interval (shaded grey area).



425

426 **Figure 6.** Relationship of *triplet* gymnosperm proportion with functional dispersion (FDis) of
 427 traits of absorptive roots in mixtures. R^2 (marginal and conditional) values were derived from
 428 linear mixed-effect models. The solid line represents a simple linear regression including a 95 %
 429 confidence interval (shaded grey area).

430 **The influence of phylogeny on fine-root traits**

431 Mean trait values of absorptive roots varied considerably among species (Table S6) and clear
 432 differences between gymnosperms and angiosperms became apparent (Table 2). At all sites
 433 where both gymnosperms and angiosperms were sampled, absorptive roots in gymnosperms were
 434 characterised by larger average diameters, lower SRL and SRA compared to angiosperm roots. In
 435 hemiboreal (Poland) and mountainous beech forests (Romania), angiosperms had significantly
 436 higher RLDs compared to gymnosperms. In boreal forests (Finland), EcM colonisation intensity
 437 was significantly higher in angiosperm (i.e. *Betula pendula*) than gymnosperm species, whereas
 438 gymnosperms had a higher infection rate than angiosperms in hemiboreal forests. Angiosperm
 439 roots in hemiboreal forests also had significantly higher N concentrations (and lower root C/N
 440 ratio) than gymnosperms roots.

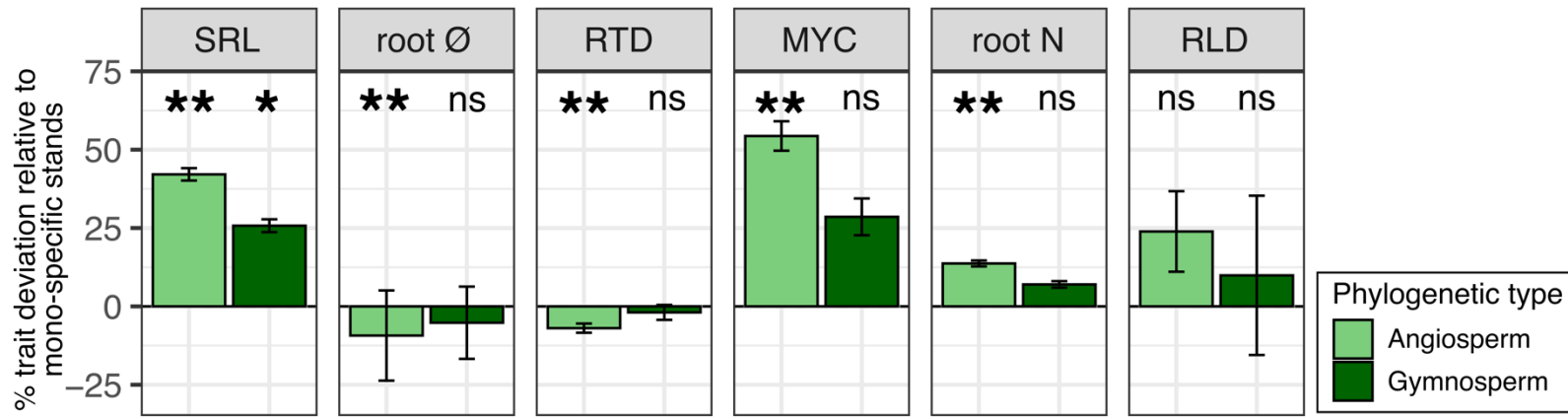
			SRL	SRA	RTD	RLD	Dia	Myc	C/N	N	
			Specific root length	Specific root area	Root tissue density	Root length density	Root diameter	EcM colonisation intensity	Root C/N conc.	Root N conc.	
Site	Phylogenetic group	Tree species richness	N	m g ⁻¹	cm ² g ⁻¹	g cm ⁻³	cm cm ⁻³	mm	n cm ⁻¹	%	
Boreal forest (Finland)	<i>Angiosperm</i>	1	2	38.9±1.2	371±7	0.36±0	0.31±0.04	0.31±0.01	3.03±0.23	43.5±1.92	1.1±0.02
		3	3	48.3±3	440±13	0.32±0.02	0.44±0.07	0.32±0.03	2.63±0.28	38.6±2.78	1.28±0.09
	<i>Gymnosperm</i>	1	4	16.9±1.4	230±15	0.46±0.08	0.28±0.07	0.44±0.01	2.16±0.14	51.4±3.63	0.94±0.07
		3	3	20.5±0.6	271±6	0.36±0.02	0.46±0.16	0.43±0.01	2.24±0.27	40.5±1	1.16±0.02
Hemiboreal mixed forest (Poland)	<i>Angiosperm</i>	1	3	38.2±6.4	378 ±46	0.34±0.03	0.34±0.05	0.33±0.02	2.32±0.37	29.3±3.68	1.63±0.15
		3	13	60.1±2.9	507±17	0.32±0.01	0.47±0.06	0.28±0.01	2.86±0.15	22.8±0.45	1.99±0.04
	<i>Gymnosperm</i>	1	3	20.4±3.7	277±27	0.32±0.01	0.16±0.02	0.46±0.05	2.53±0.23	27.4±2.51	1.69±0.14
		3	11	26.8±1.7	341±13	0.34±0.04	0.13±0.02	0.42±0.01	3.99±0.65	26.0±0.75	1.73±0.04
Mountainous beech forest (Romania)	<i>Angiosperm</i>	1	4	33.4±2	341±11	0.37±0.02	0.49±0.12	0.34±0.02	1.39±0.24	30.8±1.84	1.51±0.11
		3	8	38.4±1.5	376±13	0.4±0.04	0.61±0.14	0.32±0.01	3.52±0.34	29.5±1.21	1.49±0.05
	<i>Gymnosperm</i>	1	4	11±1.1	188±11	0.37±0.02	0.18±0.03	0.56±0.02	2.51±0.35	32.4±2.62	1.35±0.1
		3	8	13.2±0.8	214±9	0.39±0.04	0.16±0.04	0.54±0.01	2.46±0.21	31.9±1.09	1.33±0.05
Mediterranean thermophilous	<i>Angiosperm</i>	1	10	23.2±2.5	240±18	0.54±0.04	0.23±0.05	0.35±0.01	1.79±0.16	47.7±3.42	1.03±0.06
		3	18	34.8±1.9	330±13	0.43±0.02	0.19±0.03	0.31±0.01	2.6±0.21	42.8±1.61	1.11±0.03

forest (Italy)

Table 2. Mean trait values (\pm SE) of absorptive fine roots (pooled across the entire soil profile, except for chemical traits) by phylogenetic group, richness and site. Bold letters indicate significant differences between angiosperm and gymnosperm trait values across both richness levels.

441 Angiosperms and gymnosperms showed differences in intraspecific trait plasticity (Fig. 7 &
442 Table S7). Across sites, angiosperms had a significantly higher SRL, EcM colonisation intensity,
443 root N and lower average root diameters and RTD in mixtures compared to mono-specific stands.
444 For gymnosperms, trait values did not differ between the two stand types except for a higher SRL
445 in mixtures than in mono-specific stands. Both total RLD of gymnosperms and angiosperms did
446 also not significantly differ between mixed and mono-specific stands. Moreover, gymnosperm
447 FRB was negatively related to total mycorrhizal biomass (LMM, $P=0.04$) and tended to be
448 negatively related to total mycorrhizal diversity ($P=0.09$), whereas angiosperm FRB was neither
449 significantly related to mycorrhizal diversity nor biomass (both $P>0.05$, Table S4).

450



451 **Figure 7.** Plasticity index (\pm SE) for traits of absorptive fine roots of angiosperms and
452 gymnosperms across sites. Asterisks indicate significant intraspecific trait deviation between
453 mono-specific and mixed stands tested with linear mixed-effect models (** P <0.01; * P <0.05; ns
454 P >0.1). Abbreviations of the fine-root traits are shown in Table 2.

455 **Discussion**

456 Here, we demonstrated that the overall reduction in biomass of absorptive fine roots in mixtures
457 across four major European forest types reported in a previous study (Wambsganss et al., 2021) is
458 associated with morphological and chemical adaptations of fine-root traits. These changes in root
459 trait values suggest a shift towards a *faster* foraging strategy of fine roots in mixtures. The higher
460 root EcM colonisation intensity and the higher diversity of EcM in soils in mixtures further
461 indicate positive biotic feedbacks. Our analyses also showed that these mixing effects depended
462 on interspecific functional root trait dissimilarity as well as the diversity of EcM pointing to
463 complementary soil resource acquisition strategies. We observed the strongest fine-root biomass
464 reduction in mixtures with the lowest root trait dissimilarity, while such effects were nearly non-
465 existent in stands with higher root trait dissimilarity. Since root trait dissimilarity was strongly
466 positively related to gymnosperm proportion in the mixture, this result can be explained by the
467 generally stronger response of angiosperm than gymnosperm species to tree mixing observed
468 here.

469 ***Soil exploitation strategy***

470 Supporting hypothesis 1a, tree mixing significantly affected fine-root traits, including higher root
471 N concentrations and lower RTD (Fig. 2). Mixtures were also characterised by higher SRL and
472 lower root diameter compared to mono-specific stands. Contrasting our hypothesis, the length of
473 roots deployed across the three soil layers (RLD) was not significantly higher in mixtures, despite
474 an increased RLD observed in the topsoil only (Wambsganss et al., 2021). Our results strengthen
475 previous findings of increasing SRL owing to interspecific interactions in forests (Bolte &
476 Villanueva, 2006; Bu et al., 2017; Germon et al., 2018; Salahuddin et al., 2018) and suggests that
477 other morphological root changes also occur simultaneously.

478 In accordance with recent studies (Bergmann et al., 2020; Ding et al., 2020; Erktan et al., 2018;
479 Wang et al., 2018), a large share of the variation in our data was arranged along two axes (Figs 1
480 & S1), suggesting a trade-off between SRL and diameter – the *fungus collaboration* gradient - as
481 well as a trade-off between RTD and root N – the *resource acquisition-conservation* gradient.
482 The differences between morphological and chemical traits in mixtures compared to mono-
483 specific stands could also be interpreted as a shift towards a more *do-it-yourself* strategy and
484 concurrently a *fast/acquisitive* rather than a *slow/conservative* resource uptake (Bergmann et al.,

485 2020). The positive relationship between total standing FRB and a root foraging strategy
486 favouring thicker and shorter roots suggests that these trait adaptations may be linked to the
487 negative net diversity effects on FRB reported for these mixtures (Wambsganss et al., 2021). Yet,
488 the EcM colonisation intensity appeared to be independent of the SRL-diameter trade-off
489 represented by our PCAs. Instead, it was coordinated along an independent, second dimension
490 (Fig. 1) indicating a potential trade-off with RLD (discussed in the next section). This suggests
491 that in contrast to the fungal *collaboration* gradient (Bergmann et al., 2020), the trade-off
492 between SRL and diameter shown by both of our PCAs should rather be interpreted as a gradient
493 in root foraging strategies independent of EcM associations, as proposed by Ding et al. (2020). It
494 is noteworthy that this trend may result from our focus on EcM species. Whereas positive
495 relationships between fine-root diameter and mycorrhizal colonization have been found relatively
496 consistently for AM species (e.g., Kong et al., 2014; Ma et al., 2018; McCormack & Iversen,
497 2019), there is no such clear picture for EcM species (e.g., Ding et al., 2020; Kong et al., 2014;
498 McCormack & Iversen, 2019). One possible explanation lies in the increased potential for EcM
499 colonisation of thinner roots with many root tips (i.e., increased root branching intensity; Ding et
500 al. 2020). Therefore, it cannot be excluded that the framework proposed by Bergmann et al.
501 (2020), where mycorrhizal colonisation intensity aligns with fine-root diameter, may not
502 adequately account for the trade-off in root morphology observed among EcM species.

503 A high SRL is often used as an indicator for a higher soil exploitation efficiency, i.e. less C
504 required per root length deployed and thus soil volume explored (Eissenstat, 1991). Yet, a higher
505 foraging efficiency and fast strategy, do not necessarily relate to a reduction in below-ground C
506 investments, as high-SRL roots are often characterised by a shorter lifespan (McCormack et al.,
507 2012; Weemstra et al., 2020). Similarly, a lower overall RTD implies a reduction in root
508 construction costs, but also reduces root structural defence capacity possibly resulting in a shorter
509 lifespan (Eissenstat et al., 2015; Eissenstat et al., 2000). The increased root N concentrations are
510 also associated with increased root respiration rates (Reich et al., 2008) and a lower root lifespan
511 (Bergmann et al., 2020; McCormack et al., 2012). Therefore, the shift in fine-root trait values
512 from a less efficient resource foraging and *slow* strategy in mono-specific stands to more efficient
513 foraging and *faster* strategy in mixtures may have maintained (or even increased) the capacity of
514 trees to acquire nutrients. Yet, the decrease in FRB cannot be directly translated into lower C
515 investments into nutrient acquisition without additional information on respiration and turnover
516 rates of fine roots (Weemstra et al., 2020).

517 The shift in fine-root traits with species mixing may be explained in several ways. First, the
518 generally thinner and longer roots in mixtures may be explained by shifts in C allocation patterns
519 from below- to above-ground biomass (Poorter et al., 2012), which is then counterbalanced by
520 shifts in root morphology (Freschet et al., 2015; Weemstra et al., 2020) and root traits related to
521 soil resource uptake (Freschet et al., 2018). The observed above-ground overyielding of wood
522 production across the pan-European plot network this study is part of (Jucker et al., 2014)
523 suggests that this mechanism might have occurred here. Concurrently, the potentially higher soil
524 nutrient availability in mixtures at these plots (Gillespie, L. et al., unpublished data) also indicates
525 a higher community-level resource-use efficiency, e.g. faster cycling and lower losses of nutrients
526 (Richards et al., 2010), reducing below-ground C investment.

527 Second, since plastic reactions of fine roots in response to changes in water and nutrient
528 availability are well documented (Hodge, 2004), the morphological and chemical adaptations in
529 mixtures could also be linked to alterations of soil resource availability and distribution. Higher P
530 contents and smaller C/N ratios of the forest floor in mixtures at our sites (Gillespie, L. et al.,
531 unpublished data) suggest enhanced nutrient availability. Indeed, increasing root N
532 concentrations and decreasing RTD with increasing soil nutrient availability have been observed
533 for tree roots along broad environmental gradients (Ding et al., 2020; Ostonen et al., 2017) and
534 increased P availability was shown to increase SRL and decrease RTD (Li et al., 2019). However,
535 contrasting observations were also made (e.g. Freschet et al., 2018).

536 Third, the spatial distribution of soil resources may also partly explain our observations. A weak
537 but positive tree diversity effect on abundance and diversity of earthworms was observed across
538 the FunDivEUROPE plots (De Wandeler et al., 2018), indicating an increased bioturbation and
539 redistribution of soil nutrients in mixtures (Meysman et al., 2006; Patoine et al., 2020). As a
540 response, trees could have formed higher-SRL roots to enhance their chance of encountering
541 nutrient-rich spots (Chen et al., 2016). A higher concentration of nutrients in earthworm burrows
542 (Cameron, Cahill, & Bayne, 2014) could have generally led to the development of fewer roots in
543 search for nutrients. Alternatively, fine-root turnover could have increased and standing FRB
544 decreased, as earthworms may also consume living fine roots (Cortez & Bouche, 1992).

545 ***Positive biotic feedbacks***

546 The overall higher root EcM colonisation intensity and diversity in soil samples in mixtures
547 compared to pure stands (Figs 2 & 4) corroborate our hypothesis 1b regarding positive biotic
548 feedbacks. However, as traits related to the hosting of mycorrhizae (i.e. higher SRL and lower
549 root diameter) did not change as expected with an increased EcM colonisation, our hypothesis is
550 only partially supported.

551 Tree diversity can increase mycorrhizal diversity (Hanif et al., 2019; Kernaghan et al., 2003;
552 Tedersoo et al., 2016), possibly owing to a greater host-diversity, a greater diversity of organic
553 inputs (Hättenschwiler, Tiunov, & Scheu, 2005) or more favourable micro-climatic conditions
554 (e.g. higher soil moisture) (Joly et al., 2017). Here, the higher EcM diversity in mixtures could be
555 linked to higher EcM colonisation intensity of absorptive fine roots, as a higher diversity of EcM
556 may also increase the potential of root tip infection (Bzdyk et al., 2019). A higher diversity of
557 mycorrhizal fungi indicates complementary fungal nutrient exploitation strategies (Agerer, 2001;
558 Kernaghan, 2005) and thus enhanced nutrient supply to their hosts through positive biotic
559 feedbacks. Morphological root trait dissimilarity among tree species may also be related to
560 different EcM associations and EcM hyphal proliferation strategies and hence complementarity in
561 resource uptake (Cheng et al., 2016). Positive biotic feedbacks from EcM increasing soil resource
562 uptake by trees may also explain the apparent reduction of C allocated in FRB but do not
563 necessarily imply a lower overall below-ground C investment, as C transfer to mycorrhizae can
564 be substantial (Eissenstat, 1992).

565 The increased EcM colonisation intensity in mixtures found here contradicts two related studies,
566 reporting the opposite effect (Salahuddin et al., 2018; Yan et al., 2019). The shift towards a more
567 *do-it-yourself* strategy in mixtures seems to contrast the higher EcM colonisation rate, as high-
568 SRL roots are assumed to invest less C in mycorrhizal partners (McCormack & Iversen, 2019,
569 Bergmann et al., 2020). The presence of the fungal sheath surrounding root tips should
570 theoretically increase RTD (Reich, 2014), further contradicting the shift towards a *faster* fine-root
571 resource acquisition. Yet, a trade-off between mycorrhizal colonisation rate and root diameter (or
572 SRL) has mainly been observed for AM species, whereas such evidence for EcM species is
573 scarce (Kong et al., 2014; McCormack & Iversen, 2019). A recent study even reported a negative
574 relationship between root diameter and mycorrhizal colonisation rate in EcM conifer species
575 (Ding et al., 2020) and the authors speculated that the higher root branching intensity of thinner
576 roots led to an increased EcM infection probability. Still, the concomitant increase in mycorrhizal

577 colonisation intensity and SRL or decrease in diameter and tissue density are unlikely to occur in
578 combination on the same tree species and may rather be explained by a substantial increase in
579 these trait values on distinct tree species (Table S6).

580 As mentioned in the previous section, the shifts in root EcM colonisation intensity appeared to be
581 decoupled from shifts in root diameter and SRL but instead are negatively related with RLD (Fig.
582 1; Fig. S3). It has previously been observed that EcM tree species enhanced their nutrient
583 foraging by investing in mycorrhizal fungal hyphae production rather than in root length (Chen et
584 al., 2016). Hence, increasing EcM foraging precision could indicate another response of trees to
585 changes in soil nutrient availability in mixtures.

586 *Complementary acquisition strategies*

587 The average fine-root trait values varied considerably among tree species and in particular
588 between angiosperms and gymnosperms (Table 2), which is in agreement with other studies (e.g.
589 Bauhus & Messier, 1999; Tobner et al., 2013; Salahuddin et al., 2018), and suggests different soil
590 exploitation strategies.

591 The decrease in FRB with increasing fine-root trait diversity (Fig. 5) indicates that in addition to
592 the other mechanisms discussed before, resource partitioning among species in mixtures may also
593 play a role, corroborating hypothesis 1c.

594 In general, a higher trait diversity may relate to a greater niche differentiation and thus a more
595 complete or more efficient resource uptake (Barry et al., 2019). A higher functional diversity of
596 absorptive fine roots may indicate complementary soil resource acquisition strategies among
597 different tree species, possibly resulting in competitive reduction. The positive correlation
598 between fine-root trait diversity and total soil mycorrhizal diversity, as well as EcM Shannon
599 diversity (Table S4), further indicates that the dissimilarity in fine-root traits may also be related
600 to diversity in mycorrhizal resource exploitation strategies (Agerer, 2001; Cheng et al., 2016).

601 Fine-root trait diversity was further positively related to the gymnosperm proportion in tree
602 triplets across sites (Fig. 6), where the gymnosperm-dominated mixtures had the highest trait
603 diversity. Owing to their evolutionary background, trait differences within angiosperms are
604 usually greater than within gymnosperms. Here, the mixtures with the highest gymnosperm
605 proportion still included one angiosperm species, positively influencing the dissimilarity in fine-
606 root trait values. Consequently, the gymnosperm-dominated mixtures were characterised by more

607 neutral net diversity effects on standing FRB (Wambsganss et al., 2021). In contrast, low trait
608 diversity in the angiosperm mixtures may indicate similar uptake strategies and more intense
609 competition for soil resources among species. Our results could suggest that tree species with
610 similar fine-root resource acquisition strategies adjusted these in mixture (as described above) to
611 avoid competition (Hodge, 2004), resulting in the negative net diversity effects on standing FRB.
612 In addition, these adaptations in angiosperm-dominated mixtures may have also been caused by
613 changes in soil nutrient distributions in these stands, where decomposition rates (Joly et al., 2017)
614 and earthworm abundance (De Wandeler et al., 2018) increased with deciduous leaf litter
615 proportion. Yet, it has to be kept in mind that due to the imbalanced study design (i.e. stands with
616 the highest angiosperm proportion occurring in Italy and the stands with the lowest angiosperm
617 proportion occurring in Finland) we cannot clearly disentangle site effects from phylogenetic
618 identity effects in this study.

619 *The influence of phylogeny on the response of fine-root traits to mixing*

620 In accordance with our second hypothesis, thin-rooted angiosperms changed root morphology
621 more strongly in response to tree mixing (increased SRL, decreased RTD and diameter) than
622 thick-rooted gymnosperms (Fig. 5). Neither gymnosperm nor angiosperm species deployed
623 significantly higher root lengths in mixed compared to mono-specific stands. Thus only
624 qualitative root traits (i.e. how roots are built) changed in response to tree mixing, except for
625 RLD in the topsoil (Wambsganss et al., 2021).

626 These responses were also reflected at the community level, where a higher mixture proportion of
627 gymnosperms was related to a slower root foraging strategy and a higher angiosperm proportion
628 to a faster root foraging strategy (Table 1).

629 In contrast to gymnosperms, angiosperms may have adapted their fine-root soil exploitation
630 strategy in mixtures to optimize resource acquisition (Chen et al., 2016, 2018; Cheng et al.,
631 2016)– a response likely attributable to their evolutionary background (Ma et al., 2018; Wang et
632 al., 2019). This greater plasticity of angiosperm roots was observed under the relatively small
633 range of conditions the trees experienced between mixed and mono-specific stands at our study
634 sites. This pattern may change under a greater range of environmental conditions, as great
635 morphological plasticity has also been shown for gymnosperm roots along broader environmental
636 gradients (e.g. Zadworny et al., 2016).

637 In contrast to the assumedly lower dependence on mycorrhizae of thin angiosperm roots, our
638 results indicate that they were characterised by a strong increase in EcM colonisation intensity in
639 mixtures compared to mono-specific stands (Fig. 7). Yet, gymnosperm FRB was overall
640 negatively related to total mycorrhizal biomass and diversity, while angiosperm FRB was not
641 (Table S4). This suggests a trade-off between C investment in FRB vs. investment in
642 mycorrhizae for gymnosperms, supporting their commonly reported greater dependence on
643 mycorrhizae for soil exploitation (Ma et al., 2018). In contrast, the less clear balance between
644 FRB and EcM colonisation intensity of angiosperms may suggest that angiosperms employ more
645 different ways to increase their nutrient uptake capacity (e.g. specific root uptake rate, Miller &
646 Cramer, 2004; root hair length and density, Forde & Lorenzo, 2001).

647 ***Conclusions***

648 This study demonstrates that in tree species mixtures several potential non-exclusive and partially
649 contradicting underlying mechanisms may simultaneously contribute to a lower below-ground
650 biomass. Consequently, an underyielding of fine-root biomass in tree species mixtures does not
651 necessarily reflect negative below-ground species interactions and a lower performance of
652 mixtures. Here, integrating the role of mycorrhizal symbionts and further fine-root traits related
653 to fine-root soil exploitation was key to shed light on these typically overlooked underlying
654 patterns of biodiversity effects on plant biomass production.

655 Future tree diversity studies could further disentangle different drivers of net diversity effects on
656 fine-root biomass by considering gradients of abiotic (i.e. soil resource availability) and biotic
657 properties (i.e. mycorrhizal symbionts).

658 We conclude that below-ground biomass by itself is not a suitable variable to represent tree
659 community performance. Hence, we strongly recommend using integrative approaches that
660 incorporate a range of traits and C costs of above- and below-ground plant compartments to shed
661 light on the underlying mechanisms of tree diversity effects on ecosystem functioning.

662

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1002 **Supporting Information**

1003 Additional supporting information may be found in the online version of this article.

1004 **Appendix S1.** Supporting Information.