Running title: Forest type effects on microbial communities

Title: Mixing conifers and deciduous trees (*Fagus sylvatica*): response of soil microorganisms

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Abstract

Coniferous and deciduous trees growing in pure and mixed forests are likely to supply different resources to soil microorganisms thereby shaping microbial communities. However, effects of forest type on microorganisms and their variations with site conditions remain poorly studied. We investigated microbial basal respiration, microbial biomass (substrate-induced respiration method) and phospholipid-derived fatty acids (PLFAs) in the litter and soil at four nutrient-rich and four nutrient-poor sites in Lower Saxony, Germany. At each site, pure stands of European beech (*Fagus sylvatica*), Norway spruce (*Picea abies*) and Douglas-fir (*Pseudotsuga menziesii*), as well as mixed species stands of beech and spruce, and beech and Douglas-fir were investigated, i.e. a total of 40 forest stands. Forest type only affected soil microorganisms at nutrient-poor sites, but not at nutrient-rich sites. In coniferous and mixed forests at nutrient-poor sites, basal respiration and microbial biomass was lower and stress indicators were higher than in beech forests. Compared to beech, microbial biomass (-59%) and stress indicators (+46 – +87%) in Douglas-fir forests were particularly affected both in litter and in soil at nutrient-poor sites. The results suggest that higher resources in beech forests beneficially affect microorganisms in litter and soil at nutrient-poor sites, whereas Douglas-fir forests are characterized by low resources as indicated by low microbial specific respiration and fungal PLFA. The results indicate that in particular at nutrient-poor sites long-term effects of planting non-native tree species such as Douglas-fir on ecosystem functioning need further attention before planting pure or mixed forests of Douglas-fir with beech at large spatial scale. By contrast, from the perspective of microbial communities and their functioning planting Douglas-fir at nutrient-rich sites appear of little concern. Overall, the results highlight the importance of resources in determining microbial community structure and functioning, and document the sensitivity of soil microorganisms to planting non-native tree species differing in the provisioning of these resources from native species.

Mixing conifers and deciduous trees (*Fagus sylvatica*): response of soil microorganisms

Introduction

Studying the linkage between plants and microorganisms has great potential to improve our understanding of the factors that drive the composition and functioning of microorganisms in soil (Anderson and Domsch, 2010; Balvanera et al., 2006; Bardgett and van der Putten, 2014; Graham et al., 2016; van der Heijden et al., 2008). Trees as long-lived plants may affect microbial communities through several pathways, most notably by the quantity and quality of litter and root exudates (Högberg and Read, 2006; Wardle et al., 2004). These resources are likely to shape the community structure of soil microorganisms as dissimilar carbon resources favor different guilds of microorganisms (Ekblad and Nordgren, 2002; Fanin et al., 2019; Fierer et al., 2003). For example, Gram$^-$ bacteria are benefiting in particular from labile carbon compounds, whereas Gram$^+$ bacteria are adapted to use more recalcitrant carbon resources (Fanin et al., 2019; Fierer et al., 2003; Waller et al., 2020). Similarly, niches vary between fungal groups. While saprotrophic fungi are major decomposers of plant litter, mycorrhizal fungi are more abundant in deeper soil and receive carbon from plant roots (Lindahl et al., 2007; Nacke et al., 2016). The composition of microbial communities may change in response to variations in tree species composition, associated with changes in litter quality, root derived resources and mycorrhizal associations (L. Chen et al., 2019).

Coniferous and deciduous trees growing in pure and mixed forests likely differ in above-ground and belowground resources supply to soil microorganisms, but the relative importance of litter and root-derived resources for structuring microbial communities still is controversial (Bluhm et al., 2019; Schmidt et al., 2011; Wang et al., 2016). Accumulation of organic matter in coniferous forests has been attributed to the recalcitrance of conifer needles, but there is increasing evidence that roots and mycorrhizal fungi also affect the storage of soil carbon (Averill et al., 2014; Clemmensen et al., 2013; Schmidt et al., 2011). Further, the response of microbial communities to mixed forests is poorly understood, especially in the mixture of native and exotic tree species (L. Chen et al., 2019). Tree identity has been suggested to outweigh tree diversity as structuring forces of soil microbial communities, but the mixture of litter may improve resource availability for soil microorganisms and facilitate litter decomposition in mixed forests (L. Chen et al., 2019; Dawud et al., 2016; Fujii et al., 2020; Hättenschwiler et al., 2005; Scheibe et al., 2015; Schwarz et al., 2015). Specially, exotic plants potentially perform better in novel environments due to the lack of co-evolutionary history with local biota, resulting in reduced attack by antagonists (F. Chen et al., 2013; Kourtev et al., 2003, 2002; Wardle et al., 2011). This likely also affects their role in shaping soil microbial communities. Overall, it

remains unclear how soil microorganisms are affected by the identity and diversity of tree species, either coniferous or deciduous, native or exotic, single or mixed species (L. Chen et al., 2019).

Further, few studies have examined to what extent effects of tree species composition on soil microorganisms vary with site conditions such as soil type and nutritional status. According to the stress-gradient hypothesis, facilitation among plants is more pronounced at more nutrient-poor sites, but the role of microorganisms mediating such facilitation has largely been overlooked (David et al., 2020; Defossez et al., 2011). Plants do not passively tolerate environmental stress but respond and adapt in various ways to unfavorable growth conditions by induced plasticity (de Vries et al., 2019). Generally, plants allocate more resources to roots under nutrient-poor conditions (Kawaletz et al., 2013). Moreover, de Vries et al. (2019) recently showed that herb species may change the quality rather than quantity of root exudates and induce higher microbial respiration under drought conditions, presumably facilitating nutrient capture via stimulating microorganisms. Therefore, the role of tree species composition as driver of microbial community composition and functioning may be more pronounced under nutrient-poor than nutrient-rich conditions, but this has not been investigated until today until today.

A better understanding of the effects of tree species composition and site conditions on soil microorganisms has important implications for forest management, especially in temperate and boreal regions where tree species richness is low and managed forests typically are dominated by only a few species (Bauhus et al., 2010; Hu et al., 2009; Knoke et al., 2008). While Norway spruce currently is the most important timber species in Central Europe, its monocultures have been severely damaged by extreme weather and the outbreak of bark beetles in recent years, and such events are predicted to occur more frequently and at higher severity in the future (Biedermann et al., 2019; Brandl et al., 2020). For reducing the risks of damage and maintaining economic gains, planting of mixed forests including non-native species such as Douglas-fir is increasingly discussed (Ammer et al., 2018; Pretzsch et al., 2017). However, the impact of planting Douglas-fir on biodiversity and functioning of forests is little studied, and this applies in particular to the belowground system, although e.g., Douglas-fir has been suggested to affect soil chemistry in a similar way than spruce (Schmid et al., 2014). Taken together, we lack a comprehensive evaluation of different forest types on the structure and functioning of soil microbial communities across nutritional gradients.

Here, we investigate the structure and functioning of microbial communities in litter and soil of five types of forests at nutrient-rich and nutrient-poor sites. Forest types include pure stands of European beech (*Fagus sylvatica*), Norway spruce (*Picea abies*), Douglas-fir (*Pseudotsuga menziesii*), and the mixtures of the two coniferous species with beech. European beech forms the climax tree species in lowland and lower montane regions of Central Europe (Leuschner et al., 2017). Norway spruce originally only occurred at higher mountain ranges and boreal regions in Central Europe, but it has been planted across wide areas of Central

Europe including lowlands, and today it is the most important timber species in Central Europe (Knoke et al., 2008; Schmid et al., 2014). Douglas-fir was introduced into Germany from North America more than 150 years ago and has become the most abundant non-native tree species cultivated in Central Europe (Schmid et al., 2014). To better understand the response of microorganisms in litter and soil to planting different tree species in monoculture and mixture, we combined physiological (respiration based) and community approaches (phospholipid fatty acids analysis), and quantified microbial basal respiration, microbial biomass, as well as community structure and stress indicators of microorganisms (Anderson and Domsch, 2010). Physiological response variables and stress indicators were used as functional indicators of microbial communities. Generally, we assumed the structure of microbial communities to be linked to their functioning. Specifically, we hypothesized (1) coniferous trees to detrimentally affect microbial basal respiration, microbial biomass and stress indicators, with the effects being similar in Norway spruce and Douglas-fir forests, but alleviated in mixed forests with beech. Further, we hypothesized (2) effects of forest types on microbial community structure and functioning to be present at both nutrient-rich and nutrient-poor sites, but to be stronger at nutrient-poor sites. Since the litter layer is less buffered against environmental conditions than the organic and mineral soil, we also hypothesized that (3) the effects of forest type on the structure and functioning of microbial communities are more pronounced in litter than in soil.

Methods

Field sites

The study was conducted in 40 forest stands located in the south and north of Lower Saxony in Germany (eight sites x five forest types; Fig. S1). Eight sites were selected to cover a range of soil nutrients and water conditions based on prior knowledge of soil types and annual total rainfall. Four sites in the south stock on fertile soil and receive higher precipitation. Parental rock material is either loess influenced Triassic sandstone or Paleozoic shares of greywacke, sandstone, quartzite and phyllite, leading to soil types of partly podsolic Cambisol and Luvisol. Mean annual precipitation is 821 – 1029 mm (calculated from German weather service since 1981). The four northern sites are located on out-washed sand with the soil type of Podzol. The mean annual precipitation is 672 – 746 mm (Table S1). Each site (quintet) comprised three pure stands of European beech (*Fagus sylvatica* L.; Be), Douglas-fir (*Pseudotsuga menziesii* [Mirbel] Franco.; Do), Norway spruce (*Picea abies* [L.] Karst.; Sp) and two conifer-beech mixtures (Douglas-fir/European beech and Norway spruce/European beech; Do/Be and Sp/Be, respectively). On average trees were more than 50 years old in 2017. Within quintets, the distance between plots ranged from 76 m to 4600 m, and the distance of quintets ranged from 5 to 190 km. Within each stand, plots of 2500 m^2 were established, mostly in

137 rectangular shape (50 x 50 m). Tree species in pure stands comprised more than 90% of the total basal area,
138 and that in mixed stands 33 – 53% for beech and 53 – 60% for conifers.

139 Soil sampling and properties

140 Samples were taken in the eight quintets between November 2017 and January 2018. Soil cores of 5 cm
141 diameters were taken by a metal cylinder, and were separated into litter, 0 – 5 and 5 – 10 cm soil depth. In
142 each plot, three cores spaced by 5 m were taken. Samples from the same depth were pooled, resulting in a
143 total of 120 samples (40 plots × 3 depths). The soil was sieved through 2 mm mesh, and the litter was
144 fragmented into pieces using scissors (approximately < 25 mm^2) (Maraun and Scheu, 1995). Roots and
145 stones were removed. Samples were frozen at -20°C and thawed at 4°C before the analyses. The pH (KCl) of
146 litter and soil was determined using a ratio of sample to solution of 1:10, 1:5 and 1:2.5 (g/v) for litter, 0 – 5
147 and 5 – 10 cm soil, respectively. Dried soil (70°C, 48 h) was grinded in a ball mill and then total C and N
148 concentrations were determined by an elemental analyzer (NA 1110, CE-instruments, Rodano, Milano, Italy).

149 Microbial basal respiration and biomass.

150 Microbial respiration was measured by O_2 consumption using an automated micro-respirometer. The system
151 absorbs CO_2 produced by microorganisms using KOH solution, and the O_2 consumption by microorganisms is
152 replaced through electrolytic release of O_2 from $CuSO_4$ solution (Scheu, 1992). Readings were taken every
153 hour. Microbial basal respiration was measured as mean consumption of O_2 during 10 – 23 h after
154 attachment of the vessels to the respirometer system (BR; µg O_2 g^{-1} h^{-1}). Microbial biomass was determined
155 by substrate-induced respiration after the addition of glucose to saturate microbial glycolytic enzymes based
156 on the maximum initial respiratory response 4 – 7 h after the addition of glucose (*MIRR*; µl O_2 g^{-1} h^{-1}) and
157 converted to microbial biomass (C_{mic}; µg C_{mic} g^{-1}) as 38 × *MIRR* (Anderson and Domsch, 1978; Beck et al.,
158 1997). Fresh litter and soil from 0 – 5 and 5 – 10 cm depth equivalent to approximately 0.3, 1 and 3.5 g dry
159 mass was supplemented with 80, 20 and 8 mg glucose solved in 400 µl H_2O, respectively. Microbial specific
160 respiration was calculated as the ratio between microbial basal respiration and microbial biomass (qO_2; µg
161 O_2 μg^{-1} C_{mic} h^{-1}).

162 Phospholipid fatty acid analysis

163 To quantify the composition of phospholipid-derived fatty acid (PLFA) composition, lipids from leaf litter and
164 soil were extracted using a modified Bligh and Dyer method (Frostegard et al., 1993) as described in Pollierer
165 et al. (2015). Lipids were fractionated into glycolipids, phospholipids and neutral lipids by elution through
166 silica acid columns using chloroform, acetone and methanol, respectively (0.5 g silicic acid, 3 ml; HF BOND
167 ELUT-SI, Varian, Inc., Darmstadt, Germany). Phospholipids were subjected to mild alkaline methanolysis, and
168 fatty acid methyl esters (FAMEs) of phospholipids were identified by chromatographic retention time

compared to a general reference standard and a bacteria-derived standard (FAME CRM47885, C11 to C24; BAME 47080-U, C11 to C20; Sigma-Aldrich, Darmstadt, Germany) using a GC-FID Clarus 500 (PerkinElmer Corporation, Norwalk, USA) equipped with an Elite 5 column (30 m × 0.32 mm inner diameter, film thickness 0.25 µm). The temperature program started with 60 °C (hold time 1 min) and increased with 30 °C per min to 160 °C, and then by 3 °C per min to 280 °C. The injection temperature was 250 °C and helium was used as carrier gas. About 2 g of fresh litter and 4 g of fresh soil were used for the extraction.

Stress indicators and fatty acid markers

The ratio of cyclopropyl PLFAs to their monoenoic precursors (cy/pre) and the ratio of saturated to monounsaturated PLFAs (sat/mono) were used as indicators of physiological or nutritional stress ([(cy17:0 + cy19:0)/(16:1ω7 + 18:1ω7)]; [(14:0 + 15:0 + 16:0 + 17:0 + 18:0)/(16:1ω7 + 17:1 + 18:1ω9 + 18:1ω7)]; respectively). The ratio of Gram-positive to Gram-negative bacteria ($Gram^+$/ $Gram^-$) was used as indicator of carbon availability (Fanin et al., 2019; Moore-Kucera and Dick, 2008; Pollierer et al., 2015). Linoleic acid 18:2ω6,9 was used as fungal marker (Frostegård and Bååth, 1996). The saturated fatty acids i15:0, a15:0, i16:0, i17:0 were used as markers for $Gram^+$ bacteria, and the fatty acids cy17:0, cy19:0, 16:1ω7 and 18:1ω7 were assigned as markers for $Gram^-$ bacteria (Fanin et al., 2019; Frostegård and Bååth, 1996; Mitchell et al., 2010; Richter et al., 2018; Zelles, 1999). Bacterial biomass was represented by the sum of $Gram^+$ and $Gram^-$ bacteria. Total amount of PLFAs were summed up from all identified PLFAs (n = 40; nmol g^{-1} dry weight) and was used to calculate PLFA proportions. All stress indicators and FA markers were analyzed from proportions (mole percentage).

Statistical analyses

For univariate analysis, we first fitted linear mixed models (LMMs) to log-transformed response variables and then applied planned contrasts to estimate the effect size of forest type and site condition (Piovia-Scott et al., 2019). All LMMs included Forest type (European beech, Douglas-fir, Douglas-fir/European beech, Norway spruce, Norway spruce/European beech), Quintet group (nutrient-rich and nutrient-poor sites) and Depth (litter, 0 – 5 and 5 – 10 cm) as fixed effects. Backwards model selection was applied to full models, and minimal models included all main effects and the interaction of forest type and quintet group. The 40 forest plots were included as random effects to account for non-independence of samples of different depth from the same plot by forcing values from the same plot to have the same intercept (Harrison et al., 2018). In addition, the eight quintets were included as random effect depending on model selection (Zuur et al., 2009). To meet the assumption of homogeneity of variance, the mean - variance relationship was accounted for by a dispersion parameter according for depth or forest type. All model selections followed the Akaike Information Criterion (AIC) (Zuur et al., 2009).

201 Contrasts for the effect size of forest type were designed to compare coniferous and mixed forests to beech
202 forests. European beech, the climax tree species in lowland and lower montane regions of Central Europe,
203 was used as reference (Leuschner et al., 2017). Due to the property of log-transformed response variables,
204 the planned contrasts are analog to log response ratios (Piovia-Scott et al., 2019). Further, the log response
205 ratios were back transformed into response ratios and were used as effect size. Effect sizes of forest type
206 were estimated separately for nutrient-poor and nutrient-rich sites. Additionally, we applied contrasts
207 between nutrient-poor vs. nutrient-rich sites in a similar way for the effect size of quintet group. Univariate
208 response variables included microbial basal respiration, microbial biomass, microbial specific respiration,
209 stress indicators and PLFA markers.

210 For multivariate analysis of variance (MANOVA), PLFAs first were arcsine root transformed and then
211 dimensions of fatty acids were reduced by principal component analysis (PCA). Separate MANOVAs for each
212 sample depth were calculated to inspect for effects of Forest type, Quintet group and their interactions. Soil
213 properties (pH, C/N ratio) were included as covariates in multivariate analysis of covariance (MANCOVA)
214 (Langerhans et al., 2007). Significant principal components (PCs) were determined by broken stick criterion
215 (MacArthur, 1957). Only fatty acids ≥ 0.2% mean mole proportion were included in the analyses.

216 All analyses were done in R version 3.6.1 with R studio interface (V.1.2.5033; R Core Team 2019). We used
217 the "nlme" package to fit the linear mixed-effects models (Pinheiro 2019), and the "emmeans" package to
218 conduct planned contrasts (Lenth 2019). All mixed models met the assumptions of normality of residuals and
219 homogeneity of variance (Johnson and Omland, 2004). Raw data and codes will be uploaded to gfbio.org as
220 soon as the manuscript is accepted.

221 Results

222 Functional indicators of microorganisms

223 Forest type consistently affected microbial basal respiration, microbial biomass and stress indicators at
224 nutrient-poor but not at nutrient-rich sites (Forest type x Quintet group interactions; all $P < 0.083$; Table 1,
225 Figs 1 and 2), and this was generally true across the studied soil layers (Fig. S2). At nutrient-poor sites,
226 microbial basal respiration and microbial biomass was lower in coniferous and mixed forests than in the
227 beech forests. This was most pronounced in Douglas-fir forests where at nutrient-poor sites basal respiration
228 and microbial biomass were 65% and 59% lower compared to beech, respectively; similarly, basal respiration
229 and microbial biomass in the two conifer-beech mixtures were 46 – 56% lower than in pure beech. Microbial
230 basal respiration and microbial biomass in spruce forests were also lower than in beech forests, but the
231 reduction was less pronounced (-33 to -36%; Fig. 1). In addition, in Douglas-fir forests at the nutrient-poor
232 sites the microbial specific respiration was 19% lower compared to beech.

233 In contrast to basal respiration and microbial biomass, at nutrient-poor sites stress indicators, the cy/pre
234 ratio, the sat/mono ratio as well as the $Gram^+/Gram^-$ bacteria ratio, all were lower in beech forests than in
235 the other forest types (Fig. 2). The difference in the cy/pre and sat/mono ratio was most pronounced in
236 Douglas-fir (+87% and +74%) and less in spruce forests (+39% and +43%). Similar to spruce forest, the cy/pre
237 and sat/mono ratio in conifer-beech mixtures was on average 37 – 46% higher than in beech forests. Effects
238 of forest type on the $Gram^+/Gram^-$ bacteria ratio also was strongest in Douglas-fir forests (+46 %) and less
239 pronounced in the other forest types (+21 – 29%). Stress indicators generally did not differ at nutrient-rich
240 sites ($P > 0.31$; Fig. 2, Fig. S2, Table S2).

241 The response of microorganisms to changes in site conditions also varied between forest types. Differences
242 between nutrient-rich and nutrient-poor sites were most prominent in Douglas-fir forests and not significant
243 in beech forests (Figs 3 and 4, Table S3). At nutrient-poor sites, microbial basal respiration in Douglas-fir
244 forests was 57% lower and microbial biomass in Douglas-fir pure and mixed forests both were 47% lower
245 compared to nutrient-rich sites. Similarly, but less strong, microbial biomass in spruce mixed forests was 36%
246 lower at nutrient-poor sites than nutrient-rich sites. Conversely, in Douglas-fir forests the stress indicator
247 cy/pre ratio at nutrient-poor sites exceeded that of nutrient-rich sites by 82% (Fig. 4, Table S3). Similarly, in
248 Douglas-fir forests the sat/mono and $Gram^+/Gram^-$ bacteria ratio at nutrient-poor sites exceeded that at
249 nutrient-rich sites by 38 – 40%. Further, in Douglas-fir mixed forests the $Gram^+/Gram^-$ bacteria ratio at
250 nutrient-poor sites exceeded that at nutrient-poor sites by 34%.

251 ## Microbial community structure

252 As indicated by PLFA patterns, microbial community composition in beech forest differed from that in
253 Norway spruce and Douglas-fir forests, with mixed forests being intermediate between the respective single
254 species stands (Fig. 5). However, effects of forest type varied among soil layers and were most pronounced
255 in litter and 0 – 5 cm soil, but only marginally significant in 5 – 10 cm soil (Table 2). Despite strong turnover
256 between nutrient-rich and nutrient-poor sites (Quintet group effects $p < 0.001$), microbial community
257 structure in beech at nutrient-poor and nutrient-rich sites was similar, and this was most pronounced in 0 –
258 5 cm soil depth (Forest type × Quintet group interaction; Table 2, Fig. 5).

259 A number of fatty acids contributed to the separation of microbial communities of the different forest types
260 and quintet groups. Beech forests were characterized by unsaturated fatty acids at all soil depths, in
261 particular by 16:1ω7, 18:1ω7, 18:1ω9 (Fig. 5). Norway spruce forests were associated with long-chain
262 saturated fatty acids (22:0, 24:0), and Douglas-fir forests with bacterial marker fatty acids (2OH-14:0, cy17:0).
263 There also were clear differences in fatty acid patterns between nutrient-rich and nutrient-poor sites. Litter
264 at nutrient-rich sites was associated with unsaturated fatty acids such as 18:1ω9 and the fungal marker
265 18:2ω6,9. By contrast, nutrient-poor sites had higher saturated and branched fatty acids, with many of them

266 being bacterial markers, such as 14:0 and cy17:0 and cy19:0. Whereas the litter layer at nutrient-rich sites
267 was richer in fungi than at nutrient-poor sites, the opposite was true for the soil (Quintet group × Depth
268 interaction; Table 1, Figs S3 and S4).

269 As indicated by PLFA markers, the relative abundance of fungi and bacteria generally did not differ among
270 forest types, but consistently the relative abundance of fungi decreased, whereas the abundance of bacteria
271 increased with soil depth (Table 1, Fig. S3). Further, in Douglas-fir forests the abundance of fungi in soil was
272 lower than in beech forests and this was more pronounced in 5 – 10 cm soil compared to litter and 5 – 10 cm
273 soil at nutrient-poor sites (Fig. S4).

274 The northern nutrient-poor sites also were more acidic and more limited by nitrogen as indicated by lower
275 soil pH and higher C/N ratio, and the main effects of Quintet group and Forest type became non-significant
276 when pH and C/N ratio were included as covariates in the multivariate analysis in soil (Table 2, Fig. 5). In
277 addition, soil pH significantly explained the structure of microbial communities particularly in 0 – 5 cm depth
278 (Table 2).

279 Discussion

280 The functioning of microbial communities in single and mixed coniferous forests differed from those in
281 beech forests at nutrient-poor sites, but not at nutrient-rich sites. The surprising similar basal respiration,
282 microbial biomass and stress indicators at nutrient-rich sites partially contradicting our first hypothesis, and
283 suggest that at favorable environmental conditions soil microbial communities are rather irresponsive to
284 changes in tree species. By contrast, at nutrient-poor sites, microbial basal respiration, microbial biomass
285 and stress indicators were all detrimentally affected in coniferous and mixed forests, and the effects differed
286 between Douglas-fir and Norway spruce, with the effects being particularly strong in pure stands of Douglas-
287 fir but less pronounced in those of Norway spruce and mixed stands, partly supporting our second
288 hypothesis. Notably, microbial biomass and microbial stress indicators in beech forests did not differ
289 between nutrient-poor and nutrient-rich sites indicating that beech is able to maintain soil microbial
290 communities and their functioning regardless of site conditions. Admixture of beech in mixed stands with
291 Douglas-fir alleviated the detrimental effect of Douglas-fir on microbial biomass and microbial stress
292 indicators, but this was not the case in mixed stands of beech and Norway spruce, suggesting that the
293 consequences of admixture of beech to conifer plantations for microorganisms differ markedly between
294 conifer species, with beech reducing microbial stress in Douglas-fir but not in Norway spruce forests. Exotic
295 plants lack co-evolutionary history with native species and therefore, complementary effects between native
296 and exotic plants may be stronger and more likely to occur than between native species (Kawaletz et al.,

297 2013). This may also explain why alleviation of microbial stress in mixture with Douglas-fir than in those with
298 Norway spruce.

299 The site- and tree species-specific responses of microorganisms are likely caused by varying supply of energy
300 in the different forest types at nutrient-poor sites. In fact, the higher $Gram^+/Gram^-$ bacteria ratio in
301 coniferous and mixed forests at nutrient-poor sites suggests lower supply of labile carbon at nutrient-poor
302 compared to nutrient-rich sites (Fanin et al., 2019). Labile carbon compounds have been suggested to favor
303 $Gram^-$ bacteria, whereas $Gram^+$ bacteria better cope with recalcitrant carbon resources (Fanin et al., 2019;
304 Kramer and Gleixner, 2008; Waller et al., 2020). More pronounced carbon limitation in Douglas-fir was
305 further supported by its lower microbial specific respiration, which is largely due to its lower microbial basal
306 respiration compared to beech stands (Anderson and Domsch, 2010). More pronounced carbon limitation
307 has also been suggested to favor oligotrophic microbial communities characterized by low respiration rates
308 and biomass (Fanin et al., 2019; Potthast et al., 2010). This is consistent with our findings of stronger carbon
309 limitation as well as lower basal respiration and microbial biomass in single and mixed coniferous forests at
310 nutrient-poor sites. Moreover, the ratios of cy/pre and sat/mono were higher in pure species coniferous as
311 well as mixed forests at nutrient-poor compared to nutrient-rich sites, also indicating increased nutrient
312 (and/or water) stress in the former (Moore-Kucera and Dick, 2008; Pollierer et al., 2015). The fatty acids
313 16:1ω7 and 18:1ω7 are precursors of cyclopropyl fatty acids and as monounsaturated fatty acids, and they
314 form part of the nominator of the calculated stress indicators. The more pronounced stress response of
315 microorganism at nutrient-poor sites was largely caused by lower concentration of these two ω7 fatty acids
316 in coniferous and mixed forests. Indeed, it has been suggested that under stress conditions $Gram^-$ bacteria
317 change the composition of their cell membranes from 16:1ω7 and 18:1ω7 to cyclopropane fatty acids
318 associated with slower growth rates (Guckert et al., 1986; Kieft et al., 1994; Lundquist et al., 1999). The
319 similarity of microbial indicators in beech forests at nutrient-rich and nutrient-poor sites indicates that,
320 compared to coniferous stands, beech maintained microbial community structure and functioning and
321 avoided stress conditions of microorganisms by providing more labile carbon resources at nutrient-poor
322 conditions, presumably by releasing high amounts of root-derived resources.

323 The conclusion that beech is able to alleviate microbial stress via maintaining or even increasing the release
324 of root-derived resources at nutrient-poor sites is supported by the following findings. First, recent studies at
325 our study sites showed that at nutrient-poor sites the density of fine roots is higher in beech forests than in
326 pure and mixed coniferous forests (diameter < 2 mm; pers. comm. A. Lwila). Generally, it is well established
327 that plants allocate more resources into roots if the availability of soil nutrients is declining and this is
328 associated with increased root exudations (Eisenhauer et al., 2017; Laliberté et al., 2017). Recently, this also
329 was confirmed for beech forests documenting increased root exudation at more acidic and nitrogen

330 deficient stands (Meier et al., 2020). These findings support our conclusion that beech indeed maintained or
331 even increased its root exudates at nutrient-poor sites of our study which in fact were characterized by more
332 acidic conditions and higher C/N ratio in soil, and this alleviated microbial resource deficiency and stress.
333 Second, trenching and girdling experiments confirmed that reducing or omitting the flux of root-derived
334 resources into the soil strongly reduces fungal and bacterial biomass, demonstrating the importance of root-
335 derived resources for maintaining soil microbial biomass (Bluhm et al., 2019; Högberg et al., 2007; Kaiser et
336 al., 2010). Notably, we sampled litter and soil during autumn / winter after beech had shed its leaves.
337 Nevertheless, the evidence provided above suggests that the impact of roots on microorganisms still was
338 strong indicating that not only recent photosynthates but also stored carbohydrates contribute to providing
339 root-derived resources to soil microorganisms (Druebert et al., 2009). Further, these findings suggest that
340 beech has the potential to facilitate soil microorganisms at environmental stress conditions by maintaining
341 or even increasing its root exudates contrasting both of the studied conifer species. This ability presumably
342 contributed to the mitigation of the detrimental effects of Douglas-fir on microorganisms at nutrient-poor /
343 acidic sites in mixed forests. More broadly, it may even explain the observed overyielding in mixed stands of
344 beech and conifers at nutrient-poor sites (Ammer, 2019; Toïgo et al., 2015).

345 Contrary to our third hypothesis, forest type did not affect microorganisms stronger in litter than in soil. At
346 nutrient-poor sites, forest type affected microbial basal respiration, microbial biomass and microbial stress
347 in both litter and soil, with the strength of effects generally being similar in litter and soil. This supports our
348 conclusion that the effect of forest type was not mainly due to differences in litter quantity and quality, but
349 in particular to differences in root-derived resources. This contrasts earlier suggestions that microorganisms
350 in litter are more sensitive to changes in environmental conditions than those in soil because litter is less
351 buffered against environmental harshness (Pollierer et al., 2015). Supporting our conclusion that
352 microorganisms in soil sensitively respond to environmental changes, microbial stress indicators generally
353 increased while microbial biomass generally decreased with soil depth. This sensitivity presumably is related
354 to the fact that carbon availability generally decreases with soil depth resulting in stronger resource
355 limitation (Fierer et al., 2003).

356 We assumed that microbial functioning ultimately relies on its microbial community structure, and this is
357 supported by the consistent turnover in microbial community composition as well as the change in microbial
358 functions between nutrient-rich and nutrient-poor sites. Further, in contrast to coniferous and mixed forests,
359 microbial community structure and functional indices in beech forests differed little between nutrient-rich
360 and nutrient-poor sites, suggesting that among the tree species studied only beech is better able to maintain
361 and stabilize microbial community structure and functioning in litter and soil irrespective of site conditions.
362 Notably, differences in microbial community structure between forest types at nutrient-rich sites tended to

be small in 5 – 10 cm soil depth, further suggesting that the influence of tree species fades deeper in soil. By contrast, at nutrient-poor sites, effects of forest type were relatively strong in soil compared to that at nutrient-rich sites, again supporting our conclusion that at nutrient-poor sites effects of tree species on microorganisms in soil are predominantly due to differences in root-derived resources. Interestingly, the differences in microbial community structure between beech and coniferous forests in 5 – 10 cm soil depth at nutrient-poor sites were most pronounced in Douglas-fir and carbon limitation also was most prominent in 5 – 10 cm soil of Douglas-fir forests as indicated by a lower microbial specific respiration. This implies that among the tree species studied Douglas-fir provides the lowest amount of root-derived resources to soil microorganisms at nutrient-poor sites resulting in pronounced resource shortage and marked changes in microbial community composition. Such changes in microbial community structure may significantly impact the functioning of soil microbial communities as the availability of carbon resources and carbon limitation affect the vertical distribution of saprotrophic fungi and decomposition processes, e.g. by shifting the competition between saprotrophic and mycorrhizal fungi (Gadgil and Gadgil, 1971; Lindahl et al., 2007).

Interestingly, differences in microbial community composition were more pronounced between sites than among forest types. As indicated by the fungal marker FA 18:2ω6,9, the abundance of fungi in spruce and beech forests at nutrient-poor sites was higher than at nutrient-rich sites supporting earlier suggestions that the fungal energy channel dominates in low fertile systems (Wardle et al., 2004). However, in contrast to beech and spruce, the abundance of fungi in pure and mixed forests of Douglas-fir was not higher at nutrient-poor sites compared to nutrient-rich sites. Further, at nutrient-poor sites the abundance of fungi was lower in Douglas-fir than in beech forests and this was most pronounced in 5 – 10 cm depth. Potentially, the lower abundance of fungi in soil of Douglas-fir forests is due to a mismatch between local and home (native range of Douglas-fir) fungal communities (Schmid et al., 2014). In fact, in soil of introduced plant species only a subset of fungal species present in their native range has been recorded in soil of sites where they had been introduced (Schmid et al., 2014). Future studies need to examine in more detail the taxonomic composition of microbial communities in different forest types, including introduced and native tree species such as Douglas-fir and beech in particular at nutrient-poor sites deeper in soil.

In addition to quality and quantity of litter- and root-derived resources, tree species may affect microbial community structure in soil by modifying chemical soil properties. In our study, soil properties, especially pH, well explained changes in the community structure of soil microorganisms. These findings are consistent with results of continental scale studies as well as local liming experiments suggesting that soil pH is among the most prominent driver of microbial community composition (Fierer and Jackson, 2006; Frostegård et al., 1993). Further, soil pH generally correlates negatively with soil nutrient availability, supporting the importance of nutrients in the mediation of microbial responses (Meier et al., 2020). However, factors other

than soil pH and soil nutrient status may have contributed to the observed differences between nutrient-rich and nutrient-poor sites. In fact, nutrient-poor sites also were characterized by lower precipitation and this is reflected by higher $\delta^{13}C$ values (Peuke et al., 2006) of litter at nutrient-poor compared to nutrient-rich sites, potentially resulted from increased water stress at nutrient-poor compared to nutrient-rich sites (J. Lu unpubl. data). Presumably, in addition to pH, both nutrients and water stress contributed to the observed differences in microbial community composition and functioning between nutrient-poor and nutrient-rich sites. This is supported by the fact that soil water conditions are intricately linked to soil nutrient dynamics and the uptake of nutrients by plants (de Vries et al., 2019). Future studies need to disentangle the relative importance of water deficiency, nutrient availability and soil pH as driving factors of the interrelationship between tree species and soil microbial community composition and functioning. Focusing on nutrient-poor / low pH stands is most promising for resolving these interrelationships.

Conclusion

The present study documented that effects of forest type on soil microbial communities vary among tree species and mixed stands, but this strongly depends on the nutrient status of the sites. Notably, variations between sites were least pronounced in native beech and most pronounced in introduced Douglas-fir. Compared to beech, Douglas-fir strongly impacted microbial community structure and functioning at nutrient-poor sites. Therefore, in the long-term planting non-native tree species such as Douglas-fir may compromise ecosystem functioning on nutrient-poor sites, and this needs to be studied in more detail as it may not only question the establishment of pure Douglas-fir forests, but also the enrichment of beech forests by Douglas-fir at nutrient-poor sites. However, as planting Douglas-fir in single species and mixed stands with beech hardly impacted the structure and functioning of soil microbial communities at nutrient-rich sites, planting Douglas-fir may provide an alternative option to Norway spruce at least at nutrient-rich sites. Such options are urgently needed since Norway spruce forests are increasingly threatened due to global climate change and associated outbreaks of bark beetles especially in lowland regions of Europe. However, more information is needed for gaining a holistic understanding on the consequences of planting single species or mixed stands of Douglas-fir on ecosystem properties and processes, such as below- and aboveground biodiversity of other taxa, and the decomposition of litter. Future studies need to focus on potential facilitation or inhibition of planting mixed forests of beech and Douglas-fir on soil microorganisms and timber yield in particular at nutrient-poor sites. Overall, our findings highlight that tree species differently shape the structure and functioning of soil microbial communities with their effects vary strongly with soil conditions.

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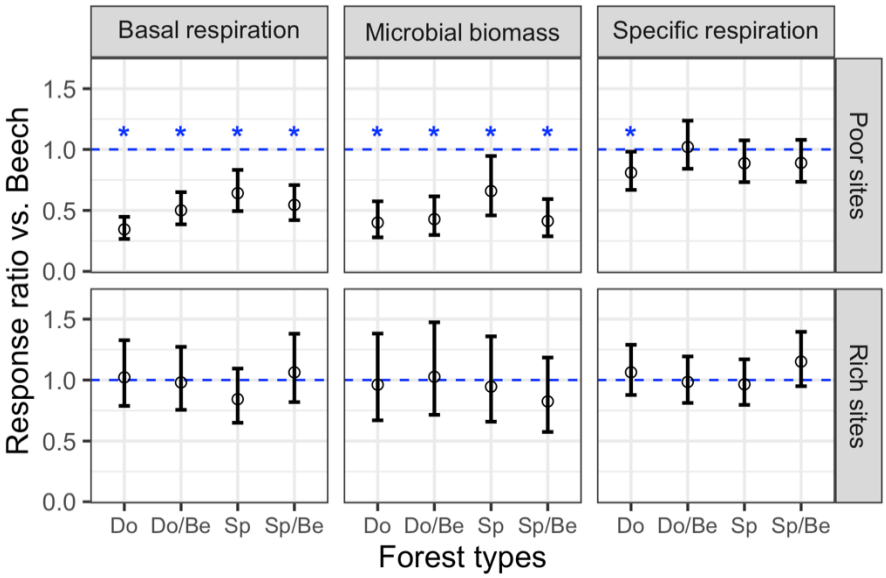
655 Figure 1



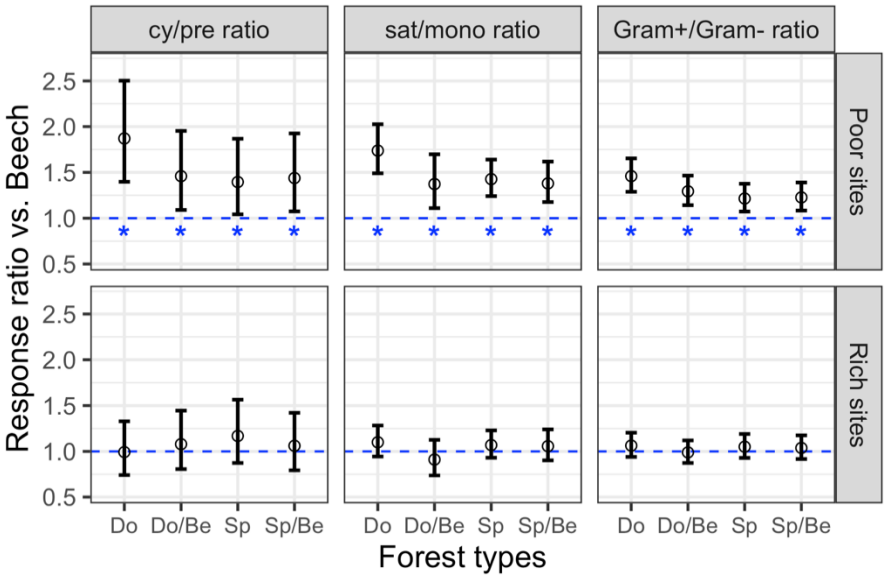
656

657 Figure 1. Effects of conifers and conifer-beech mixtures (Douglas-fir [Do], Douglas-fir with beech [Do/Be],
658 Norway spruce [Sp] and Norway spruce with beech [Sp/Be]) on microbial basal respiration (µg O_2 g^{-1} C h^{-1}),
659 microbial biomass (µg C_{mic} g^{-1} C) and microbial specific respiration (µg O_2 $µg^{-1}$ C_{mic} h^{-1}) at nutrient-poor and
660 nutrient-rich sites. Effect sizes are given as back transformed log response ratios compared to beech forests
661 [ln (value in coniferous or mixed forest types / values in beech)]. Effect sizes were averaged across three
662 depths (litter, 0–5 cm and 5–10 cm soil). Values smaller than 1 indicate higher values in beech. Asterisks
663 indicate significant effects ($p < 0.05$). Bars represent 95% confidence intervals (n=12).

664

665

666 Figure 2

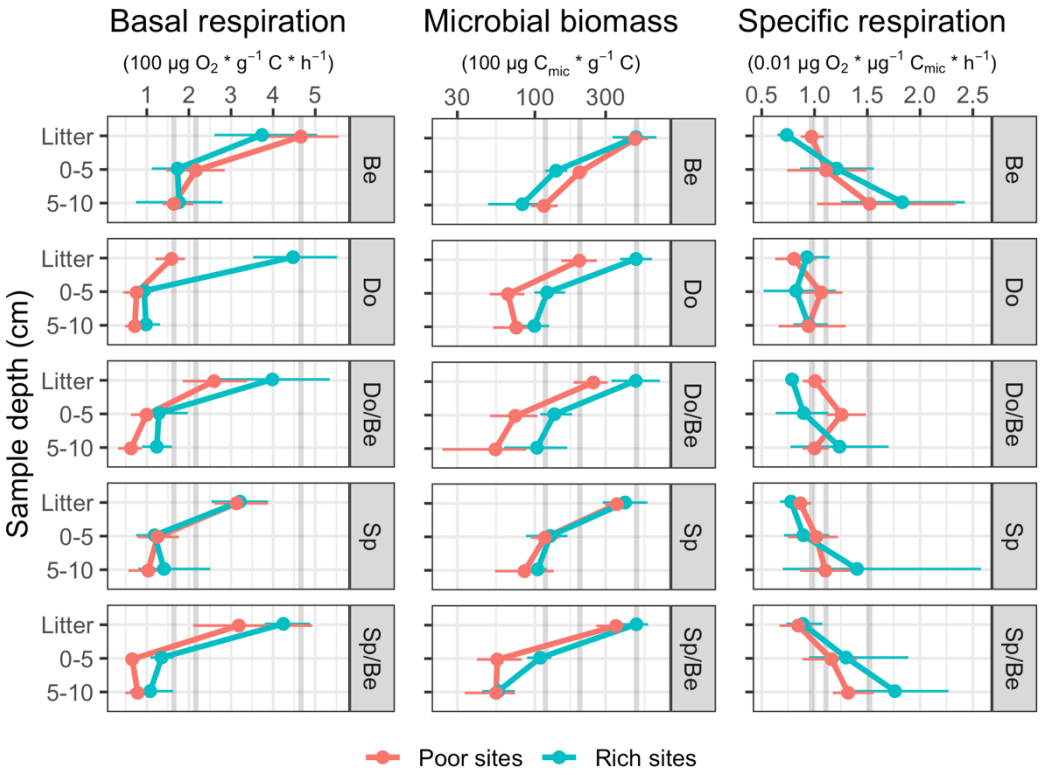


667

668 Figure 2. Effects of conifers and conifer-beech mixtures (Douglas-fir [Do], Douglas-fir with beech [Do/Be],
669 Norway spruce [Sp] and Norway spruce with beech [Sp/Be]) on stress indicators: ratio of cyclopropyl PLFAs
670 to its monoenoic precursors, ratio of saturated to monounsaturated PLFAs, and ratio of Gram positive to
671 Gram negative bacteria (Cy/pre, SAT/MONO and Gram+/Gram-, respectively) at nutrient-poor and nutrient-
672 rich sites. Effect sizes are given as back transformed log response ratios compared to beech forests [ln (value
673 in coniferous or mixed forest types / values in beech)]. Effect sizes were averaged across three depths (litter,
674 0–5 cm and 5–10 cm soil). Values larger than 1 indicate lower values in beech. Asterisks indicate significant
675 effects ($p < 0.05$). Bars represent 95% confidence intervals (n=12).

676

677 Figure 3

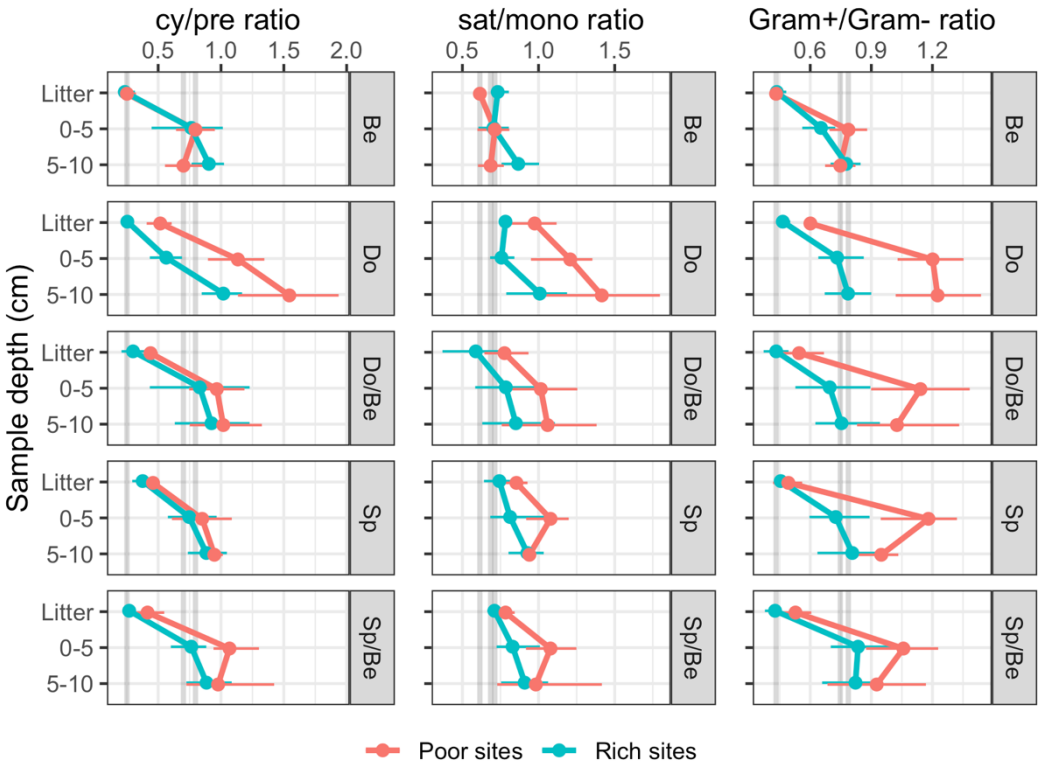


678

679 Figure 3. Changes in microbial basal respiration (µg O_2 g^{-1} C h^{-1}), microbial biomass (µg C_{mic} g^{-1} C) and
680 microbial specific respiration (µg O_2 $µg^{-1}$ C_{mic} h^{-1}) with soil depth (litter and 0–5 cm and 5–10 cm soil) in five
681 forest types (European beech [Be], Douglas-fir [Do], Douglas-fir with beech [Do/Be], Norway spruce [Sp] and
682 Norway spruce with beech [Sp/Be]) at nutrient-poor and nutrient-rich sites. Points and horizontal bars
683 represent means and standard errors (n=4). The grey vertical bars represent respective values in beech
684 forests at nutrient-poor sites in litter, 0–5 cm and 5–10 cm soil depth. Note log scale for microbial biomass.

685

686 Figure 4



687

688 Figure 4. Changes in stress indicators (ratio of cyclopropyl fatty acids to its precursors, ratio of saturated to
689 monounsaturated fatty acids and ratio of Gram-positive to Gram-negative bacteria [Cy/pre, SAT/MONO and
690 Gram+/Gram-, respectively]) with soil depth (litter and 0–5 cm and 5–10 cm soil) in five forest types
691 (European beech [Be], Douglas-fir [Do], Douglas-fir with beech [Do/Be], Norway spruce [Sp] and Norway
692 spruce with beech [Sp/Be]) at nutrient-poor and nutrient-rich sites. Points and horizontal bars represent
693 means and standard errors (n=4). The grey vertical bars represent respective values in beech forests at
694 nutrient-poor sites in litter, 0–5 cm and 5–10 cm depth.

695

696

Figure 5

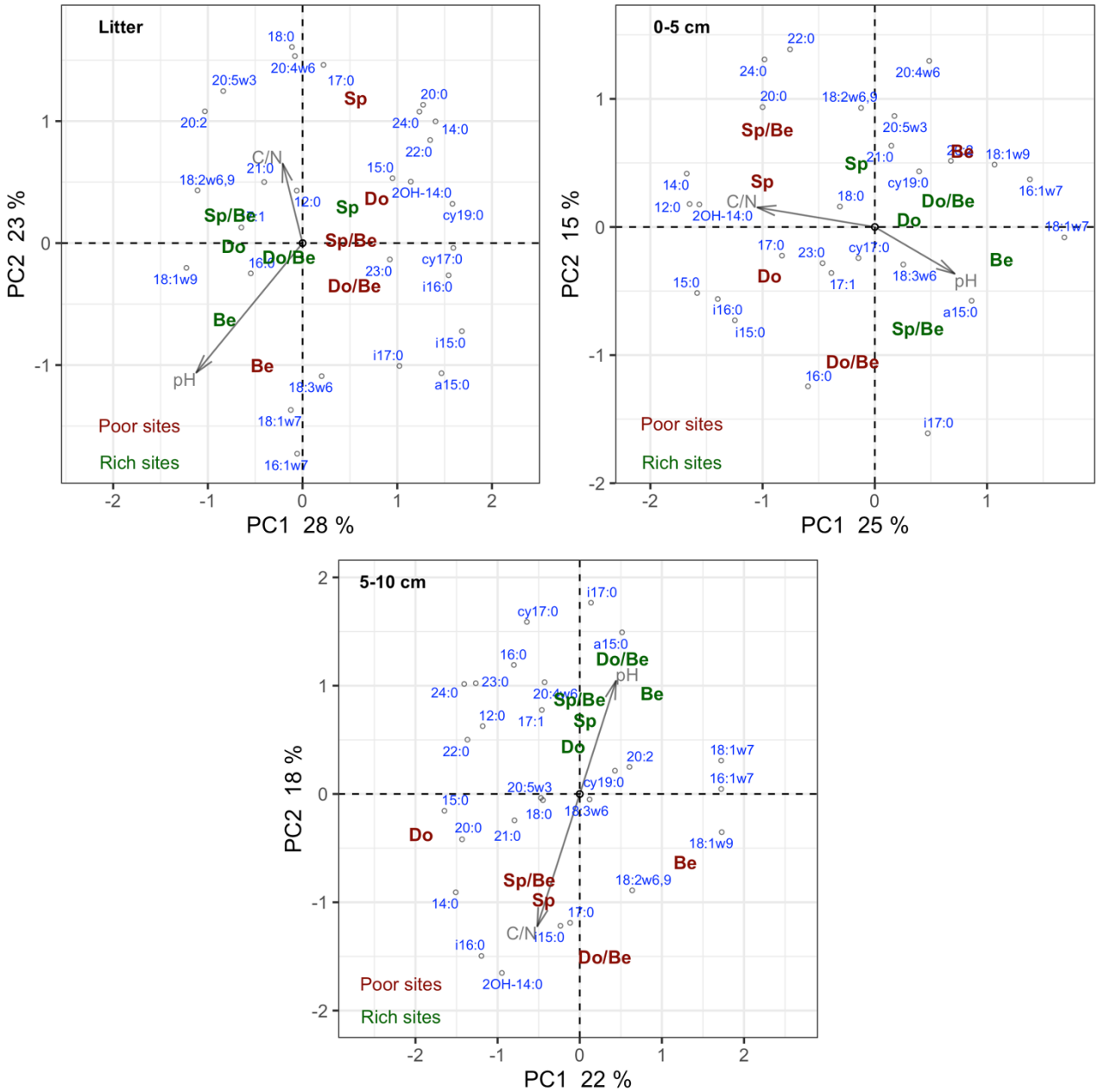


Figure 5. Principal component analysis of the microbial community structure (as indicated by phospholipid-derived fatty acids) in five forest types (European beech [Be], Douglas-fir [Do], Douglas-fir with beech [Do/Be], Norway spruce [Sp] and Norway spruce with beech [Sp/Be]) at nutrient-poor and nutrient-rich sites in litter, 0–5 cm and 5–10 cm soil depth. Positions of forest types represent centroids (n=4). Nutrient-poor sites are given in red and nutrient-rich sites in green. C/N and pH were post-fitted into the plot.

Table 1. F- and P-values of linear mixed effects models on the effect of Forest type (European beech, Douglas-fir, Norway spruce, mixture of European beech with Douglas-fir and mixture of European beech with Norway spruce), Quintet group (nutrient-poor and nutrient-rich sites) and Depth (litter, 0–5 cm and 5–10 cm) on (a) basal respiration, microbial biomass and microbial specific respiration, (b) stress indicators (Cy/pre, SAT/MONO, $Gram^+/Gram^-$ ratios) and (c) percentages of fungal, bacteria, and fungi/bacteria ratio. Significant effects are given in bold ($p < 0.05$).

(a)	Basal respiration			Microbial biomass			Specific respiration		
Factor	df	F	P	df	F	P	df	F	P
Forest type (F)	4,24	7.51	**<0.001**	4,30	6.03	**0.001**	4,24	0.92	0.47
Quintet group (Q)	1,6	3.27	0.12	1,30	16.06	**<0.001**	1,6	0.52	0.49
Depth (D)	2,78	181.58	**<0.001**	2,78	231.82	**<0.001**	2,78	17.37	**<0.001**
F × Q	4,24	8.83	**<0.001**	4,30	4.54	**0.006**	4,24	2.36	0.08
(b)	cy/pre ratio			sat/mono ratio			$Gram^+/Gram^-$ ratio		
Factor	df	F	P	df	F	P	df	F	P
Forest type (F)	4,24	2.75	0.052	4,24	8.278	**<0.001**	4,24	3.39	**<0.001**
Quintet group (Q)	1,6	3.48	0.11	1,6	0.195	0.67	1,6	8.17	**0.030**
Depth (D)	2,68	238.82	**<0.001**	2,76	5.551	**0.006**	2,76	228.51	**<0.001**
F × Q	4,24	2.70	0.055	4,24	3.650	**0.019**	4,24	2.27	**0.012**
Q × D	2,68	6.20	**0.003**	2,76	10.71	**<0.001**	2,76	7.51	**0.001**
F × D	8,68	3.72	**0.001**	-	-	-	-	-	-
(c)	% Fungi			% Bacteria			Fungi/Bacteria ratio		
Factor	df	F	P	df	F	P	df	F	P
Forest type (F)	4,30	1.77	0.16	4,30	2.19	0.094	4,24	0.83	0.52
Quintet group (Q)	1,30	6.46	**0.02**	1,30	4.34	**0.046**	1,6	4.98	0.07
Depth (D)	2,68	982.11	**<0.001**	2,78	149.47	**<0.001**	2,78	623.37	**<0.001**
F × Q	4,30	1.99	0.12	4,30	0.733	0.58	4,24	1.60	0.21
Q × D	2,68	3.61	**0.002**	-	-	-	-	-	-
F × D	8,68	18.54	**<0.001**	-	-	-	-	-	-

Table 2. Results of two-way MANOVAs of PLFA composition in litter, 0–5 cm and 5–10 cm soil. Factors include Forest type (European beech, Douglas-fir, Norway spruce and mixture of European beech with Douglas-fir and mixture of European beech with Norway spruce), Quintet group (nutrient-rich and nutrient-poor sites) and their interactions. MANOVA was based on principal components from each sample depth (significant PCs were determined by broken stick criterion; see Methods). Type I sum of square was used for models without soil parameters, and type II sum of squares for those with soil parameters. Significant effects are given in bold ($p < 0.05$).

		Without soil parameters				With soil parameters			
Soil depths	Factors	df	Wilk's lambda	F	P	df	Wilk's lambda	F	P
Litter	Forest type (F)	4,30	0.32	3.29	**0.001**	4,28	0.42	2.23	**0.019**
	Quintet group (Q)	1,30	0.42	12.77	**<0.001**	1,28	0.61	5.57	**0.004**
	F × Q	4,30	0.73	0.77	0.677	4,28	0.74	0.69	0.757
	pH	-	-	-	-	1,28	0.75	2.85	0.057
	C/N	-	-	-	-	1,28	0.82	1.95	0.146
0 – 5 cm	Forest type (F)	4,30	0.19	2.80	**0.001**	4,28	0.34	1.57	0.081
	Quintet group (Q)	1,30	0.39	8.14	**<0.001**	1,28	0.80	1.18	0.350
	F × Q	4,30	0.36	1.57	0.078	4,28	0.31	1.68	0.054
	pH	-	-	-	-	1,28	0.47	5.41	**0.002**
	C/N	-	-	-	-	1,28	0.66	2.44	0.063
5 – 10 cm	Forest type (F)	4,30	0.41	1.76	0.051	4,28	0.45	1.45	0.142
	Quintet group (Q)	1,30	0.45	8.15	**<0.001**	1,28	0.82	1.34	0.285
	F × Q	4,30	0.69	0.68	0.805	4,28	0.74	0.51	0.936
	pH	-	-	-	-	1,28	0.84	1.22	0.326
	C/N	-	-	-	-	1,28	0.75	2.08	0.114