Running title: Forest type effects on microbial communities

Title: Mixing coniferous and deciduous trees (*Fagus sylvatica*): Site-specific response of soil microorganisms

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Abstract

Coniferous and deciduous trees growing in pure and mixed forests supply different resources to soil microorganisms thereby shaping microbial communities. However, effects of different forest types including non-native species on microbial communities and their variation with site conditions such as soil type and nutritional status remain poorly understood. We quantified functional (substrate-induced respiration and stress indicators) and structural (phospholipid-derived fatty acids) responses of microbial communities to forest types at four nutrient-rich and four nutrient-poor sites. Five types of forests were investigated at each site, including pure stands of native European beech (*Fagus sylvatica*), range-expanding Norway spruce (*Picea abies*) and non-native Douglas-fir (*Pseudotsuga menziesii*), as well as mixed species stands of beech with spruce or with Douglas-fir, i.e., 40 forest plots. Forest type only affected soil microorganisms at nutrient-poor sites, but not at nutrient-rich sites. At nutrient-poor sites, both pure and mixed coniferous forests, in particular pure Douglas-fir forests, detrimentally affected soil microorganisms compared to beech forests. By contrast, structural and functional indicators of microorganisms in beech forests at nutrient-poor sites resembled those at nutrient-rich sites, likely because beech maintained the provisioning of high amounts of root-derived resources. The results indicate that long-term effects of planting exotic tree species such as Douglas-fir either in pure or in mixed stands on ecosystem functioning need further attention especially at nutrient-poor sites, but planting Douglas-fir at nutrient-rich sites appear of little concern from the perspective of microbial communities. Overall, the results highlight the potential importance of root-derived resources in determining microbial community structure and functioning, and document the sensitivity of soil microorganisms to planting non-native tree species that may differ in the provisioning of these resources from native species.

1. Introduction

Studying the linkage between plants and soil microorganisms has great potential to improve our understanding of the factors that drive microbial community composition (Balvanera et al., 2006; van der Heijden et al., 2008; Anderson and Domsch, 2010; Bardgett and van der Putten, 2014; Graham et al., 2016). Trees as long-lived plants may affect microbial communities through several pathways, most notably by the quantity and quality of litter and root exudates (Wardle et al., 2004; Högberg and Read, 2006). 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; Fierer et al., 2003; Fanin et al., 2019). For example, Gram$^-$ bacteria are benefiting in particular from labile carbon compounds, whereas Gram$^+$ bacteria are adapted to use more recalcitrant carbon resources (Fierer et al., 2003; Fanin et al., 2019; 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). Therefore, 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).

Conifers and deciduous trees growing in pure and mixed stands likely differ in the supply of resources to soil microorganisms. Accumulation of organic matter in the floor of coniferous forests has been attributed to the recalcitrance of conifer needles, but roots and mycorrhizal fungi also affect the storage of soil carbon (Schmidt et al., 2011; Clemmensen et al., 2013; Averill et al., 2014). Below-ground processes, such as root exudation and the competition of saprotrophic and mycorrhizal fungi, maybe more important for structuring microbial communities than the amount and quality of above-ground litter (Scheu et al., 2003; Pollierer et al., 2007; Schmidt et al., 2011; Augusto et al., 2015; Wang et al., 2016; Bluhm et al., 2019; Jones et al., 2019). Further, the response of microbial communities to mixed forests is poorly understood. Litter mixture may improve resource availability for soil microorganisms and facilitate litter decomposition (Hättenschwiler et al., 2005; Fujii et al., 2020), although tree identity has been suggested to outweigh tree diversity as structuring force of soil microbial communities (Scheibe et al., 2015; Dawud et al., 2016; L. Chen et al., 2019). Mixing of tree species has also been shown to increase both above-ground and below-ground biomass due to interspecific facilitation and improved resource partitioning, resulting in higher complementarity in space and time in mixed forests (Cardinale et al., 2007; Ma and Chen, 2016; Emmett Duffy et al., 2017). These interactions likely affect the way forests shaping soil microbial communities. Overall, however, the role of identity and diversity of tree species, either coniferous or deciduous, in shaping the structure and functioning of soil microorganisms is little studied.

The relationship between tree species and microbial communities strongly depends on environmental context (Ratcliffe et al., 2017; X. Chen et al., 2019), but little is known to what extent effects of tree species composition on soil microorganisms vary with site conditions, such as soil type and nutritional status. Facilitation among plants is more pronounced in nutrient-limited environments according to the stress-gradient hypothesis, and such positive interspecific interactions at nutrient-poor conditions are likely to be mediated by soil microorganisms (Defossez et al., 2011; Harpole et al., 2016; David et al., 2020). In fact, plants do not passively tolerate environmental stress, but adapt in various ways to unfavorable growth conditions by induced plasticity (Callaway et al., 2003; de Vries et al., 2019). As a trade-off, plants generally allocate more resources to roots than to shoots under nutrient-poor conditions (Kawaletz et al., 2013; Yan et al., 2016), and this likely modifies soil microbial communities. Indeed, de Vries et al. (2019) recently showed that under drought conditions herb species change the quality rather than quantity of root exudates and induce higher microbial respiration, 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.

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 one or a few species (Knoke et al., 2008; Hu et al., 2009; Bauhus et al., 2010). While Norway spruce currently is the most popular high-yielding timber species in Central Europe, in recent years it has have been severely damaged by extreme conditions of weather and the outbreak of bark beetles, and such events are predicted to occur more frequently and at higher severity in the future (Biedermann et al., 2019; Brandl et al., 2020; Pettit et al., 2020). For reducing the risk of damage and maintaining economic gains, planting of mixed forests including non-native species such as Douglas-fir is increasingly discussed (Pretzsch et al., 2017; Ammer et al., 2018). To date, 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 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 forest types 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 (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 understand the response of microorganisms to planting different tree species in single and mixed stands, physiological (respiration based) and community approaches (phospholipid fatty acid analysis) were combined. We quantified microbial basal respiration, microbial biomass, and community structure as well as 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.

2. Methods

2.1. 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; Glatthorn 2017). 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 rectangular shape (50 x 50 m). Tree species in pure stands comprised more than 90% of the total basal area, and that in mixed stands 33 – 53% for beech and 53 – 60% for conifers.

2.2. Soil sampling and properties

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

2.3. Microbial basal respiration and biomass.

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

2.4. Phospholipid fatty acid analysis

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

2.5. 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) (Moore-Kucera and Dick, 2008; Pollierer et al., 2015). The ratio of $Gram^+$ to $Gram^-$ bacteria was used as indicator of carbon availability (Fanin et al., 2019). 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 (Frostegård and Bååth, 1996; Zelles, 1999; Mitchell et al., 2010; Richter et al., 2018; Fanin et al., 2019). 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).

2.6. 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 quintet group (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). Based on Akaike Information Criterion (AIC), the eight quintets were included as random effect, and the mean - variance relationship was also accounted for by a dispersion parameter to meet the assumption of homogeneity of variance (Johnson and Omland, 2004; Zuur et al., 2009). Further, fixed effects were selected based on likelihood ratio test.

Contrasts for the effect size of forest type were designed to compare coniferous and mixed forests to beech forests. European beech, the climax tree species in lowland and lower montane regions of Central Europe, was used as reference (Leuschner et al., 2017). Due to the property of log-transformed response variables,

198 the planned contrasts are analog to log response ratios (Piovia-Scott et al., 2019). Further, the log response
199 ratios were back transformed into response ratios and were used as effect size. Effect sizes of forest type
200 were estimated for nutrient-poor and nutrient-rich sites. Additionally, we applied contrasts between
201 nutrient-poor vs. nutrient-rich sites in a similar way for the effect size of quintet group. Univariate response
202 variables included microbial basal respiration, microbial biomass, microbial specific respiration, stress
203 indicators and PLFA markers.

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

210 All analyses were done in R with RStudio interface (R Core Team, 2019; RStudio Team, 2019). We used the
211 "nlme" package to fit the linear mixed-effects models (Pinheiro et al., 2019), and the "emmeans" package to
212 conduct planned contrasts (Lenth, 2020). All mixed models met the assumptions of normality of residuals
213 and homogeneity of variance.

214 3. Results

215 3.1. Functional indicators of microorganisms

216 Forest type consistently affected microbial basal respiration, microbial biomass and stress indicators at
217 nutrient-poor but not at nutrient-rich sites (Forest type x Quintet group interactions; all $P < 0.08$; Table 1,
218 Figs 1 and 2), and this was generally true across the studied soil layers (Fig. S2). At nutrient-poor sites,
219 microbial basal respiration and microbial biomass was lower in coniferous and mixed forests than in beech
220 forests. This was most pronounced in Douglas-fir, where at nutrient-poor sites basal respiration and
221 microbial biomass were 65% and 59% lower compared to pure beech, respectively; similarly, basal
222 respiration and microbial biomass in the two conifer-beech mixtures were 46 – 56% lower than in beech.
223 Microbial basal respiration and microbial biomass in spruce forests were also lower than in beech forests,
224 but the difference was less pronounced (-33 to -36%; Fig. 1). In addition, in Douglas-fir forests at the
225 nutrient-poor sites microbial specific respiration was 19% lower compared to beech.

226 In contrast to basal respiration and microbial biomass, at nutrient-poor sites stress indicators, the cy/pre
227 ratio, the sat/mono ratio as well as the $Gram^+/Gram^-$ bacteria ratio, all were lower in beech forests than in
228 the other forest types (Fig. 2). The difference in the cy/pre and sat/mono ratio was most pronounced in
229 Douglas-fir (+87% and +74%) and less in spruce forests (+39% and +43%). Similar to spruce forest, the cy/pre

230 and sat/mono ratio in conifer-beech mixtures was on average 37 – 46% higher than in beech forests. Effects
231 of forest type on the $Gram^+/Gram^-$ bacteria ratio also was strongest in Douglas-fir forests (+46 %) and less
232 pronounced in the other forest types (+21 – 29%). Stress indicators generally did not differ at nutrient-rich
233 sites ($P > 0.31$; Fig. 2, Fig. S2, Table S2).

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

244 3.2. Microbial community structure

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

252 A number of fatty acids contributed to the separation of microbial communities of the different forest types
253 and quintet groups. Beech forests were characterized by unsaturated fatty acids at all soil depths, in
254 particular by 16:1ω7, 18:1ω7, 18:1ω9 (Fig. 5). Norway spruce forests were associated with long-chain
255 saturated fatty acids (22:0, 24:0), and Douglas-fir forests with bacterial marker fatty acids (2OH-14:0, cy17:0).
256 There also were clear differences in fatty acid patterns between nutrient-rich and nutrient-poor sites. Litter
257 at nutrient-rich sites was associated with unsaturated fatty acids such as 18:1ω9 and the fungal marker
258 18:2ω6,9. By contrast, nutrient-poor sites had higher saturated and branched fatty acids, with many of them
259 being bacterial markers, such as 14:0 and cy17:0 and cy19:0. Whereas the litter layer at nutrient-rich sites
260 was richer in fungi than at nutrient-poor sites, the opposite was true for the soil (Quintet group × Depth
261 interaction; Table 1, Figs S3 and S4).

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

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

4. Discussion

Overall, the results only in part support our first and second hypotheses. Forest types only affected the functioning of microbial communities at nutrient-poor sites, but not at nutrient-rich sites. At nutrient-poor sites, microbial basal respiration, microbial biomass and stress indicators all were detrimentally affected in pure and mixed coniferous forests, and, contrasting our first hypothesis the effects differed between Douglas-fir and Norway spruce, with the effects being particularly strong in Douglas-fir, but less pronounced in Norway spruce and mixed stands. By contrast, similar basal respiration, microbial biomass and stress indicators at nutrient-rich sites across the studied forest types contradict our second hypothesis and suggest that, at favorable environmental conditions, soil microbial communities are rather irresponsive to changes in tree species. Notably, microbial biomass and microbial stress indicators in beech forests did not differ between nutrient-poor and nutrient-rich sites indicating that beech is able to maintain the functioning of soil microbial communities regardless of site conditions. Admixture of beech in mixed stands with Douglas-fir or Norway spruce both detrimentally affected microbial biomass and microbial stress indicators, suggesting that caution is needed when mixing beech with conifers. However, results of the present study also indicate that compared to pure Douglas-fir forests, mixed Douglas-fir forests with beech mitigate the detrimental effects of pure Douglas-fir forests on soil microbial communities. More broadly, exotic plants lack co-evolutionary history with native species and therefore, complementary effects between native and exotic plants may be stronger and more likely to occur than between native species (Kawaletz et al., 2013). This may also explain the more pronounced alleviation of microbial stress in mixtures of Douglas-fir with beech than in those of Norway spruce with beech.

The site- and tree species-specific responses of microorganisms are likely caused by varying energy supply in different forest types at nutrient-poor sites. In fact, at nutrient-poor sites, the higher $Gram^{+}/Gram^{-}$ bacteria

294 ratio in coniferous and mixed forests suggests lower supply of labile carbon than in beech forests (Fanin et
295 al., 2019). Labile carbon compounds have been suggested to favor Gram$^-$ bacteria, whereas Gram$^+$ bacteria
296 better cope with recalcitrant carbon resources (Kramer and Gleixner, 2008; Fanin et al., 2019; Waller et al.,
297 2020). Among the forest types studied, Douglas-fir forests were particularly limited by labile carbon as
298 further supported by its lower microbial specific respiration, which was largely due to lower microbial basal
299 respiration compared to beech stands (Anderson and Domsch, 2010). Limited supply of labile carbon also
300 has been suggested to favor oligotrophic microbial communities characterized by low respiration rates and
301 biomass (Potthast et al., 2010; Fanin et al., 2019). This is consistent with our findings of strong carbon
302 limitation as well as low basal respiration and microbial biomass in single and mixed coniferous forests at
303 nutrient-poor sites. Moreover, at nutrient-poor sites, both the cy/pre and sat/mono ratios were higher in
304 pure species coniferous as well as mixed forests than in pure beech forests, also indicating higher nutrient
305 (and/or water) stress in the former (Moore-Kucera and Dick, 2008; Pollierer et al., 2015). The Gram$^-$ bacteria
306 marker fatty acids 16:1ω7 and 18:1ω7 are precursors of cyclopropyl fatty acids and as monounsaturated
307 fatty acids. They form part of the nominator of the calculated stress indicators. The more pronounced stress
308 response of microorganism in coniferous and mixed forests at nutrient-poor sites was largely caused by
309 lower concentration of these two ω7 fatty acids. Indeed, it has been suggested that under stress conditions
310 Gram$^-$ bacteria change the composition of their cell membranes from 16:1ω7 and 18:1ω7 to cyclopropane
311 fatty acids associated with slower growth rates (Guckert et al., 1986; Kieft et al., 1994; Lundquist et al., 1999).
312 The similarity of microbial indicators in beech forests at nutrient-rich and nutrient-poor sites indicates that,
313 compared to coniferous stands, beech maintained microbial community functioning and avoided stress
314 conditions of microorganisms by providing more labile carbon resources at nutrient-poor conditions,
315 presumably by releasing high amounts of root-derived resources.

316 The conclusion that beech is able to alleviate microbial stress via maintaining or even increasing the release
317 of root-derived resources at nutrient-poor sites is supported by the following findings. First, current studies
318 at our study sites showed that at nutrient-poor sites the biomass of fine roots is higher in beech forests than
319 in pure and mixed coniferous forests while at nutrient-rich sites root biomass varies little among forest types
320 (A. Lwila, pers. comm.). Generally, it is well established that plants allocate more resources into roots if the
321 availability of soil nutrients is declining and this is associated with increased root exudation (Eisenhauer et al.,
322 2017; Laliberté et al., 2017). Recently, this also was confirmed for beech forests where higher root exudation
323 was found in more acidic and nitrogen deficient stands (Meier et al., 2020). These findings support our
324 conclusion that beech indeed maintained or even increased its root exudates at nutrient-poor sites which in
325 fact were characterized by more acidic conditions and higher C/N ratio in soil. High root-derived resources
326 presumably also were responsible for the alleviated microbial resource deficiency and stress in beech forests
327 at nutrient-poor sites. Second, trenching and girdling experiments confirmed that reducing or omitting the

flux of root-derived resources into the soil strongly reduces fungal and bacterial biomass, demonstrating the importance of root-derived resources for maintaining soil microbial biomass (Högberg et al., 2007; Kaiser et al., 2010; Bluhm et al., 2019). Notably, we sampled litter and soil during autumn / winter after beech had shed its leaves. Nevertheless, the evidence provided above suggests that the impact of roots on microorganisms still was strong indicating that not only recent photosynthates but also stored carbohydrates contribute to providing root-derived resources to soil microorganisms (Druebert et al., 2009). Further, these findings suggest that beech has the potential to facilitate soil microorganisms at environmental stress conditions by maintaining or even increasing its root exudates contrasting both of the studied coniferous species. This ability presumably contributed to the mitigation of the detrimental effects of Douglas-fir on microorganisms at nutrient-poor / acidic sites in mixed forests. More broadly, it may even explain the observed overyielding in mixed stands of beech and conifers at nutrient-poor sites, highlighting the potential role of microorganisms in the facilitation of plants under environment stress conditions (Toïgo et al., 2015; Ammer, 2019; David et al., 2020).

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

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

361 were relatively large in soil, again supporting our conclusion that at nutrient-poor sites effects of tree species
362 on microorganisms in soil are predominantly due to differences in root-derived resources. Interestingly, the
363 differences in microbial community structure between beech and coniferous forests in 5 – 10 cm soil depth
364 at nutrient-poor sites were most pronounced in Douglas-fir, and carbon limitation also was most prominent
365 in 5 – 10 cm soil of Douglas-fir forests as indicated by lower microbial specific respiration. This implies that
366 among the tree species studied Douglas-fir provides the lowest amount of root-derived resources to soil
367 microorganisms at nutrient-poor sites resulting in pronounced resource shortage and marked changes in
368 microbial community composition. Such changes in microbial community structure may significantly impact
369 the functioning of soil microbial communities as the availability of carbon resources and carbon limitation
370 affect the vertical distribution of saprotrophic fungi and decomposition processes, e.g. by shifting the
371 competition between saprotrophic and mycorrhizal fungi (Gadgil and Gadgil, 1971; Lindahl et al., 2007).

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

385 In addition to root-derived resources, tree species may affect microbial community structure in soil by
386 modifying chemical properties. In our study, soil properties, especially pH, well explained changes in the
387 community structure of microorganisms in soil. This finding are consistent with results of continental scale
388 studies as well as local liming experiments suggesting that soil pH is among the most prominent driver of
389 microbial community composition (Frostegård et al., 1993a; Fierer and Jackson, 2006). Further, soil pH
390 generally correlates negatively with soil nutrient availability, supporting the importance of nutrients in the
391 mediation of microbial responses (Meier et al., 2020). However, factors other than soil pH and soil nutrient
392 status may have contributed to the observed differences between nutrient-rich and nutrient-poor sites. In
393 fact, nutrient-poor sites also were characterized by lower precipitation and this is reflected by higher $\delta^{13}C$

values of litter at nutrient-poor compared to nutrient-rich sites (J.-Z. Lu, unpubl. data), likely reflecting higher water stress at nutrient-poor compared to nutrient-rich sites (Peuke et al., 2006). 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.

5. 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 at 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 varying strongly with soil conditions.

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Figures and Tables

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

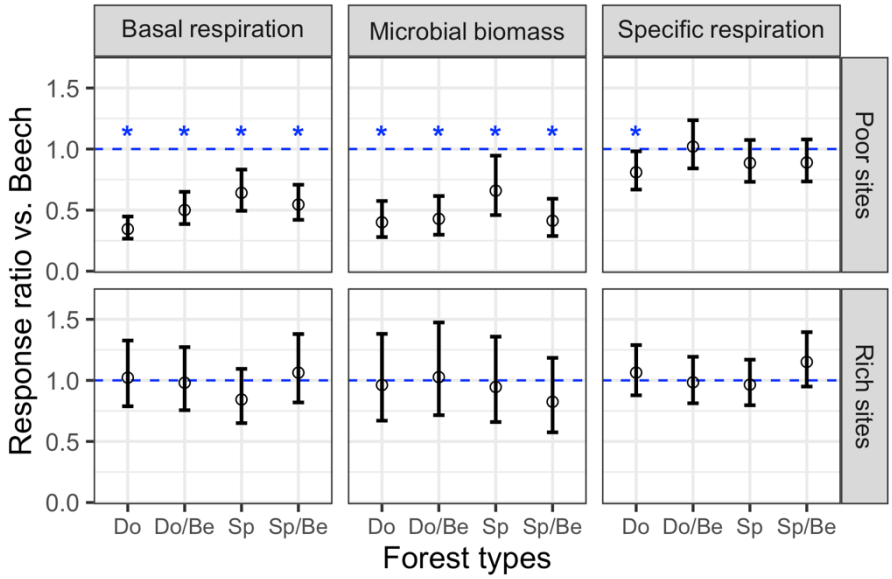
Figure 2. Effects of conifers and conifer-beech mixtures (Douglas-fir [Do], Douglas-fir with beech [Do/Be], Norway spruce [Sp] and Norway spruce with beech [Sp/Be]) on stress indicators: ratio of cyclopropyl PLFAs to its monoenoic precursors, ratio of saturated to monounsaturated PLFAs, and ratio of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

Figure 3. Changes in microbial basal respiration (µg O_2 g^{-1} C h^{-1}), microbial biomass (µg C_{mic} g^{-1} C) and microbial specific respiration (µg O_2 µg^{-1} C_{mic} h^{-1}) with soil depth (litter and 0–5 and 5–10 cm soil) 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. Points and horizontal bars represent means and standard errors (n=4). The grey vertical bars represent respective values in beech forests at nutrient-poor sites in litter, 0–5 and 5–10 cm soil depth. Note log scale for microbial biomass.

Figure 4. Changes in stress indicators (ratio of cyclopropyl fatty acids to its precursors, ratio of saturated to monounsaturated fatty acids and ratio of $Gram^+$ to $Gram^-$ bacteria [cy/pre, sat/mono and $Gram^+/Gram^-$, respectively]) with soil depth (litter and 0–5 and 5–10 cm soil) 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. Points and horizontal bars represent means and standard errors (n=4). The grey vertical bars represent respective values in beech forests at nutrient-poor sites in litter, 0–5 and 5–10 cm depth.

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 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.

724 Figure 1



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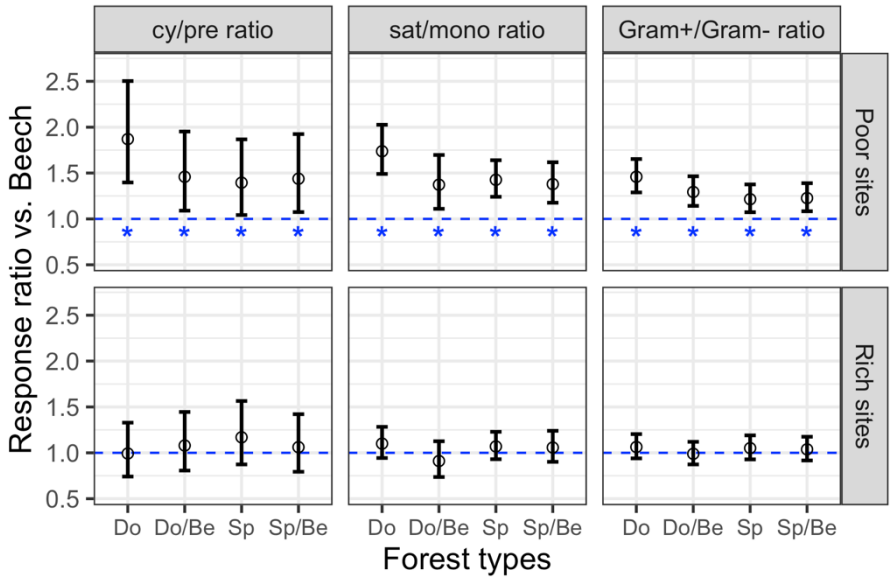
727 Figure 2



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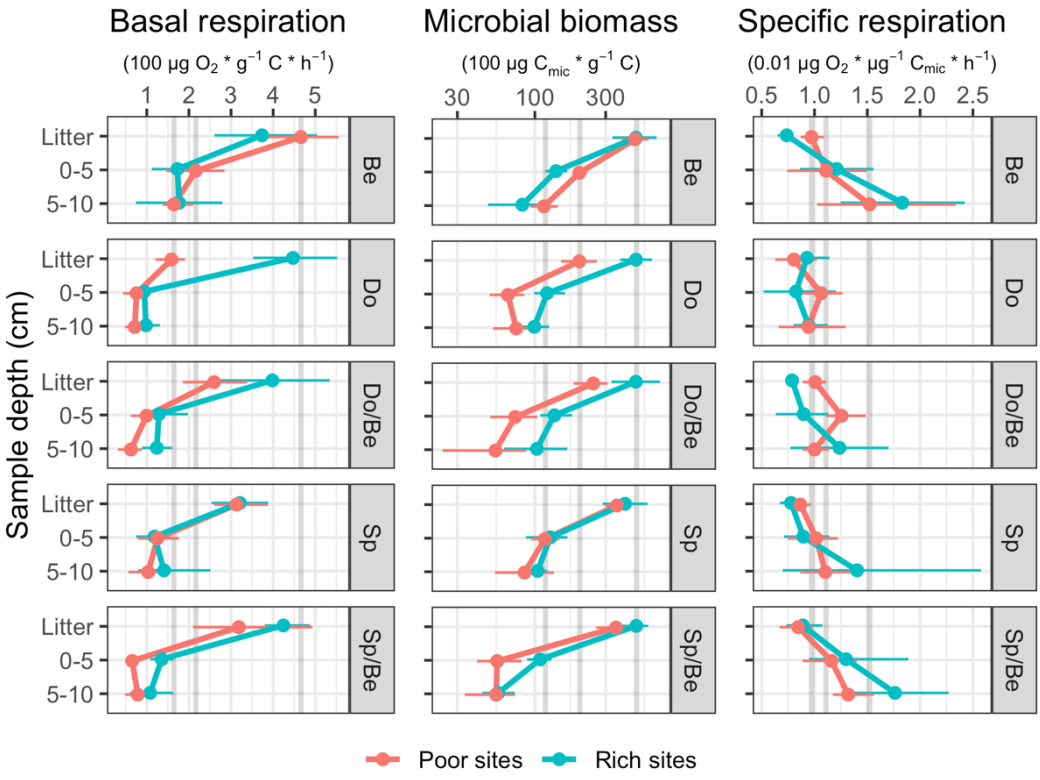
730 Figure 3



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733 Figure 4

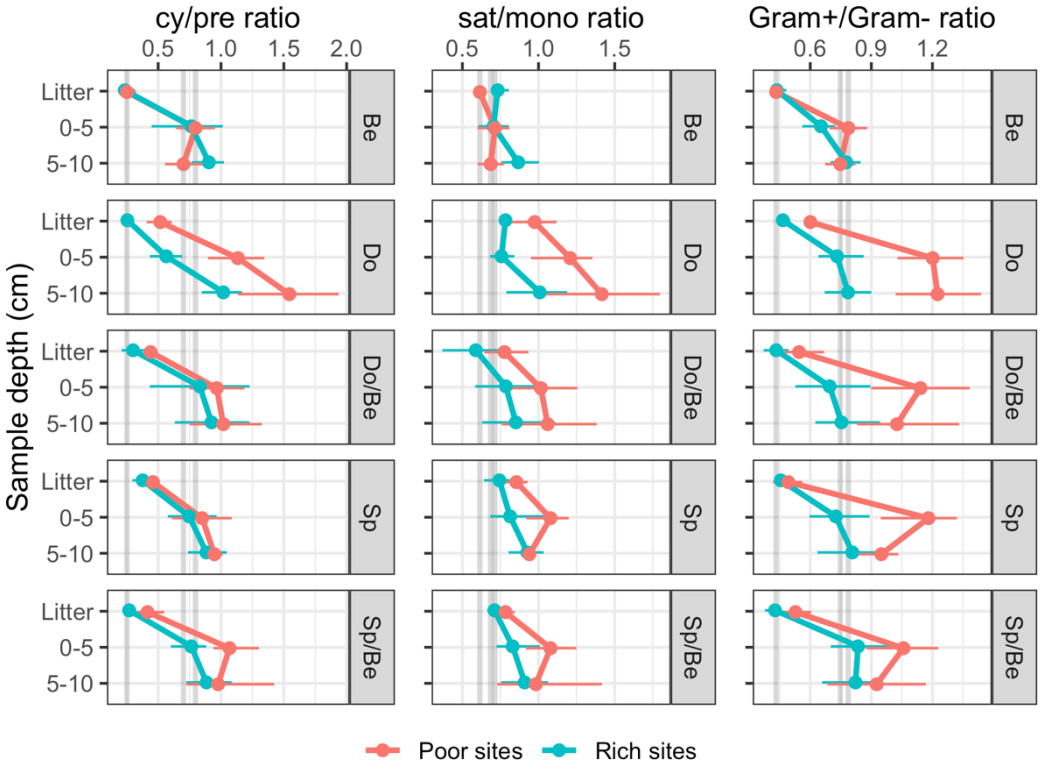

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Figure 5

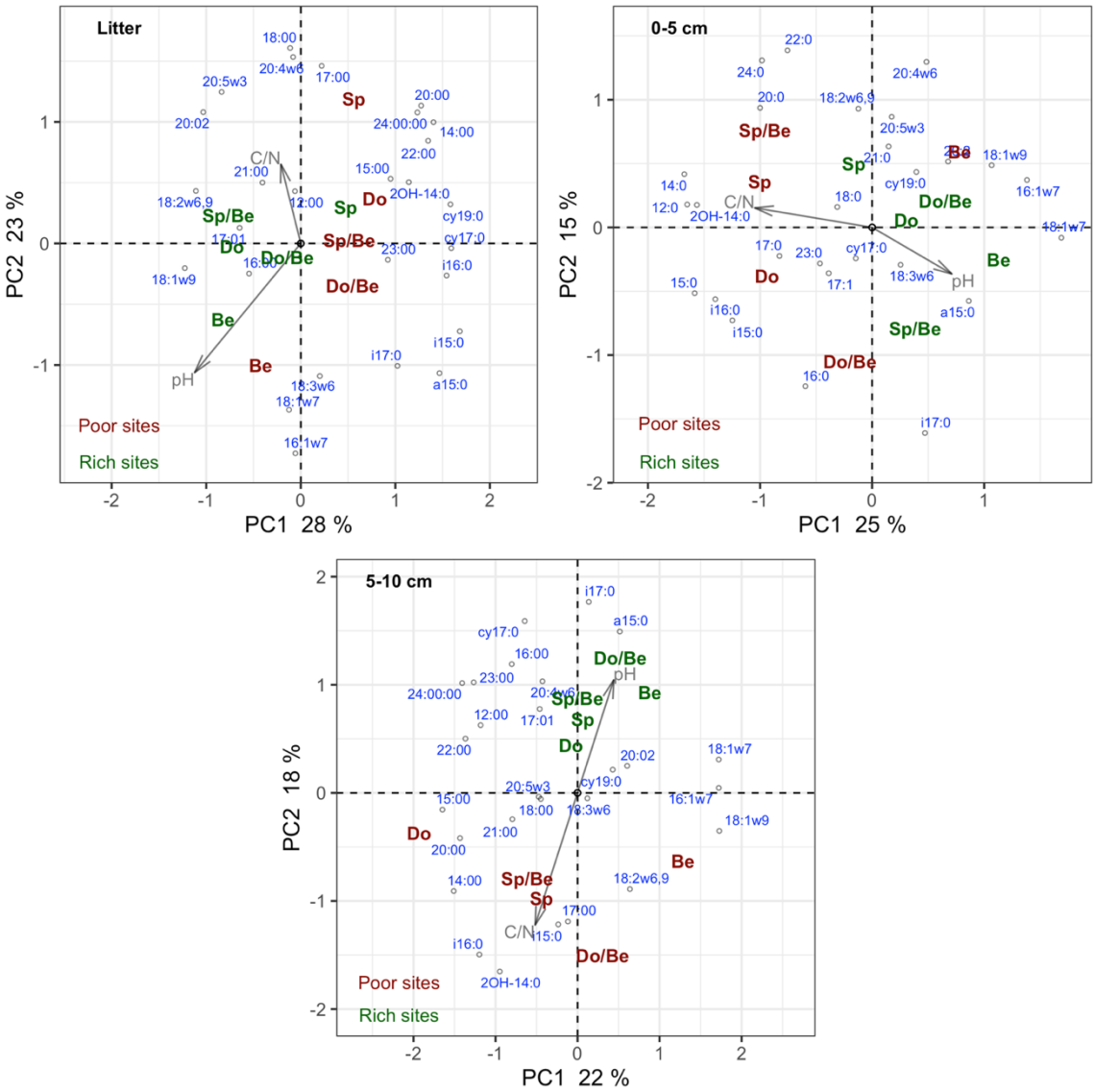
Litter
PC1 28 %
PC2 23 %
Poor sites
Rich sites
C/N
pH
Sp
Do
Sp/Be
Do/Be
Be
0-5 cm
PC1 25 %
PC2 15 %
5-10 cm
PC1 22 %
PC2 18 %

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 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	5.95	**0.001**	4,24	0.92	0.47
Quintet group (Q)	1,6	3.27	0.12	1,30	16.93	**<0.001**	1,6	0.52	0.49
Depth (D)	2,78	181.58	**<0.001**	2,70	271.27	**<0.001**	2,78	17.37	**<0.001**
F × Q	4,24	8.83	**<0.001**	4,30	4.61	**0.005**	4,24	2.36	0.08
F × D	-	-	**-**	8,70	2.15	**0.042**	-	-	-

(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	4,30	0.83	0.52
Quintet group (Q)	1,30	6.46	**0.02**	1,30	4.34	**0.046**	1,30	4.98	**0.033**
Depth (D)	2,68	982.11	**<0.001**	2,78	149.47	**<0.001**	2,68	995.4	**<0.001**
F × Q	4,30	1.99	0.12	4,30	0.733	0.58	4,30	1.60	0.20
Q × D	2,68	3.61	**0.002**	-	-	-	2,68	16.92	**<0.001**
F × D	8,68	18.54	**<0.001**	-	-	-	8,68	2.84	**0.009**

Table 2. Results of (a) two-way MANOVAs and (b) MANCOVA of PLFA composition in litter, 0–5 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 ($p < 0.05$).

		(a) Without soil parameters				(b) 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