Running title: Forest type effects on microbial communities

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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 likely supply different resources to soil microorganisms thereby shaping microbial communities. However, effects of forest types including non-native species and their variation with site conditions on microbial communities 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. Each site comprised five forest types, 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 stands. 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 at nutrient-poor sites, but planting Douglas-fir at nutrient-rich sites may be 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 from native species in the provisioning of these resources.

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).

The accumulation of organic matter in coniferous forests has been attributed to the recalcitrance of coniferous 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 between saprotrophic and mycorrhizal fungi, maybe more important for structuring microbial communities than the input of above-ground litter (Scheu et al., 2003; Pollierer et al., 2007; Schmidt et al., 2011; Augusto et al., 2015; Yan et al., 2016; Bluhm et al., 2019; Jones et al., 2019). Despite that coniferous and deciduous trees likely differ in the supply of litter and root-derived resources to soil microorganisms, the response of microbial communities to mixed forests is little studied. Litter mixture may improve resource availability to soil microorganisms and facilitate litter decomposition (Hättenschwiler et al., 2005; Fujii et al., 2020), although tree species 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 also has 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 shape 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 to what extent effects of tree species composition on soil microorganisms vary with site conditions, such as nutrient status and water availability, is little known. Facilitation among plants is more pronounced in unfavorable environments according to the stress-gradient hypothesis, and such positive interspecific interactions 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 respond in various ways to unfavorable growth conditions, such as by allocating more resources to roots than to shoots under nutrient-poor conditions (Callaway et al., 2003; Kawaletz et al., 2013; Yan et al., 2016). Further, 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 unfavorable than favorable environmental 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 damaged severely by extreme weather conditions and the outbreak of bark beetles, events that are predicted to occur more frequently and at higher severity in 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 environmental gradients.

Here, we investigate the structure and functioning of microbial communities in litter and soil of five forest types at sites differing in environmental conditions, in particular in soil nutrients but also rainfall and pH. Forest types include pure stands of European beech (*Fagus sylvatica*), Norway spruce (*Picea abies*), Douglas-fir (*Pseudotsuga menziesii*), and the mixtures of the respective

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 has been planted across wide areas of Central Europe including lowlands (Knoke et al., 2008; Schmid et al., 2014). Douglas-fir has been 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 with half of the stands located in four sites in the south and the other half in four sites in the north of Lower Saxony, Germany, covering a range of environmental conditions (eight sites x five forest types; Fig. S1). The four sites in the south stock on fertile soil and receive higher precipitation. Parental rock is either loess influenced Triassic sandstone or Paleozoic shares of greywacke, sandstone, quartzite and phyllite, resulting in 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 sites in the north are located on out-washed sand with the soil type of Podzol. The mean annual precipitation is 672 – 746 mm. The southern sites are richer in nutrients than the northern sites as reflected by lower C/N ratio, higher total soil P, higher pH and higher cation exchange capacity (CEC) (Table 1; Foltran et al., 2020). Hereafter, we refer to the southern sites as nutrient-rich sites and to the northern sites as nutrient-poor sites.

Each site 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 sites, the distance between stands ranged from 76 m to 4600 m, and the distance between sites ranged from 5 to 190 km. Within each stand, plots of 2500 m^2 were established, mostly in rectangular shape (50 m 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 between November 2017 and January 2018. Soil cores of 5 cm diameter 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 (< 25 mm^2) (Maraun and Scheu, 1995). Roots > 2 mm in diameter and stones were removed. Samples were frozen at -20°C and thawed at 4°C before further analyses. The pH of litter and soil was determined using a ratio of sample to solution (KCl, 1M) of 1:10, 1:5 and 1:2.5 (g/v) for litter, 0 – 5 and 5 – 10 cm soil, respectively. Moisture content was measured by drying samples at 105°C for 48 h. Dried samples were grinded in a ball mill and then total carbon and nitrogen concentrations were determined by an elemental analyzer (NA 1110, CE-instruments, Rodano, Milano, Italy). Biomass of litter was estimated by weighing samples that had been dried at 50°C for > 48 h (Macfadyen, 1961). Water content did not differ consistently between nutrient-rich and nutrient-poor sites across layers, with higher water content in litter but lower water content in soil at nutrient-poor than at nutrient-rich sites (interaction of Site condition and Depth; $F_{2,68} = 26.21$, $P < 0.001$); further site characteristics including soil texture and soil chemical properties (percentage of carbon and nitrogen, C/N ratio, cation exchange capacity, pH, concentration of phosphorus and Ca^{2+}) are given in Table 1.

2.3. Microbial basal respiration and biomass

Field moist samples were weighed and preincubated at room temperature (20°C) overnight before placement in an automated micro-respirometer system (Scheu, 1992). The system absorbs CO_2 produced by microorganisms using KOH solution, and O_2 consumed by microorganisms is replaced through electrolytic release of O_2 from $CuSO_4$ solution. Microbial basal respiration was measured as mean consumption of O_2 during 10 – 23 h after attachment of the vessels to the respirometer system ($\mu g\ O_2\ g^{-1}\ h^{-1}$). Microbial biomass was determined by substrate-induced respiration after the addition of glucose to saturate microbial glycolytic enzymes based on the maximum initial

177 respiratory response 4 – 7 h after the addition of D-glucose (*MIRR*; μl O_2 g^{-1} h^{-1}) and converted to
178 microbial biomass (C_{mic}; μg C_{mic} g^{-1}) as 38 × *MIRR* (Anderson and Domsch, 1978; Beck et al.,
179 1997). Basal respiration and substrate-induced respiration were measured at 22°C in a water bath.
180 Fresh litter and soil from 0 – 5 and 5 – 10 cm depth equivalent to approximately 0.3, 1 and 3.5 g dry
181 mass was supplemented with 80, 20 and 8 mg glucose solved in 400 μl H_2O, respectively. The ratio
182 between microbial basal respiration and microbial biomass was taken as microbial specific
183 respiration (qO_2; μg O_2 μg^{-1} C_{mic} h^{-1}).

184 2.4. Phospholipid fatty acid analysis

185 To quantify the composition of phospholipid-derived fatty acid (PLFA) composition, lipids from leaf
186 litter and soil were extracted using a modified Bligh and Dyer method (Frostegård et al., 1993) as
187 described in Pollierer et al. (2015). Lipids were fractionated into glycolipids, phospholipids and
188 neutral lipids by elution through silica acid columns using chloroform, acetone and methanol,
189 respectively (0.5 g silicic acid, 3 ml; HF BOND ELUT-SI, Varian, Inc., Darmstadt, Germany).
190 Phospholipids were subjected to mild alkaline methanolysis and fatty acid methyl esters (FAMEs)
191 were identified by chromatographic retention time compared to a general reference standard and a
192 bacteria-derived standard (FAME CRM47885, C11 to C24; BAME 47080-U, C11 to C20; Sigma-
193 Aldrich, Darmstadt, Germany) using a GC-FID Clarus 500 (PerkinElmer Corporation, Norwalk, USA)
194 equipped with an Elite 5 column (30 m × 0.32 mm inner diameter, film thickness 0.25 μm). The
195 temperature program started with 60 °C (hold time 1 min) and increased by 30 °C per min to
196 160 °C, and then by 3 °C per min to 280 °C. The injection temperature was 250 °C and helium was
197 used as carrier gas. Approximately 2 g of fresh litter and 4 g of fresh soil were used for the
198 extraction.

199 2.5. Stress indicators and fatty acid markers

200 The ratio of cyclopropyl PLFAs to their monoenoic precursors (cy/pre) and the ratio of saturated to
201 monounsaturated PLFAs (sat/mono) were used as indicators of physiological or nutritional stress
202 ([(cy17:0 + cy19:0)/(16:1ω7 + 18:1ω7)]; [(14:0 + 15:0 + 16:0 + 17:0 + 18:0)/(16:1ω7 + 17:1 +
203 18:1ω9 + 18:1ω7)]; respectively) (Moore-Kucera and Dick, 2008; Pollierer et al., 2015). The ratio of
204 $Gram^+$ to $Gram^-$ bacteria was used as indicator of carbon availability (Fanin et al., 2019). The
205 saturated fatty acids i15:0, a15:0, i16:0, i17:0 were used as markers for $Gram^+$ bacteria, and the
206 fatty acids cy17:0, cy19:0, 16:1ω7 and 18:1ω7 were assigned as markers for $Gram^-$ bacteria
207 (Frostegård and Bååth, 1996; Zelles, 1999; Mitchell et al., 2010; Richter et al., 2018; Fanin et al.,
208 2019). Bacterial biomass was represented by the sum of $Gram^+$ and $Gram^-$ bacteria. Linoleic acid
209 18:2ω6,9 was used as fungal marker (Frostegård and Bååth, 1996). Total amount of PLFAs were
210 summed up from all identified PLFAs (n = 40; nmol g^{-1} dry weight) and was used to calculate PLFA

211 proportions. All stress indicators and FA markers were analyzed from proportions (mole
212 percentage).

213 2.6. Statistical analyses

214 For univariate analysis, we first fitted linear mixed models (LMMs) to log-transformed response
215 variables and then applied planned contrasts to estimate the effect size of Forest type and Site
216 condition (Piovia-Scott et al., 2019). All LMMs included Forest type (European beech, Douglas-fir,
217 Douglas-fir/European beech, Norway spruce, Norway spruce/European beech), Site condition
218 (nutrient-rich and nutrient-poor sites) and Depth (litter, 0 – 5 and 5 – 10 cm) as fixed effects. Full
219 models were selected stepwise by likelihood ratio test, and minimal models included all main effects
220 and the interaction of Forest type and Site condition. The 40 forest plots were included as random
221 effects to account for non-independence of samples of different depth from the same plot by forcing
222 values from the same plot to have the same intercept (Harrison et al., 2018). Based on Akaike
223 Information Criterion (AIC), the eight sites were included as random effect, and the mean-variance
224 relationship was accounted for by a dispersion parameter to meet the assumption of homogeneity of
225 variance (Johnson and Omland, 2004; Zuur et al., 2009).

226 Contrasts for the effect size of forest type were designed to compare coniferous and mixed forests
227 to beech forests. European beech, the climax tree species in lowland and lower montane regions of
228 Central Europe, was used as reference (Leuschner et al., 2017). Due to the property of log-
229 transformed response variables, the planned contrasts are analog to log response ratios (Piovia-
230 Scott et al., 2019). Further, the log response ratios were back transformed into response ratios and
231 were defined as effect sizes. Effect sizes of forest type were estimated for nutrient-poor and
232 nutrient-rich sites based on minimal models. Additionally, we applied contrasts between nutrient-
233 poor vs. nutrient-rich sites in a similar way for the effect sizes of Site condition. Univariate response
234 variables included microbial basal respiration, microbial biomass, microbial specific respiration,
235 stress indicators and PLFA markers.

236 For multivariate analysis of variance (MANOVA), PLFAs first were arcsine root transformed and
237 then dimensions of fatty acids were reduced by principal component analysis (PCA). Separate
238 MANOVAs for each sample depth were calculated to inspect for effects of Forest type, Site
239 condition, and their interactions. Further, selected environmental variables were included into
240 MANOVAs as covariates to test whether they explain effects of main factors in the models
241 (Langerhans et al., 2007; Bennett et al., 2020). Significant principal components (PCs) were
242 determined by broken stick criterion (MacArthur, 1957). Covariates were selected according to

243 significance in permutation tests based on adjusted R^2 in redundancy analyses (RDA) (Oksanen et
244 al., 2019). Only fatty acids ≥0.2% mean mole proportion were included in the analyses.

245 All analyses were done in R with RStudio interface (R Core Team, 2019; RStudio Team, 2019). We
246 used the "nlme" package to fit LMMs (Pinheiro et al., 2019), and the "emmeans" package to conduct
247 planned contrasts (Lenth, 2020). All mixed models met the assumptions of normality of residuals
248 and homogeneity of variance.

249 3. Results

250 3.1. Functional indicators of microorganisms

251 Forest type consistently affected microbial basal respiration, microbial biomass and stress indicators
252 at nutrient-poor but not at nutrient-rich sites (Forest type x Site condition interactions; all $P < 0.08$;
253 Table 2, Figs 1 and 2), and this was generally true across soil layers (Fig. S2). At nutrient-poor sites,
254 microbial basal respiration and microbial biomass was lower in coniferous and mixed forests than in
255 beech forests. This was most pronounced in Douglas-fir, where basal respiration and microbial
256 biomass at nutrient-poor sites were 65% and 59% lower compared to pure beech, respectively;
257 similarly, basal respiration and microbial biomass in the two conifer-beech mixtures were 46 – 56%
258 lower than in beech. Microbial basal respiration and microbial biomass in spruce forests were also
259 lower than in beech forests, but the difference was less pronounced (-33 to -36%; Fig. 1). In
260 addition, in Douglas-fir forests at nutrient-poor sites microbial specific respiration was 19% lower
261 compared to beech forests.

262 In contrast to basal respiration and microbial biomass, at nutrient-poor sites stress indicators, the
263 cy/pre ratio, the sat/mono ratio as well as the $Gram^+/Gram^-$ bacteria ratio, all were lower in beech
264 forests than in the other forest types (Fig. 2). The difference in the cy/pre and sat/mono ratio was
265 most pronounced in Douglas-fir (+87% and +74%) and less in spruce forests (+39% and +43%).
266 Similar to spruce forests, the cy/pre and sat/mono ratio in conifer-beech mixtures were on average
267 37 – 46% higher than in beech forests. Effects of forest type on the $Gram^+/Gram^-$ bacteria ratio also
268 was strongest in Douglas-fir forests (+46 %) and less pronounced in the other forest types (+21 –
269 29%). Stress indicators generally did not differ at nutrient-rich sites ($P > 0.31$; Fig. 2, Fig. S2, Table
270 S1).

271 The response of microorganisms to changes in site conditions also varied between forest types.
272 Differences between nutrient-rich and nutrient-poor sites were most prominent in Douglas-fir forests
273 and not significant in beech forests (Figs 3 and 4, Table S2). At nutrient-poor sites, microbial basal
274 respiration in Douglas-fir forests was 57% lower and microbial biomass in Douglas-fir pure and

275 mixed forests both were 47% lower compared to nutrient-rich sites. Similarly, but less strong,
276 microbial biomass in spruce mixed forests was 36% lower at nutrient-poor sites than nutrient-rich
277 sites. Conversely, in Douglas-fir forests the stress indicator cy/pre ratio at nutrient-poor sites
278 exceeded that of nutrient-rich sites by 82% (Fig. 4, Table S2). Similarly, in Douglas-fir forests the
279 sat/mono and $Gram^+/Gram^-$ bacteria ratio at nutrient-poor sites exceeded that at nutrient-rich sites
280 by 38 – 40%. Further, in Douglas-fir mixed forests the $Gram^+/Gram^-$ bacteria ratio at nutrient-poor
281 sites exceeded that at nutrient-rich sites by 34%. Further, the response of microbial stress indicators
282 to changes in site conditions varied with soil depth; in particular, the responses of the sat/mono ratio
283 and the $Gram^+/Gram^-$ ratio were most pronounced in 0 – 5 cm soil depth (Fig. 4, Table 2).

284 3.2. Microbial community structure

285 As indicated by PLFA patterns, microbial community composition in beech forests differed from that
286 in Norway spruce and Douglas-fir forests, with mixed forests being intermediate between the
287 respective single species stands (Fig. 5). Across layers, effects of forest type were significant in litter
288 and 0 – 5 cm soil, and marginally significant in 5 – 10 cm soil (Table 3). Despite strong turnover
289 between nutrient-rich and nutrient-poor sites (Site condition effects; $p < 0.001$), beech maintained
290 similar microbial community structure between nutrient-poor and nutrient-rich sites, and this was
291 most apparent in 0 – 5 cm soil depth (Forest type × Site condition interaction; Table 3, Fig. 5).

292 A number of fatty acids contributed to the separation of microbial communities of the different forest
293 types and site conditions. Beech forests were characterized by unsaturated fatty acids at all soil
294 depths, in particular by 16:1ω7, 18:1ω7, 18:1ω9 (Fig. 5). Norway spruce forests were associated
295 with long-chain saturated fatty acids (22:0, 24:0), and Douglas-fir forests with bacterial marker fatty
296 acids (2OH-14:0, cy17:0). There also were clear differences in fatty acid patterns between nutrient-
297 rich and nutrient-poor sites. Litter at nutrient-rich sites was associated with unsaturated fatty acids
298 such as 18:1ω9 and the fungal marker 18:2ω6,9. By contrast, litter at nutrient-poor sites had higher
299 saturated and branched fatty acids, with many of them being bacterial markers, such as 14:0 and
300 cy17:0 and cy19:0.

301 Litter at nutrient-rich sites was generally richer in fungi than at nutrient-poor sites, but the opposite
302 was true for the soil (Site condition × Depth interaction; Table 2, Fig. S3). As indicated by PLFA
303 markers, the relative abundance of fungi and bacteria generally did not differ among forest types,
304 but the relative abundance of fungi consistently decreased, whereas the abundance of bacteria
305 increased with soil depth (Figs S3 and S4). Further, based on the contrast of Site condition, at
306 nutrient-poor sites the relative abundance of fungi in pure beech and spruce forests was higher than

307 at nutrient-rich sites. By contrast, in pure and mixed Douglas-fir forests at nutrient-poor sites the
308 relative abundance of fungi in soil was similar to that at nutrient-rich sites (Table S2, Fig. S3).

309 Nutrient-poor sites were more limited by nitrogen as indicated by higher C/N ratio but also had lower
310 soil pH (Fig. 5). Soil C/N ratio and pH as well as soil moisture were among the most important
311 environmental variables in explaining variations in PLFA composition, but their contribution varied
312 with soil depth (Table S3). Among the environmental variables studied (pH, C/N ratio, water content,
313 carbon content, litter mass), pH accounted for 8% out of the total of 13% explained by all
314 environmental variables in litter, whereas in 0 – 5 and 5 – 10 cm soil depth C/N ratio accounted for
315 22% and 13% out of the total of 37% and 15% of the variation explained by all environmental
316 variables (Table S3). Further, the main effects of Site condition became non-significant in soil after
317 including C/N ratio, pH and water content (0 – 5 cm soil depth) and C/N ratio (5 – 10 cm soil depth)
318 as covariates in the MANOVAs, but the effects of forest type remained marginally significant in both
319 0 – 5 and 5 – 10 cm soil depth (Table 3, Fig. 5). By contrast, in litter, including pH as covariate into
320 the MANOVA did not significantly affect the effects of Forest type and Site condition.

321 4. Discussion

322 Overall, the results only in part support our first and second hypotheses. Forest types only affected
323 the functioning of microbial communities at nutrient-poor sites, but not at nutrient-rich sites. At
324 nutrient-poor sites, microbial basal respiration, microbial biomass and stress indicators all were
325 detrimentally affected in pure and mixed coniferous forests, and, contrasting our first hypothesis the
326 effects differed between Douglas-fir and Norway spruce forests, with the effects being particularly
327 strong in Douglas-fir, but less pronounced in Norway spruce and mixed stands. By contrast, similar
328 basal respiration, microbial biomass and stress indicators at nutrient-rich sites across the studied
329 forest types contradict our second hypothesis and suggest that, at favorable environmental
330 conditions, soil microbial communities are rather irresponsive to changes in tree species. Notably,
331 microbial biomass and microbial stress indicators in beech forests did not differ between nutrient-
332 poor and nutrient-rich sites indicating that beech is able to maintain the functioning of soil microbial
333 communities regardless of site conditions. Admixture of beech in mixed stands with Douglas-fir or
334 Norway spruce both detrimentally affected microbial biomass and microbial stress indicators,
335 suggesting that caution is needed when mixing beech with conifers. However, results of the present
336 study also indicate that compared to pure Douglas-fir forests, mixed Douglas-fir forests with beech
337 mitigate the detrimental effects of pure Douglas-fir forests on soil microbial communities. Exotic
338 plants lack co-evolutionary history with native species and, therefore, complementary effects

339 between native and exotic plants are more likely to occur than between native species (Kawaletz et
340 al., 2013). This may explain the alleviation of microbial stress in mixtures of Douglas-fir with beech.

341 The site- and tree species-specific responses of microorganisms are likely caused by varying
342 energy supply in different forest types at nutrient-poor sites. In fact, at nutrient-poor sites, the higher
343 $Gram^+/Gram^-$ bacteria ratio in coniferous and mixed forests suggests lower supply of labile carbon
344 than in beech forests (Fanin et al., 2019). Labile carbon compounds have been suggested to favor
345 $Gram^-$ bacteria, whereas $Gram^+$ bacteria better cope with recalcitrant carbon resources (Kramer and
346 Gleixner, 2008; Fanin et al., 2019; Waller et al., 2020). Among the forest types studied, Douglas-fir
347 forests were particularly limited by labile carbon as further supported by its lower microbial specific
348 respiration, which was largely due to lower microbial basal respiration compared to beech stands
349 (Anderson and Domsch, 2010). Limited supply of labile carbon also has been suggested to favor
350 oligotrophic microbial communities characterized by low respiration rates and biomass (Potthast et
351 al., 2010; Fanin et al., 2019). This is consistent with our findings of strong carbon limitation as well
352 as low basal respiration and microbial biomass in single and mixed coniferous forests at nutrient-
353 poor sites. Moreover, at nutrient-poor sites, both the cy/pre and sat/mono ratios were higher in pure
354 coniferous as well as mixed forests than in pure beech forests, also indicating higher nutrient
355 (and/or water) stress in the former (Moore-Kucera and Dick, 2008; Pollierer et al., 2015). The $Gram^-$
356 bacteria marker fatty acids 16:1ω7 and 18:1ω7 are precursors of cyclopropyl fatty acids and as
357 monounsaturated fatty acids they form part of the nominator of the calculated stress indicators. The
358 more pronounced stress response of microorganism in coniferous and mixed forests at nutrient-poor
359 sites largely was caused by lower proportions of these two ω7 fatty acids. Indeed, it has been
360 suggested that under stress conditions $Gram^-$ bacteria change the composition of their cell
361 membranes from 16:1ω7 and 18:1ω7 to cyclopropane fatty acids associated with slower growth
362 rates (Guckert et al., 1986; Kieft et al., 1994; Lundquist et al., 1999). The similarity of microbial
363 indicators in beech forests at nutrient-rich and nutrient-poor sites indicates that, compared to
364 coniferous stands, beech maintained microbial community functioning and avoided stress conditions
365 of microorganisms by providing more labile carbon resources at nutrient-poor conditions,
366 presumably by releasing high amounts of root-derived resources (Meier et al., 2020).

367 The suggestion that beech is able to alleviate microbial stress via maintaining or even increasing the
368 release of root-derived resources at nutrient-poor sites is supported by the following findings. First,
369 current studies at our study sites showed that at nutrient-poor sites the biomass of fine roots is
370 higher in beech forests than in pure and mixed coniferous forests while at nutrient-rich sites root
371 biomass varies little among forest types (A. Lwila, pers. comm.). Generally, it is well established that
372 plants allocate surplus carbon into roots if the availability of soil nutrients is declining and this is

373 associated with increased root exudation (Eisenhauer et al., 2017; Laliberté et al., 2017; Prescott et
374 al., 2020). Recently, this also was confirmed for beech forests where higher root exudation was
375 found in more acidic and nitrogen deficient stands (Meier et al., 2020). These findings support our
376 suggestion that beech indeed increased its root exudates at nutrient-poor sites, which in fact were
377 characterized by higher C/N ratio and more acidic conditions in soil. High root-derived resources
378 presumably also were responsible for the alleviated microbial resource deficiency and stress in
379 beech forests at nutrient-poor sites. Second, trenching and girdling experiments also confirmed that
380 reducing or omitting the flux of root-derived resources into the soil strongly reduces fungal and
381 bacterial biomass, demonstrating the importance of root-derived resources for maintaining soil
382 microbial biomass (Högberg et al., 2007; Kaiser et al., 2010; Bluhm et al., 2019). Overall, these
383 findings suggest that beech has the potential to facilitate soil microorganisms at environmental
384 stress conditions by increasing its root exudates contrasting both of the studied coniferous species.
385 This ability presumably contributed to the mitigation of the detrimental effects of Douglas-fir on
386 microorganisms at nutrient-poor / acidic sites in mixed forests. More broadly, it may explain the
387 observed overyielding in mixed stands of beech and conifers at nutrient-poor sites, highlighting the
388 potential role of microorganisms in the facilitation of plants under environment stress conditions
389 (Toïgo et al., 2015; Ammer, 2019; David et al., 2020).

390 Contrary to our third hypothesis, the effect of forest type was not stronger in litter than in soil. At
391 nutrient-poor sites, forest type affected microbial basal respiration, microbial biomass and microbial
392 stress in both litter and soil, with the strength of the effects generally being similar across layers.
393 This supports our suggestion that the effect of forest type was not mainly due to differences in litter
394 quantity and quality, but in particular to differences in root-derived resources. This contrasts earlier
395 suggestions that microorganisms in litter are more sensitive to changes in environmental conditions
396 than those in soil because litter is less buffered against environmental harshness (Pollierer et al.,
397 2015). Supporting our suggestion that microorganisms in soil sensitively respond to environmental
398 changes, microbial biomass generally decreased while microbial stress indicators generally
399 increased with soil depth. This sensitivity presumably is related to the fact that generally carbon
400 availability decreases with soil depth resulting in stronger resource limitation (Fierer et al., 2003). As
401 indicated by sat/mono and $Gram^+/Gram^-$ ratios, this increase in resource limitation is particularly
402 strong from litter to soil in pure coniferous and mixed forests at nutrient-poor sites.

403 We assumed that the functioning of microorganisms ultimately relies on its microbial community
404 structure, and this is supported by the consistent turnover in microbial community composition as
405 well as the change in microbial functions between nutrient-rich and nutrient-poor sites. Further, in
406 contrast to coniferous and mixed forests, microbial community structure and functional indices in

407 beech forests differed little between nutrient-rich and nutrient-poor sites, suggesting that among the
408 tree species studied only beech is able to maintain and stabilize microbial community structure and
409 functioning in litter and soil irrespective of site conditions. Notably, differences in microbial
410 community structure between forest types at nutrient-rich sites tended to be small in 5 – 10 cm soil
411 depth, further suggesting that the influence of tree species fades deeper in soil. By contrast, at
412 nutrient-poor sites, differences in microbial community structure between forest types were relatively
413 large in soil, again supporting our hypothesis that at nutrient-poor sites effects of tree species on
414 microorganisms in soil are predominantly due to differences in root-derived resources. Interestingly,
415 the differences in microbial community structure between beech and coniferous forests in 5 – 10 cm
416 soil depth at nutrient-poor sites were most pronounced in Douglas-fir, and carbon limitation also was
417 most prominent in 5 – 10 cm soil of Douglas-fir forests as indicated by lower microbial specific
418 respiration. This implies that among the tree species studied Douglas-fir provides the lowest amount
419 of root-derived resources to soil microorganisms at nutrient-poor sites resulting in pronounced
420 resource shortage and marked changes in microbial community composition. Such changes in
421 microbial community structure may significantly impact the functioning of soil microbial communities
422 as the availability of carbon resources and carbon limitation affect the vertical distribution of
423 saprotrophic fungi and decomposition processes, e.g. by shifting the competition between
424 saprotrophic and mycorrhizal fungi (Gadgil and Gadgil, 1971; Lindahl et al., 2007).

425 Our results are consistent with earlier findings indicating that regional factors more strongly shape
426 microbial community structure than tree species (Pollierer et al., 2015; Richter et al., 2018) as the
427 relative abundance of fungi and bacteria as well as the fungi/bacteria ratio did not vary with forest
428 types but with site conditions. As indicated by the relative abundance of the fungal marker FA
429 18:2ω6,9, fungi generally were favored in the soil of nutrient-poor sites, supporting earlier
430 suggestions that the fungal energy channel dominates in infertile systems (Wardle et al., 2004).
431 However, contrasting earlier suggestions, in Douglas-fir forests at nutrient-poor sites the fungal
432 channel did not dominate, although they were also characterized as infertile with high C/N ratio and
433 low pH (Fig. 5). In fact, at nutrient-poor sites the relative abundance of fungi in soil of Douglas-fir
434 forests was similar to that at nutrient-rich sites, resulting in lower abundance of fungi in Douglas-fir
435 compared to beech forests based on planned contrasts, particularly in 5 – 10 cm soil depth. The
436 relatively low abundance of fungi in soil of Douglas-fir forests may be due to a mismatch between
437 local and home (native range of Douglas-fir) fungal communities (Schmid et al., 2014), as in soil of
438 introduced plant species only a subset of fungal species present in their native range has been
439 recorded in soil of sites where they had been introduced (Schmid et al., 2014). Overall, the results of
440 our study support the sensitivity of PLFA analysis in detecting changes in microbial community
441 composition with forest types (Ramsey et al., 2006; Frostegård et al., 2011). However, future

442 studies using molecular techniques are needed to uncover the microbial taxa responsible for the
443 observed differences among forest types, including introduced and native tree species such as
444 Douglas-fir and beech in particular at nutrient-poor sites deeper in soil.

445 Notably, we sampled litter and soil during autumn / winter after beech had shed its leaves. The
446 hypothesis of high root-derived resources in beech forests implies that the impact of roots on
447 microorganisms still was strong in autumn / winter indicating that not only recent photosynthates but
448 also stored carbohydrates contribute to providing root-derived resources to soil microorganisms
449 (Druebert et al., 2009). However, it remains open whether the effects of forest type on microbial
450 communities get stronger or weaker with time hampering generalization of our finding across
451 seasons. Despite microbial communities may fluctuate considerably with season (Koranda et al.,
452 2013; Abramoff and Finzi, 2016; Nacke et al., 2016), comprehensive sampling in Douglas-fir forests
453 across seasons suggested that, except during summer drought, microbial stress indicators fluctuate
454 little (Moore-Kucera and Dick, 2008). In addition, as our sampling was limited to the upper 10 cm of
455 the soil, we may not have captured the full effects of roots, as e.g., roots of beech may extend
456 considerably deeper into the soil (Leuschner et al., 2004), although fine roots generally are
457 concentrated in upper soil layers (Schmid, 2002; Leuschner et al., 2004). To better understand the
458 linkage between roots and microbial communities, more information is needed on the vertical
459 distribution of fine roots as well as differences in root exudation between the studied tree species
460 across nutrient gradients.

461 In our study, among the soil chemical properties studied, C/N ratio and pH best explained the
462 variations in microbial community structure. Typically, soil pH correlates negatively with soil nutrient
463 availability (Meier et al., 2020), further supporting our conclusion on the importance of nutrients in
464 driving the responses of microorganisms to variations in site conditions. However, factors other than
465 soil nutrient status and soil pH may have contributed to the observed differences between nutrient-
466 rich and nutrient-poor sites. Despite moisture in litter and soil contributed little to microbial
467 community structure, nutrient-poor sites also are characterized by lower precipitation and this is
468 reflected by higher $\delta^{13}C$ values of litter at nutrient-poor compared to nutrient-rich sites (J.-Z. Lu,
469 unpubl. data), likely reflecting higher water stress at nutrient-poor compared to nutrient-rich sites
470 (Peuke et al., 2006). Presumably, in addition to nutrients, both pH and water stress contributed to
471 the observed differences in microbial community composition and functioning between nutrient-poor
472 and nutrient-rich sites. This is supported by the fact that soil water conditions are intricately linked to
473 soil nutrient dynamics and the uptake of nutrients by plants (de Vries et al., 2019). Future studies
474 need to disentangle the relative importance of nutrient availability, soil pH and water deficiency as
475 driving factors of the interrelationship between forest stands 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 site conditions, in particular soil nutrients. 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 site conditions.

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Figures and Tables

Figure 1. Effects of conifers and conifer-beech mixtures 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 (Douglas-fir [Do], Douglas-fir with beech [Do/Be], Norway spruce [Sp] and Norway spruce with beech [Sp/Be]). 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).

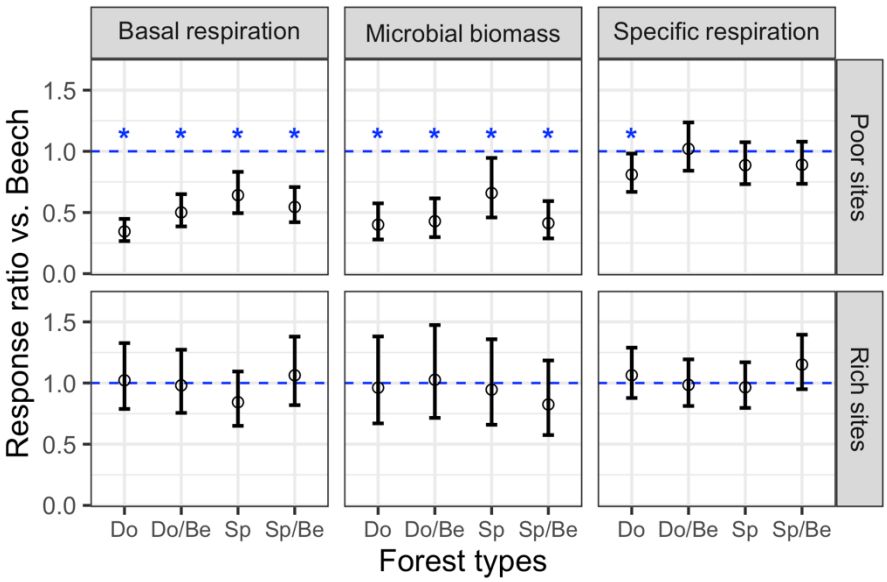
Figure 2. Effects of conifers and conifer-beech mixtures on stress indicators: ratio of cyclopropyl PLFAs to its monoenoic precursors, ratio of saturated to monounsaturated PLFAs, and ratio of $Gram^+$ to $Gram^-$ bacteria (cy/pre, sat/mono and $Gram^+/Gram^-$, respectively) at nutrient-poor and nutrient-rich sites (Douglas-fir [Do], Douglas-fir with beech [Do/Be], Norway spruce [Sp] and Norway spruce with beech [Sp/Be]). 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, pH, water content (water%), carbon content (C%) as well as litter mass were post-fitted into the plot. Significant variables selected by RDA permutation test were in bold (based adjusted R^2).

856 Figure 1



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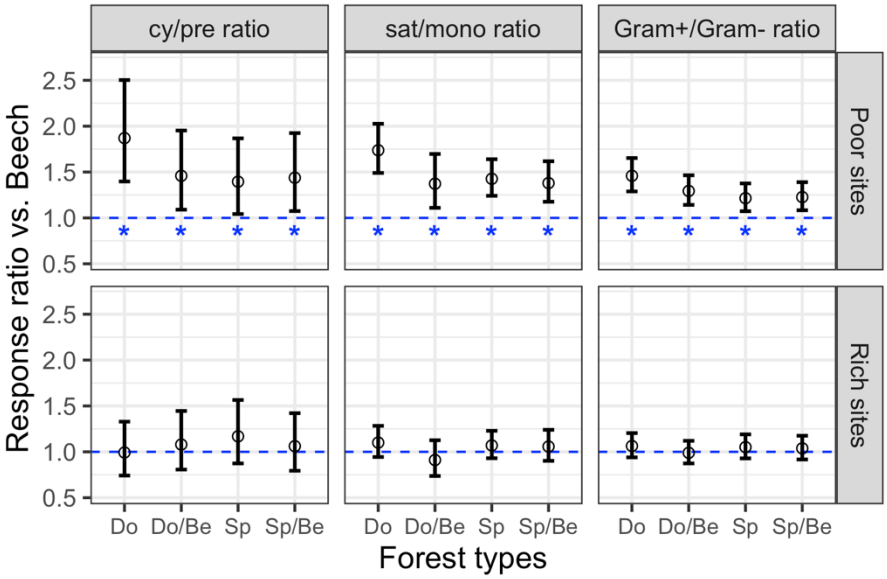
859 Figure 2



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862 Figure 3

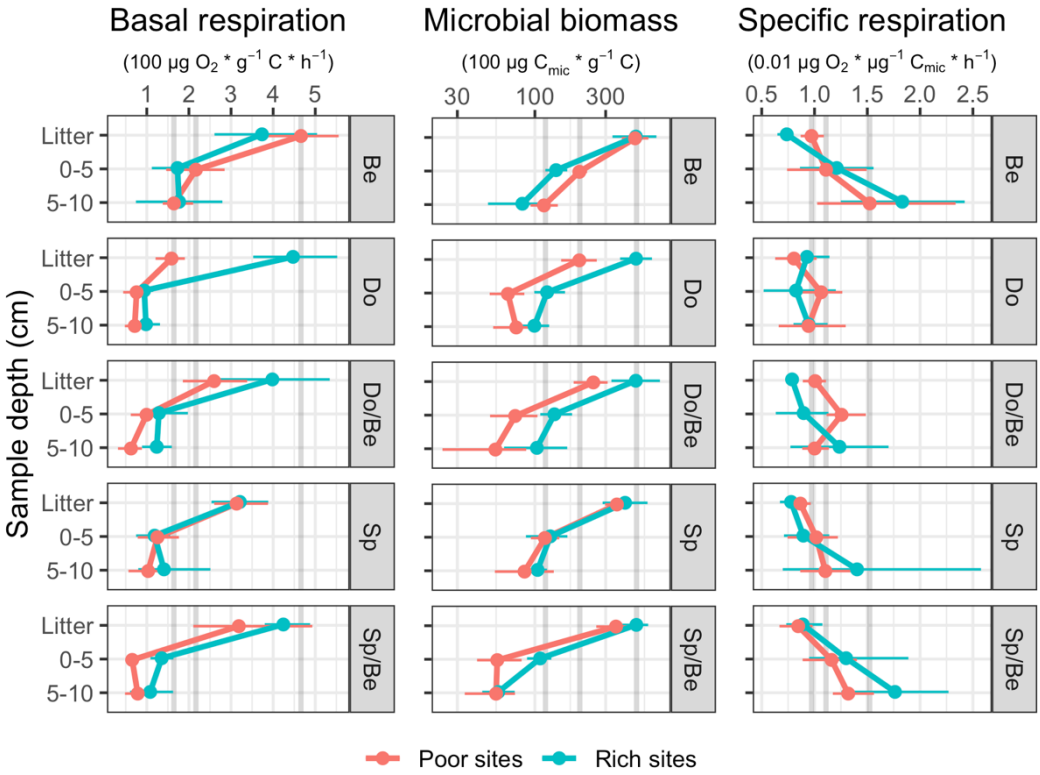

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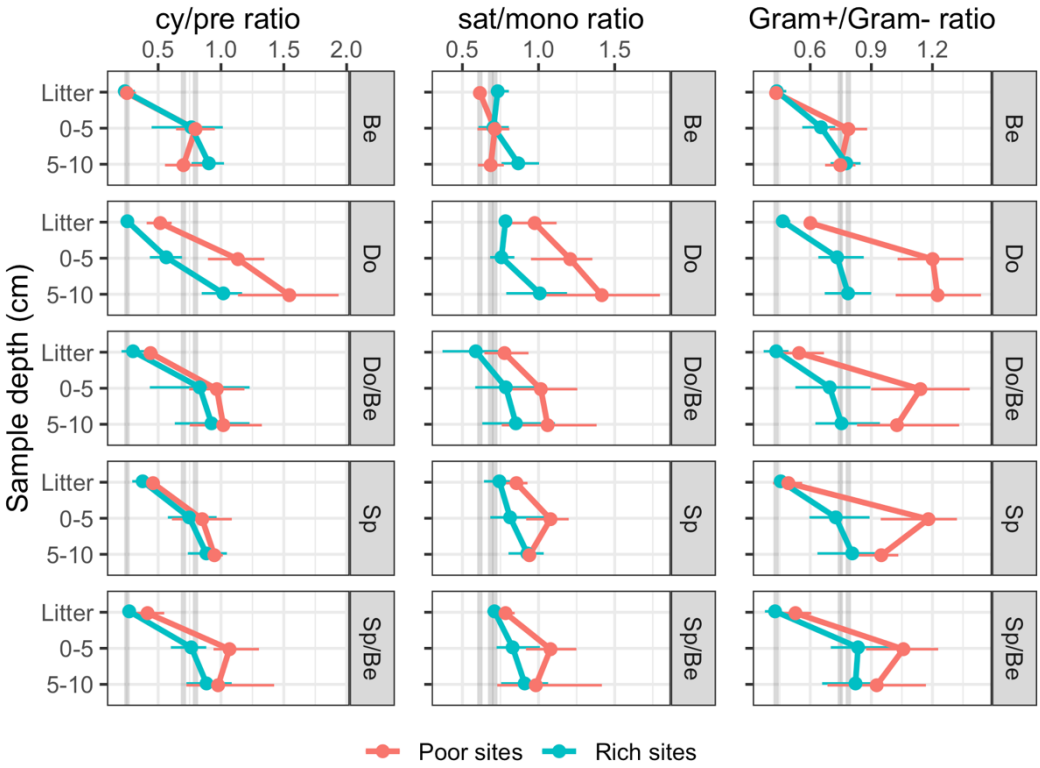
Figure 4


cy/pre ratio
sat/mono ratio
Gram+/Gram- ratio
0.5
1.0
1.5
2.0
0.6
0.9
1.2
Sample depth (cm)
Litter
0-5
5-10
Be
Do
Do/Be
Sp
Sp/Be
Poor sites
Rich sites

868 Figure 5

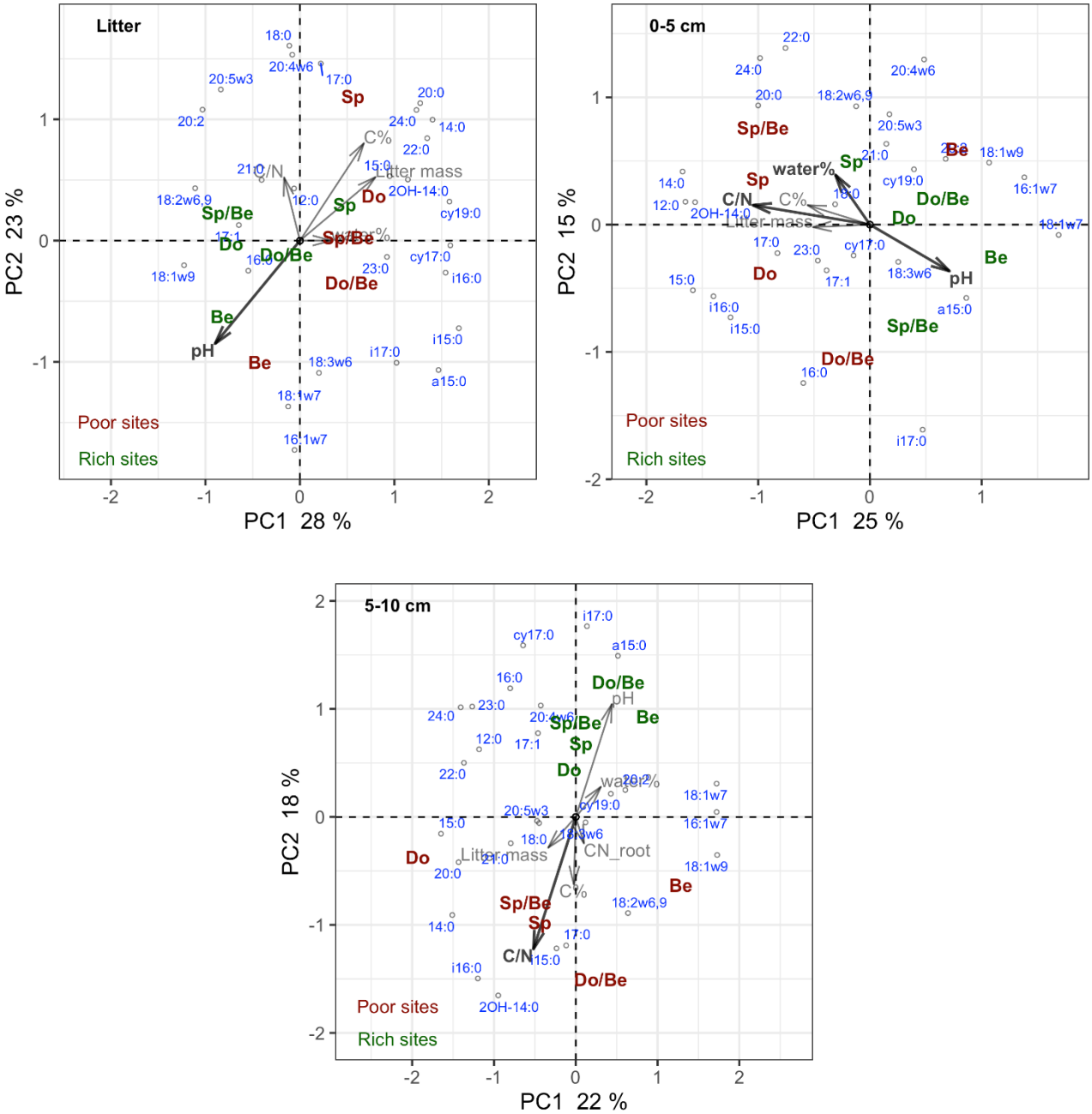


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Table 1. General information as well as physical and chemical properties of the soil of the study sites. Soil texture is based on 5–30 cm mineral soil, and soil chemical properties are based on 0–5 cm mineral soil. Soil moisture is expressed as percentage of dry mass, and was measured in litter, 0–5 and 5–10 cm soil. Ca^{2+} concentration was analyzed in exchangeable form by NH_4Cl extraction, and total P was determined by pressure digestion (for detailed see Methods and Foltran et al. 2020).

	Site condition	Rich sites				Poor sites			
	Site number	1	2	3	4	5	6	7	8
General information	Site name	Harz	Dassel	Winnefeld	Nienover	Nienburg	Unterlüß	Göhrde II	Göhrde I
	Latitude (°N)	51.770	51.726	51.662	51.696	52.621	52.837	53.127	53.201
	Longitude (°E)	10.397	9.704	9.574	9.526	9.281	10.331	10.814	10.801
	MAT (°C)	7.63	8.59	8.90	9.01	9.70	9.03	9.19	9.20
	MAP (mm)	1029.24	821.29	822.43	874.97	733.34	746.56	681.68	672.63
	Slope (°)	15.52	2.96	4.97	6.56	1.85	1.19	1.43	2.05
	Elevation (m)	510.80	426.17	349.06	323.37	92.00	161.20	129.80	120.00
Texture & moisture	Sand%	16	26	20	20	80	79	79	73
	Silt%	16	53	57	57	13	15	15	24
	Clay%	68	21	23	23	7	6	6	3
	Water% (Litter)	237.2	221.2	235.5	232.4	245.9	303.1	328.5	265.7
	Water% (0–5)	135.5	106.6	84.7	62.7	71.4	120.2	88.1	76.2
	Water% (5–10)	59.1	39.6	37.8	29.6	22.4	26.9	23.9	22.9
Chemical properties	C %	7.09	6.19	7.50	5.30	7.97	4.03	9.31	5.36
	N %	0.37	0.34	0.37	0.29	0.28	0.17	0.34	0.24
	C/N	19.16	18.21	20.27	18.28	28.46	23.71	27.38	22.33
	CEC (mmol dm^{-3})	237.31	121.26	114.91	95.96	74.81	47.79	71.10	67.72
	pH (KCl)	3.42	3.15	3.34	3.47	3.00	3.00	2.71	3.01
	P (mg kg^{-1})	0.61	0.54	0.44	0.46	0.16	0.11	0.17	0.24
	Ca^{2+} (CEC; mmol dm^{-3})	28.35	13.77	32.56	27.88	20.38	12.91	7.90	11.28

MAT: mean annual temperature
MAP: mean annual precipitation
CEC: cation exchange capacity

Table 2. 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), Site condition (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 and bacterial PLFAs (of total PLFAs), 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	8.94	**<0.001**	4,30	5.95	**0.001**	4,24	0.92	0.470
Site condition (S)	1,6	3.90	0.096	1,30	16.93	**<0.001**	1,6	0.52	0.498
Depth (D)	2,78	168.37	**<0.001**	2,70	271.27	**<0.001**	2,78	17.37	**<0.001**
F × S	4,24	10.91	**<0.001**	4,30	4.61	**0.005**	4,24	2.36	0.082
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**
Site condition (S)	1,6	3.48	0.111	1,6	0.195	0.304	1,6	8.17	**0.028**
Depth (D)	2,68	238.82	**<0.001**	2,76	5.551	**<0.001**	2,76	228.51	**<0.001**
F × S	4,24	2.70	0.055	4,24	3.650	**0.002**	4,24	2.27	**0.012**
S × D	2,68	6.20	**0.003**	2,76	10.705	**<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	0.161	4,30	2.19	0.094	4,24	0.83	0.520
Site condition (S)	1,30	6.46	**0.016**	1,30	4.34	**0.046**	1,6	4.98	**0.033**
Depth (D)	2,68	982.11	**<0.001**	2,78	149.46	**<0.001**	2,68	995.40	**<0.001**
F × S	4,30	1.99	0.121	4,30	0.73	0.577	4,24	1.60	0.207
S × D	2,68	18.54	**<0.001**	-	-	-	2,68	16.92	**<0.001**
F × D	8,68	3.61	**0.002**	-	-	-	8,68	2.84	**0.009**

Table 3. Results of two-way MANOVAs on PLFA composition (a) without covariates and (b) with covariates (pH, C/N ratio, water content) 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), Site condition (nutrient-rich and nutrient-poor sites) and their interactions. MANOVAs were based on principal components from each sample depth (significant PCs were determined by broken stick criterion; see Methods). Type I sequential sum of square was used for models with and without covariates. Significant effects are given in bold ($P < 0.05$).

		(a) Without covariates				(b) With covariates			
Depth	Factor	df	Wilk's lambda	F	P	df	Wilk's lambda	F	P
						Covariates: pH			
Litter	Forest type (F)	4,30	0.32	3.29	**0.001**	4,29	0.39	2.53	**0.008**
	Site condition (S)	1,30	0.42	12.77	**<0.001**	1,29	0.61	5.72	**0.004**
	F × S	4,30	0.73	0.77	0.677	4,29	0.75	0.68	0.767
						Covariates: C/N + pH + water%			
0–5 cm	Forest type (F)	4,30	0.19	2.80	**<0.001**	4,27	0.30	1.67	0.057
	Site condition (S)	1,30	0.39	8.14	**<0.001**	1,27	0.75	1.54	0.218
	F × S	4,30	0.36	1.57	0.078	4,27	0.33	1.53	0.096
						Covariates: C/N			
5–10 cm	Forest type (F)	4,30	0.41	1.76	0.051	4,29	0.41	1.68	0.067
	Site condition (S)	1,30	0.45	8.15	**<0.001**	1,29	0.82	1.39	0.265
	F × S	4,30	0.69	0.68	0.805	4,29	0.73	0.54	0.915