Edge and management effects on soil microbial communities in deciduous forests across Europe

Running title: Soil microbial variation across European forests

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Abstract

Forest fragmentation increases the proportion of edge area and this, in turn, induces changes in forest structure, species composition and microclimate. These factors are also strongly determined by the forest management regime. Although the interactive effects of edges and management on forest plant communities have been extensively studied, little is known about the response of the belowground communities. Here we investigated the variation of soil microbiota in 45 deciduous broadleaved forests along a latitudinal gradient from Italy to Norway at a continental scale across Europe. Phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) were used to map the microbial community in the forest edge and interior across three forest types (dense, intermediate, open forest). Microbial community composition was only affected by forest edge effects and not by forest management type. We did not find any interaction effects between forest type and distance-to-edge. Arbuscular mycorrhizal fungi (AMF) were significantly more abundant in edges and Gram-negative bacteria more abundant in interiors, respectively. The microbial community composition was closely related to soil pH, soil potassium and nitrogen, texture (percent sand) and soil temperature. In sum, we reveal the edaphic properties-dependence of soil microbial composition and also highlight the effects of forest edges on the belowground communities. More research is still required to understand the consequences of forest edges and forest management for forest functioning and all components of biodiversity.

Keywords: Microbial community, edge effect, forest type, PLFA, deciduous forests

1. Introduction

Humans have transformed forests into other land uses for thousands of years, which largely increased the level of forest fragmentation (Kaplan et al., 2009). Consequently, the proportion of interior forest habitat to the total amount of forest area has decreased steeply to the benefit of increasing forest edge area across many forested parts of the globe (Fischer et al., 2021). Based on the proportion of forest in the neighborhood of a forest pixel, an approach to define forest interior as described as Riitters et al. (1997), the global net loss rate of forest interior area is more than three times the global net loss rate of all forest habitat loss between 2000 and 2012 (Riitters et al., 2016). It has been estimated that nearly 20% of the global forested area is positioned within 100 m of a forest edge (Haddad et al., 2015), while approximately 3.6% of deciduous forests across Europe within 4.5 m of a forest edge (Meeussen et al., 2021).

In forest edges, the environmental conditions, including the temperature, light, soil moisture, and nutrient inputs are different from those away from the edges and towards the interiors of the forest fragments, and these local changes can strongly impact biological communities (Cadenasso and Pickett, 2001; Pardini, 2004). These so-called “edge-effects” within forest fragments are multi-faceted and depend on the contrasting characteristics between edges and interiors as well as edge and adjacent land (Harper et al., 2005). For example, forest edges are generally exposed to more sunlight, higher wind speed, and stronger air mixing, leading to a higher evapotranspiration and temperature variability than the interiors of the forest (Chen et al., 1993; Young and Mitchell, 1994; Didham and Lawton, 1999; Davies-Colley et al., 2000). These initial responses to edge effects will affect the recruitment, growth, mortality, and species interactions at the edge, which will have a profound influence on community structure and ecosystem functioning (Fagan et al.,

1999; Harper et al., 2005).

The size of edge effects are not similar but they generally depend on the size and shape of the forest fragments (Ewers and Didham, 2007), the contrast with adjacent non-forest environments (Fletcher and Koford, 2003), and other contextual factors, such as forest structure and edge orientation (Matlack and Litvaitis, 1999; Orczewska and Glista, 2005; Remy et al., 2018). Notably, forest management can strongly affect the response of community structure to forest edges as these practices could shape the community structure via general alterations of forest structure and its impact on the underlying microclimate, which would further affect the edge contrast with the surrounding landscapes (Aussenac, 2000; Crow et al., 2002). For instance, in open (recently thinned) forests, wind speeds, nutrient inputs, and seed dispersal of generalists into the interior of the forests is facilitated and thus a lower edge-interior contrast is found than in dense forests (Cadenasso and Pickett, 2001). Although edge effects among varied forest types have been studied in few studies, most of them still focused on above-ground biological communities (Karraker and Welsh, 2006; Morris et al., 2010; Girona et al., 2016; Govaert et al., 2020), therefore little is known about the dual, interactive effects of forest edges and types on belowground microbial communities.

Soil microbial communities offer great potential in forest ecosystem functioning as they are actively involved in biogeochemical processes, such as soil organic matter decomposition, nutrient immobilization, etc (Osburn et al., 2021). Due to the different sensitivity of microbial species to environmental conditions such as light, temperature, moisture, and soil nutrients, the composition of the microbial community is expected to vary due to forest types or edge effects. For example, increased light in thinned forests or forest edges may enhance the supply of carbohydrates from

vegetation to symbiotic fungal counterparts (such as mycorrhiza), thereby enhancing the colonization and growth of fungi (Louche-Tessandier et al., 1999; Fuzy et al., 2014). Similarly, decreased topsoil moisture in edges and increased nitrogen availability can be expected to impact the biomass, community composition and activity of the soil microbial groups (Allen and Schlesinger, 2004; Malmivaara-Lamsa et al., 2008; Ushio et al., 2008; Remy et al., 2018).

Here we address this knowledge gap and examined the belowground community composition responses to the edge effects within forests with different forest types (dense, intermediate and open forests) in 45 deciduous broadleaved forest edges across Europe. The edges were situated along a latitudinal gradient from Italy to Norway. Our aim was to disentangle the effects of the distance to the edge and forest type on soil microbial community composition at the continental scale, while taking variation of environmental gradients in multiple European regions into account to increase generality. We expected that the microbial community composition would change along the forest edge-interior transect, but that these effects depended on the forest types.

2. Materials and methods

2.1 Study area

Soils were sampled in 9 different regions along a latitudinal gradient in Europe, crossing the temperate, Mediterranean and boreonemoral forest biomes. Along this north-south gradient, the selected regions were in Norway, Central Sweden, Southern Sweden, Germany, Poland, Belgium, Northern France, Switzerland and Italy (Figure 1). In three of these regions, i.e., Norway, Belgium and Italy, three elevational belts were additionally sampled (i.e. low, intermediate and high elevation). Other regions only contained the lower elevational belt (i.e., lowland conditions). All

of the 15 sampling sites (9 low elevation sites, 3 intermediate elevation sites and 3 high elevation sites) contained three distinct forest types, i.e., dense forest, intermediate forest and open forests. Dense forests were characterized by a well-developed shrub layer, high basal area and dense canopy cover. This type of forest had not been managed for more than 10 years and was generally not thinned during the last 30 years. Intermediate forest had lower basal areas and canopy coverage since its last thinning event, usually occurring 5 to 10 years before sampling. Open forest was characterized by a lower basal area and higher canopy openness. These forests had been thinned during the 1–4 years before sampling. A 100 m transect from the southern forest edge to the interior were established in each forest. A total of 45 transects were included in this study (15 sites × 3 forest types). In each transect, two 3 × 3 m^2 plots were set up, one at the edge (0–3 m) and one at the interior (98–101 m away from the edge). More details regarding the study design are provided in Govaert et al. (2020) and Meeussen et al. (2020).

2.2 Soil chemical and texture analysis and soil microclimate: abiotic analysis

In all 90 plots, topsoil samples (0–10 cm depth) were collected for chemical analyses of nutrients and pH, and subsurface soil samples (10–20 cm depth) were used for texture analysis. Five random subsamples from each plot were collected and then pooled together for subsequent analysis. The mixed topsoil samples were dried and sieved through 1 mm mesh, then soil pH (in H_2O), calcium, potassium, magnesium, total carbon and nitrogen, and bioavailable phosphorus (Olsen P) were measured as described by Govaert et al. (2020). The texture was determined by sieving and sedimentation with the pipet method according to ISO 11277 (2009).

Soil moisture was gravimetrically determined by air-drying the 0-10 cm soil sample at 50°C

for 48 hours in a drying oven. Soil temperatures in the plot were measured by temperature data loggers (Lascar Easylog EL-USB-1) installed at a depth of 5 cm, and the mean temperatures during the summer (June-August) of 2018 were selected for the following analysis. See Meeussen et al. (2021) for details on the microclimate.

2.3 Determination of soil microbial community composition: biotic analysis

Additional soil samples (0-10 cm) for soil microbial analysis were also a subsample of selected samples within the plot during the June-August of 2018. These samples were immediately after sampling kept in portable cooling boxes before being transported to the laboratory, and then the samples were stored in the freezer at -18°C until phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) extraction. The PLFA and NLFA were extracted and determined according to Quideau et al. (2016). In brief, 3 g freeze-dried soil samples were extracted with Bligh and Dyer extractant, which comprised of a citrate buffer, chloroform and methanol with a ratio of 0.8:1:2 (v/v/v). Glycolipids were washed off from the polar lipids with acetone. Neutral lipids and phospholipids were eluted and collected from polar lipids by adding chloroform and methanol to the solid-phase extraction column (silica), respectively. The separated neutral lipids and phospholipids were transformed to fatty acid methyl esters (FAMEs) with methanolic KOH. N_2-dried FAMEs were resolved by adding 1 ml hexane before the gas chromatograph analysis, and the identification and quantification of each PLFA/NLFA were accomplished by gas chromatography-mass spectrometry (GC–MS, Trace GC-DSQ, Thermo Fisher, USA). Methyl nonadecanoate (MeC19:0) was used as internal standard and the concentration of each biomarker was expressed in μg/g.

A total of 23 PLFAs and 7 NLFAs were detected in this study. Only 19 PLFAs and one NLFA, which showed a high-frequency and well-recognized identity here, were selected as useful biomarkers to calculate the total microbial biomass. The PLFA biomarkers iC 15:0, iC 15:0, aC 15:0, iC 16:0, iC 17:0 and aC 17:0 were assigned to Gram-positive bacteria (Farrell et al., 2013; Kaiser et al., 2015); 16:1ω7c, cy 17:0 and cy 19:0 were assigned to Gram-negative bacteria (Mitchell et al., 2015); 10MeC16:0, 10MeC17:0 and 10MeC18:0 were assigned to Actinobacteria (Xu et al., 2019); C14:0, C15:0 , C16:0 and C17:0 were assigned to non-specific bacteria (Steinbeiss et al., 2009; Willers et al., 2015); while 18:2ω6c and 18:1ω9c were assigned to saprophytic fungi (Zhang et al., 2014). To minimize the influence of the background amounts (from bacteria) of PLFA16:1ω5c on the estimation of arbuscular mycorrhizal fungal biomass in soil, the ratio of NLFA and PLFA 16:1ω5c was used to represent arbuscular mycorrhizal fungi (Olsson, 1999; Ngosong et al., 2012). The relative abundance of each microbial group was calculated as the sum of representative PLFA/NLFA biomarkers divided by the total microbial biomass.

2.4 Statistical analyses

All statistical analyses were carried out in R (ver.4.0.3) (R Core Team, 2008). To explore correlation within the data, we first calculated a Spearman correlation matrix between pairs of edaphic properties (% sand, % silt, % clay, pH, total C and N, Olsen P, K, Mg, Ca, C/N, soil moisture and temperature) using the *rcorr* function in the *Hmisc* package (Harrell and Dupont, 2008). We used linear mixed-effects models (LMMs) to test factor variables distance-to-edge and forest type effects on edaphic properties and microbial composition, respectively. When fitting

LMMs on edaphic properties, we used log transformations for response variables: % sand, % silt, % clay, total C, Olsen P, Mg, Ca, C/N; while we used sqrt transformations for response variable total N (Table S1). When fitting LMMs on microbial composition, we used log transformations for response variable- total microbial biomass (Table 1). We tested the interaction between distance-to-edge and forest types as fixed effect in our LMMs, while the "region" factor variable (with 15 levels) was included as a random effect (random intercept) in our LMMs. The *lmer* function in *lme4* package was used to fit LMMs (Bates et al., 2014). Additionally, the magnitude of edge influence (MEI) was calculated per forest type for each microbial group. The MEI was estimated for edge plots for each transect as $(e\text{-}i)/(e+i)$, wherein e represents the relative abundance of each microbial group in the edge plot and i represents those in the interior plot (Harper et al., 2005).

Models were run for each microbial group as well as total microbial biomass separately to test the multiple edaphic properties effects on microbial composition. First, a model including predictor variables soil pH, % sand, % silt, % clay, C, N, Olsen P, K, Mg, Ca, C/N, moisture, temperature was assessed for each response variables, i.e., relative abundance of each microbial group as well as total microbial biomass. We controlled for collinearity among response variables with Spearman correlation coefficients (Figure S1) and used $r>0.7$ as a threshold to remove explanatory variables that are too much correlated to limit multicollinearity issues in the models (Dormann et al., 2013). Soil C, Mg, Ca, silt % and clay % were highly correlated with other edaphic properties (Figure S1) and thus were not used as explanatory variables in these LMMs. A LMM with the remaining explanatory variables and random effect "region" was fitted for each response variable. Second, the generated models were simplified using the *dredge* function of the

201 *MuMIn* package based on Akaike's Information Criterion (AIC) and the single best model was
202 selected for the following analyses.

203 To explore the variability of the microbial community composition along the
204 distances-to-edge and forest type gradients, non-metric multidimensional scaling (NMDS) based
205 on the Bray Curtis distance matrix were conducted in the metaMDS function in the *vegan* package
206 (Oksanen et al., 2013). The Shepard diagram of NMDS can be found in Figure S2. Permutational
207 multivariate analysis of variance (PERMANOVA) was used to test the significance in the
208 microbial community composition (for 999 permutations) along the distance-to-edge and forest
209 type gradients (function *adonis* in *vegan* package).

210 The relationship between microbial community composition and edaphic properties was
211 determined by canonical correspondence analysis (CCA). Before the analysis, edaphic properties
212 were log- or sqrt-transformed (same with Table S1) to reduce the influence of a skewed data
213 distribution on the results. The best model was selected using the *step* function, which uses AIC
214 for the model choice. The collinearity among the constraining variables were checked by Variance
215 Inflation Factor (VIF) using *vif.cca* function and the variables with high VIF (>10) was excluded
216 in the final model. The CCA model and the permutation test for CCA were performed within
217 function *cca* and *anova.cca* in the *vegan* package.

218

219 **3. Results**

220 3.1 Abiotic environmental properties

221 We first tested the interactive effects of distance-to-edge and forest type on the edaphic properties.
222 Based on the LMMs, we found no significant interactions on the edaphic properties we tested

(Table S1). Then, we tested the main effects of distance-to-edge and forest types on those properties, respectively. We found the distance to the forest edge significantly influenced several edaphic properties. For example, soil K, Mg and Ca were 34.37%, 48.52%, and 63.63% were higher in forest edges than in forest interior, respectively ($p < 0.01$). The distance-to-edge also had an impact on soil pH and temperature, with a significantly higher pH value and temperature in forest edges than those in interiors ($p < 0.01$ and < 0.05, respectively). Forest types did not affect edaphic properties significantly, with an exception that soil temperature was significantly higher in open forest than in dense and intermediate forest ($p < 0.05$).

3.2 Soil microbial abundance

Similarly, we found no significant interactions of distance-to-edge and forest type on the relative abundance of microbial groups as well as the total microbial biomass based on the LMMs (Table S2). Then we tested the main effects only, i.e., forest types and distance from edges respectively, on the soil microbial abundance. We found that the distance-to-edge exerted a significant impact on soil microbial abundance, in which arbuscular mycorrhizal fungi (AMF) showed a significantly higher relative abundance in the forest edges than interiors, while Gram-negative bacteria showed a significantly higher proportion in the forest interiors than edges instead (Figure 2, Table 1). In contrast, the microbial abundance was not significantly affected by the forest type ($p > 0.05$, Table 1). However, when further investigating the magnitude of edge influence on the microbial composition in different forest types separately, we still found some contrasting edge effects among different microbial groups and forest types. In general, we found the greatest MEI in AMF groups, irrespective of forest types (dense forest, intermediate forest and open forests) (Figure 3).

When compared with dense forests, the absolute MEI of AMF tends to decrease (close to zero) in open forests, while it tends to increase in intermediate forests. In contrast, the absolute MEI in Gram-negative bacteria increased after the thinning practice (see intermediate and open forest), while the largest effect size was also found in intermediate forests. Notably, other microbes only changed to a minor degree or highly variable between the positive and negative MEI, indicating the unstable status of edge effects.

The edaphic properties had a varying effect on the relative abundance of different microbial groups (Table 2). Soil K was the most influential factor which was retained in most of the models. However, soil K was positively correlated with the abundance of saprotrophic fungi but negatively correlated with the abundance of Gram-negative bacteria and Actinobacteria. Other edaphic factors, for example, % sand also had significant correlations with microbial groups, which was positively correlated with Gram-negative bacteria while negatively correlated with Actinobacteria. Finally, the total microbial biomass was larger in soils with higher N but decreased with Olsen P concentrations.

3.3 Soil microbial community composition

The NMDS revealed the effects of multiple groups on the soil microbial community composition (Figure 4). The NMDS ordination of PLFA/NLFA profiles produced a two-dimensional ordination with low stress (S= 0.0827) after 20 iterations. Although not significant, distance-to-edge still had an impact on the soil microbial composition to a large degree (PERMANOVA, $p = 0.078$), while neither forest type nor the interaction between distance-to-edge and forest type had observable effects on the microbial community composition ($p = 0.736$ and $p = 0.631$, respectively; Table S3).

CCA were performed to investigate which edaphic properties contributed to explain the variation in soil microbial community composition (Figure 5). According to the CCA, the first two axes explained 24.54% of the variance in microbial community composition. Variation in the microbial community was significantly related to soil pH, K, N, % sand and temperature (Table S4). The first CCA axis was strongly correlated with soil K and pH but negatively correlated with % sand and temperature, and the second CCA axis was positively correlated with soil N.

4. Discussion

4.1 Distance-to-edge effect

A different microbial PLFA/NLFA community was observed between forest interiors and edges, which could be mainly attributed to edge-to-interior gradients in soil pH, N and K concentration (Figure 5a). It is known that forest edges that border a non-forested habitat can exhibit increased aboveground productivity due to the greater light exposure (Malanson and Kupfer, 1993), therefore the altered litter input and quality in edges might increase the soil pH and fertility (see Hamberg et al., 2008 and Table S1). These results are also in line with the study of Malmivaara-Lamsa et al. (2008), who showed that edge-to-interior gradients induced alterations on soil pH and nutrient levels that could make significant contributions to shifts in the microbial community composition of boreal urban forest soils. As shown in previous literature, microclimate, including soil temperature and moisture, could also affect the microbial composition and activity (Castano et al., 2018). Studies examining the influence of soil moisture on the microbial community are consistent in that increased moisture would accelerate the decomposition rates of soil organic matter to a certain level (Wang et al., 2016; Luis Moreno et al., 2019), at least in

289 temperate forests. However, the soil moisture tested here was a snapshot in time, therefore it is
290 possible that there isn't a visible correlation between the soil moisture and microbiota. In contrast,
291 we found a significant correlation between microbial composition and soil temperature (Figure 5a
292 & Table S4). This is in agreement with the findings of a meta-analysis (Zhou et al., 2016),
293 showing that the temperature is an important determinant of the composition of bacterial and
294 fungal communities by analyzing soil samples from a wide range of temperature gradients in
295 North America. In addition, we found that texture (% sand) also played a significant role in
296 driving the different composition of the microbial PLFA community. This may be linked to plant
297 communities in oligotrophic sandy soils, which exhibited an alternative resource acquisition
298 strategy to promote their nutrient and water capture from soil (Kochsiek et al., 2013), and the
299 changes in soil nutrient supply induced by those plants may in turn affect the composition of soil
300 microbial community.

301 However, the magnitude and direction of the edge effect on microbial abundance varied
302 considerably among microbial groups. We showed a higher relative abundance of AMF in forest
303 edges than interiors (Figure 2). Given that forest edges receive more light, wind and also present
304 warmer and drier conditions than forest interiors (Chen et al., 1993), the amount of
305 drought-resistant and warm-tolerant plant species are expected to increase in forest edges (Ranney
306 et al., 1981; Tuff et al., 2016). As shown in our previous study, total species richness of plant is
307 higher in edges, which is mainly attributed to the higher generalist richness here (Govaert et al.,
308 2020). Generalists were defined as species that can be or mainly be found in open vegetation as
309 well as those true open habitat species as described in Govaert et al. (2020). The higher generalist
310 abundance in edges indicates that these plant species may form a stronger AMF affinity than the

311 species growing in the interior (specialists in closed forests). Additionally, edges and intermediate
312 forests may also be associated the higher functional diversity in the understorey due to more
313 heterogeneous environmental conditions (Magura, 2017; De Pauw et al., 2021). Furthermore,
314 AMF have been known to enhance the hosting plants tolerance to abiotic stresses, such as drought,
315 salt, pollutants (Begum et al., 2019), and also benefit plants by increasing their disease resistance
316 (Song et al., 2015). Thus, the higher AMF in forest edges revealed here may indicate the potential
317 role of AMF in helping the hosting plant survive under complex and fluctuating environments in
318 edges. In contrast, the microclimate in the forest interiors was more stable compared to the forest
319 edges, since plant tissue can serve as a barrier that alleviates the forest interior from greater
320 fluctuations in temperature, wind speeds, moisture, etc (Chen et al., 1995). Here, we found a
321 significantly higher abundance of Gram-negative bacteria in forest interiors than edges (Table 1 &
322 Figure 2). Gram-negative bacteria are thought to be less resistant to stress and harsh conditions
323 owing to their thinner cell membrane and disability to sporulate, which makes them more
324 vulnerable in an inhospitable condition (Huang and Hull, 2017). Thus, the less variable
325 microclimate conditions such as found in forest interiors could provide a more favorable habitat
326 than forest edges for Gram-negative bacteria to colonize and propagate.
327
328 4.2 Forest management effect
329 In contrast, forest management did not exhibit a significant impact on microbial abundance and
330 community composition (Table 1 and Figure 4). Compared with clear-cutting, thinning (selective
331 harvesting) has been acknowledged as an alternative way in forest management practice due to the
332 benefits in maintaining mature forest functioning as well as the above- and below-ground

biodiversity (Gundersen et al., 2006; Lohmus, 2011; Muscolo et al., 2021). As an example, Cheng et al. (2018) studied the change of microbial community after a long-term thinning practice (18 years) and found no significant differences in the overall soil microbial composition among different treatments. Besides, the soil microbial community might recover from the disturbances even faster than we expect under reasonably moderate management. For example, Shao et al. (2016) evaluated the effects of plant removal practices on the soil biotic community in a bamboo forest and found that microbial community composition only changed in the first year after the plant removal and quickly recovered in the second year. Therefore, it is reasonable to infer that the microbial communities in open forests (recently thinned) and intermediate forests (thinned) were less affected by the management, or soon recovered to the original (dense forests) composition at the moment of our sampling. Moreover, the soil microbial community is expected to change in response to environmental properties including pH, soil texture, nutrient levels, and water content (Richter et al., 2018). However, most of these factors in the present study did not significantly differ between forest types (Table S1), indicating that the soil responses appear to be insensitive to forest thinning under current operational prescriptions.

4.3 Interactive effect of distance-to-edge and forest management

We found no evidence of interactive effects of forest management and distance to the edge on any microbial response variable. The lack of interaction suggested that PLFA community level responses of these two factors operate independently of each other. This contradicts our hypothesis from our previous work on plants (Govaert et al., 2020), i.e., the most contrasting edge effects of plant richness would be found in forest with most open canopy. It has been proved that the

355 well-developed plant structure in dense forests can play a protective role on the microclimatic
356 changes between forest edge and interior, and help to preserve the original microclimate in the
357 forest interior (Matlack, 1993; Meeussen et al., 2020). In the open forests, there was more solar
358 radiation entering the forest floor, which can somewhat level off the contrasting microclimate
359 between forest edge and interior compared to those in dense forests (Wright et al., 2010). While in
360 thinned forests, the plant structure was under development, and the relatively open edges may
361 result in a steep edge-to-interior gradient, which potentially enlarges the contrasting microclimate
362 conditions between edges and interiors compared to those in dense forests. In this study, although
363 we found the most significant effect size of several microbial groups (such as AMF and
364 Gram-negative bacteria) in intermediate forests (Figure 3), the results of the belowground
365 community were weak compared with previous experimental evidence from plant communities,
366 which revealed strong evidence of plant community composition along edge-to-interior gradients
367 responding differently according to forest types (Govaert et al., 2020). Ultimately, our results
368 agreed with previous studies, in which ecological theory developed for aboveground communities
369 differs in the degree of applicability to those belowground communities living in the soil matrix
370 (Deyn and Putten, 2005; Wardle, 2010).
371
372 4.4 Conclusions
373 Our results demonstrated, over a large geographic gradient, that the overall forest structure (forest
374 types) did not affect soil microbial community composition but the distance-to-edge did,
375 suggesting an environmental selection for microbial communities along the edge-to-interior
376 gradients. Notably, AMF and Gram-negative bacteria were the two microbial groups most affected

by the edge effects. Considering the warmer microclimate and higher soil nutrient availability in edges, more plant generalists occur there, which can facilitate the enrichment of AMF in edges. Besides, the symbiosis of AMF and plant species would also lead to a change in nutrient cycling and forest ecosystem functioning in turn. We also found that the overall microbial composition is closely related to the soil pH, N, K concentration, % sand and temperature, which should be explicitly considered in affecting the soil microbial composition in European deciduous forests. Overall, this study provided a profound basis of forest belowground community responses to distance to the forest edge and forest management. More investigations are still required to validate our observations in other forest or soil types and to scrutinize the impact on forest functioning.

CRediT authorship contribution statement

J.Y., H.B., P.D.F., and K.V. conceived the ideas and designed methodology; all authors collected data; J.Y. performed statistical analyses; J.Y., with contributions from H.B., P.D.F., and K.V. wrote the paper; all authors discussed the results and commented on the manuscript drafts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data related to this manuscript are available on Figshare: https://figshare.com/s/9b6b0518ff1f6df96b82.

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Reference

Allen, A.S., Schlesinger, W.H., 2004. Nutrient limitations to soil microbial biomass and activity in loblolly pine forests. Soil Biol. Biochem. 36 (4), 581–589.

Aussenac, G., 2000. Interactions between forest stands and microclimate: Ecophysiological aspects and consequences for silviculture. Ann. Forest Sci. 57 (3), 287–301.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting linear mixed-effects models using *lme4*. J. Stat. Soft 67 (1), 1–48.

Begum, N., Qin, C., Ahanger, M.A., Raza, S., Khan, M.I., Ashraf, M., Ahmed, N., Zhang, L.X., 2019. Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. Front. Plant Sci. 10, 1068.

Cadenasso, M.L., Pickett, S.T.A., 2001. Effect of edge structure on the flux of species into forest interiors. Conserv. Biol. 15 (1), 91–97.

Castano, C., Lindahl, B.D., Alday, J.G., Hagenbo, A., Martinez de Aragon, J., Parlade, J., Pera, J., Antonio Bonet, J., 2018. Soil microclimate changes affect soil fungal communities in a Mediterranean pine forest. New Phytol. 220 (4), 1211–1221.

Chen, J., Franklin, J.F., Spies, T.A., 1993. Contrasting microclimates among clearcut, edge, and interior of old-growth Douglas-fir forest. Agr. Forest Meteorol. 63 (3-4), 219–237.

Chen, J., Franklin, J.F., Spies, T.A., 1995. Growing- season microclimatic gradients from clearcut edges into old-growth Douglas-fir forests. Ecol. Appl. 5 (1), 74–86.

Cheng, X.R., Xing, W.L., Yuan, H.J., Yu, M.K., 2018. Long-term thinning does not significantly affect soil water-stable aggregates and diversity of bacteria and fungi in Chinese fir (*Cunninghamia lanceolata*) plantations in Eastern China. Forests 9 (11), 687.

Crow, T.R., Buckley, D.S., Nauertz, E.A., Zasada, J.C., 2002. Effects of management on the

composition and structure of northern hardwood forests in Upper Michigan. Forest Sci. 48 (1), 129–145.

Davies-Colley, R.J., Payne, G.W., van Elswijk, M., 2000. Microclimate gradients across a forest edge. New Zeal. J. Ecol. 24 (2), 111–121.

De Pauw, K., Meeussen, C., Govaert, S., Sanczuk, P., Vanneste, T., Bernhardt-Romermann, M., Bollmann, K., Brunet, J., Calders, K., Cousins, S.A.O., Diekmann, M., Hedwall, P.-O., Iacopetti, G., Lenoir, J., Lindmo, S., Orczewska, A., Ponette, Q., Plue, J., Selvi, F., Spicher, F., Verbeeck, H., Vermeir, P., Zellweger, F., Verheyen, K., Vangansbeke, P., De Frenne, P., 2021. Taxonomic, phylogenetic and functional diversity of understorey plants respond differently to environmental conditions in European forest edges. J. Ecol. 109 (7), 2629–2648.

Deyn, G., Putten, W., 2005. Linking aboveground and belowground diversity. Trends Ecol. Evol. 20 (11), 625–633.

Didham, R.K., Lawton, J.H., 1999. Edge structure determines the magnitude of changes in microclimate and vegetation structure in tropical forest fragments. Biotropica 31 (1), 17–30.

Dormann, C.F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carre, G., Garcia Marquez, J.R., Gruber, B., Lafourcade, B., Leitao, P.J., Muenkemueller, T., McClean, C., Osborne, P.E., Reineking, B., Schroeder, B., Skidmore, A.K., Zurell, D., Lautenbach, S., 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography 36 (1), 27–46.

Ewers, R.M., Didham, R.K., 2007. The Effect of fragment shape and species' sensitivity to habitat edges on animal population size. Conserv. Biol. 21 (4), 927–936.

Fagan, W.F., Cantrell, R.S., Cosner, C., 1999. How habitat edges change species interactions. The Am. Nat. 153 (2), 165–182.

Farrell, M., Kuhn, T.K., Macdonald, L.M., Maddern, T.M., Murphy, D.V., Hall, P.A., Singh, B.P., Baumann, K., Krull, E.S., Baldock, J.A., 2013. Microbial utilisation of biochar-derived carbon. Sci. Total Environ. 465, 288–297.

Fischer, R., Taubert, F., Mueller, M.S., Groeneveld, J., Lehmann, S., Wiegand, T., Huth, A., 2021. Accelerated forest fragmentation leads to critical increase in tropical forest edge area. Sci. Adv. 7 (37), 7012.

Fletcher, R.J., Koford, R.R., 2003. Spatial responses of Bobolinks (*Dolichonyx oryzivorus*) near different types of edges in northern Iowa. Auk 120 (3), 799–810.

Fuzy, A., Bothe, H., Molnar, E., Biro, B., 2014. Mycorrhizal symbiosis effects on growth of chalk false-brome (*Brachypodium pinnatum*) are dependent on the environmental light regime. J. Plant Physiol. 171 (5), 1–6.

Girona, M.M., Morin, H., Lussier, J.-M., Walsh, D., 2016. Radial growth response of black spruce stands ten years after experimental shelterwoods and seed-tree cuttings in Boreal forest. Forests 7 (10), 240.

Govaert, S., Meeussen, C., Vanneste, T., Bollmann, K., Brunet, J., Cousins, S.A., Diekmann, M., Graae, B.J., Hedwall, P.O., Heinken, T., 2020. Edge influence on understorey plant communities depends on forest management. J. Veg. Sci. 31 (2), 281-292.

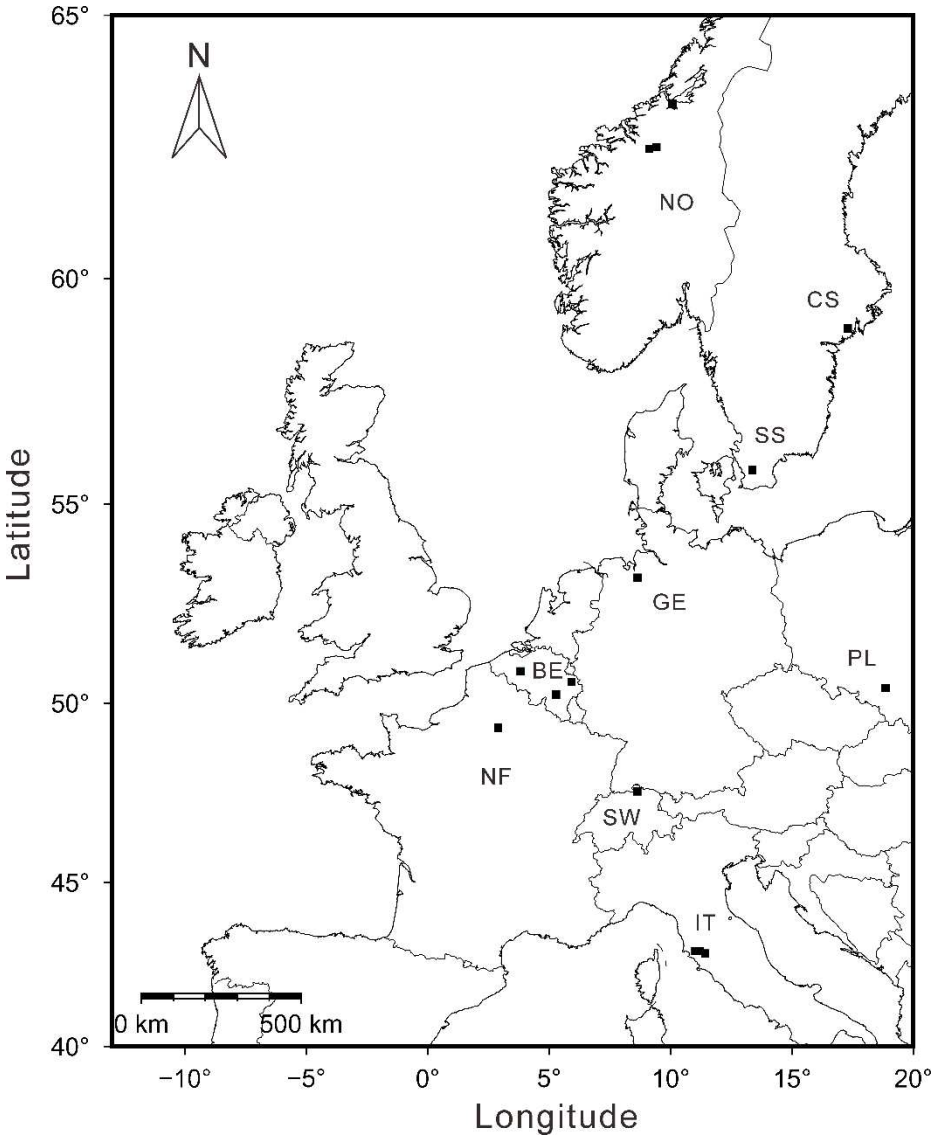
Gundersen, P., Schmidt, I.K., Raulund-Rasmussen, K., 2006. Leaching of nitrate from temperate forests-effects of air pollution and forest management. Environ. Rev. 14 (1), 1–57.

Haddad, N.M., Brudvig, L.A., Clobert, J., Davies, K.F., Gonzalez, A., Holt, R.D., Lovejoy, T.E., Sexton, J.O., Austin, M.P., Collins, C.D., Cook, W.M., Damschen, E.I., Ewers, R.M., Foster, B.L.,

475 Jenkins, C.N., King, A.J., Laurance, W.F., Levey, D.J., Margules, C.R., Melbourne, B.A.,
476 Nicholls, A.O., Orrock, J.L., Song, D.-X., Townshend, J.R., 2015. Habitat fragmentation and
477 its lasting impact on Earth's ecosystems. Sci. Adv. 1 (2), e1500052.
478 Hamberg, L., Lehvavirta, S., Malmivaara-Lamsa, M., Rita, H., Kotze, D.J., 2008. The effects of habitat
479 edges and trampling on understorey vegetation in urban forests in Helsinki, Finland. Appl. Veg.
480 Sci. 11 (1), 83–98.
481 Harper, K.A., Macdonald, S.E., Burton, P.J., Chen, J.Q., Brosofske, K.D., Saunders, S.C., Euskirchen,
482 E.S., Roberts, D., Jaiteh, M.S., Esseen, P.A., 2005. Edge influence on forest structure and
483 composition in fragmented landscapes. Conserv. Biol. 19 (3), 768–782.
484 Harrell, F.E., Dupont, C., 2016. Hmisc: Harrell Miscellaneous. Version 4.0-0 https://cran.r-project.org/
485 web/packages/ Hmisc/index.html.
486 Huang, M.W., Hull, C.M., 2017. Sporulation: how to survive on planet Earth (and beyond). Curr. Genet.
487 63 (5), 831–838.
488 Kaiser, C., Kilburn, M.R., Clode, P.L., Fuchslueger, L., Koranda, M., Cliff, J.B., Solaiman, Z.M.,
489 Murphy, D.V., 2015. Exploring the transfer of recent plant photosynthates to soil microbes:
490 mycorrhizal pathway vs direct root exudation. New Phytol. 205 (4), 1537–1551.
491 Kaplan, J.O., Krumhardt, K.M., Zimmermann, N., 2009. The prehistoric and preindustrial deforestation
492 of Europe. Quaternary Sci. Rev. 28 (27), 3016–3034.
493 Karraker, N.E., Welsh, H.H., Jr., 2006. Long-term impacts of even-aged timber management on
494 abundance and body condition of terrestrial amphibians in Northwestern California. Biol.
495 Conserv. 131 (1), 132–140.
496 Kochsiek, A., Tan, S., Russo, S.E., 2013. Fine root dynamics in relation to nutrients in oligotrophic
497 Bornean rain forest soils. Plant Ecol. 214 (6), 869–882.
498 Lohmus, A., 2011. Silviculture as a disturbance regime: the effects of clear-cutting, planting and
499 thinning on polypore communities in mixed forests. J. Forest Res. 16 (3), 194–202.
500 Louche- Tessandier, D., Samson, G., Hernandez- Sebastia, C., Chagvardieff, P., Desjardins, Y., 1999.
501 Importance of light and CO_2 on the effects of endomycorrhizal colonization on growth and
502 photosynthesis of potato plantlets (*Solanum tuberosum*) in an in vitro tripartite system. New
503 Phytol. 142 (3), 539–550.
504 Luis Moreno, J., Torres, I.F., Garcia, C., Lopez-Mondejar, R., Bastida, F., 2019. Land use shapes the
505 resistance of the soil microbial community and the C cycling response to drought in a
506 semi-arid area. Sci. Total Environ. 648, 1018–1030.
507 Magura, T., 2017. Ignoring functional and phylogenetic features masks the edge influence on ground
508 beetle diversity across forest-grassland gradient. Forest Ecol. Manag. 384, 371–377.
509 Malanson, G.P., Kupfer, J.A., 1993. Simulated fate of leaf litter and large woody debris at a riparian
510 cutbank. Canadian J. Forest Res. 23 (4), 582–590.
511 Malmivaara-Lamsa, M., Hamberg, L., Haapamaki, E., Liski, J., Kotze, D.J., Lehvavirta, S., Fritze, H.,
512 2008. Edge effects and trampling in boreal urban forest fragments-impacts on the soil
513 microbial community. Soil Biol. Biochem. 40 (7), 1612–1621.
514 Matlack, G.R., 1993. Microenvironment variation within and among forest edge sites in the eastern
515 United States. Biol. Conserv. 66 (3), 185–194.
516 Matlack, G.R., Litvaitis, J.A., 1999. Forest edges. In: M.L.Hunter (eds) Maintaining biodiversity in
517 forest ecosystems. Cambridge University Press, Cambridge, pp 210–233.
518 Meeussen, C., Govaert, S., Vanneste, T., Calders, K., Bollmann, K., Brunet, J., Cousins, S.A.,

519 Diekmann, M., Graae, B.J., Hedwall, P.-O., 2020. Structural variation of forest edges across
520 Europe. Forest Ecol. Manag. 462, 117929.
521 Meeussen, C., Govaert, S., Vanneste, T., Haesen, S., Van Meerbeek, K., Bollmann, K., Brunet, J.,
522 Calders, K., Cousins, S.A.O., Diekmann, M., Graae, B.J., Iacopetti, G., Lenoir, J., Orczewska,
523 A., Ponette, Q., Plue, J., Selvi, F., Spicher, F., Sorensen, M.V., Verbeeck, H., Vermeir, P.,
524 Verheyen, K., Vangansbeke, P., De Frenne, P., 2021. Drivers of carbon stocks in forest edges
525 across Europe. Sci. Total Environ. 759, 143497.
526 Mitchell, P.J., Simpson, A.J., Soong, R., Simpson, M.J., 2015. Shifts in microbial community and
527 water-extractable organic matter composition with biochar amendment in a temperate forest
528 soil. Soil Biol. Biochem. 81, 244–254.
529 Morris, A.D., Miller, D.A., Kalcounis-Rueppell, M.C., 2010. Use of forest edges by bats in a managed
530 pine forest landscape. J. Wildlife Manage. 74 (1), 26–34.
531 Muscolo, A., Settineri, G., Romeo, F., Mallamaci, C., 2021. Soil biodiversity as affected by different
532 thinning intensities in a pinus laricio stand of Calabrian Apennine, South Italy. Forests 12 (1),
533 108.
534 Ngosong, C., Gabriel, E., Ruess, L., 2012. Use of the signature fatty acid 16: 1ω5 as a tool to determine
535 the distribution of arbuscular mycorrhizal fungi in soil. J. Lipids 2012, 236807–236808.
536 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R., Simpson, G.L.,
537 Solymos, P., Stevens, M.H.H., Wagner, H., 2012. Package 'vegan'-Community ecology
538 package, version 2.0 http://cran.r-project.org/web/packages/vegan/vegan.pdf.
539 Olsson, P.A., 1999. Signature fatty acids provide tools for determination of the distribution and
540 interactions of mycorrhizal fungi in soil. FEMS Microbiol. Ecol. 29 (4), 303–310.
541 Orczewska, A., Glista, A., 2005. Floristic analysis of the two woodland-meadow ecotones differing in
542 orientation of the forest edge. Polish J. Ecol. 53 (3), 365–382.
543 Osburn, E.D., Badgley, B.D., Strahm, B.D., Aylward, F.O., Barrett, J.E., 2021. Emergent properties of
544 microbial communities drive accelerated biogeochemical cycling in disturbed temperate
545 forests. Ecology 102 (12): e03553.
546 Pardini, R., 2004. Effects of forest fragmentation on small mammals in an Atlantic Forest landscape.
547 Biodivers. Conserv. 13 (13), 2567–2586.
548 Quideau, S.A., McIntosh, A.C., Norris, C.E., Lloret, E., Swallow, M.J., Hannam, K., 2016. Extraction
549 and analysis of microbial phospholipid fatty acids in soils. J. Vis. Exp. 114, e54360.
550 R Core Team, 2008. R: A language and environment for statistical computing, R Foundation for
551 Statistical Computing: Vienna, Austria.
552 Ranney, J.W., Bruner, M.C., Levenson, J.B., 1981. The importance of edge in the structure and
553 dynamics of forest islands. In: Burgess R.L., Sharpe D.M. (eds) Forest Island Dynamics in
554 Man-dominated Landscapes, Springer-Verlag, New York, pp 67–96.
555 Remy, E., Wuyts, K., Verheyen, K., Gundersen, P., Boeckx, P., 2018. Altered microbial communities
556 and nitrogen availability in temperate forest edges. Soil Biol. Biochem. 116, 179–188.
557 Richter, A., Schoning, I., Kahl, T., Bauhus, J., Ruess, L., 2018. Regional environmental conditions
558 shape microbial community structure stronger than local forest management intensity. Forest
559 Ecol. Manag. 409, 250–259.
560 Riitters, K., Wickham, J., Costanza, J.K., Vogt, P., 2016. A global evaluation of forest interior area
561 dynamics using tree cover data from 2000 to 2012. Landscape Ecol. 31 (1), 137–148.
562 Riitters, K.H., Oneill, R.V., Jones, K.B., 1997. Assessing habitat suitability at multiple scales: A

563 landscape-level approach. Biol. Conserv. 81 (1–2), 191–202.
564 Shao, Y., Wang, X., Zhao, J., Wu, J., Zhang, W., Neher, D.A., Li, Y., Lou, Y., Fu, S., 2016. Subordinate
565 plants sustain the complexity and stability of soil micro- food webs in natural bamboo forest
566 ecosystems. J. Appl. Ecol. 53 (1), 130–139.
567 Song, Y.Y., Chen, D.M., Lu, K., Sun, Z.X., Zeng, R.S., 2015. Enhanced tomato disease resistance
568 primed by arbuscular mycorrhizal fungus. Front. Plant Sci. 6, 786.
569 Steinbeiss, S., Gleixner, G., Antonietti, M., 2009. Effect of biochar amendment on soil carbon balance
570 and soil microbial activity. Soil Biol. Biochem. 41 (6), 1301–1310.
571 Tuff, K.T., Tuff, T., Davies, K.F., 2016. A framework for integrating thermal biology into fragmentation
572 research. Ecol. Lett. 19 (4), 361–374.
573 Ushio, M., Wagai, R., Balser, T.C., Kitayama, K., 2008. Variations in the soil microbial community
574 composition of a tropical montane forest ecosystem: Does tree species matter? Soil Biol.
575 Biochem. 40 (10), 2699–2702.
576 Wang, D., He, N., Wang, Q., Lu, Y., Wang, Q., Xu, Z., Zhu, J., 2016. Effects of Temperature and
577 Moisture on Soil Organic Matter Decomposition Along Elevation Gradients on the Changbai
578 Mountains, Northeast China. Pedosphere 26 (3), 399–407.
579 Wardle, D.A., 2010. Communities and Ecosystems: Linking the Above-ground and Below-ground
580 Components. Austral Ecol. 29 (3), 358–359.
581 Willers, C., Jansen van Rensburg, P., Claassens, S., 2015. Phospholipid fatty acid profiling of microbial
582 communities–a review of interpretations and recent applications. J. Appl. Microbiol. 119 (5),
583 1207–1218.
584 Wright, T.E., Kasel, S., Tausz, M., Bennett, L.T., 2010. Edge microclimate of temperate woodlands as
585 affected by adjoining land use. Agr. Forest Meteorol. 150 (7–8), 1138–1146.
586 Xu, Y., Seshadri, B., Bolan, N., Sarkar, B., Ok, Y.S., Zhang, W., Rumpel, C., Sparks, D., Farrell, M.,
587 Hall, T., 2019. Microbial functional diversity and carbon use feedback in soils as affected by
588 heavy metals. Environ. Int. 125, 478–488.
589 Young, A., Mitchell, N., 1994. Microclimate and vegetation edge effects in a fragmented
590 podocarp-broadleaf forest in New Zealand. Biol. Conserv. 67 (1), 63–72.
591 Zhang, B., Li, Y., Ren, T., Tian, Z., Wang, G., He, X., Tian, C., 2014. Short-term effect of tillage and
592 crop rotation on microbial community structure and enzyme activities of a clay loam soil. Biol.
593 Fert. Soils 50 (7), 1077–1085.
594 Zhou, J., Deng, Y., Shen, L., Wen, C., Yan, Q., Ning, D., Qin, Y., Xue, K., Wu, L., He, Z., Voordeckers,
595 J.W., Van Nostrand, J.D., Buzzard, V., Michaletz, S.T., Enquist, B.J., Weiser, M.D., Kaspari,
596 M., Waide, R., Yang, Y., Brown, J.H., 2016. Temperature mediates continental-scale diversity
597 of microbes in forest soils. Nat. Commun. 7, 12083.



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599 **Figure 1** Overview of study regions along the latitudinal gradient in Europe. Norway (NO),
600 Central Sweden (CS), Southern Sweden (SS), Germany (GE), Poland (PL), Belgium (BE),
601 Northern France (NF), Switzerland (SW) and Italy (IT).

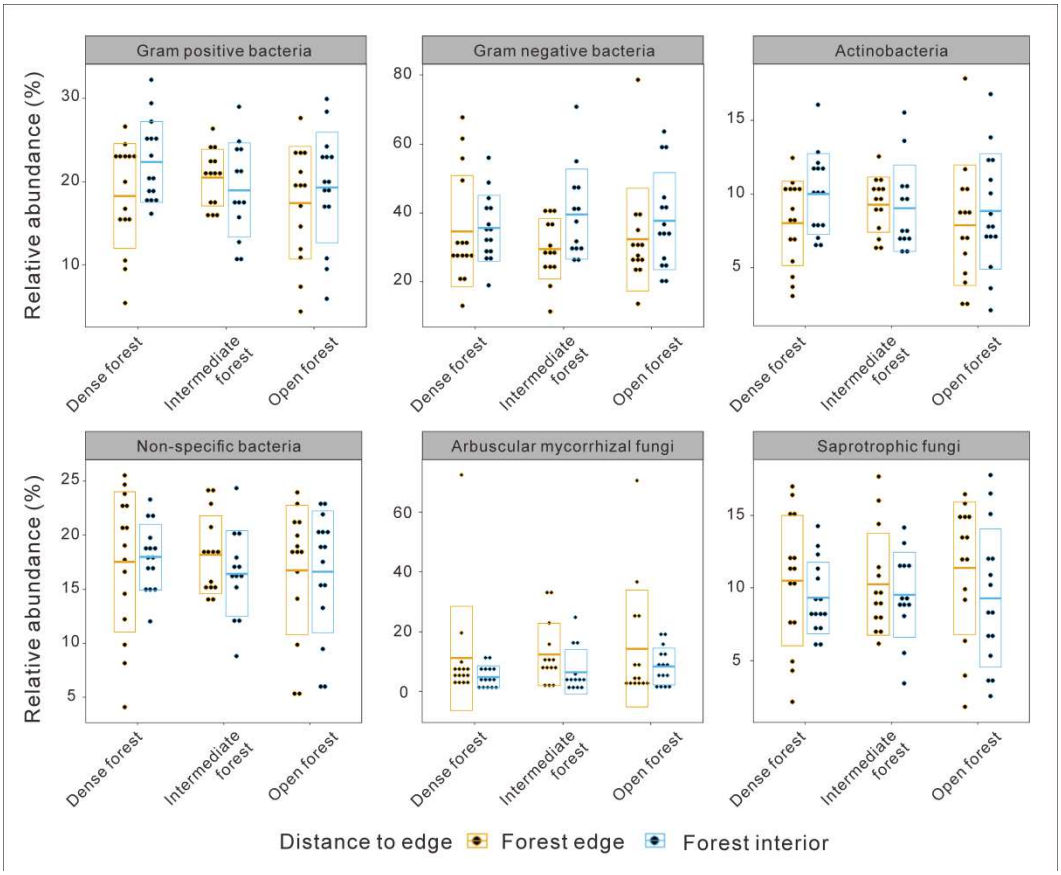
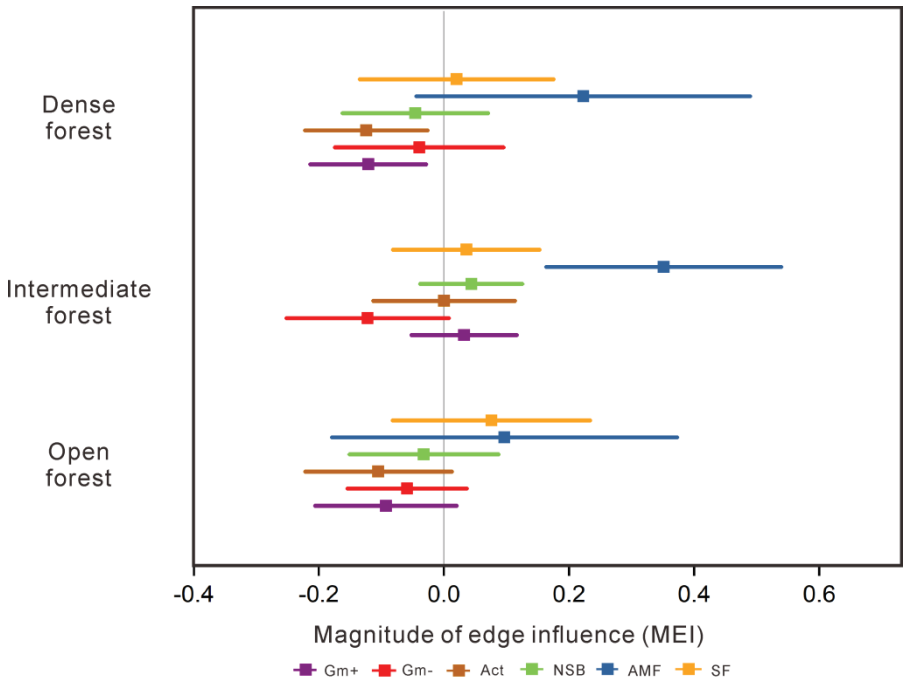


Figure 2 Relative abundances of microbial groups in the three forest types (dense, intermediate and open forests) and at the edge or in the interior. The relative abundance of each microbial group was calculated as the sum of representative PLFA or NLFA biomarkers divided by the total microbial biomass.



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609 **Figure 3** Magnitude of edge influence (MEI) of each microbial group in three different forest
610 types. Error bars indicate 95% confidence intervals. Gm+: Gram-positive bacteria; Gm-:
611 Gram-negative bacteria; Act: Actinobacteria; NSB: Non-specific bacteria; AMF: Arbuscular
612 mycorrhizal fungi; SF: Saprotrophic fungi.

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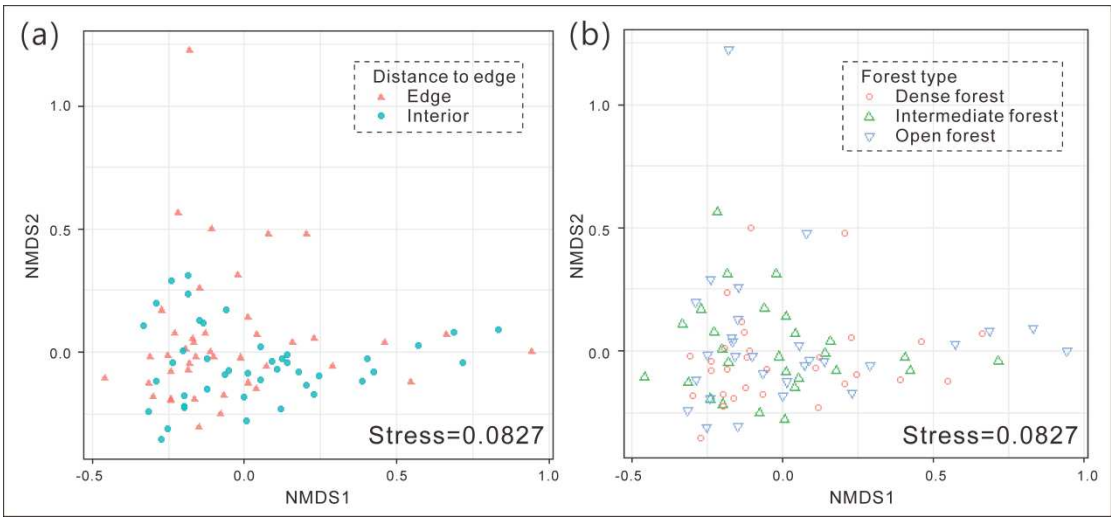

Figure 4 Non-metric multidimensional scaling (NMDS) results of the microbial composition based on the PLFA/NLFA biomarkers. Variation of the microbial composition between different distances to forest edge (a) and forest types (b) based on the Bray-Curtis distance matrix.

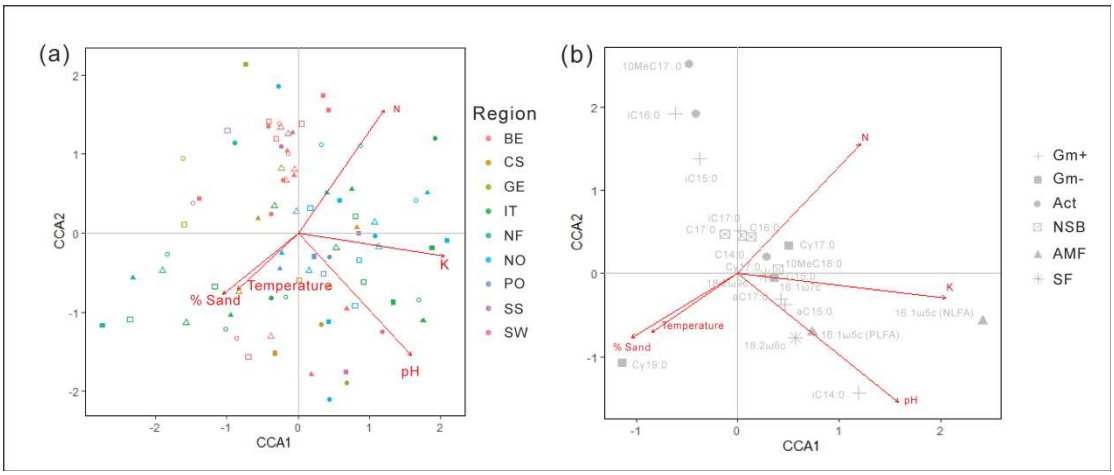

Figure 5 The relationship between microbial community composition and microclimate soil temperature and soil properties based on canonical correspondence analysis (CCA). The first axis accounted for 20.15% of the variability and the second axis accounted for 4.39%. The significance levels of CCA1 and CCA2 were P=0.001 and P=0.165, respectively (permutation test). (a) The distribution of samples collected in different regions. (b) The distribution of single PLFA or NLFA biomarkers. Different symbols in this panel indicated the classified microbial groups. Gm+: Gram-positive bacteria; Gm-: Gram-negative bacteria; Act: Actinobacteria; NSB: non-specific bacteria; AMF: Arbuscular mycorrhizal fungi; SF: Saprotrophic fungi.

631 **Table 1** Effects of forest type and distance from the edge on the relative abundance of microbial
632 groups and total microbial biomass based on linear mixed-effect models. Chi-square values of
633 each specific microbes and total microbial biomass were calculated based on ANOVA results of
634 each model. Statistically significant Chi-square values were highlighted in bold.

	Distance from edge			Forest types		
	Chi-square value	Conditional R^2 (%)	Marginal R^2 (%)	Chi-square value	Conditional R^2 (%)	Marginal R^2 (%)
Gram positive	1.7105 ns	4.73	1.92	1.8681 ns	4.66	2.10
Gram negative	**3.8504** *↑	10.60	4.06	0.078 ns	6.24	0.09
Actinobacteria	2.5482 ns	16.61	2.51	1.3623 ns	15.05	1.36
Non-specific bacteria	0.1645 ns	8.77	0.18	0.8328 ns	9.81	0.88
Arbuscular mycorrhizal fungi	**5.3332***↓	5.90	5.90	1.0751 ns	1.25	1.25
Saprotrophic fungi	3.0074 ns	12.18	3.12	0.2355 ns	8.64	0.25
Total microbial biomass §	2.6376 ns	11.31	2.76	1.6341 ns	10.01	1.73

635 Asterisks indicate significance (*p < 0.05; ns, non-significant). §, log-transformed. The formulas
636 used for distance from edge and forest type were 'Y ~ distance from forest edge + (1 | region)' and
637 'Y~ forest type + (1 | region)', respectively.

Table 2 Effects of soil pH, % sand, soil N, Olsen P, K content, C/N, soil moisture, and soil temperature on the relative abundance of each microbial group and total biomass based on linear mixed effect models. Chi-square values of each specific microbes and total microbial biomass were reported. Statistically significant Chi-square values were highlighted in bold.

	pH	% Sand §	N (mg/kg) £	Olsen.P (mg/kg)§	K (mg/kg) §	C/N §	Moisture %	Temperature ℃	Conditional R^2 (%)	Marginal R^2 (%)
Gram positive	1.7583						**8.3735**** ↑	2.7036	16.35	13.49
Gram negative		**8.5289**** ↑			**6.5015*** ↓	**4.3730*** ↓		2.4495	22.59	20.05
Actinobacteria		**5.2099*** ↓	**7.5980**** ↑	**7.6963**** ↑	**9.0984**** ↓				26.25	25.24
Non-specific bacteria			**6.5811*** ↑						8.09	7.28
Arbuscular Mycorrhizal Fungi					2.2676				25.98	25.98
Saprotrophic Fungi	**5.7219*** ↑				**4.0215*** ↑				17.83	17.57
Total microbial biomass §			**14.9839***** ↑	**4.1619*** ↓					17.91	16.36

*p<0.05,**p<0.01,***p<0.001.