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Effects of Forest Management Practices in Temperate Beech Forests on Bacterial and Fungal Communities Involved in Leaf Litter Degradation

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Abstract Forest management practices (FMPs) significantly influence important ecological processes and services in Central European forests, such as leaf litter decomposition and nutrient cycling. Changes in leaf litter diversity, and thus, its quality as well as microbial community structure and function induced by different FMPs were hypothesized to be the main drivers causing shifts in decomposition rates and nutrient release in managed forests. In a litterbag experiment lasting 473 days, we aimed to investigate the effects of FMPs (even-aged timber management, selective logging and unmanaged) on bacterial and fungal communities involved in leaf litter degradation over time. Our results showed that microbial communities in leaf litter were strongly influenced by both FMPs and sampling date. The results from nonmetric multidimensional scaling (NMDS) ordination revealed distinct patterns of bacterial and fungal successions over time in leaf litter. We demonstrated that FMPs and sampling dates can influence a range of factors, including leaf litter quality, microbial macronutrients, and pH, which significantly correlate with microbial community successions.

Keywords Forest management · Fungal diversity · Bacterial diversity · Microbial community · Lignocellulose decomposition · Decomposition rates · Nutrient release

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Introduction

Forest management practices (FMPs) alter the composition and performance of most forest biota including plants, animals, and microorganisms [1–5]. Intensive forest management can change the quantity and quality of leaf litter by modifying the diversity and community structure of higher plants in the tree, shrub, and herb strata [2, 6, 7]. Changes in physical and chemical properties of leaf litter are known to affect leaf litter decomposition rates and consequently impact nutrient cycling and carbon sequestration rates in forest ecosystems [8–10]. It has been proposed that a managed forest (such as even-aged timber management) has higher decomposition and nutrient release rates compared to unmanaged forests as a result of

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highly heterogeneous litter quality [7]. Several studies have indicated that an increase in plant diversity and, thus, a larger spectrum of leaf litter qualities causes nonadditive decomposition rates, meaning that litter mixtures decompose at a different rate compared to that predicted from decomposition rates of single litter types [11, 12]. Alternatively, Chapman et al. [12] suggested that the alteration of microbial succession could be the mechanism for nonadditive effects in litter decomposition.

In Central Europe as well as elsewhere in the Northern hemisphere, even-aged forests are widespread. In North America, and elsewhere in the palearctic boreal forest belt, even-aged timber management includes clear cutting by heavy machinery, especially in softwood stands [13]. However, in Central Europe, clear cutting without intended abandonment of land-use as forest is normally not allowed or restricted to small areas of few hectares. Nevertheless, even-aged forests in different areas are all subjected to intensive thinning operations and wood harvesting and lead to an even age of timber trees [14]. Thinning operations and forest structure significantly affect the microclimate such as light penetration and temperature. Wood harvesting and transport can significantly impact soil physical characteristics, e.g., through compaction, that in turn could significantly influence soil microbial communities. Selective logging (in German known as "Plenterwald") is encouraged as eco-friendly forest management system in hardwood forests [14]. Although selectively logged forests have uneven-aged forest structures, they can nevertheless differ strongly from unmanaged forest. If many trees are harvested, this forest management practice can be considered as intensive. Selectively logged forests have been suggested to be similarly efficient in maintaining diversity of different groups of saproxylic organisms including both fungi and invertebrates [4, 5] as unmanaged forests; however, to the best of our knowledge the effect of selective logging on microbial communities in leaf litter is still unknown. Due to the wide distribution of even-aged forests and the increasing interest for eco-friendly forest management practices, even-aged timber management and selective logging were chosen for our study.

In managed forest ecosystems, large amounts of wood are often harvested. Thus, leaf litter may be considered as the major source of nutrients that return to the soil [7]. Effects on nutrient dynamics and decomposition rates can significantly affect the nutrient status, productivity, and sustainability of such managed forests. Microbial communities, both bacterial and fungal, play important roles in decomposition processes in leaf litter as well as other substrates [4, 5, 7]. Thus, investigating microbial community successions in leaf litter under different FMPs may provide crucial information to explain the differences in leaf litter decomposition rates in forests with different FMPs [12]. In our study, we hypothesized that nonadditive effects in litter decomposition rates in even-aged and selectively logged forests can be partly explained by differences of microbial community successions as compared to unmanaged forest [12].

To test this hypothesis, this study aimed at (i) investigating the effects of different FMPs on bacterial and fungal communities in leaf litter over time and (ii) testing whether alteration of microbial community development could be the mechanism for nonadditive decomposition rates occurring in mixed leaf litter under different FMPs. Therefore, we carried out a 15-month litterbag experiment and investigated microbial diversity patterns at four time points using automated ribosomal intergenic spacer analysis (ARISA) of the bacterial intergenic spacer (IGS) and the fungal nuclear ribosomal internal transcribed spacer (nrITS) regions [4, 5, 15]. ARISA is a widely used cost-effective molecular culture-independent technique for analyzing microbial diversity and community structure in various types of habitats encompassing both terrestrial and aquatic ecosystems [4, 5, 15–20]. We carried out multivariate analysis to investigate how different factors under different FMPs, including leaf litter quality parameters (carbon/nitrogen and lignin/N ratios) and other abiotic factors in leaf litter (pH, water content, concentrations of microbial macronutrients and micronutrients) shaped the bacterial and fungal communities and their succession.

Materials and Methods

Study Area and FMPs

Leaf litter samples used in this study were obtained from a 473-day litterbag experiment carried out in the Hainich-Dün region in Central Germany (about 1300 km^2; 51°16′ N 10°47′ E), part of the German Biodiversity Exploratories (http://www.biodiversity-exploratories.de), as described by Purahong et al. [7]. The three FMPs used in this study were (i) European beech even-aged forest (BA, semi-natural forest with natural regeneration, even-age forest structure), (ii) European beech selectively logged forest (BS, close-to-nature forest management with natural regeneration, uneven-age forest structure), and (iii) unmanaged deciduous forest reserves dominated by European beech (BU, uneven-age forest structure). All sites were dominated by European beech (*Fagus sylvatica*) but, depending on the management, had varying degrees of admixed additional tree species (Table S1). The BA was dominated by three tree species (85 % *F. sylvatica*, 10 % *Acer* sp., and *Fraxinus* sp. 5 %), whereas the BS was dominated by two tree species (90 % *F. sylvatica* and 10 % *Acer* sp.) and the BU by *F. sylvatica* alone. All study sites are located within a 30-km radius and thus varied little in geographic and climatic conditions. The soil of all sites has been characterized as Stagnosol [21], with a pH of 5.1 ± 1.1 [22] and a thin litter layer (2–5 cm depth).

Experimental Design and Litterbag Experiment

Within each FMP area (10,000 m^2 each), we assigned three plots (2×8 m) located on available flat land; these represented three replicates. The distances between the plots were at least 2.5 m. See Purahong et al. [7] for detailed information on the experimental design.

A total of 135 litterbags were used for the experiment. Each litterbag (25×25 cm, 2-mm nylon mesh size) contained 10 g of mixed local leaves, representative of the litter composition of the deciduous tree species at each site (Table S1) [7]. Freshly fallen leaves were collected from the forest floor during the peak litter fall period. At the end of the litter fall period (13 November 2009), 108 litterbags were placed horizontally among fallen leaves in the upper litter horizon of the study plots (12 bags per plot resulting in 36 litterbags per management type); 27 litter bags (9 per treatment) were retained to determine the initial dry mass (oven-dried at 105 °C≥24 h until constant weight). Three litterbags per plot were retrieved on four sampling dates: in 2010 on 10 February (89 days, winter), 12 May (180 days, spring), and 24 August (284 days, summer) and in 2011 on 1 March (473 days, winter). Leaf litter from the three litterbags retrieved from the same plot and treatment were pooled. Thus, three composite samples from each site were obtained for each treatment and sampling date. Each composite sample was homogenized and divided into subsamples for DNA extraction and determination of physical properties, nutrient concentrations (microbial macronutrients: C, N, P, K, Ca, Mg, Fe; microbial micronutrients: Mn, Co, Cu, V), and lignin content. Samples were stored at −20 °C until analysis. Further details on the experimental procedure can be found in supporting information.

Physicochemical Analyses of Leaf Litter

The water content was analyzed by oven drying the leaf litter samples at 105 °C≥24 h or until constant weight. Leaf litter pH was determined in H_2O and $CaCl_2$ [23] using 0.5 g of ground litter sample and 10 ml demineralized water after overnight incubation (see more detailed information in supporting information). Total C and N were determined by dry combustion at 1000 °C with an Elementar Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Nutrient ions (Mg, K, P, Ca, Fe, Cu, V, Mn, and Co) were determined using inductively coupled plasma (ICP) optical emission spectrometry (ICP-OES) and mass spectrometry (ICP-MS) according to manufacturers' specifications (PerkinElmer Inc., Waltham, MA, USA). Klason lignin content was determined gravimetrically as the dry mass of solids after sequential hydrolysis with sulfuric acid (72 % *w/w*); in a second step, acid soluble lignin was measured by UV spectrophotometry in 4 % H_2SO_4 [24, 25]. Total lignin was obtained by summing acid-insoluble (Klason) lignin and acid-soluble lignin [26]. All physicochemical analyses were conducted in triplicate on the same subsample.

DNA Extraction and Determination of Bacterial and Fungal ARISA Patterns

DNA was extracted from 100 mg of each of the homogenized freeze-dried leaf litter samples using a ZR Soil Microbe DNA MiniPrep kit according to the manufacturer's instructions (Zymo Research, Irvine, CA, USA) [4, 5]. The presence and quantity of genomic DNA were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany), and the extracts were then stored at −20 °C.

B-ARISA (bacterial) and F-ARISA (fungal) were conducted under the conditions described by Frossard et al. [15] and Purahong et al. [4], respectively. Operational taxonomic units (OTUs) derived from ARISA were defined as described previously by Green et al. [27] and Jones et al. [16] and may differ from those OTU definitions relying on sequences. Briefly, B-ARISA polymerase chain reaction was performed in duplicate reactions under the conditions described by Borneman and Triplett [28] and modified according to Frossard et al. [15] and Purahong et al. [29]: the PCR mixture (20 μl) contained 1 μl DNA template (~20 ng DNA template as determined by NanoDrop); 10 μM of primer 1406f (5′-TGYACACACCGCCCGT-3′) labeled with FAM at 5′-end and an unlabeled 23Sr primer (5′-GGGTTBCCCCATTC RG-3′); 4 μl FIREPol 5× Master Mix (Solis BioDyne, Tartu, Estonia); and water to 20 μl. PCR was carried out with an initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 35 s, 55 °C for 45 s, and 72 °C for 2 min, with a final extension at 72 °C for 5 min. F-ARISA amplification was done in duplicate under the conditions described by Purahong et al. [4]: the PCR mixture (20 μl) contained 1 μl DNA template (~20 ng DNA template as determined by NanoDrop); 10 μM of fungal-specific, plant-excluding primer ITS1F (5′- CTTGGTCATTTAGAGGAAGTAA-3′, Gardes and Bruns [30]) labeled with FAM at 5′-end and an unlabeled ITS4 primer (5′-TCCTCCGCTTATTGATATGC-3′, White et al. [31]); 4 μl FIREPol 5× Master Mix (Solis BioDyne, Tartu, Estonia); and water to 20 μl. PCR was carried out with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 60 s, 55 °C for 60 s, and 72 °C for 75 s, with a final extension at 72 °C for 7 min. The PCR products were purified using a PCRExtract Mini Kit (5PRIME, Hamburg, Germany). A standardized quantity of 40 ng (B-ARISA) or 20 ng (F-ARISA) DNA, as determined by NanoDrop, was mixed with 14 μl of deionized Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) and 0.1 μl of internal-size standard Map Maker 1500 ROX (50–1500 bp) (BioVentures, Inc, Murfreesboro, TN, USA). The mixture was denatured for 5 min at 95 °C and chilled on ice for at least

10 min before being further processed using a capillary sequencer (ABI PRISM 3730xl Genetic Analyzer, Applied Biosystems). DNA normalization was done twice in ARISA (before the initial PCR and before the separation of DNA fragments using capillary electrophoresis) to make this standard ARISA robust for inferring changes in community structure [4, 5, 32].

All peaks of the fragments between 200 and 1500 bp that were present in two analytical PCR replicates were used for further analyses [15]. The two independent PCR replicates were highly correlated. OTU binning was carried out using an interactive custom binning script in R version 2.14.1 [32]. The total peak area per sample was normalized to 1, and the relative fluorescent intensity (RFI) was calculated. All peaks with RFI values lower than 0.09 % were discarded as background noise. A strategy involving a binning size of 2 bp was applied to the bacterial and fungal ARISA data, and the binning frame that gave the highest pairwise similarity among samples was used for further statistical analyses.

Statistical Analyses

Decomposition rates for each forest type were determined by fitting a single negative exponential decay equation [33]. Our experimental design may contain potential sources of dependencies among samples as each plot within one replicate site represents only one stand per FMP. We analyzed the data as suggested by Yannarell et al. [34] by treating the experiment as a split-plot design, with FMP as a whole-plot factor replicated three times, the forest plots as the plot factor, and time as the within-plot factor. B-ARISA and F-ARISA fingerprint data were analyzed according to this split-plot design with permutational multivariate analysis of variance (PERMANOVA) using the "adonis" function of the "vegan" package in R [35]. Our null distribution was generated based on 1999 permutations of the rows of the sample-by-OTU data tables according to our restricted permutation scheme. Non-metric multidimensional scaling (NMDS), based on Bray–Curtis distance measurements (including plotting correlations with environmental variables), was carried out using the software PAST to visualize the patterns of bacterial and fungal community succession over time and to investigate the factors related to bacterial and fungal community assemblages [36]. Goodness-of-fit statistics (R^2) of environmental variables fitted to the NMDS ordination of bacterial and fungal communities were calculated using the "envfit" function in the "vegan" package, with P values based on 999 permutations [35]. All significant environmental variables ($P<0.05$) were plotted in the NMDS ordination using PAST. Ordinations of detailed bacterial and fungal communities in leaf litter under different forest management practices at each sampling date were analyzed using principle component analysis (PCA) with the biplots option of PAST.

Results

Effect of FMPs on Bacterial and Fungal Community Structure

Microbial communities were strongly influenced by FMPs (bacterial community: pseudo-F=4.65, P=0.001; fungal community: pseudo-F=6.76, P=0.001) and sampling dates (bacterial community: pseudo-F=10.23, P=0.001; fungal community: pseudo-F=13.69, P=0.001; Table 1, Fig. 1a, b). Bacterial communities from the BA forest formed a distinct cluster in the NMDS biplot (Fig. 1a). Bacterial communities in unmanaged beech (BU) and BS forests were more similar to each other; however, bacterial communities from both forests at the same sampling date showed no overlap in NMDS ordination space (Fig. 1a). Fungal communities in the BA forest also showed distinct community succession patterns compared to those of BU and BS forests (Fig. 1b). Communities changed more slowly and/or were resistant to temporal changes. Fungal communities from BU and BS forests developed over time, overlapping on two sampling dates (180 and 284 days, Fig. 1b). Ordination plots of bacterial and fungal communities in leaf litter under different FMPs at each sampling date are given in the supporting information (Figures S1, S2, respectively).

Overall, 267 bacterial and 220 fungal OTUs were detected in the three forest types. Approximately 54–58 % of the bacterial and 44–55 % of the fungal OTUs were shared between all forest types (Table 2). Patterns of bacterial OTU richness in leaf litter from managed forests (BA and BS) were similar and increased over time but differed from that of the BU forest (Fig. 2a). Bacterial OTU richness in the BU forest was higher compared to BA and BS forests at two sampling dates (89 and 180 days, Fig. 2a). Distinct patterns of fungal OTU richness in leaf litter were observed in different FMPs (Fig. 2b).

Factors Corresponding with Bacterial and Fungal Community Successions

We explored the relationships between environmental variables and bacterial and fungal community succession patterns by fitting leaf litter physicochemical properties to the NMDS ordination of bacterial and fungal communities [34, 35]. For bacterial communities, we found that litter quality parameters (C/N and lignin/N ratios), macronutrients in leaf litter (total C, total N, K, and Ca) and pH were strongly related to bacterial community succession ($P<0.01$, Table 3). Litter Fe was marginally related to bacterial community composition (P= 0.081). In the case of fungi, similar factors that corresponded with bacterial communities (C/N and lignin/N ratios, total C, total N, K, Ca and pH) also significantly correlated with the fungal communities (Table 3). In addition, total lignin (P= 0.006) and litter Fe (P=0.003) concentrations were significantly related to fungal community succession.

Table 1 Permutational multivariate analysis of variance for bacterial and fungal community structure

Microbial community	Source	Pseudo-*F*	R^2	*P* value
Bacterial community	Overall sampling date	10.23	0.21	0.001***
	Sampling date (only BA)	4.12	0.29	0.001***
	Sampling date (only BS)	6.00	0.38	0.001***
	Sampling date (only BU)	8.01	0.45	0.001***
	Overall FMP	4.65	0.09	0.001***
	FMP (only 1st sampling date)	1.90	0.21	0.033*
	FMP (only 2nd sampling date)	1.94	0.22	0.017*
	FMP (only 3rd sampling date)	3.74	0.35	0.001***
	FMP (only 4th sampling date)	3.88	0.36	0.001***
	Sampling date×FMP	2.71	0.05	0.007**
Fungal community	Overall sampling date	13.69	0.25	0.001***
	Sampling date (only BA)	7.39	0.42	0.002**
	Sampling date (only BS)	7.30	0.42	0.001***
	Sampling date (only BU)	4.76	0.32	0.001***
	Overall FMP	6.76	0.12	0.001***
	FMP (only 1st sampling date)	6.86	0.50	0.004**
	FMP (only 2nd sampling date)	8.54	0.55	0.003**
	FMP (only 3rd sampling date)	2.53	0.27	0.013*
	FMP (only 4th sampling date)	2.73	0.28	0.005**
	Sampling date×FMP	3.14	0.06	0.011*

P values were based on 1,999 restricted permutation of samples

*$P<0.05$, **$P<0.01$, ***$P<0.001$

FMP forest management practice, *BA* beech even-aged forest, *BS* beech selectively logged forest, *BU* beech unmanaged forest

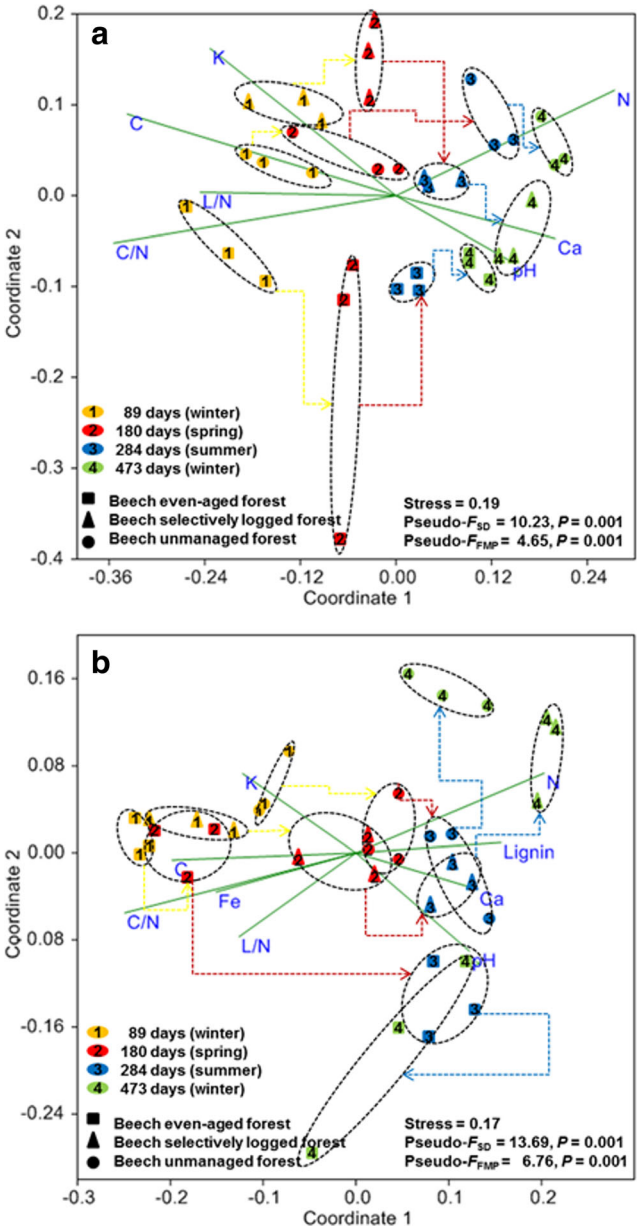

Fig. 1 NMDS ordination biplots showing microbial community succession in leaf litter in forests with different forest management practices (*FMPs*): bacterial communities (**a**) and fungal communities (**b**). Permutational multivariate analysis of variance was conducted to test the effects of sampling date (*SD*) and FMP on bacterial and fungal community structure (*P* values were based on 1999 permutations). Environmental variables were fitted to the nonmetric multidimensional scaling (*NMDS*) ordination of bacterial and fungal communities. The significance was based on 999 permutations and only significant variables ($P<0.05$) are shown

Discussion

FMPs influenced tree species richness that in turn significantly affected the leaf litter quality in forests with different FMPs. The initial chemical composition of dried leaf litter under different forest management practices are shown in Table S2. Decomposition rates of leaf litter in even-aged (BA) and selectively logged (BS) beech forests were strongly increased (31–72 %) when small fractions (5–10 %) of leaf litter from *Acer* sp. and/or *Fraxinus* sp. were mixed with *F. sylvatica* leaf litter (Tables S1 and S3) [7]. Overall, our results support the idea that different microbial community succession could be a mechanism explaining the nonadditive decomposition rates of mixed leaf litter in forests under different FMPs [12]. Some studies have provided experimental evidence of the effects of substrate quality on decomposers [37–41]. Interestingly, both synergistic and antagonistic interactions have been observed in litter mixtures [42–44]. Obviously in our study, synergistic interactions played an important role and may be explained, for example, by a positive feedback of soil invertebrates and decomposers due to diverse habitat and energy sources and the nutrient transfer (leaching) from nutrient-rich to nutrient-poor (e.g., *Fagus* sylvatica) litter species [42, 43].

Table 2 Pairwise comparison of bacterial and fungal OTUs from different forest management practices

Microbial group	Pairwise comparison	Number of OTUs		
		Only in 1st forest	Only in 2nd forest	Shared
Bacteria	Beech unmanaged vs. beech even-aged	47	52	139
	Beech unmanaged vs. beech selectively logged	58	50	128
	Beech selectively logged vs. beech even-aged	47	60	131
Fungi	Beech unmanaged vs. beech even-aged	65	44	87
	Beech unmanaged vs. beech selectively logged	47	39	105
	Beech selectively logged vs. beech even-aged	55	42	89

Our study showed that bacterial and fungal communities were strongly influenced by FMPs and incubation time of leaf litter material (sampling dates). The succession patterns of both bacterial and fungal communities differed significantly under different FMPs. This may be explained by the fact that forest management can greatly alter tree species richness, quality and quantity of leaf litter input, forest structure, and microclimate [4–6, 45] that, in turn, could significantly affect microbial communities [4, 46]. In addition, we found that FMPs also affect the initial bacterial and fungal community structure (Figure S3). Thus, it is conceivable that any differences in litter among

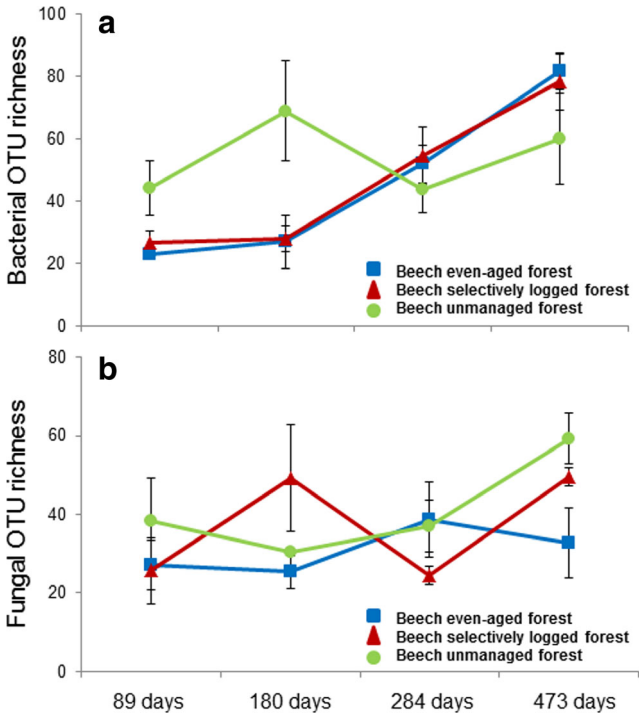

Fig. 2 Patterns of bacterial (**a**) and fungal (**b**) OTU richness in leaf litter across different forest management practices over time

Table 3 Goodness-of-fit statistics (R^2) of environmental variables fitted to the nonmetric multidimensional scaling (NMDS) ordination of bacterial and fungal communities

Variables	Bacterial community		Fungal community	
	R^2	*P* value	R^2	*P* value
Total C	0.754	0.001***	0.410	0.001***
Total N	0.435	0.001***	0.506	0.001***
C/N ratio	0.741	0.001***	0.699	0.001***
Lignin/N ratio	0.408	0.001***	0.240	0.011*
pH ($CaCl_2$)	0.298	0.004**	0.232	0.011*
Mg	0.092	0.219	0.104	0.155
K	0.464	0.002**	0.204	0.027*
Ca	0.301	0.004**	0.193	0.026*
P	0.090	0.214	0.098	0.156
Mn	0.073	0.292	0.001	0.984
Fe	*0.1301*	*0.081*	0.257	0.003**
Cu	*0.160*	*0.051*	0.003	0.955
Co	0.020	0.730	0.060	0.372
V	0.041	0.498	0.087	0.233
Water content	0.068	0.285	0.352	0.533
Total lignin	0.021	0.723	0.240	0.006**

*$P<0.05$, **$P<0.01$, ***$P<0.001$

Marginally significant variables $P<0.10$ are shown in italics

FMPs are related to the assembly or activity of these initial microbial communities. The combination of our results on microbial community structure (especially fungi) and previous work on oxidative enzyme activities from the same samples [7] could provide some indication of which aspects of community succession influence decomposition rates. The fungal communities from BA forest at the early decomposition stage (sampling dates, 89 and 180 days) differed from those of BU and BS forests (Figs. 1b and S2), which was reflected in differences in enzyme activities in the same samples. BA forest samples had high activities of manganese peroxidase, an exclusively fungal, extracellular enzyme pivotal for enzymatic lignin decomposition [7]. On the contrary, BU and BS litter samples showed low or no manganese peroxidase activity but high activities of the weaker oxidative enzyme laccase, an extracellular enzyme of bacterial or fungal origin which only plays an inferior role in enzymatic lignin degradation [7]. It is known that lignin decomposition is the rate-limiting step in litter decomposition; thus, samples of leaf litter in which lignin is decomposed faster will likely have higher decomposition rates [7]. Nevertheless, using microbial community structure to predict microbially mediated ecological functions such as patterns of enzyme activities may not give a definitive answer. A recent study reported a disconnection between microbial community structure and function in forest ecosystems due to functional redundancy within the microbial community and the differences between the drivers of microbial growth and those of microbial function [47]. To

further investigate the functional redundancy, studies using sequence-based approaches and microbial ecosystem function analysis are needed.

It is noteworthy from our study that litter quality, microbial macronutrients, and pH are important factors shaping the microbial community in leaf litter under different FMPs. Nutrient elements are able to affect microbial growth, activity, and survival [48, 49]. Specifically, C and N are important elements in all macromolecules including carbohydrates, proteins, lipids, and nucleic acids [49]. K and Ca are required for the activity of enzymes as cofactors [49]. Highly concentrated transition metals may be toxic to microorganisms; however, this was not the case in our study because all transition metal concentrations we measured were below the toxic levels reported for microbes [50]. Of all the transition metals, iron (Fe) had the highest impact on both bacterial and fungal community development in leaf litter. Fe is required in large amounts as it is involved in many important metabolic processes [49]. Ligninolytic peroxidases (i.e., aforementioned manganese peroxidase, lignin peroxidase, and versatile peroxidase) are secreted, exclusively fungal heme proteins that indeed depend on Fe^{3+} as the central ion in the catalytically active porphyrin ring. This also applies to most monoxygenases and dioxygenases (e.g., P450 and Fe–S cluster enzymes) that are the responsible biocatalysts in the intracellular metabolism of aromatics [51].

Interestingly, the total lignin content only significantly correlates with fungal communities. This may be related to the ability of fungi (in particular of certain basidiomycetes) to degrade and chemically modify the recalcitrant lignin polymer to a substantial extent [7, 52]. Such fungi were reported to secrete a range of ligninolytic and lignin-modifying enzymes: high-redox potential ligninolytic peroxidases as mentioned as well as laccase (copper-containing phenol oxidases) [52]. In contrast, few bacteria are able to produce extracellular laccase, and they are generally lacking manganese-oxidizing peroxidases that initiate lignin oxidation [52, 53]. All these enzymes can catalyze the oxidation of phenolic lignin moieties; however, the more recalcitrant nonphenolic lignin accounting for 90 % of the aromatic rings in the lignin polymer [54] can only be attacked by the high-redox potential peroxidases, while laccase has a generally lower oxidative strength [55, 56] and is therefore only considered as a lignin-modifying rather than a ligninolytic enzyme [57]. Overall, our results support the hypothesis that different FMPs could alter bacterial and fungal community succession patterns, which are involved in leaf litter degradation and explain the different ligninolytic enzyme activities and leaf litter decomposition rates, which were found under different FMPs [7].

Conclusion and Outlook

We conclude that FMPs significantly affect bacterial and fungal community succession and richness patterns. FMPs influence bacterial and fungal community succession in leaf litter over time via a number of factors, including leaf litter quality, microbial macronutrients, and pH. Alteration of microbial community succession is one of the mechanisms that could explain nonadditive decomposition rates occurring in mixed leaf litter under different FMPs. Further studies using sequence-based approaches may be needed in the future to unravel the major responders to management and time factors.

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