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# Uncoupling of microbial community structure and function in decomposing litter across beech forest ecosystems in Central Europe

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Correspondence and requests for materials should be addressed to J.L.M.G. (jgut@umn.edu) or D.Krüger (nasowasnun@googlemail.com)

\* These authors contributed equally to this work.

† Current address: Department of Soil, Water, and Climate, University of Minnesota, 439 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA.

Witoon Purahong<sup>1,2\*</sup>, Michael Schloter<sup>3\*</sup>, Marek J. Pecyna<sup>4</sup>, Danuta Kapturska<sup>1,4</sup>, Veronika Däumlich<sup>1</sup>, Sanchit Mital<sup>1</sup>, François Buscot<sup>1,5,6</sup>, Martin Hofrichter<sup>4</sup>, Jessica L. M. Gutknecht<sup>1</sup> † & Dirk Krüger<sup>1</sup>

<sup>1</sup>UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany, <sup>2</sup>Technical University of Munich, Chair for Soil Science, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany, <sup>3</sup>Helmholtz Zentrum München, Research Unit for Environmental Genomics, Ingolstädter Landstr. 1, D-85758 Oberschleissheim, Germany, <sup>4</sup>Dresden University of Technology (TU Dresden) - International Institute (IH) Zittau, Markt 23, D-02763 Zittau, Germany, <sup>5</sup>University of Leipzig, Institute of Biology, Johannisallee 21-23, D-04103 Leipzig, Germany, <sup>6</sup>German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany.

**The widespread paradigm in ecology that community structure determines function has recently been challenged by the high complexity of microbial communities. Here, we investigate the patterns of and connections between microbial community structure and microbially-mediated ecological function across different forest management practices and temporal changes in leaf litter across beech forest ecosystems in Central Europe. Our results clearly indicate distinct pattern of microbial community structure in response to forest management and time. However, those patterns were not reflected when potential enzymatic activities of microbes were measured. We postulate that in our forest ecosystems, a disconnect between microbial community structure and function may be present due to differences between the drivers of microbial growth and those of microbial function.**

A major portion of Europe's forest cover (approx. 57% if the Russian Federation is excluded) is managed for woody biomass production<sup>1</sup>. Forest system management practices (FMPs) can significantly alter soil physical, biological and chemical properties, as well as microclimatic conditions. These in turn can have profound effects for many ecosystem functions including productivity, carbon storage and biodiversity conservation<sup>2-4</sup>. However despite their importance as drivers for most ecosystem functions, the influence of different forest management practices on microbial communities in different soil compartments and their functional traits is still not well understood. It is generally believed that the structure of microbial communities determines their function<sup>5-9</sup>. However, they also propose that there may be, in some instances, a disconnection between microbial community structure and its functions. For instance, findings from several ecosystems have demonstrated different responses between microbial community structure and functions<sup>9,10</sup>. Thus it can be difficult to predict how changes in environmental factors will affect microbial community structure and microbially-mediated ecosystem functions in different ecosystems because there may be differences in the resistance to change at either the the community and functional levels, and because of functional redundancy within the microbial community<sup>11</sup>. The aims of this study were to investigate the patterns of and connections between microbial community structure and microbially-mediated functions across different FMPs as a function of time.

Leaf litter degradation is a very interesting case study for examining microbial structure-function relationships. It provides an important basis for the nutrient supply of trees in most forest ecosystems and thus leaf litter degradation and recycling, which is mainly catalyzed by microbes, determines productivity to a large degree<sup>12</sup>. Forest system management practices can potentially change the chemical composition of leaf litter by changing the dominant tree species as well as tree species richness, abundance and performance<sup>2,12</sup>. In addition, microbial communities colonizing leaf litter undergo major temporal changes, both seasonally and over the course of litter decomposition<sup>13,14</sup>. In temperate forests, the bulk of the litter input (for both leaf and fine root litter) occurs in the



autumn and the concentrations of easily degradable substrates from fresh leaf-litter including carbohydrates cellulose, proteins, starch and tannins are the highest in soil at this time. This presence of labile substrates may in turn stimulate microbial biomass in winter<sup>15–18</sup>. Plant and soil microbial seasonal cycles can also significantly influence nutrient availability in forest soil, also potentially affecting microbial community composition and feedbacks on microbial decomposition of leaf litter<sup>5</sup>. In this study we investigated microbial communities colonizing the litter material as well as selected functional traits over time in leaf litter from unmanaged stands and stands subject to different forest management regimes (different FMPs). The tested regimes involved age-class and selection cutting, both of which leave rather many forest gaps that enable the establishment of diverse tree species<sup>12,19</sup>. The unmanaged stands had fewer gaps and were only dominated by European beech (*Fagus sylvatica*), which produces low-quality leaf litter with a high C:N ratio<sup>12</sup>. Microbial community structure was determined broadly using phospholipid fatty acids. The functional traits of microbial communities were evaluated by measuring the potential activities of specific microbial enzymes involved in the biogeochemical cycling of important elements such as carbon, nitrogen and phosphorus<sup>10</sup>. We hypothesized that FMP and time of litter incubation in soil (days after incubation, DAI) both have significant effects on microbial community structure and potential enzyme activities. However the extent to which microbial community structure and function will be influenced will differ leading to a decoupling of structure–function relationships in this soil compartment. FMP and DAI may strongly affect microbial community structure, however, different microbial communities from different FMPs and/or DAI may perform similar enzyme activities (functional redundancy)<sup>10</sup>. Furthermore, differences between the drivers of microbial growth and enzyme activities may also be responsible for the disconnection of microbial community structure and function<sup>20</sup>. We used multivariate analysis to identify leaf litter properties with significant effects on the overall microbial community and enzyme activity patterns. Factors considered in this analysis included leaf litter quality parameters (C:N and lignin:N ratios) and other abiotic properties of the litter such as its pH, water content, and concentrations of microbial macronutrients and micronutrients (trace elements).

## Results

**Effects of FMP and DAI on microbial community structure and potential enzyme activities.** Abundance of phospholipid fatty acid (PLFA) indicators were significantly influenced by FMP and temporal (DAI) changes, and also by the interaction between FMP and DAI ( $P < 0.001$  in all cases, Table S1, Figure 1). Overall in the leaf litter of managed forests (beech age-class, BA and beech selection cutting, BS) the highest amounts of PLFA (both total and key groups) were detected in winter (89 and 473 DAIs) while the lowest PLFA amounts were measured in spring and summer (180, 284 DAIs) (Figure 1). Similar results were obtained for litter material from the unmanaged beech forest (BU). However, during the second winter the microbial biomass in the BU forest did not increase to the same extent as in the BA and BS forests. The fungal: bacterial ratio decreased over time under all FMPs (Figure 1). At 89 and 180 DAI, the leaf litter from BU forest had a significantly higher fungi: bacteria ratio than that observed in the BA and BS forests (89 DAI: BU = 5.76, BS = 2.82, BA = 2.72; 180 DAI: BU = 3.43, BS = 2.42, BA = 2.78). PLFA indicators for all bacterial groups except Gram negative bacteria varied significantly with FMP in spring. In summer, no significant differences among different PLFA indicators were ascertained for bacterial groups, except for Gram positive bacteria whose indicators were highest in BS forest and lowest in BA forest. NMDS analysis of PLFA patterns revealed four distinct clusters representing samples taken at different DAI ( $R_{DAI} = 0.97$ ,  $P < 0.001$ , Figure 2a). The different FMPs also affected microbial

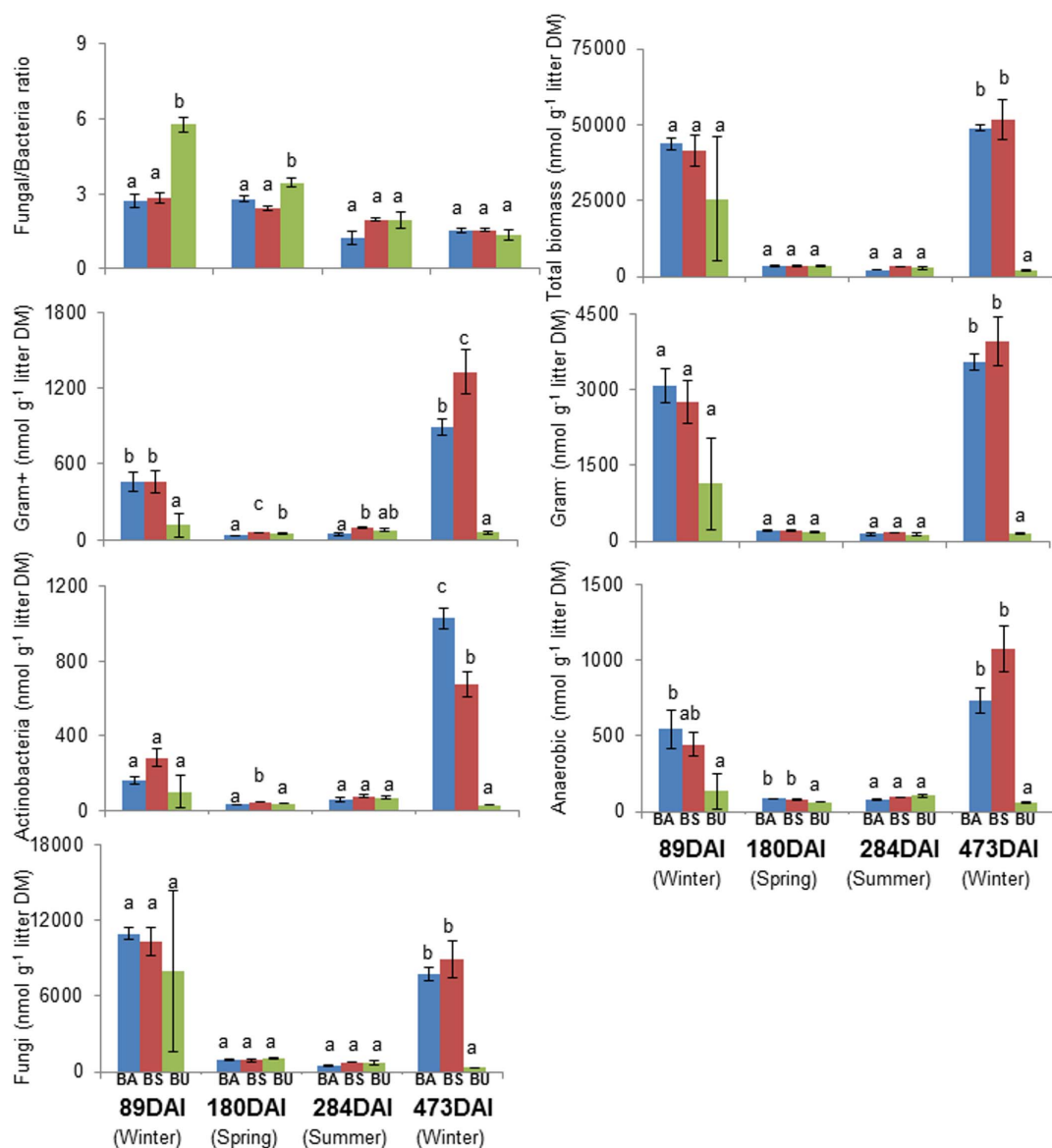
community structure, as shown by the existence of one cluster corresponding to samples from managed forests (BA and BS) and another corresponding to the BU forest at specific DAI values ( $R_{FMP} = 0.69$ ,  $P < 0.001$ , Figure 2a).

The potential activities of the enzymes investigated in this study did not follow the same trends as lipid biomass or lipid indicators (Tables S1 and S2, Figures 2b and 3). Mantel tests revealed an insignificant correlation between the PLFA and enzyme activity matrices (Mantel  $R$  (Euclidean distance matrix) = 0.05,  $P > 0.05$ , Mantel  $R$  (Bray-Curtis distance matrix) = 0.09,  $P > 0.05$ ). Enzyme activities did not vary according to the microbial biomass (Table S2). Enzyme activities in leaf litter under different forest management practices are presented in detail in Figure 3. Hydrolytic enzymes, cellobiohydrolase and  $\beta$ -glucosidase were significantly influenced by DAI and the interaction between FMP and DAI ( $P < 0.05$ , Table S1) but were not significantly influenced by FMPs. Xylosidase activity was significantly influenced by FMP and DAI ( $P < 0.01$ , Table S1). Xylosidase activity was generally high in BU (low forest management intensity) compared with BA (high forest management intensity). N-acetylglucosaminidase and acid phosphatase activities were significantly influenced by FMP and the interaction between FMP and DAI ( $P < 0.05$ , Table S1). The activities of the studied oxidative enzymes also behaved differently compared to microbial biomass: laccase activity was significantly affected by FMP and DAI ( $P < 0.05$ , Table S1), peroxidase by DAI ( $P < 0.01$ , Table S1), and manganese peroxidase by FMP ( $P < 0.01$ , Table S1). However, the interaction between FMP and DAI did not significantly affect the activities of any studied oxidative enzyme ( $P > 0.05$ , Table S1). Overall the observed NMDS patterns for microbial community structure were not reproduced in the NMDS analysis of enzyme activities where there was no recognizable clustering of samples representing either different FMPs or DAI values (Figure 2b). Although the different FMPs and DAI significantly influenced potential enzyme activity patterns, the degree of influence indicated by ANOSIM  $R$  values were much lower than for the microbial lipid abundances ( $R_{FMP} = 0.36$ ,  $R_{DAI} = 0.44$ ,  $P < 0.001$  in both cases). In some cases, microbial communities collected from forests subject to different FMPs or collected at different times exhibited similar enzyme activities (Figure 2b).

**The effects of the physicochemical properties of leaf litter on microbial community structure and function.** Our results indicated that microbial community structure and potential enzymatic activities are regulated and controlled by different physicochemical properties of the leaf litter. The structure of the microbial communities was significantly influenced by all leaf litter parameters (i.e. C:N and lignin:N ratios), the levels of most microbial macronutrients other than P (C, N, Mg, K, Ca, Fe), the levels of one microbial micronutrient (Mn), and the pH (Table 1). In contrast, only C, K, P, Mn, and pH significantly influenced enzyme activities. Leaf litter quality parameters (C:N and lignin:N ratios) that had very significant effects on the structure of the microbial community ( $P < 0.01$ ) did not significantly affect the pattern of enzymatic activities ( $P > 0.05$ ) (Table 1). Changes in leaf litter chemistry over time are presented in Figure S1.

## Discussion

In this experiment, we examined the patterns of and connections between microbial community structure and function across different forest management practices and as a function of time using leaf litter as an example. PLFA analysis was used for characterizing the microbial community structure. This method provides a broad estimation of the viable biomass of different microbial functional groups<sup>21</sup>. Some phospholipids (such as the fungal indicators 18:1 $\omega$ 9, 18:2 $\omega$ 6,9 and 18:3 $\omega$ 3,6,9) or potential enzymatic activities could be derived from plants<sup>22</sup>. While this consideration was taken into account for our interpretations, we assume that residual con-



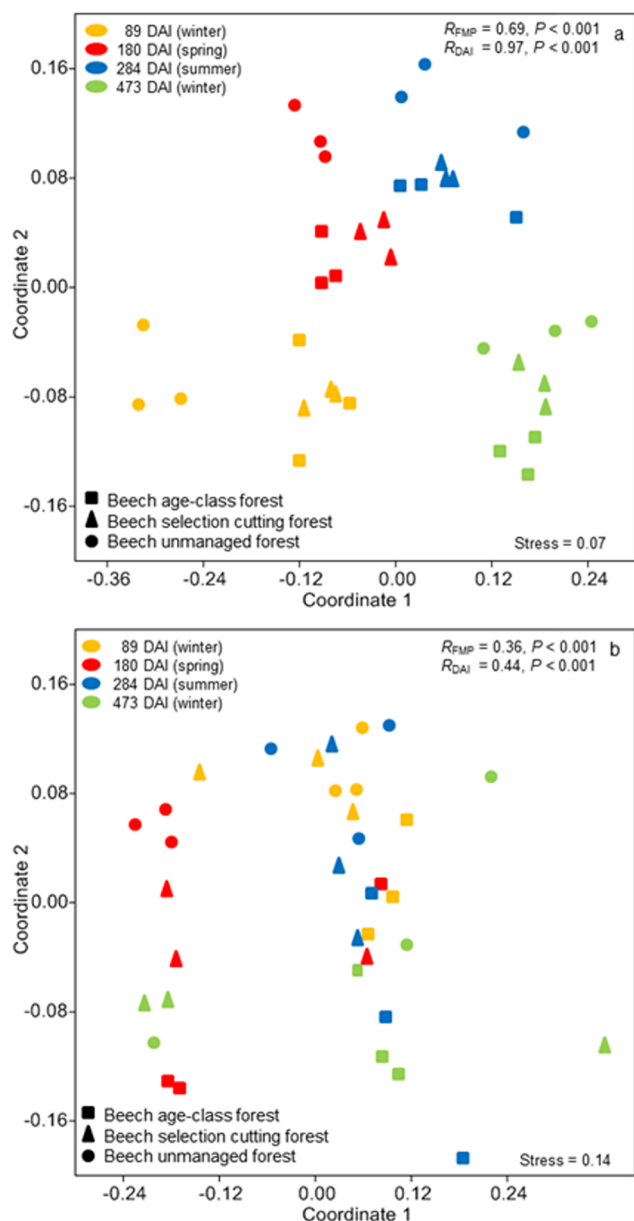
**Figure 1** | Fungal: bacterial ratio and microbial PLFA abundances (both total and key groups) (mean  $\pm$  SE) in leaf litter samples collected at four sampling dates (days after incubation commenced, DAI). European beech age-class forest (BA, blue), European beech selection cutting forest (BS, red) and unmanaged deciduous forest reserves dominated by European beech (BU, green). Litter DM = litter dry mass. Different letters indicate significant differences ( $P < 0.05$ ) between different treatments at each DAI according to one-way ANOVA and Fisher's Least Significant Difference (from 89 DAI to 473 DAI).

tributions from plants would be far surpassed by new microbial growth. This assumption is based on sample timing at 89 DAI to 473 DAI. By 89 DAI, a residual effect from PLFA or enzymes from the residual litter would be minimal, especially because the litter was dried and processed before plot establishment<sup>23</sup>. Based on the results of leaf litter decomposition rates of the same experiment, we showed that forest management practices significantly influence the decomposition kinetics (BA forest has higher decomposition rate than BU forest)<sup>12</sup>. Thus, a caveat to this dataset is that at the same DAIs, leaf litter from different FMPs (BA and BU forests) may not be at the same decomposition stages. This may also affect the patterns of enzyme activities and PLFA in leaf litter at the same DAI (different FMPs).

As expected, high microbial biomass was observed for all FMPs in winter (89 and/or 473 DAI)<sup>15–18</sup>. Our biomass analyses indicated that fungi were the dominant leaf litter-decomposing organisms under all of the studied FMPs and DAI. On the other hand, bacteria increased

in abundance as decomposition progressed. Fungi are generally considered to be the primary decomposers of leaf litter in temperate forest ecosystems<sup>13</sup> and they rapidly colonize leaf litter during the early stages of decomposition<sup>24</sup>. Biological fragmentation caused by the action of fungi and invertebrates and physical fragmentation results in an increased leaf litter surface area and the release of nutrients during the later stages of leaf litter decomposition<sup>25</sup>, which may enhance the activity of bacteria during litter decomposition<sup>26</sup>.

We identified clear differences between the microbial communities from leaf litter produced under different FMPs (i.e. from managed vs. unmanaged forests) and between microbial communities collected at different time points within the 473 day sampling period. This strongly suggests that the structure of microbial communities in the leaf litter of the studied forest systems is sensitive to forest management practices, the stage of litter decomposition, and seasonal factors. Similar conclusions have been drawn in a number of previous studies examining various types of disturbance: in general, the



**Figure 2** | Nonmetric multidimensional scaling (NMDS) ordination of (a) microbial community structure inferred from PLFA analysis and (b) activities of 8 different enzymes. FMP = forest system management practice; DAI = days after incubation commenced;  $R$  = degree of separation between test groups ranging from  $-1$  to  $1$ ;  $R = 0$ , not different;  $R = 1$ , completely different;  $P$  values were based on 999 permutations.

structures of microbial communities are sensitive to many factors and not immediately resilient to disturbance<sup>11</sup>. In our studied sites, FMPs affect tree species composition that, in turn, significantly alters leaf litter quality and nutrients in BA and BS forests as compared to BU forest<sup>12</sup>. The results from multivariate analysis (Table 1) demonstrate that leaf litter quality and most macronutrients are significantly correlated with changes in microbial community composition. BU forest is located in a forest reserve area, which, for at least 60 years, has not undergone thinning, wood harvesting, or other serious disturbances that would have resulted in the loss of large numbers of trees. Thus the BU had fewer gaps and were only dominated by European beech (*Fagus sylvatica*), which produces low-quality leaf litter<sup>12</sup>. On the other hand, thinning operation and woody biomass harvesting in BA (whole stand harvesting) and BS

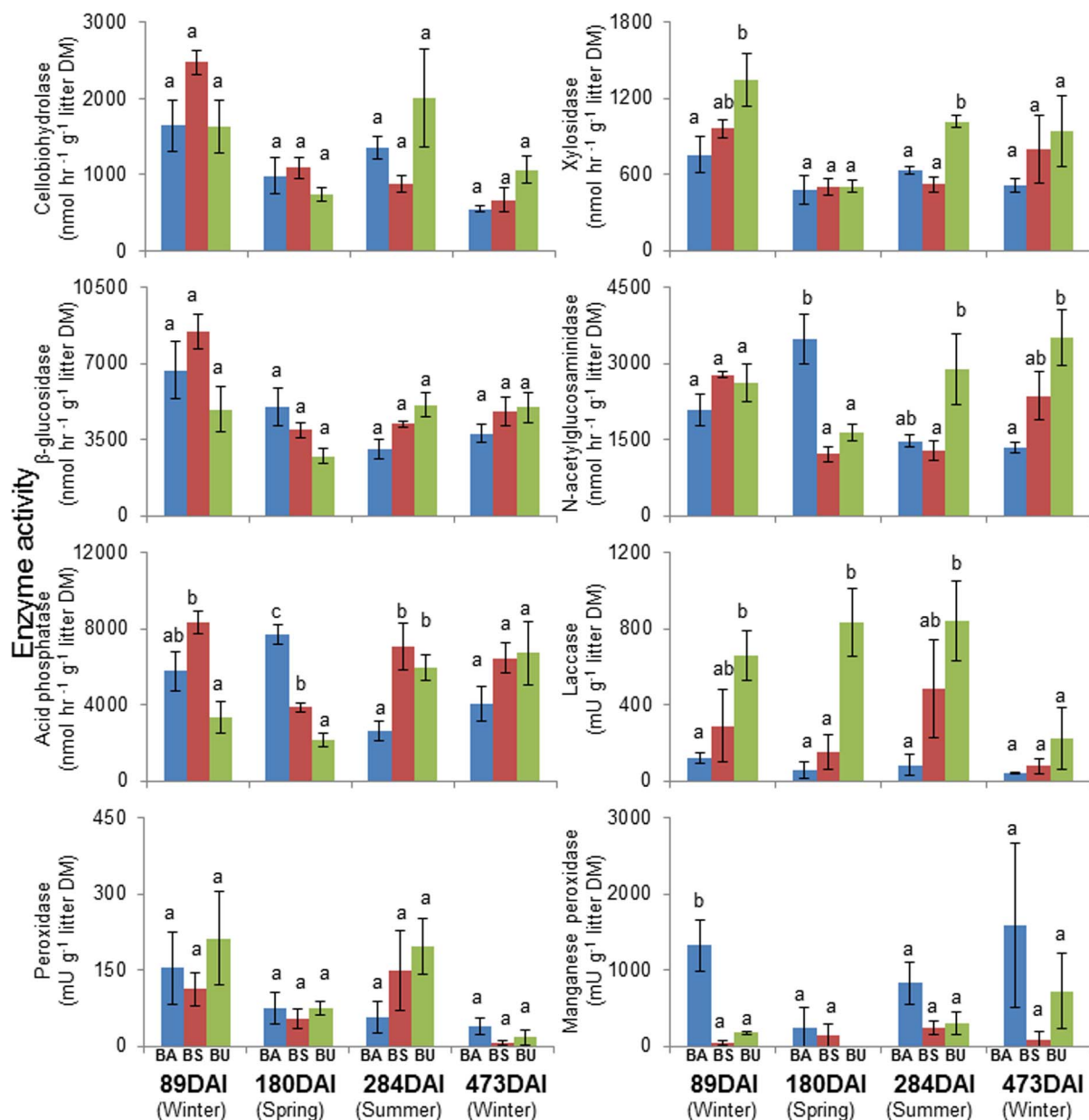
(individual tree harvesting) could generate the forest gaps that are important for natural tree regeneration and the maintenance of plant diversity. Thus, *Acer* sp. successfully established by natural regeneration in both BA and BS forests (Table S3). In addition, a small fraction of *Fraxinus* sp. was also found in BA forest (Table S3). *Acer* sp. and *Fraxinus* sp. leaf litter are known to have better quality and nutrients compared with *Fagus sylvatica* leaf litter<sup>12</sup>.

In contrast, we did not identify any clear changes in the patterns of potential enzyme activities in leaf litter samples due to FMPs or temporal factors. The responses of enzymatic activities to DAI and FMPs varied and were not always significant. Our results therefore indicate a decoupling of microbial community structure and the metabolic functions of potential microbial enzymatic activities across FMPs and temporal changes in temperate forest ecosystems. This might be explained, at least in part, by the widespread occurrence of hydrolytic enzymes (including polymer-disintegrating cellulases and xylanases) among different groups of microbes (fungi, bacteria, protists) so that they can replace each other in the course of community changes (functional redundancy)<sup>27</sup>. For example, the cellulases of fungi may disappear along with their producers during leaf litter succession, but they can be replaced by similarly active enzymes of actinobacteria or other Gram-positive bacteria<sup>28</sup>. In the case of oxidative enzymes, the situation is somewhat different. Oxidative enzymes were reported to be more organism-specific. Both laccase and generic peroxidases are primarily secreted by fungi (both ascomycetes and basidiomycetes)<sup>29,30</sup>. This is especially true for Mn-peroxidase that, in soil environments, is exclusively produced by specific basidiomycetous fungi causing a kind of white-rot in leaf litter<sup>31,32</sup>. Thus, the activities of oxidative enzymes may not be as widely spread as those of hydrolytic enzymes. This means, if appropriate fungi are present in a litter bag, there will be a high level of oxidative enzymes but if not, this activity will be lacking.

Insofar as our results deviate from the generally notion that structure determines function, these findings together with those of Frossard et al. can be regarded as supporting a paradigm shift<sup>10</sup>. Frossard and colleagues demonstrated a decoupling of bacterial community structure and function (represented by the activities of 10 separate enzymes) across temporal changes in nascent stream corridors. The disconnection between microbial community structure and function in these two cases may be due to functional redundancy within the studied microbial communities<sup>10</sup>. Our results show that different microbial communities (from either different FMPs or different points in time) can produce similar enzyme activities, thereby we speculate that at least some level of functional redundancy may occur. In addition to microbial functional redundancy, we demonstrated that differences in the drivers of microbial growth versus function can be used to explain the uncoupling between microbial community structure and the metabolic activity of microbial enzymes<sup>20</sup>. While all of the studied litter quality parameters and the levels of microbial macronutrients (except P) significantly influenced microbial community structure, only a few of these factors were found to significantly affect the overall pattern of potential enzyme activities. *Vice versa* P concentration did not significantly influence the microbial community structure but did have significant effects on the overall patterns of enzyme activity.

Based on our results, we suggest that there is some degree of functional redundancy in the microbial community. However, we also detected differences in litter decomposition rates and nutrient dynamics between forests with different FMPs (especially between unmanaged and age-class forests)<sup>12</sup>. This is due to the time lag in functional similarity. Our results show that microbial communities from different FMPs perform similar enzyme activities at different points in time. We have previously shown that the rates of leaf litter decomposition are highest in systems that decompose lignin most rapidly (i.e. those with high levels of manganese peroxidase, an efficient ligninolytic enzyme)<sup>12</sup>. Thus, leaf litter produced under FMPs





**Figure 3 | Enzyme activities (mean  $\pm$  SE) in leaf litter samples collected at four sampling dates (days after incubation commenced, DAI).** European beech age-class forest (BA, blue), European beech selection cutting forest (BS, red) and unmanaged deciduous forest reserves dominated by European beech (BU, green). Litter DM = litter dry mass. Data on oxidative enzyme activities are derived from Purahong et al.<sup>12</sup>. Different letters indicate significant differences ( $P < 0.05$ ) between different treatments at each DAI according to one-way ANOVA and Fisher's Least Significant Difference (from 89 DAI to 473 DAI).

that are more amenable to rapid colonization by microbial communities producing efficient lignolytic enzymes may decompose relatively quickly.

In summary, the structure of microbial communities and microbial ecological functions of metabolic enzymes are dependent on the complex interactions between numerous environmental factors. In temperate forest ecosystems, forest management is one of the most important factors affecting both microbial community structure and its ecological function.

## Methods

**Study site.** The study was conducted at three forest sites in the Hainich-Dün region of Central Germany (about 1,300 km<sup>2</sup>; 51°16'N 10°47'E), which is part of the German Biodiversity Exploratories<sup>33</sup>. The forests in this region grow on soils over limestone bedrock<sup>33</sup>. The main soil type in the study area according to the German soil classification system and the World Reference Base of Soil Resources is a Stagnosol<sup>33</sup>.

The soil pH is weakly acidic ( $5.1 \pm 1.1$ ; mean  $\pm$  SD)<sup>34</sup>. Thin litter (in the range 2–5 cm) were recorded at all three forest sites<sup>12</sup>. The annual mean temperature and precipitation ranged, respectively, from 6.5–8°C and 500–800 mm<sup>33</sup>. All information pertaining to these forests has been described in detail elsewhere<sup>12,33</sup>.

Three forest sites (10,000 m<sup>2</sup> each) were selected based on their forest system management practices with each site representing one treatment. All sites were dominated by European beech (*Fagus sylvatica*) and located less than 30 km apart, to reduce the influence of variations in geography and climate. Within each forest site, we assigned three plots (2  $\times$  8 m<sup>2</sup>) located on flat land to represent three experimental replicates of each forest management practice. The three FMPs considered in this study were: (i) European beech age-class forest (BA, semi-natural forest with natural regeneration, even-age forest structure); (ii) European beech selection cutting forest (BS, near-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)<sup>12</sup>. These three forest sites differ in terms of the silvicultural management intensity indicator (SMI)<sup>35</sup>. The SMI was low for the BU and BS forests but high in the BA forest<sup>35</sup>. The litter composition for individual forest sites and the initial chemical composition of the litter material are given in Tables S3 and S4.



**Table 1 | Goodness-of-fit statistics ( $R^2$ ) of environmental factors fitted to the nonmetric multidimensional scaling (NMDS) ordination of microbial community structure and enzyme activities. The significance was based on 999 permutations. Significant factors ( $P < 0.05$ ) are indicated in bold: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$**

Variables	Microbial biomass		Enzyme activities	
	$R^2$	$P$	$R^2$	$P$
Total C	<b>0.701</b>	<b>0.001***</b>	<b>0.266</b>	<b>0.007**</b>
Total N	<b>0.424</b>	<b>0.001***</b>	0.073	0.309
C:N ratio	<b>0.717</b>	<b>0.001***</b>	0.032	0.566
Lignin:N ratio	<b>0.280</b>	<b>0.006**</b>	0.090	0.209
pH (CaCl <sub>2</sub> )	<b>0.460</b>	<b>0.001***</b>	<b>0.315</b>	<b>0.002**</b>
pH (H <sub>2</sub> O)	0.086	0.258	0.025	0.634
Mg	<b>0.235</b>	<b>0.009**</b>	0.010	0.872
K	<b>0.396</b>	<b>0.001***</b>	<b>0.608</b>	<b>0.001***</b>
Ca	<b>0.288</b>	<b>0.006**</b>	0.063	0.354
P	0.052	0.414	<b>0.352</b>	<b>0.002**</b>
Mn	<b>0.271</b>	<b>0.010**</b>	<b>0.218</b>	<b>0.013*</b>
Fe	<b>0.383</b>	<b>0.001***</b>	0.000	0.999
Cu	0.006	0.910	0.026	0.650
Co	0.073	0.265	0.009	0.919
V	0.001	0.988	0.119	0.149
Water content	0.103	0.166	0.085	0.216
Total lignin	0.094	0.211	0.086	0.218

**Experimental design and sampling.** At each forest site, freshly fallen leaves of deciduous species were collected from the forest floor in October, 2009. The leaves from each plot were separated according to species and air dried to constant weight at room temperature. Ten grams of local mixed leaves, representative of the litter composition of the deciduous tree species at each respective site were then placed in litterbags (25 × 25 cm, 2 mm nylon mesh size) for deployment<sup>12</sup>. At the end of the litter fall period (13 November 2009), 12 replicate litter bags were placed in each plot, for a total of 36 litterbags per management type and 108 litterbags in total. 27 additional litterbags (9 per treatment) were retained to determine the initial dry mass (oven-dried at 105°C ≥ 24 h until constant weight), nutrient element concentrations, and lignin content of the litter mixtures (Data supplement, Table S4). To preserve the situation in the temperate forest, we allowed the fresh leaf canopy litter to fall above the litter bags. Litterbags were retrieved on four sampling dates: in 2010 on 10 February (89 days after incubation commenced, DAI), 12 May (180 DAI), 24 August (284 DAI), and in 2011 on 1 March (473 DAI). On each sampling occasion, 3 replicate litter bags per plot (nine litterbags per treatment) were randomly removed, placed into a separate clean plastic bag to reduce the loss of small fragments, and transported on ice (0°C) to the laboratory within 4 h. Once in the laboratory, litter bags were processed immediately. First, the three replicate litterbags retrieved from the same plot and treatment were pooled and their wet weight was determined. Thus, three composite samples from each site were obtained for each treatment and DAI. Each composite sample was then homogenized and subsampled for determination of dry mass, nutrient element concentrations, lignin content, phospholipid fatty acids analysis (PLFA) and enzyme activities.

**Details of phospholipid fatty acid analysis (PLFA).** Microbial community structure was assessed using the PLFA method described by Wu et al.<sup>36</sup>. Briefly, phospholipids were first extracted three times from leaf litter using a methanol, chloroform, citric acid solution. Phospholipids were then separated using silica columns and methylated by alkaline methanolysis. A total of 42 lipids with 20 or fewer carbon atoms each were used to calculate the total microbial lipid abundance (nmol lipid g litter DM<sup>-1</sup>). Several individual PLFAs were used as indicators for key groups of microbes: 15:0 iso for Gram-positive bacteria; 16:1ω7c for Gram-negative bacteria; 17:0 cyclo and 19:0 cyclo for anaerobic bacteria; 10Me16:0 for Actinobacteria and 18:2ω6,9c for general fungi. In our study, 3–5 individual PLFA markers that can be used to quantify the abundances of Gram-positive bacteria (15:0 iso, 15:0 ante, 16:1ω7c and 17:0 ante), Gram-negative bacteria (16:1ω5c, 16:1ω7c and 16:1ω9c) and general fungi (18:1ω9, 18:2ω6,9c and 18:3ω3,6,9c) were highly correlated ( $R = 0.93–1.00$ ,  $P < 0.001$ ; Table S5)<sup>5,37–39</sup>. Thus, we found that results were similar whether we used one *versus* more indicators for each microbial group. For our final analysis, we only used one indicator (except for anaerobic bacteria).

**Determination of potential enzymatic activities.** A total of 8 enzyme activities were measured. Specifically, five hydrolytic enzymes which are important for carbon (β-glucosidase (EC 3.2.1.21), cellobiohydrolase (EC 3.2.1.91), and xylosidase (EC 3.2.1.37), nitrogen (N-acetylglucosaminidase, EC 3.1.6.1), and phosphorus (acid phosphatase, EC 3.1.3.2) acquisition were assessed based on methods described by Sinsabaugh et al. and German et al.<sup>40,41</sup>. Three oxidative enzymes important for lignin

degradation (laccase (EC 1.10.3.2), general peroxidase (EC 1.11.1.7), and manganese peroxidase (EC 1.11.1.13), MnP) were measured as described by Purahong et al.<sup>12</sup>. Manganese peroxidase activities were not captured by the assay for general peroxidases because, without sufficient amounts of Mn(II) in the assay solution, manganese peroxidase cannot work. Thus in this experiment we found no correlation between the activities of these two enzymes ( $R = 0.09$ ,  $P = 0.582$ ) (Table S2).

**Measurements of physicochemical parameters of the leaf litter.** The water content and pH (in H<sub>2</sub>O and 0.01 M CaCl<sub>2</sub>) of all leaf litter samples were also measured. The pH measurement in 0.01 M CaCl<sub>2</sub> was found to provide more consistent and reproducible results than pH measured in H<sub>2</sub>O. Total C and N were determined by dry combustion at 1,000°C with an Elementar Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) (DIN/ISO 10694 (Aug. 1996)). Nutrient ions (Mg, K, P, Ca, Fe, Cu, V, Mn, Co) were determined using inductively coupled plasma (ICP) optical emission spectrometry (ICP-OES) and mass spectrometry (ICP-MS) according to manufacturers' specifications. Total lignin was calculated by summing Klason lignin (acid insoluble lignin) and acid soluble lignin<sup>42</sup>. 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 UV-photometrically in 4% H<sub>2</sub>SO<sub>4</sub><sup>43,44</sup>. All physicochemical analyses were conducted in triplicate on the same subsample.

**Statistical analysis.** Total microbial biomass, the abundance of microbial indicators, and enzyme activities were analyzed using two-way (FMP and DAI) analysis of variance (ANOVA) with  $P < 0.05$  considered significant, implemented in the PAST program<sup>12,45</sup>. All data were also analyzed using a one-way ANOVA to determine the differences ( $P < 0.05$ ) among different treatments at each DAI<sup>12</sup>. When the differences were significant, they were further analyzed using a post-hoc test (Fisher's Least Significant Difference,  $P < 0.05$ ). All datasets were tested for normality using Shapiro-Wilk and Jarque-Bera tests and the equality of group variances was examined using Levene's test. ANOVA residuals were also plotted to determine the normal probability using the Shapiro-Wilk W test. A Log<sub>10</sub> transformation ( $\log_{10}(x + 1)$ ) was applied to all data sets that did not meet the parametric assumptions. Non-metric multidimensional scaling (NMDS) based on a Bray-Curtis similarity measures (100 random restarts) were performed on a matrix of PLFAs information (expressed as mole percentages) and a matrix including information on all enzyme activities (Log<sub>10</sub> transformation) using PAST program<sup>45</sup> and vegan package of R version 2.13.1<sup>46</sup>. Two-way (FMP and DAI) Analysis of Similarity (ANOSIM) based on Bray-Curtis measure was conducted to test for differences among clusters<sup>45</sup>. Correlation between microbial community structure (PLFA matrix) and function (enzyme activity matrix) were analyzed using Mantel tests in PAST program<sup>45</sup>. To investigate the influence of leaf litter physicochemical factors on patterns of microbial community and enzyme activities, we fitted the physicochemical factors onto NMDS ordinations and calculated goodness-of-fit statistics ( $R^2$ ) using the envfit function in the vegan package<sup>46</sup>.  $P$  values were based on 999 permutations<sup>46</sup>.

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## Author contributions

D. Krüger, F.B., M.H., J.G., D. Kapturska, M.P. and W.P. conceived and designed the experiments. M.P., D. Kapturska, W.P., V.D. and S.M. performed the experiments. W.P. analyzed the data. D. Krüger, F.B., M.H., J.G. and W.P. contributed reagents/materials/analysis tools. W.P., M.S., M.P., D. Krüger, M.H., J.G. wrote the paper.

## Additional information

**Supplementary information** accompanies this paper at <http://www.nature.com/scientificreports>

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