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Extrinsic rather than intrinsic factors determine microbial colonization of deadwood

Keywords: grassland, forest, microclimate, bacteria, fungi, mass loss, decomposition, metabarcoding, amplicon sequencing

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Abstract

Deadwood decomposition is primarily attributed to wood-colonizing fungi and bacteria, driven mainly by intrinsic (e.g. tree species identity) rather than by extrinsic factors. A recent cross-ecosystem study, using gamma-sterilized wood blocks of different coniferous and deciduous tree species placed at 150 forest and 150 grassland sites, revealed that intrinsic factors most strongly influenced rate of decomposition. These results raised the question of whether the wood-colonizing microbial biodiversity follows similar assembly patterns. For this purpose, we used metabarcoding to analyse the fungal and bacterial communities colonizing the wood blocks. We discovered that the wood-colonizing communities were more strongly determined by extrinsic factors such as the ecosystem type and microclimate (air humidity, soil pH, soil moisture, soil temperature) than by intrinsic factors (tree species identity, wood pH, wood mass loss). Although overall these results seem to be more pronounced for fungi, both communities comprised highly specialized wood colonizers in both ecosystems. For instance, the fungal genus *Mycena* and the bacterial genus *Granulicella* were detected more frequently in forests, whereas *Exophiala* and *Sphingomonas* were more abundant in grasslands. Wood mass loss exhibited a stronger correlation with reduced fungal diversity, while bacterial richness displayed no association with mass loss, both within and across forest and grassland sites. However, the composition of both colonizers' communities was consistently linked to wood mass loss. Our study system suggests that the environment selects distinct wood-colonizing communities that differ greatly in their decomposition efficiency; this result highlights the importance of cross-ecosystem analyses to assess ecological patterns.

1 Introduction

The decomposition of organic matter is a key process of the carbon (C) cycle in terrestrial ecosystems. Deadwood alone contains approx. 73 billion tons of C, and is considered an important C stock in forest ecosystems, contributing to the flow of nutrients into the soil (Pan et al., 2011; Bani et al., 2018). The decomposition of deadwood presents significant challenges because it contains recalcitrant organic compounds, such as lignin, which can only be utilized by a limited number of microorganisms. In addition, the process is hindered by the presence of limited nitrogen (N) concentrations (Bani et al., 2018). Among the organisms that can make use of lignin are saprobic fungi including white-rot basidiomycetes that rapidly colonize litter and wood by degrading both cellulose and, extensively, lignin, and brown-rot basidiomycetes that predominantly degrade cellulose by marginally modifying lignin (Hatakka and Hammel, 2010; Krishna and Mohan, 2017; Kipping et al., 2024). However, metabolically less specialized fungi (e.g., xylariaceous soft-rot fungi), which are generally restricted to the degradation of cellulose and low-molecular-weight compounds, are also prominent deadwood colonizers (Hatakka and Hammel, 2010; Krishna and Mohan, 2017). Bacteria are considered to contribute indirectly to decomposition by providing N to other colonizers for their stoichiometric requirements (Bani et al., 2018; Tláskal et al., 2021), but they are also capable of directly utilizing a broad range of plant-derived substrates (López-Mondéjar et al., 2020; Algora et al., 2022; Qi et al., 2023). Both groups are expected to play complementary roles in wood decomposition (Gómez-Brandón et al., 2020; Tláskal et al., 2021).

The efficiency of the decomposition process varies according to several factors and is linked to the diversity and activity of colonizing microorganisms (Kahl et al., 2017; Hu et al., 2018; Purahong et al., 2022). Their ability to colonize deadwood is, in turn, affected by both extrinsic and intrinsic factors. Among the latter are host tree-related factors such as log diameter, C or lignin content or pH value, which impact both the colonizing community and the decomposition process (Hoppe et al., 2015; Zanne et al., 2015; Kahl et al., 2017; Hu et al., 2018; Moll et al., 2018; Leonhardt et al., 2019). Fungal and bacterial colonizers, including saprotrophs (i.e. microbes that can decompose deadwood), also differ greatly between host tree species (Purahong et al., 2018a; Lee et al., 2020; Moll et al., 2021a). However, extrinsic factors such as air temperature, precipitation or habitat structure regulate colonization patterns and decomposition processes as well (Merganičová et al., 2012; Bradford et al., 2014; Parisi et al., 2018; Lee et al., 2020; Edelmann et al., 2023). Moreover, soil pH has been found to be a good

predictor of wood decomposition, with faster decomposition in soil with higher pH (Hu et al., 2021). So far, when comparing intrinsic and extrinsic factors, host tree-related factors have been identified as the main drivers of decomposition, even on a global scale (Zanne et al., 2015; Hu et al., 2018), and also as the main drivers for the colonizing community (Krah et al., 2018; Lee et al., 2020; Müller et al., 2020; Rieker et al., 2022).

Although the colonizing community and the decomposition process are inextricably linked, this causal relationship and the resulting dependencies are difficult to explore. Generally, one can hypothesize two scenarios: (i) certain attributes of the community, such as species richness, are linked to decomposition rates. Thus, higher species richness is expected to promote decomposition rates as species simultaneously utilize different fractions of the substrate (resource partitioning); however, neutral or negative diversity-decomposition relationships have also been observed (Bell et al., 2005; Hättenschwiler et al., 2005; Fukami et al., 2010; Nielsen et al., 2011; de Graaff et al., 2015). (ii) community composition instead of species richness is linked to decomposition rates as specific organisms are more important for this ecosystem process than others (Nielsen et al., 2011). However, under natural conditions, linking diversity and decomposition remains challenging due to the complexity of the system.

All these factors have mainly been studied in the context of natural deadwood in forest ecosystems, and knowledge and observations beyond this ecosystem remain limited. While temperate forests are characterized by high canopy cover and large amounts of woody plant material, grasslands that are dominated by grasses and herbaceous plant species have limited to missing tree canopy covers. This significantly impacts environmental properties on the plot and landscape level, such as microclimate in terms of air temperature, precipitation and moisture (Breshears, 2006). However, grasslands are not detached from adjacent forest sites, can include woody plants such as individual trees or shrubs and grassland plant species contain lignin as well, albeit to a lesser extent (Koukoura et al., 2003; Klaus et al., 2011). Of course, woody plant material in non-forests sites is also colonized and decomposed, but it has recently been shown that the decomposition efficiency slows with increasing distance from forests (Smith and Peay, 2021). Decomposition has been shown to be negatively correlated with fungal diversity and with changes in colonizers' community composition (Smith and Peay, 2021). However, whether this community change is consistent across different host tree species and whether deadwood-colonizing communities in non-forest sites still exhibit strong host tree specificity was not investigated. Knowledge about related colonizing patterns and mechanisms is still pending.

In a previous study in which standardized and sterilized wood blocks were placed on the floor of 150 forest and 150 grassland sites, we found that host tree-related factors (i.e. tree species identity) was the main driver for deadwood mass loss and overall higher decomposition rates in forest sites (Kipping et al., 2022). These results raised the question of whether differences in decomposition are also reflected by the colonizing communities, i.e. are the wood blocks on the grassland sites also colonized by tree species-specific decomposers and whether these, in turn, are actual wood saprotrophs. To answer this, we identified fungal and bacterial communities colonizing the wood blocks of beech (*Fagus sylvatica*), spruce (*Picea abies*) and pine (*Pinus sylvestris*) via DNA-based metabarcoding in a similar highly replicated setup. In line with our previous findings, we hypothesize (i) that there are distinct colonizing communities associated with the different wood substrates (tree species) and between the two ecosystems (forest vs. grassland) and (ii) that those communities are more strongly correlated to intrinsic factors, i.e. host tree-related factors (tree species identity, wood pH, wood mass loss) than to the extrinsic factors (ecosystem type and microclimate). Further, if our hypotheses are confirmed and specialized wood-colonizing communities are observed, we assume that these are the main actors of wood decomposition and (iii) correlate with the mass loss of wood.

2 Methods

2.1 Study area and experimental setup

This study was conducted as a part of the Biodiversity Exploratories project (<http://www.biodiversity-exploratories.de>) (Fischer et al., 2010). Its unique design allows detailed analysis of bacterial and fungal communities along a regional north-south gradient in Germany in terms of different ecosystem patterns (forest *versus* grassland). The present study was conducted at 150 grassland and 150 forest sites in the three different regions (Exploratories). These Exploratories vary in location: Schorfheide-Chorin in Brandenburg, North-Eastern Germany (52°47'25''–53° 13'26''N/13°23'27''–14°08'53''E, 3–140 m a.s.l.); Hainich-Dün with the surrounding area in Thuringia, Central Germany (50°56'14''–51°22'43''N/10°10'24''–10°46'45''E, 285–550 m a.s.l.); and Swabian Alb in Baden-Wuerttemberg, South-Western Germany (48°20'28''–48°32'02''N/9°10'49''–09°35'54''E, 460–860 m a.s.l.). Schorfheide-Chorin is characterized by mean annual temperatures of 8–8.5 °C and mean annual precipitation of 500 to 600 mm, Hainich-Dün by 6.5–8°C and 500 to 800 mm, and Swabian Alb by 6–7°C and 700 to 1000 mm. Forest plots measure 100x100 m and are dominated by one of the following tree species: (1) *Fagus sylvatica* (European beech) – mostly dominant in Hainich, (2) *Picea abies* (Norway spruce), (3) *Pinus sylvestris* (pine) and to a lesser extent (4) *Quercus petraea/Quercus robur* (oak). Grassland plots measure 50x50 m. Environmental and microclimatic parameters such as soil pH, soil temperature (C°) 5 cm below soil surface, soil moisture (%) 10 cm below soil surface, soil type, air temperature (C°) 10 cm above and relative air humidity 2 m above the soil surface were determined for each of the 300 plots during the year of exposure, as previously described (Schöning et al., 2021; Hänsel et al., 2024). The annual mean value was calculated for each variable and used for further statistical analyses.

In order to contrast the importance of intrinsic and extrinsic factors on the colonizing communities, 1,800 wood blocks from the three main tree species of temperate forests (*Fagus sylvatica*, *Pinus sylvestris* and *Picea abies*) were sterilized by gamma radiation of 70 kGy (Gammaservice, Radeberg, Germany) as outlined previously (Muszynski et al., 2021) and then sealed in packages. Thereafter, wood blocks were precisely weighed under kiln-dried conditions (48 h at 103°C) to record the initial wood weight. In April 2017, two wood blocks of each tree species were placed aboveground in contact with the soil. After 397–414 days of environmental exposure, wood blocks were cleaned with a pencil brush on-site (Kipping et al., 2022). One

wood block of each tree species was shock frozen with liquid N₂ at the field sites, while the other was used for mass loss and pH determination. Shock-frozen wood blocks were transported with dry ice to the laboratory. Uncooled wood blocks were then dried for 48 h in a climatic chamber at 103°C ±2°C and then placed in a desiccator. Thereafter, the weight of each wood block was measured, and drying was repeated to constant weight. The percentage mass loss was determined by dividing the dry weight after exposure by the initial dry weight for every single wood block (Maurer et al., 2021; Kipping et al., 2022). The pH of wood was measured by an extraction method according to Sandermann and Rothkamm (1959) with some modifications. Briefly, 2 g ± 0.2 g of each wood block were ground into sawdust, and then immediately 20 mL of 0.2 M NaCl solution (pH 6) were added. The extracts were agitated overnight, and the pH was measured with a glass electrode (Mettler Toledo, Gießen, Germany) according to the manufacturers' instructions. The extraction method was repeated in independent triplicates, which demonstrated that there was no significant difference between the measurements.

2.2 Library preparation, sequencing, bioinformatics

Shock-frozen wood blocks were ground into sawdust by adding liquid N₂ using a swing mill (Retsch, Haan, Germany). Community DNA was isolated from approximately 100 mg of each homogenized wood sample using a Quick-DNA Fecal/Soil Microbe kit (Zymo Research, Irvine, CA, USA). The fungal ITS2 region was amplified using the primers fITS7/ITS4 as previously described by Leonhardt et al. (2019). The V4 region of the prokaryotic 16S rRNA gene was amplified using the primers 515F/806r according to Moll et al. (2018). PCR products were then barcoded and pooled in equimolar amounts as described in Moll et al. (2021b). The final library was used for 2 x 300 bp paired end sequencing (MiSeq Reagent kit v3) on an Illumina MiSeq system (Illumina Inc., San Diego, CA, United States) at the Department of Soil Ecology of the Helmholtz Centre for Environmental Research – UFZ.

Raw reads were processed using the Dadasnake pipeline (Weißbecker et al., 2020), which is based on DADA2 (Callahan et al., 2016). Both primer sequences were cut using cutadapt (v1.18). Fungal reads with expected error rates (maxEE) lower than 3 and a minimum base quality of 9 were further considered. Fungal reads were not truncated to a specific length in order to take into account the length variability of the ITS region. The reads obtained were then merged with an overlap of 20 nt and one mismatch was allowed. For prokaryotes, forward and reverse reads were cut to a length of 180 and 140 nt, respectively, and a minimum base quality of 9 was required. Reads with expected error rates higher than 0.5 were discarded and the

quality checked reads were merged with an overlap of 12 nt and 0 mismatches. For both groups, chimera removal was performed based on the consensus algorithm. Subsequently, fungal and prokaryotic ASVs (amplicon sequence variants) were subjected to post-clustering at 97 % sequence similarity using VSEARCH (Version 2.10.4) (Rognes et al., 2016) to consider potential intraspecific sequence variation. Resulting fungal and bacterial OTUs (Operational Taxonomic Units) were taxonomically assigned using the Bayesian Classifier implemented in mothur (Schloss et al., 2009) against the UNITE database (version 9.0) and against the SILVA database (SSU Ref, version 138), respectively. For fungi, only sequences with a length of 200-550 bp were reported in the final OTU table. For the prokaryotic V4 region, only OTUs of 240-265 bp, assigned to the kingdom Bacteria and not identified as chloroplasts or mitochondria, were finally reported in the OTU table. In total, 802 wood blocks (406 forest, 396 grassland) were recovered and successfully sequenced resulting in 38,365,996 quality filtered fungal sequences and 40,672,659 bacterial sequences.

2.3 Statistical analyses

All statistical analyses were performed using R Version 4.2.3. (R Core Team, 2023) and the interface RStudio (Version, RStudio Inc., Boston, USA). Sequencing depth was normalized based on subsampling to the sample with the lowest number of sequences using the function 'rarefy_even_depth' of the 'phyloseq' package (McMurdie and Holmes, 2013). This resulted in 21,648 and 23,962 sequences per sample, and 3,811 and 11,599 OTUs for fungi and bacteria, respectively. Taxonomic composition from phylum down to family level (down to order level for bacteria) was visualized via heat trees using the package 'metacoder' (Foster et al., 2017). In order to analyse colonizers' alpha-diversity for differences between host tree species (*F. sylvatica*, *P. sylvestris* or *P. abies*) and ecosystem type (forest versus grassland), the number of observed OTUs was calculated and tested using the non-parametric Kruskal-Wallis test ('kruskal.test' function).

Due to high abundance of individual fungal OTUs in some samples (> 90 %), fungal abundances were log-transformed ($\log_{10}(x+1)$) for all further analyses. Differences in colonizers' community compositions between host tree species and ecosystem type were tested by permutational multivariate analysis of variance (PERMANOVA) based on 999 permutations using the w 'adonis2' function of the 'vegan' package (Oksanen et al., 2022). Community composition was further analysed and visualized by Principal Coordinates Analysis (PCoA) based on Bray-Curtis distance using the function 'ordinate' and 'plot_ordination' of the 'phyloseq' package.

In order to quantify the relative contribution of the intrinsic versus extrinsic factors on colonizers' community composition, two-way variation partitioning (function 'varpart') on Bray-Curtis distance was conducted. For this analysis microclimatic predictors further characterizing the respective fraction were included: The first 'intrinsic' fraction consisted of tree species identity, wood pH and mass loss, whereas the second 'extrinsic' fraction consisted of ecosystem type and microclimatic parameters: soil pH, soil temperature, soil type, soil moisture, relative air humidity and air temperature. Pearson correlation between predictor variables was used to check for collinearity (Dormann et al., 2013) resulting in a coefficient <0.7 for all pairs. Significance of both fractions was tested using distance-based redundancy analysis based on Bray-Curtis distance, using the function 'dbrda' of the 'vegan' package. Afterwards, each microclimatic parameter was individually tested for significant correlation with the fungal and bacterial Bray-Curtis distance matrix using Mantel tests and spearman rank correlation (function 'cal_mantel' within the 'microeco' package) (Liu et al., 2020). *P* values were corrected by the FDR approach (false discovery rate) using the function 'p.adjust'. For some samples, wood pH determination was not possible as only one wood block was found after exposure and this was used for amplicon sequencing. This explains why 759 samples were processed for variance partitioning and mantel tests including wood pH as a predictor.

Genus-based community analysis is a useful approach as many fungi and bacteria can be grouped in relation to their occurrence and/or ecological function on this taxonomic level. We, therefore, analysed the 20 most abundant genera in relation to ecosystem type and host tree species using the non-parametric two-way Scheirer–Ray–Hare test, using the function 'scheirerRayHare'. We used FUNGuild (Nguyen et al., 2016) and FungalTrait (Polme et al., 2021) databases to identify fungal wood and/or litter saprotrophs and genera known to have endophytic capability across these dominant taxa. Likewise, we employed FAPROTAX to assign functions to bacterial taxa (Louca et al., 2016) and examined the 20 most abundant bacterial genera using current literature (see Table S1). We assessed their potential ability for atmospheric N fixation and complex organic compound degradation and determined whether these genera had previously been detected in deadwood .

In order to analyse the relationship between wood mass loss and observed OTU richness, Kendall rank correlation was performed separately for each host tree species (*F. sylvatica*, *P. sylvestris* or *P. abies*) within the two ecosystem types (grassland or forest) and over the entire data set. Wood mass loss data were square root transformed to improve normality. Accordingly, the relationship between community composition and wood mass loss was investigated separately for each host tree species within the two ecosystem types and over the entire data

263 set using PERMANOVA based on Bray-Curtis distance and 999 permutations. In addition, as
264 Agaricomycetes accounts for a high number of wood-saprotrophs and the bacterial order
265 *Rhizobiales* for a high number of nitrogen-fixing species, we analysed the relative abundance of
266 both groups in relation to wood mass loss over the entire data set separately for each host tree
267 species using Kendall rank correlation.

268 Figures were generated using 'ggplot2' and 'patchwork' (Wickham, 2016; Pedersen, 2020)
269 and partly optimized using CorelDRAW® Graphics Suite X8 (Corel Corporation, Ottawa,
270 Canada).

3 Results

All wood blocks of *F. sylvatica*, *P. sylvestris* and *P. abies* had been colonized by diverse communities (Fig.1). Based on sequence abundance data, fungal communities in grassland wood blocks mainly contained Ascomycota (~82%), whereas Basidiomycota accounted for ~51% (~40% Agaricales) in forest ecosystems (Fig.1A, B). Bacterial communities were dominated by Proteobacteria (~60%), followed by Actinobacteriota (15%), Bacteroidota (12%) and Acidobacteriota (5%) without showing such a clear distinction between ecosystem types at the phylum level (Fig.1C, D). Overall, grassland ecosystems harboured a higher number of wood-colonizing OTUs than forests for both investigated groups (fungi – 2,845 vs. 2,042 OTUs, bacteria – 8,900 vs. 7,488 OTUs). Wood blocks in grassland sites had been colonized by significantly higher numbers of fungi compared to forests ($\chi^2 = 206.2$, $p < 0.001$), while the number of bacterial colonizers did not differ between the ecosystems ($\chi^2 = 3.7$, $p > 0.05$). The number of observed colonizers (OTU richness) differed between host tree species (*F. sylvatica*, *P. sylvestris* or *P. abies*) for both groups (fungi - $\chi^2 = 27.5$, $p < 0.001$, bacteria - $\chi^2 = 263.1$, $p < 0.001$), resulting in lower numbers for beech wood compared to both conifers (Fig. 2A, D).

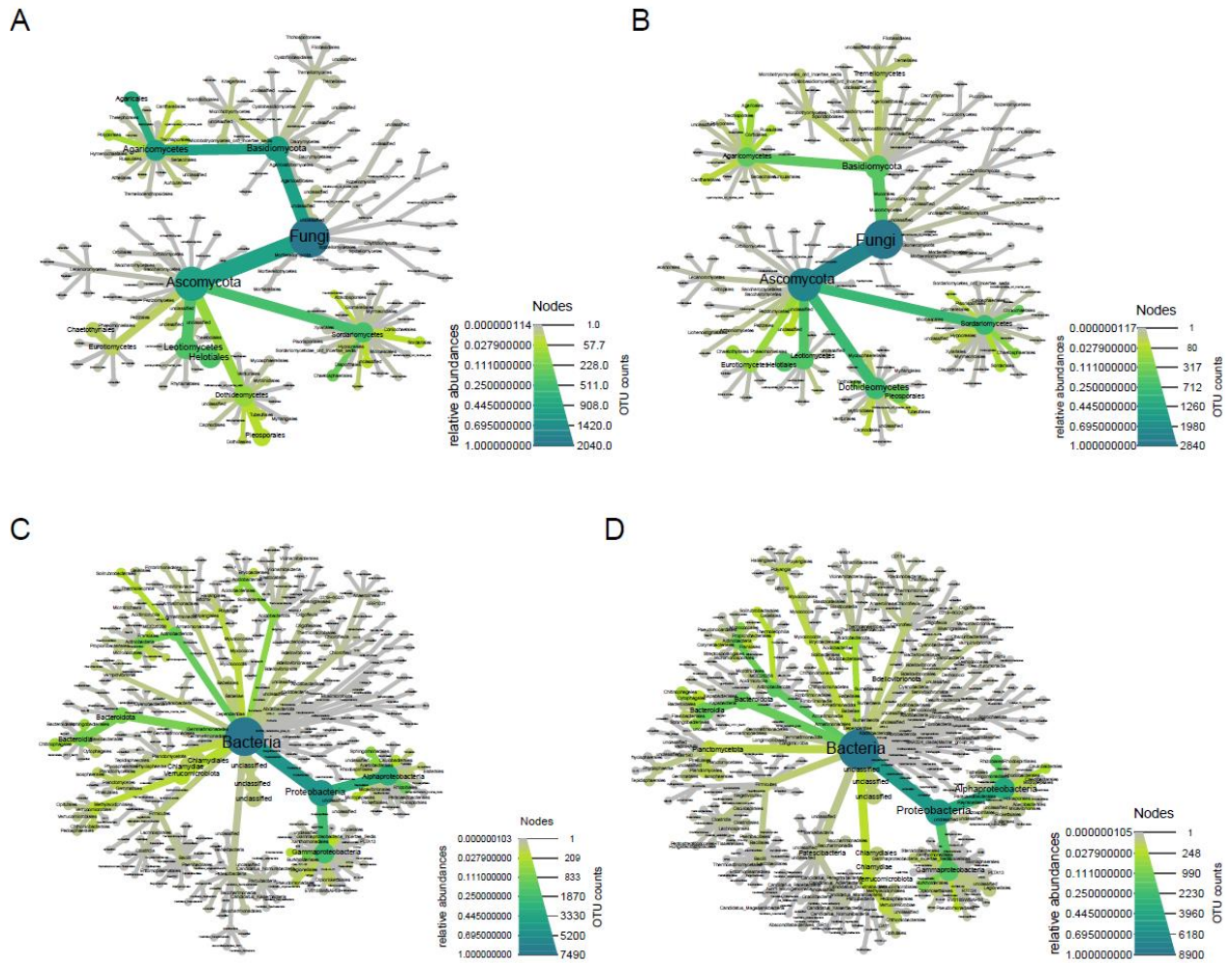


Figure 1: Taxonomic diversity of wood-colonizing fungi (A&B) and bacteria (C&D) in forest (A&C) and grassland ecosystems (B&D); colours of nodes and edges represent the relative OTU abundance of each taxonomic rank, node size represents number of OTUs of each taxonomic rank.

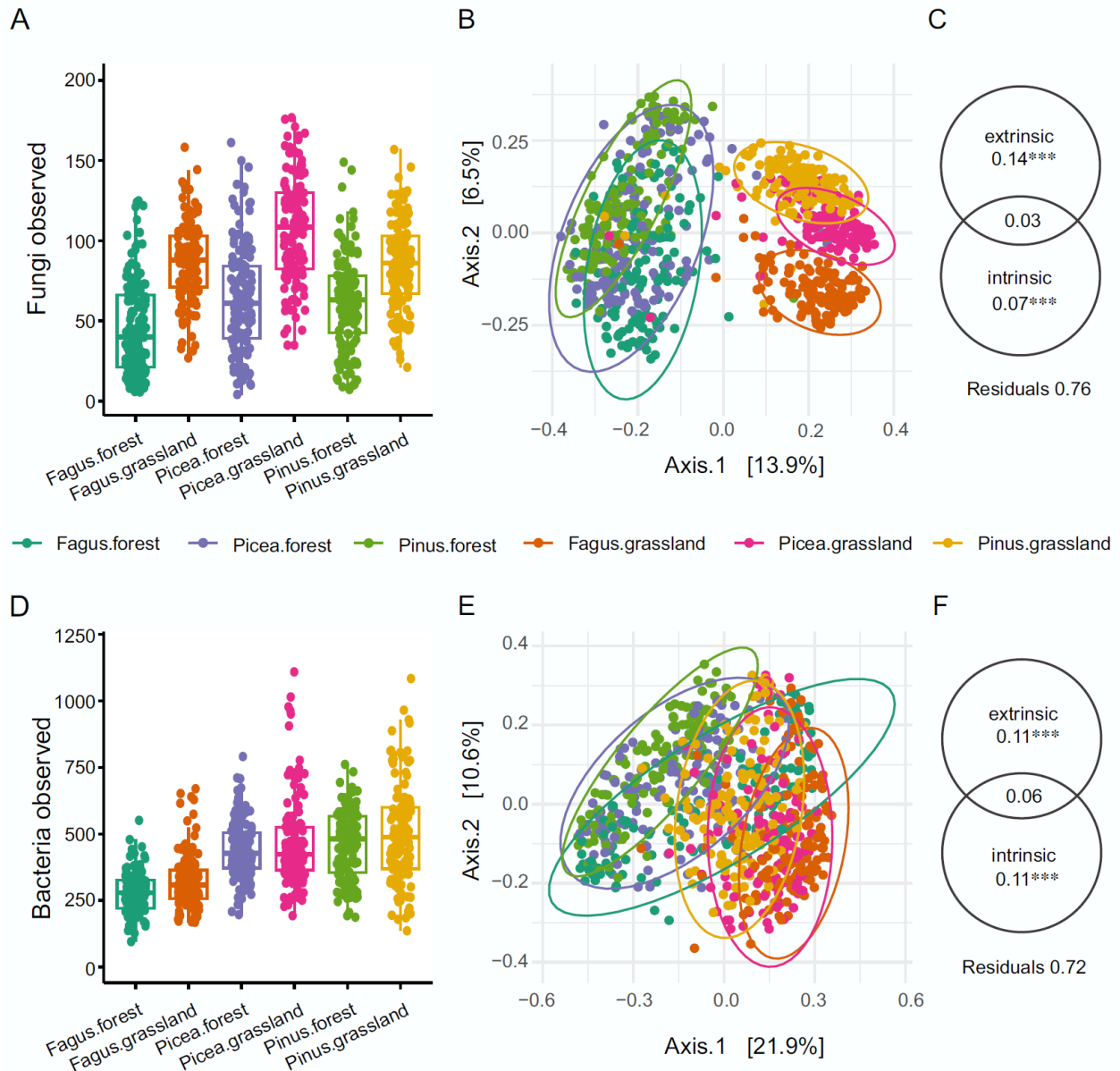


Figure 2: Community attributes for fungi (A, B, C) and bacteria (D, E, F). Observed number of OTUs colonizing different host tree species (*Fagus sylvatica*, *Pinus sylvestris* or *Picea abies*) within the respective ecosystem types (grassland or forest) (A&D). First two axes of Principal Coordinate Analysis (PCoA) showing wood-colonizers' communities separated by host tree species and ecosystem type (B&E). Results of two-way variation partitioning on Bray-Curtis distance showing the explained variation (adjusted R^2) of the intrinsic fraction (~host tree species + wood pH + wood mass loss) and the extrinsic fraction, i.e. environment (~ecosystem + soil pH + soil temperature + soil type + soil moisture + relative air humidity + air temperature) (C&F).

Community compositions of both colonizer groups differed between the two ecosystem types (fungi – pseudo- $F = 108.63$, $p = 0.001$, bacteria - pseudo- $F = 104.78$, $p = 0.001$) and between host tree species (fungi – pseudo- $F = 29.58$, $p = 0.001$, bacteria - pseudo- $F = 42.22$, $p = 0.001$, Table S2). This was further confirmed by PCoA ordination showing wood-colonizers' communities separated by ecosystem type and host tree species (Fig. 2B&E). Variation partitioning revealed a stronger effect of the extrinsic fraction ($R^2 = 0.14$ for ecosystem, soil pH, soil temperature, soil type, soil moisture, air temperature and air humidity) than the intrinsic fraction ($R^2 = 0.07$ for host tree species, wood pH and wood mass loss) on fungal community variation, whereas for bacteria, variation was similar for the two fractions (intrinsic $R^2 = 0.11$, extrinsic $R^2 = 0.11$) (Fig. 2C&F). All measured intrinsic and extrinsic variables significantly correlated with fungal and bacterial community compositions (Table 1).

Table 1: Relationship between fungal and bacterial community compositions and variables attributed to intrinsic (wood pH, mass loss) and extrinsic (soil pH, soil temperature, soil moisture, air temperature and air humidity) factors revealed by Mantel test on Bray Curtis distance.

Variables	Fungi		Bacteria	
	Spearman	adj. P	Spearman	adj. P
	ρ	value	ρ	value
a) Intrinsic variables				
Mass loss	0.26	0.0012	0.15	0.0014
Wood pH	0.20	0.0012	0.28	0.0014
b) Extrinsic variables				
Air temperature	-	n.s.	0.02	0.044
Air humidity	0.08	0.0012	0.05	0.0014
Soil pH	0.36	0.0012	0.22	0.0014
Soil moisture	0.22	0.0012	0.15	0.0014
Soil temperature	0.07	0.0012	0.04	0.0128

These results were also reflected by the most abundant colonizing genera, which differed strongly depending on ecosystem type (forest or grassland) and host tree species (*F. sylvatica*, *P. sylvestris* or *P. abies*) (Fig. 3). For instance, genera such as *Mycena* (mean grassland $0.2 \pm$ standard deviation of 0.8% , mean forest $6.5 \pm 9.3\%$) or *Hyaloscypha* (mean grassland $1.3 \pm 1.6\%$, mean forest $3.4 \pm 2.4\%$) were detected in forest ecosystems, whereas *Exophiala* (mean

grassland $4.5 \pm 2.1\%$, mean forest $1.4 \pm 1.8\%$) or *Dinemasporium* (mean grassland $1.6 \pm 1.8\%$, mean forest $0.03 \pm 0.3\%$) were more abundant in wood blocks exposed in grasslands (Fig. 3A). In addition, the abundance of many fungal genera significantly differed between host tree species (e.g. *Lophium* on *P. sylvestris* in both ecosystems, Fig. 3A, Table S3). For bacteria, the abundance of several genera differed strongly between ecosystems as well (Fig. 3B, Table S4). For instance, the relative proportions of *Sphingomonas* (mean grassland $11.0 \pm 5\%$, mean forest $6.3\% \pm 3.6\%$) *Methylobacterium*-*Methylorubrum*-complex (mean grassland $4.5 \pm 3.6\%$, mean forest $0.5 \pm 0.8\%$) and *Amnibacterium* (mean grassland $1.7 \pm 1.6\%$, mean forest $0.3 \pm 0.5\%$) were higher in grassland, whereas *Puia* (mean grassland $0.7 \pm 1.2\%$, mean forest $2.5 \pm 1.8\%$) and *Acidibacter* (mean grassland $0.5 \pm 0.7\%$, mean forest $2.4\% \pm 1.7\%$) were higher in forest ecosystems. Other bacterial genera were more abundant in wood from a specific host tree species independent of ecosystem type (e.g. *Luteibacter* [mean grassland $2.3 \pm 2.4\%$, mean forest $1.9 \pm 2.5\%$] or *Terriglobus* [mean grassland $1.9 \pm 1.5\%$, mean forest $1.4 \pm 1.2\%$] in *F. sylvatica*). These results were further supported by community-based analyses using FungalTrait and FAPROTAX, which showed differences in potentially relevant functions between tree species, but mainly between ecosystems for fungi and bacteria (Figure S1 & Figure S2). For instance, more fungal wood saprotrophs could be annotated in forests, (Figure S1), while methanotrophic and methylotrophic bacteria could be more frequently assigned in grasslands. (Figure S2).

The community composition was linked to wood mass loss for both colonizer groups for all host tree species ($p = 0.001$ each, Table S5). This correlation was also observed within ecosystems ($p < 0.05$ each, Table S6). Fungal OTU richness (number of observed OTUs in wood blocks) within ecosystems was not linked to mass loss (Fig. 4A). Considering both ecosystems, fungal OTU richness was correlated with wood mass loss for all host tree species displaying weak negative correlation for both conifers (*Picea*: Kendall's $\tau - 0.21$, $p < 0.001$, *Pinus*: -0.17 , $p < 0.001$) and a moderate correlation coefficient for *Fagus* (Kendall's $\tau - 0.32$, $p < 0.001$) (Fig.4A). Bacterial OTU richness was not correlated with wood mass loss (Fig.4B).

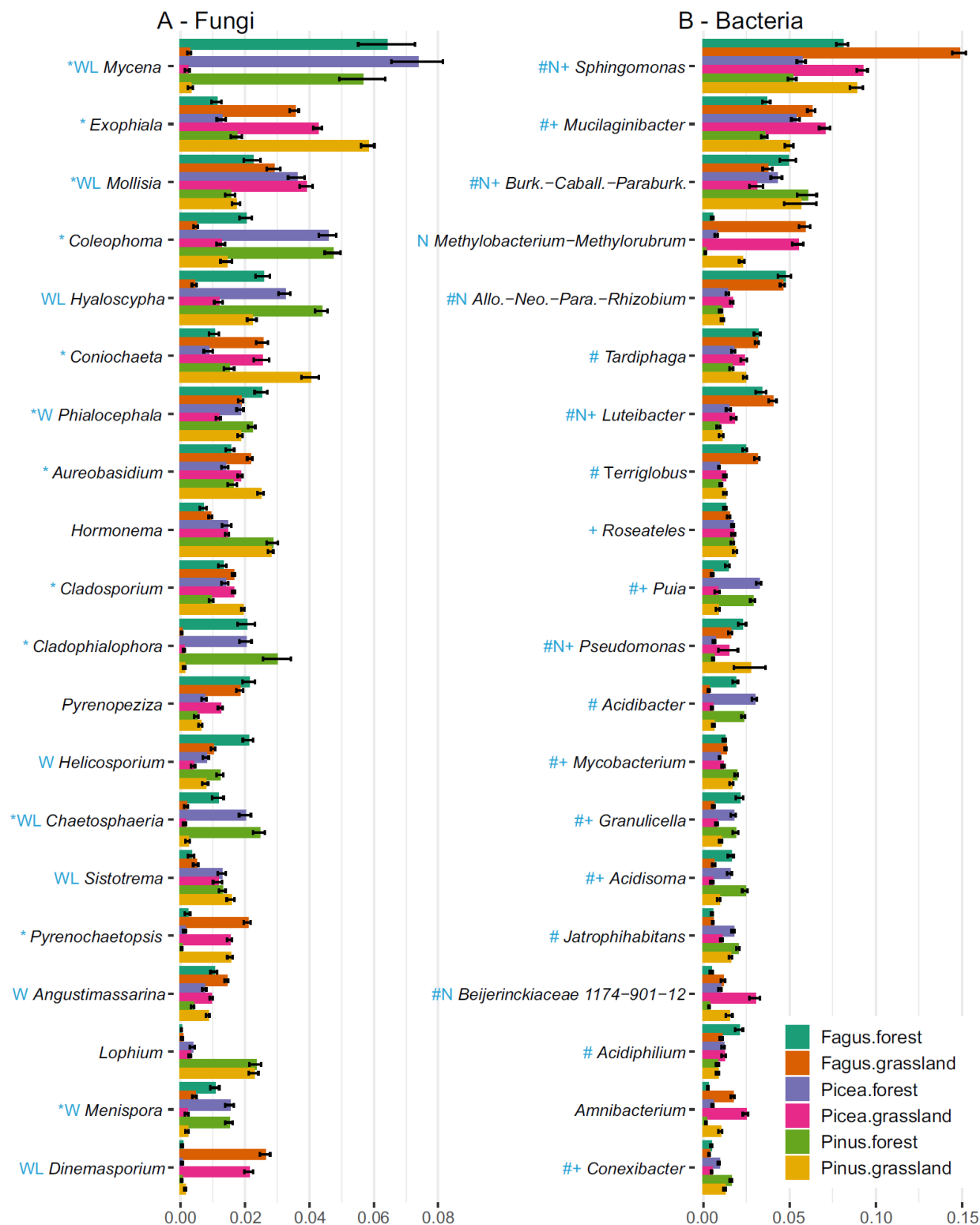


Fig. 3: Relative abundances of most abundant (A) fungal and (B) bacterial genera colonizing different host tree species (*Fagus sylvatica*, *Pinus sylvestris* or *Picea abies*) within the respective ecosystem (grassland or forest). W: fungal wood saprotrophs; L: fungal litter saprotrophs; *: fungal genera with endophytic capability; #: bacterial wood colonizers; N: genus described as

potential N-fixing bacteria; +: genus including bacterial degraders of complex compounds. Burk.- Caball.-Paraburk. = *Burkholderia-Caballeronia-Paraburkholderia* complex; Allo.-Neo.-Para.- Rhizobium = *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* complex.

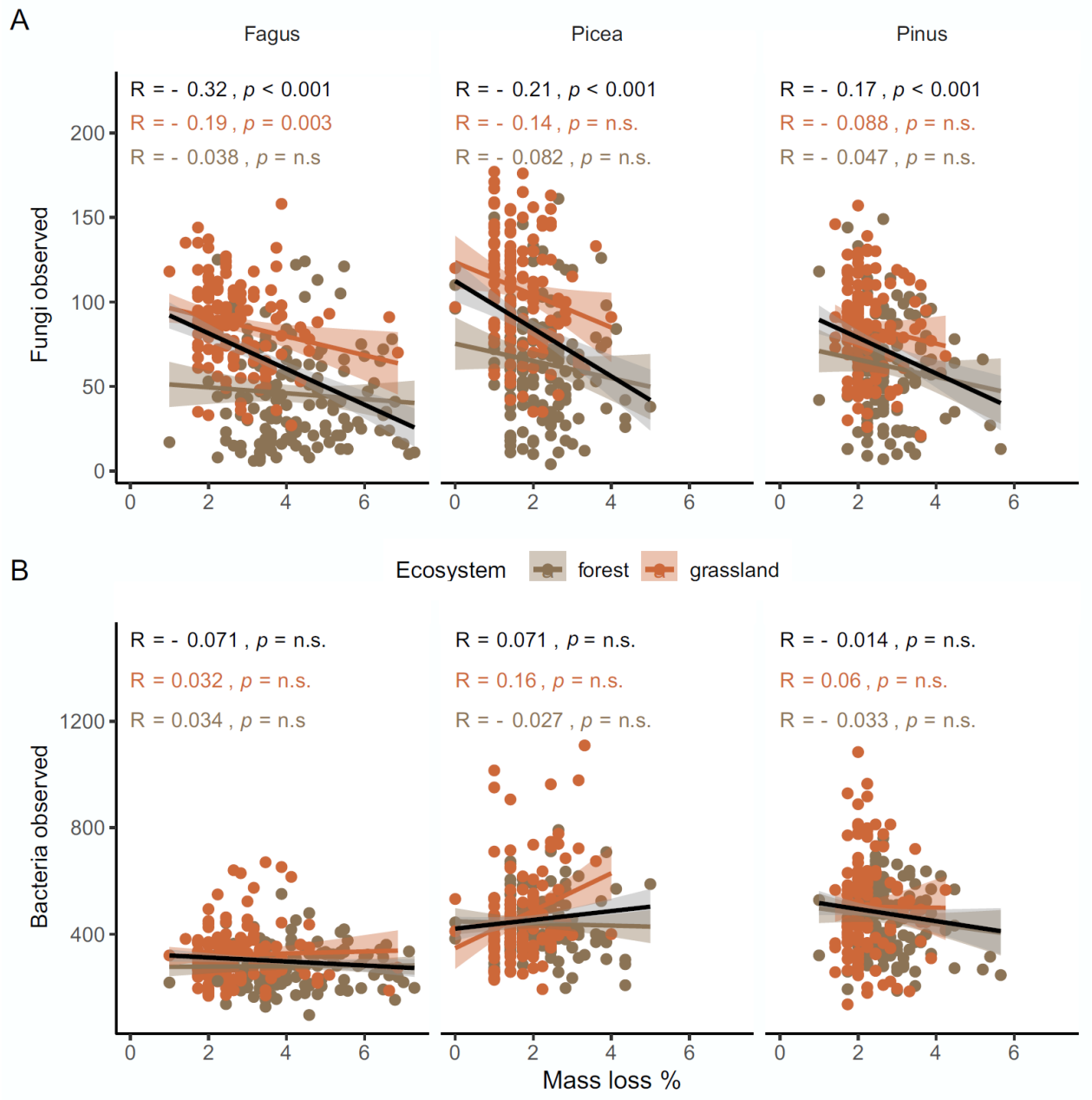


Fig. 4: Colonizers' OTU richness (number of observed OTUs in wood blocks) in relation to wood mass loss (square root transformed) separately for each host tree species (*Fagus sylvatica*, *Pinus sylvestris* or *Picea abies*), for the entire data set (black line) and for each ecosystem type separately (forest: brown, grassland: orange). Lines and confidence intervals based on linear regression ($x \sim y$) are drawn for clarity. Coefficients are based on Kendall rank correlation.

4 Discussion

From previous studies in forest ecosystems we have learned that the efficiency of the decomposition process and the diversity, composition and variation of decomposer communities appear to be determined by intrinsic rather than extrinsic factors (Hu et al., 2018; Krah et al., 2018; Lee et al., 2020; Müller et al., 2020; Kipping et al., 2022; Rieker et al., 2022). However, apart from finding that both host tree and environment-related factors (hypothesis (i)) controlled fungal and bacterial diversity patterns, the latter outweighed the importance of intrinsic factors, in contrast to our hypothesis (ii). Hence, extrinsic factors led to distinct diversity patterns in deadwood-colonizing microbial communities in forest and grassland ecosystems, with forest ecosystems exhibiting higher decomposition efficiency.

4.1 Scale of investigation explaining diversity patterns

The observed impact of host tree-related factors on determining fungal, bacterial and beetle communities is well known. Certain wood properties, such as pH, lead to distinct and specialized species communities, and this is particularly noticeable in differences between coniferous and deciduous tree species (Wende et al., 2017; Purahong et al., 2018a; Purahong et al., 2018b; Moll et al., 2021a). Among these are many taxa that rely on wood as a nutrient resource, which serves as an explanation for the strong association (Heilmann-Clausen et al., 2016; Wende et al., 2017; Moll et al., 2021a; Purahong et al., 2022). Studies contrasting intrinsic and extrinsic factors as predictors for wood-colonizing communities have usually been conducted within forest ecosystems (Bässler et al., 2010; Krah et al., 2018; Lee et al., 2020; Rieker et al., 2022). Even though it has been shown that these deadwood-colonizing communities are structured by extrinsic factors, host tree-related factors surpassed the effect of the environment (Krah et al., 2018; Lee et al., 2020). Hence, one reason for the opposite effect observed in the present study (Figs. 2, 3) is likely to be the scale of the investigation (Levin, 1992). This may reveal two different community assembly mechanisms when going beyond the forest ecosystem: dispersal limitation and environmental filtering. Knowledge about dispersal ecology of wood-colonizing fungi is scarce and contradictory. Dispersal is reported to be unrestricted at large scales (Komonen and Müller, 2018) or even restricted at small scales (Norros et al., 2012). Outside of forest ecosystems, however, dispersal seems to be limited (Peay and Bruns, 2014; Smith and Peay, 2021). Spores and mycelia from wood colonizers known from forest habitats may not be omnipresent at the scale of our study. Accordingly, members of Agaricales, i.e. *Mycena* or

Tubaria, were significantly more abundant (Table S3) in forest wood blocks. However, these diversity patterns could also be caused by environmental filtering, supporting the hypothesis that “everything is everywhere and the environment selects” (stated by Lourens Bass-Becking 1934 (Whitfield, 2005)). This means that although these organismic groups are not dispersal limited, their proliferation or colonization ability seems to be strongly limited under existing environmental conditions, while other wood saprotrophs such as *Dinemasporium* seem to prevail in colonizing wood blocks exposed in grassland. Much less is known about the dispersal ability of wood-colonizing bacteria, but due to their small size and ubiquitous occurrence, they are thought to be present at broad spatial scales (Fenchel and Finlay, 2004; O'Malley, 2007). Indeed, over great environmental gradients within a forest ecosystem, dispersal limitation has not been observed (Müller et al., 2020; Rieker et al., 2022). At the scale of our study, bacterial colonizer patterns seem to be similar to those of fungi, showing clear differences between forests and grasslands, but also between tree species. Several dominant bacterial genera, like *Mucilaginibacter*, *Luteibacter* and *Pseudomaonas*, have been previously detected in deadwood and are known for their ability to degrade complex organic compounds (Hoppe et al., 2015; Tláskal and Baldrian, 2021; Purahong et al., 2022; Ren et al., 2022). Interestingly, dominant genera such as *Sphingomonas* or the *Methylobacterium–Methylobacterium* complex, which include potential N-fixers, have been found to be more abundant in grasslands (Videira et al., 2009; Grossi et al., 2020; Purahong et al., 2022; Ren et al., 2022).

Members of several fungal and bacterial genera primarily colonized the deadwood substrate in one or the other ecosystem (Tables S3 & S4). For microbes in particular, environmental filtering seems to be a prominent mechanism in determining community assembly processes and thus diversity patterns (Kivlin et al., 2014; Cao et al., 2016; Glassman et al., 2017), here supported by the significant correlation between environmental parameters and both colonizers' communities (Table 1). It is well known that the composition of soil microbial communities is strongly correlated with edaphic factors such as pH value or water content (Rousk et al., 2010; Brockett et al., 2012). As lying deadwood is located on the floor and thus is in direct contact with the soil surface it is considered to represent a species pool for deadwood-colonizing microbes. Therefore, these factors may act as a selective filter, indirectly affecting wood communities, as previously described (Hu et al., 2021). Based on our data, neither assembly mechanism (dispersal or filtering) can be disentangled from each other and both may contribute to the observed patterns at the investigated scale across ecosystems.

4.2 Role of endophytes explaining diversity patterns

A further explanation for the separation of wood colonizers between the two ecosystems may be the inactivation of potential endophytes known to be present in the wood of living trees (Jeremic et al., 2004; Parfitt et al., 2010; Song et al., 2017; Liu et al., 2019). Some of these fungal and bacterial species are expected to change their ecological strategies to saprotrophic lifestyles after tree death, in order to directly exploit the resource (Parfitt et al., 2010; Song et al., 2017). The presence of these species can change decomposition efficiency by impairing the development and establishment of species that arrive later (Hiscox et al., 2015; Cline et al., 2018). Such 'priority effects' are assumed to occur through niche pre-emption and niche modification, e.g. changes of pH, water content or release of secondary metabolites (Fukami, 2015; Hiscox et al., 2018). Several of the dominant fungal genera are known to comprise foliar or root-endophytes/associated species (e.g. *Mycena*, *Exophiala*, *Coleophoma*), thus demonstrating both endophytic and saprotrophic capabilities. Although the ecology of wood endophytes is not well studied, their communities seem to differ in relation to host tree species and wood traits (de Errasti et al., 2010; Lee et al., 2019). For litter, the presence of endophytes has gained increasing attention in relation to the "home-field-advantage" (Fanin et al., 2021). Veen et al. (2019) observed distinct litter colonizing fungal communities between 'home' and 'away'. Interestingly, these were mainly explained by the soil source (extrinsic factor) when using sterilized litter (i.e. removing endophytes) and by litter type (intrinsic factor) when non-sterilized litter was used. Hence, the presence of endophytes would probably increase the wood substrate effect size for colonizing communities of decaying wood. In contrast, in our present study, sterilized deadwood eradicates such endophytic activities and thereby probably strengthens the extrinsic (environmental) impact on fungal and bacterial assembly processes. Nevertheless, using standardized wood substrates of the same origin and size enabled us to follow mechanisms of wood colonization to elucidate extrinsic factors under various environmental conditions as this allowed us to eliminate key organismal intrinsic factors.

4.3 Wood mass loss and community patterns

In accordance with hypothesis (iii), wood mass loss correlated with the colonizing microbial community. A negative correlation between fungal diversity and mass loss was observed when considering both ecosystems. In particular, high fungal species richness in grassland ecosystems corresponded to lower mass loss while low species richness in forest ecosystems correlated with higher mass loss, which explains this negative relationship. (Fig. 4A). However,

these findings were not restricted to forests or grasslands, indicating that the ecosystem is the main cause of this observation and thus emphasizing the effect of investigated scale. Accordingly for bacteria, where similar numbers of species (OTUs) have colonized the deadwood substrate in forest and grassland ecosystems, neither positive nor negative richness-mass loss relationships were observed (Fig. 4B). By manipulating the initial fungal community, various studies have shown that subsequent fungus to fungus interactions determine the secondary colonizing community, resulting in negative correlations between diversity and decomposition (Fukami et al., 2010; Hiscox et al., 2015; Fukasawa and Matsukura, 2021). However, the extent to which these observations, which were made under controlled laboratory conditions with small blocks of wood or wood powder, can be directly transferred to natural communities is still unclear. Fukasawa and Matsukura (2021) anticipated increased decomposition in more species rich communities under natural conditions, as resource partitioning and facilitation may compensate for competition. Indeed, other studies using deadwood logs under natural conditions have shown a positive relationship between fungal species richness and wood decay (Rajala et al., 2015; van der Wal et al., 2015). Hence, we cannot rule out the possibility that the semi-natural wood blocks used here partly explain the negative diversity-decomposition relationship. Nevertheless, community compositions of both decomposer groups correlated with wood mass loss, suggesting a changing community during the process and the contribution of specific fungal and bacterial taxa to decomposition. Notably, several forest samples were strongly dominated by fungal wood degraders such as *Mycena* (Agaricomycetes). Their presence had a positive impact on mass loss (Fig. S3A), and in turn, seems to contribute to the negative relationship between diversity and decomposition (Fig. S3B). However, there were also several samples that showed a high proportion of Agaricomycetes despite there being only a low level of degradation. This supports the suggested combative strength of wood degraders in occupying the substrate, as previously described (Boddy, 2000), probably first investing energy in exploiting the resource and eliminating other colonizers before starting effective utilization. Bacterial communities have been shown to differ between wood decay stages and to underlie succession during wood decomposition in relation to chemical and physical changes to the substrate (Hoppe et al., 2015; Rinta-Kanto et al., 2016; Tláškal and Baldrian, 2021). They are assumed to play a complementary role in wood decomposition by providing N to fungal wood colonizers (Tláškal et al., 2021; Purahong et al., 2022). Indeed, Rhizobiales, the bacterial group that is expected to include free-living atmospheric dinitrogen fixing microorganisms, was consistently observed in each wood sample, slightly but significantly decreasing with increasing mass loss (Fig. S4).

4.4 Conclusions

Within our study system, distinct colonizing communities varying between wood substrates and ecosystems were identified. In contrast to our expectations, these communities were more strongly controlled by extrinsic than by intrinsic factors. A link between colonizers and mass loss was detected for all tree species, resulting in similar patterns for all wood substrates. Hence, our findings suggest that both grassland and forest ecosystems select distinct wood-colonizing communities that vary greatly in their decomposition abilities, although known wood saprotrophs have been observed in both ecosystems. Wood-colonizing communities within grasslands show the same host tree specificity as in forest sites. The relative strength of these drivers was similar for fungi and bacteria. Our study highlights the dependence on the investigated scale (ecosystem type) when assessing ecological patterns. Therefore, enhancing environmental variability can decrease the strong substrate effect size known for wood-colonizing fungi and bacteria. The role of wood endophytes remains uncertain in this context and requires further research comparing wood substrate effect size with and without endophyte inactivation.

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535

6 Data availability

All raw reads have been submitted to the NCBI short read archive (SRA, <https://www.ncbi.nlm.nih.gov/sra/>) and are accessible under bioproject PRJNA1091617. This work is based on data elaborated by 'BLD-MultiFuncDiv' & 'FunWood' from the Biodiversity Exploratories program (DFG Priority Program 1374, NO 834/5-4, BU 941/126-3, MO 4586/1-4). The datasets on wood mass loss, weather and soil conditions are publicly available in the Biodiversity Exploratories Information System (<http://doi.org/10.17616/R32P9Q>), (Maurer et al., 2021; Schöning et al., 2021; Hänsel et al., 2024). The OTU tables and wood pH values have also been submitted to the Biodiversity Exploratories Information System under IDs 31639 and 31647 and 31879 and will be made publicly available after an embargo period to give data owners and collectors time to perform their analysis.

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