

Hidden in the Hollow: First Insights into Fungal Communities of Tree Hollow wood mold

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Abstract

Tree hollows containing wood mold are long-lasting microhabitats supporting diverse communities, yet their fungal assemblages remain poorly characterized. We investigated fungal diversity and composition across a decomposition gradient of wood mold from 36 tree hollows in Germany (2025) using fungal ASVs, applying coverage-based standardization and indicator species analyses. Fungal richness showed a non-significant but consistent increase with decomposition, peaking in advanced stages. Abundance-weighted diversity remained largely stable, with pronounced compositional shifts only in the most advanced decay stage, suggesting late-stage changes in resource limitation and competitive dynamics. Geographic distance had no detectable effect. Community turnover was mainly driven by rare and moderately abundant taxa, whereas dominant taxa remained comparatively stable. Indicator taxa reflected a subtle successional transition from early saprotrophs to cellulose-degrading and increasingly soil-associated fungi, including ectomycorrhizal species. Overall, decomposition stage structured fungal communities in tree hollows, highlighting the importance of maintaining hollows across decay stages to preserve fungal diversity.

Introduction

Fungi in forest ecosystems represent an exceptionally diverse and functionally important group, occupying a wide range of ecological niches and contributing to multiple key ecosystem processes (Runnel et al. 2025). Their diversity spans a broad spectrum of lifestyles, including mutualistic, parasitic, and saprotrophic interactions, which are closely linked to fundamental processes such as primary production and decomposition (Sudharsan et al. 2023; Marcot 2017). Through these roles, fungi form complex interactions across habitats and trophic levels, making them central components of forest ecosystem functioning, e.g. primary drivers of organic matter turnover in forest ecosystems (Lonsdale et al. 2008; Stokland et al. 2012).

Trees are among the most important and diverse hosts for fungi, providing a wide range of ecological niches that vary across spatial and temporal scales (Singavarapu et al. 2024). During their living stage, trees associate with diverse fungal symbionts, including mycorrhizal fungi and endophytes. In particular, foliar (leaf) endophytes can have beneficial effects on their host trees, for example by enhancing stress tolerance or resistance to pathogens (Arnold et al. 2003; Sudharsan et al. 2023). In addition, mycorrhizal fungi play a crucial role in tree nutrition by improving the uptake of water and essential nutrients (Smith und Read 2010). In contrast, many epiphytic fungi occurring on tree surfaces are components of lichens; these represent mutualistic associations primarily between fungi and photosynthetic partners such as algae or cyanobacteria, rather than direct mutualistic interactions with the tree itself (Hawksworth et al. 1984).

Following tree death, woody substrates are colonized by saprotrophic fungi that drive decomposition processes and nutrient cycling (Boddy 2001). However, this dichotomous view of living versus dead tree-associated fungal communities is overly simplistic. Even living trees contain a diversity of microhabitats

that differ strongly in their physical structure and resource availability. Among these, specialized structures such as phytotelmata and tree hollows represent distinct ecological niches with unique environmental conditions (Schauer et al. 2025; Schmidl et al. 2008).

Despite extensive research on fungal communities associated with living trees and deadwood, fungal diversity and community assembly within tree-associated microhabitats remain poorly understood. Tree hollows constitute a largely unexplored habitat for fungal communities. While these structures are well recognized as biodiversity hotspots for saproxylic invertebrates, birds, and mammals (Schauer et al. 2025; Goldingay 2009; Micó 2018), their role as habitats for fungi—and especially the processes structuring fungal communities within them—has not been explicitly investigated.

Tree hollows are long-lasting, stable structures in forest ecosystems that are regarded as keystone structures for a wide range of organisms (Micó 2018; Schauer et al. 2025; Lindman et al. 2023). These cavities form gradually through mechanical damage, fungal decay, and biological activity, leading to the accumulation of partially decomposed organic material commonly referred to as wood mold. Wood mold represents a complex and dynamic substrate composed of fragmented wood, plant litter, faunal remains, and microbial biomass. Hollows often maintain buffered microclimatic conditions, including relatively stable moisture regimes and reduced temperature fluctuations (Lindman et al. 2023). In addition, wood mold accumulates organic inputs from surrounding vegetation and fauna, resulting in gradual nutrient enrichment and increasing structural heterogeneity (Stokland et al. 2012).

As decomposition progresses, dead wood undergoes substantial physical and chemical transformation, ultimately contributing to nutrient cycling and habitat formation within forest ecosystems (Gessner et al. 2010; Valentín et al. 2014). Fungi play a central role in wood decomposition and are among the primary drivers of organic matter turnover in forest ecosystems (Lonsdale et al. 2008; Stokland et al. 2012). Through the production of extracellular enzymes capable of degrading lignin, cellulose, and hemicellulose, fungi initiate and regulate the conversion of woody tissue into humified organic matter (Stokland et al. 2012). The assembly of fungal communities during wood decomposition is commonly described as a successional process, in which early colonizers modify substrate properties, thereby facilitating or inhibiting the establishment of later-arriving taxa (Fukasawa und Matsukura 2021; Boddy 2001). Classical models of fungal succession in decaying wood propose a shift from fast-growing pioneer species capable of exploiting relatively intact woody substrates toward more specialized decomposers adapted to increasingly complex and nutrient-enriched environments (Brabcová et al. 2022; Saine et al. 2024). However, these successional dynamics have been studied primarily in fallen logs or standing deadwood. Despite increasing attention to forest microbial ecology, fungal community assembly within tree hollow wood mold remains unexplored. To our knowledge, no studies have explicitly investigated fungal communities inhabiting tree hollows, and the ecological processes structuring these communities have not been addressed.

From a theoretical perspective, community assembly during decomposition may be governed by both deterministic and stochastic ecological processes (Chen et al. 2025; van der Wal et al. 2015). Habitat

filtering is expected to play a major role as the physical and chemical characteristics of woody substrates change over time, selecting fungi with specific functional traits related to substrate utilization, stress tolerance, and competitive ability (Oechler et al. 2026; Hoppe et al. 2016). At the same time, neutral processes such as dispersal limitation and stochastic colonization may influence fungal community composition, particularly in spatially isolated microhabitats such as tree hollows (Cat et al. 2026; Kivlin et al. 2014). Disentangling the relative influence of environmental gradients and spatial processes remains a key challenge in microbial ecology, especially in structurally complex habitats where microenvironmental conditions vary over small spatial scales.

Tree hollow wood mold presents a particularly interesting system for investigating these processes because it differs substantially from other decomposing wood substrates. As decomposition progresses, the substrate may transition from wood-like material to a soil-like environment, potentially allowing colonization by fungal taxa with diverse ecological strategies, including saprotrophic, and mycorrhizal species. These characteristics suggest that fungal communities in hollow wood mold may follow successional trajectories that differ from those observed in exposed deadwood systems.

Despite the ecological importance of tree hollows, several critical knowledge gaps remain. First, most studies examining fungal succession in decaying wood have focused on coarse woody debris, logs or stump decomposition, leaving fungal diversity patterns of tree hollows largely uncharacterized. Second, while tree hollows are known to support diverse saproxylic communities (Schauer et al. 2025; Micó 2018), the extent to which fungal community assembly is structured by decomposition stage versus spatial factors has rarely been investigated. Third, little is known about how fungal taxa are distributed across decomposition stages within hollow wood mold or whether specific taxa can serve as indicators of successional transitions. Addressing these gaps is essential for understanding the ecological processes shaping biodiversity within hollow tree habitats and for evaluating their role in forest ecosystem functioning and conservation.

Patterns of biodiversity along decomposition gradients can be evaluated using Hill numbers, which provide a unified framework for assessing diversity across multiple orders that emphasize rare, common, or dominant taxa. Such an approach allows detection of whether community shifts are driven by changes in overall richness, redistribution of relative abundances, or turnover among rare species (Chao et al. 2023). Rare fungal taxa are increasingly recognized as important components of ecosystem functioning, potentially acting as reservoirs of functional diversity and responding sensitively to environmental change (Stokland et al. 2012; Mouillot et al. 2013; Jain et al. 2014). However, their contribution to successional processes in wood mold ecosystems remains largely unexplored.

In this study, we investigated fungal community dynamics in wood mold from tree hollows across a gradient of decomposition using high-throughput sequencing of fungal amplicon sequence variants (ASVs). Specifically, we aimed to (i) quantify fungal diversity across decomposition stages using Hill numbers, (ii) evaluate the relative influence of decomposition stage and geographic distance on fungal community dissimilarity, and (iii) identify fungal taxa associated with specific stages of wood mold

decomposition. By integrating diversity metrics, indicator species analysis, and distance-based modelling, this study provides new insights into fungal succession and community assembly within tree hollow ecosystems and contributes to a broader understanding of microbial biodiversity in structurally complex forest microhabitats.

Material and Methods

Study area and sampling method

The study was conducted in July 2025 in the northern Steigerwald region (Bavaria, Germany, Fig. 1). We sampled 36 European beech (*Fagus sylvatica*) trees containing tree hollows with at least 2 cm of accumulated detritus (wood mold). This threshold was applied to ensure that only well-developed hollows were included, as the presence of wood mold is a defining characteristic of established tree hollows. In contrast, early-stage cavities lacking sufficient detritus were excluded, as they do not yet represent fully developed hollow habitats. Wood mold was collected at a depth of 2 cm below the surface of the mold to 7 cm depth. Mold was classified in three degrees of decomposition (Dod). We used three parameters to determine the degree of decomposition (color, texture of the wood mold, and visible wood parts, Jarzabek 2005). Following literature, we differentiated three stages.

Dod 1 was characterized by yellow to light brown in color, visible wood parts of bigger size, low decay, Dod 2 by light brown to brown in color, visible wood parts of smaller size, medium decay, and Dod 3 by dark brown to black in color, no visible wood parts, high decay.

Identification of fungi via DNA metabarcoding

The mold samples were immediately cooled in the field with cooling blocks and boxes, then frozen within a few hours after collection. We used metabarcoding to identify the species of fungi in each mold sample. Wood mold samples (5 g) were freeze dried for 90h at -50°C (Christ Alpha 1–4 LSCplus, Osterode am Harz, Germany) and afterwards homogenized in vibrating mill with 28 Hz for 5 minutes (Retsch MM 400, Haan, Germany). Metagenomic DNA was extracted from 0.25 g of the homogenate using the NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol and diluted to each 5 ng/μl. The ITS2 region was amplified using gITS7 and ITS4 primers (Ihrmark et al. 2012; White et al. 1990) fused to Illumina adapter sequences. In particular, the gITS7 primer was prepared as a phased-primer mixture where zero, one (C), or two (TC) bases were added in between the Illumina adapter and the gITS7 sequences, respectively, and mixed in equimolar amounts. Each amplification reaction contained 12.5 μl KAPA HiFi HotStart ReadyMix (Roche Diagnostics, Mannheim, Germany), 0.2 μM of forward and reverse primers, 2.5 μl of metagenomic DNA in a total volume of 25 μl. The amplicon PCR were performed in a 96-well thermocycler (Mastercycler epgradient S: Eppendorf, Hamburg, Germany) using the following cycling protocol: initial activation of KAPA polymerase at 95°C for 3 min, followed by 25 cycles at 95°C for 30 sec, annealing at 50°C for 30 sec, extension at 72°C for 30

sec, respectively, and a final extension at 72°C for 5 min. The amplicon PCR products were purified using the NucleoMag NGS clean-up kit (Macherey-Nagel) and quality-checked by capillary electrophoresis (Fragment Analyzer 5200: Agilent, Waldbronn, Germany). Sample specific DNA indices were added to each sample library (2.5 µl of purified amplicon PCR product) in a second PCR with unique index primer combination taken from the Nextera XT Index kit v2 (Illumina, Berlin, Germany). The index PCR was performed in a 96-well thermocycler using the following cycling protocol: initial activation of KAPA polymerase at 95°C for 3 min, followed by 12 cycles at 95°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 30 sec, respectively, and a final extension at 72°C for 5 min. Index PCR products were purified and quality-checked by capillary electrophoresis as described above. Sample-specific libraries were combined at equimolar amounts in one library pool and were size-selected from 300 to 750 bp (Pippin Prep: Biozym, Hess. Oldendorf, Germany). The pooled library was sequenced on an iSeq-100 sequencing machine (Illumina) in 300 bp single-end mode (R1, corresponding to the forward primer direction). Illumina adapter sequences were trimmed, and raw reads were demultiplexed by the iSeq-100 software at the end of the NGS run.

Microbiome bioinformatics was performed using Qiime2 2025.4 (Bolyen et al. 2019). Amplicon-specific primer sequences were removed from raw demultiplexed sequence data using Cutadapt (Martin 2011) (via q2-cutadapt trim-single). The trimmed reads were denoised, dereplicated and filtered for chimeras using DADA2 with the pseudo pooling method (Callahan et al. 2016) (via q2-dada2 denoise-single). Possible contaminating sequences were identified using PCR control samples and the prevalence method and of DECONTAM (Lafond-Lapalme et al. 2017) (via q2-decontam-identify) and removed from the ASV sequences and abundance table. Taxonomic classification was obtained using a naïve-bayes trained classifier against the UNITE 10 reference database (Abarenkov et al. 2024) (via q2-feature-classifier classify-sklearn). ASVs that could not be taxonomically classified to any known reference taxon, were removed from the dataset.

Data analysis

All statistical analyses were carried out in R version 4.5.2 (R Core Team 2025). Fungal alpha diversity was assessed using a coverage-based rarefaction and extrapolation approach implemented in the R package iNEXTbeta.3D (Chao et al. 2023). Diversity estimates were calculated using the estimatedD function, which standardizes samples by sample coverage (SC), i.e. the estimated completeness of sampling. The standardized coverage level was set to SC = 0.9993, corresponding to the minimum sample coverage observed across all samples, thereby enabling reliable comparisons among assemblages despite differences in sampling completeness. Diversity was evaluated using Hill numbers, including species richness ($q = 0$), Shannon diversity ($q = 1$), and Simpson diversity ($q = 2$), thereby capturing complementary aspects of community diversity and evenness.

Differences in coverage-standardized diversity estimates among treatments were evaluated using linear models. Although Hill numbers (effective numbers of species) are conceptually derived from count data, exploratory analyses indicated that their distribution was approximately normal. We therefore applied

linear models, while also verifying robustness with alternative count-based approaches (e.g. negative binomial models), which yielded qualitatively consistent results. Model assumptions were assessed using diagnostic residual plots, and residual distributions were further validated using simulated residual diagnostics implemented in the DHARMa package (Hartig 2024).

Following confirmation that model assumptions were adequately met, the effects of degree of decomposition of the wood mold on fungal diversity were tested with linear models. In addition, pairwise comparisons among treatment levels were conducted using estimated marginal means to identify potential differences between specific treatment groups, using the R package *emmeans* (Lenth and Piaskowski 2025), accounting for multiple testing using Tukey correction.

Residual spatial autocorrelation was assessed using Moran's I on model residuals based on a k-nearest neighbor spatial weights matrix ($k = 5$) using the R package *spdep* (Pebesma und Bivand 2023). No significant spatial autocorrelation was detected for any of the Hill numbers. Consequently, no spatial correction was used for all models.

Taxonomic beta diversity among samples was quantified using Hill-number-based dissimilarity metrics using the R package *iNEXTbeta.3D* (Chao et al. 2023). Maximum theoretical diversity $C_{\max}$ was calculated and pairwise beta diversity between samples was subsequently computed using abundance-based dissimilarity metrics standardized by $C_{\max}$ (0.9994). Three complementary dissimilarity measures corresponding to Hill numbers were derived following Chao et al. (2023): Sørensen-type dissimilarity for $q = 0$ (incidence-/richness-based), Horn-type dissimilarity for $q = 1$ (abundance-weighted), and Morisita–Horn-type dissimilarity for $q = 2$ (dominant-species weighted). Pairwise values were converted into distance matrices and expressed as dissimilarity values ($1 - \text{similarity}$).

To investigate differences in community composition among degree of decomposition, Non-metric multidimensional scaling (NMDS) was then performed using the R package *vegan* (Jari Oksanen et al. 2025), with four dimensions ($k = 4$), a maximum of 500 random starts (*trymax* = 500), and 100 initial tries.

To evaluate the influence of degree of decomposition and space on community dissimilarity, Multiple Regression on distance Matrices (MRM) was applied with R package *ecodist* (Goslee and Urban 2007). Environmental distance was calculated as Euclidean distance based on differences in degree of decomposition. Geographic distance between sampling locations was calculated using Euclidean distances derived from spatial coordinates. Separate MRM models were fitted for each Hill-number-based dissimilarity matrix to assess whether taxonomic turnover was associated with environmental differences while accounting for spatial distance among sites. Statistical significance of regression coefficients was assessed using permutation tests with 999 permutations.

Indicator species analysis was conducted to identify fungal taxa significantly associated with degradation categories. Indicator values were calculated using the R package “*indicspecies*” (De

Cáceres and Legendre 2009). Decomposition stages were used as grouping variables. Fungal lifestyles have been derived from FungalTraits Database (Pöhlme et al. 2021).

Results

Mean fungal richness (Hill number $q = 0$) increased along the decomposition gradient, with the lowest mean number of ASVs observed in degree of decomposition stage 1 (195.75 ± 78.55 SD), followed by stage 2 (237.25 ± 97.35 SD) and stage 3 (290.67 ± 164.44 SD). Decomposition stage 1 had 183 unique ASVs, stage 2 303 ASVs, and stage 3 606 ASVs (Fig. 2). Despite this increasing trend, linear modelling revealed no statistically significant effect of decomposition stage on species richness ($F_{2,33} = 1.87$, $p = 0.17$), although richness at degree of decomposition stage 3 tended to be higher than at stage 1 ($p = 0.06$) (Fig. 3a) (for whole species list see Supplement Table 1, for model details see Supplement Table 2).

Patterns were consistent when diversity was assessed using abundance-weighted Hill numbers. Shannon diversity ($q = 1$) showed a gradual increase across degree of decomposition, with estimated marginal means of 21.70 ± 4.17 SE, 28.30 ± 4.17 SE, and 30.30 ± 4.17 SE for degree of decomposition 1, 2, and 3, respectively. However, degree of decomposition did not significantly influence Shannon diversity ($F_{2,33} = 1.18$, $p = 0.32$), and no pairwise contrasts were statistically significant (all Tukey-adjusted $p \geq 0.32$) (Fig. 3b, for model details see Supplement Table 3).

Similarly, Simpson diversity ($q = 2$) did not differ significantly among degree of decomposition ($F_{2,33} = 0.89$, $p = 0.42$). Estimated marginal means ranged from 10.10 ± 1.84 SE at degree of decomposition 1, to 13.50 ± 1.84 SE at degree 2, and 11.10 ± 1.84 SE at degree 3. Pairwise comparisons among degrees of decomposition were not significant (all Tukey-adjusted $p \geq 0.41$) (Fig. 3c, for model details see Supplement Table 4).

The NMDS ordinations revealed broadly similar patterns in community composition across all Hill number orders (see supplement Figure S1). Stress values were comparable among analyses ($q_0 = 0.193$, $q_1 = 0.193$, $q_2 = 0.191$). At $q = 0$ the degree of decomposition (Dod1, Dod2, and Dod3) showed noticeable but overlapping separation, with relatively high dispersion within groups. At $q = 1$ overlap among groups remained evident. At $q = 2$ the ordination showed tighter clustering patterns and clearer shifts in group positions. Overall, the ordination patterns indicate subtle but evident differences in community composition among treatments, with the influence of degree of decomposition becoming more apparent as greater weight was given to dominant species ($q = 2$).

Multiple regression on distance matrices (MRM) was used to assess the relative influence of distance of degree of decomposition and geographic distance on community dissimilarity across diversity orders ($q = 0, 1$, and 2). For $q = 0$, which emphasizes rare taxa, community dissimilarity was significantly associated with distances of degree of decomposition. Greater similarity among samples was observed with increasing distance of degree of decomposition ($\beta = -0.013$, $p = 0.005$) (Fig. 4a.). In contrast, geographic distance [m] did not significantly explain variation in community composition ($\beta =$

$2.717287e^{-07}$, $p = 0.811$) (Fig. 4b.). The overall model was significant ($R^2 = 0.014$, $p = 0.027$), although the amount of explained variation was low.

For $q = 1$, which weights taxa according to their relative abundance, differences in degree of decomposition remained weakly associated with community dissimilarity ($\beta = -0.012$, $p = 0.049$) (Fig. 5a). However, geographic distance [m] again showed no significant relationship with community composition ($\beta = 7.119399e^{-07}$, $p = 0.636$) (Fig. 5b). The overall model was not statistically significant ($R^2 = 0.007$, $p = 0.160$).

For $q = 2$, which emphasizes dominant taxa, neither the degree of decomposition ($\beta = -0.007$, $p = 0.252$) (Fig. 6a) nor geographical distance ($\beta = 0.159$, $p = 0.234$) (Fig. 6b) significantly explained patterns of community dissimilarity. Consistent with these results, the overall model was not significant ($R^2 = 0.005$, $p = 0.243$).

The indicator species analysis identified several fungal taxa that were significantly associated with specific degree of decomposition ($p \leq 0.05$). Although taxonomic resolution of ITS data at the species level is limited and might be uncertain, we keep species-level annotations for consistency while interpreting these results cautiously. Overall, four taxa were associated with degree of decomposition stage 1, eight with stage 2, and 20 with stage 3, suggesting a progressive increase in decomposition stage-specific indicator taxa along the decomposition gradient (Table 1).

Degree of decomposition stage 1 was characterized by strong associations with three saprotrophic species: *Penicillium brevicompactum*, *Allomusicillium domschii*, *Oxyporus corticola*, and *Pleurotus ostreatus*, all of which exhibited high indicator values and significant group specificity. Among these, *Penicillium brevicompactum* and *Allomusicillium domschii* showed the strongest associations with this decomposition category.

Degree of decomposition stage 2 was primarily characterized by saprotrophic fungal taxa, including *Coprinopsis alnivora*, *Cephalotrichum stemonitis*, *Petriella guttulata*, *Fusarium avenaceum*, *Phialemonium inflatum*, and *Aspergillus pseudogracilis*. *Coprinopsis alnivora* showed the strongest indicator value for this degree of decomposition, suggesting a particularly strong association with intermediate decomposition conditions.

The highest number of indicator taxa was observed at degree of decomposition stage 3. This category was characterized by diverse fungal taxa including ectomycorrhizal species such as *Rhizopogon vinicolor* and *Rhizopogon rudus*, as well as saprotrophic fungi such as *Tausonia pullulans*, *Goffeauzyma gastrica*, and *Solicoccozyma terricola*. Several taxa, including *Fusicolla septimanifiniscientiae*, *Bolbitius reticulatus*, and *Lycoperdon* species, exhibited particularly strong associations with this degree of decomposition stage.

Table 1

Indicator species analysis of fungal taxa across the degrees of decomposition (Dod). Indicator values represent the association strength of each species with the respective decomposition category. Values are partitioned into three components corresponding to the relative association with each group. Species with highest indicator value per Dod are printed in boldface.

Species	Dod	Indicator value 1	Indicator value 2	Indicator value 3	p-value	Lifestyle
<i>Allomusicillium domschii</i>	1	0.692	0.095	0.065	0.032	saprotrophic
<i>Oxyporus corticola</i>	1	0.577	9.63E + 09	0.002	0.005	saprotrophic
Penicillium brevicompactum	1	0.737	0.171	0.093	0.034	saprotrophic
<i>Pleurotus ostreatus</i>	1	0.333	0.000	0.000	0.030	saprotrophic
<i>Aspergillus pseudogracilis</i>	2	0.041	0.427	0.004	0.029	saprotrophic
<i>Cephalotrichum stemonitis</i>	2	0.002	0.826	0.056	0.027	saprotrophic
Coprinopsis alnivora	2	0.000	0.833	1.03E + 09	0.001	saprotrophic
<i>Coprinopsis mitrispora</i>	2	0.000	0.457	0.054	0.034	saprotrophic
<i>Fusarium avenaceum</i>	2	0.002	0.407	0.000	0.038	saprotrophic, parasitic
<i>Petriella guttulata</i>	2	0.068	0.648	0.057	0.012	saprotrophic
<i>Phialemonium inflatum</i>	2	0.013	0.498	0.142	0.018	endophytic
<i>Volvariella bombycina</i>	2	0.009	0.448	1.16E + 09	0.012	endophytic
<i>Apodus deciduus</i>	3	0.000	0.002	0.329	0.041	saprotrophic
<i>Atractospora decumbens</i>	3	0.003	0.000	0.496	0.013	saprotrophic
<i>Beauveria caledonica</i>	3	0.000	0.000	0.332	0.049	pathogenic
<i>Bolbitius reticulatus</i>	3	0.002	0.000	0.745	0.027	saprotrophic
<i>Chrysosporium carmichaelii</i>	3	0.005	0.011	0.421	0.044	saprotrophic
<i>Clitocybe nebularis</i>	3	0.043	0.100	0.464	0.049	saprotrophic, ectomycorrhiza
<i>Coprinellus xanthothrix</i>	3	0.000	0.000	0.579	0.004	saprotrophic

Species	Dod	Indicator value 1	Indicator value 2	Indicator value 3	p-value	Lifestyle
<i>Cyphellophora endoradicis</i>	3	0.001	0.046	0.499	0.020	endophytic
<i>Exophiala moniliae</i>	3	0.000	0.003	0.403	0.018	saprotrophic
Fusicolla septimanifiniscientiae	3	0.006	0.080	0.797	0.009	saprotrophic
<i>Goffeauzyma gastrica</i>	3	0.000	0.005	0.324	0.025	saprotrophic
<i>Lycoperdon perlatum</i>	3	0.017	0.031	0.673	0.028	saprotrophic
<i>Lycoperdon pyriforme</i>	3	0.003	0.007	0.646	0.006	saprotrophic
<i>Mycosylva setosa</i>	3	0.002	0.000	0.490	0.018	saprotrophic
<i>Rhizopogon rudus</i>	3	0.000	0.000	0.333	0.030	ectomycorrhiza
<i>Rhizopogon vinicolor</i>	3	3.13E + 09	0.001	0.664	0.006	ectomycorrhiza
<i>Rhodocollybia butyracea</i>	3	0.003	0.023	0.342	0.020	ectomycorrhiza
<i>Saitozyma podzolica</i>	3	0.000	0.000	0.333	0.027	saprotrophic
<i>Solicoccozyma terricola</i>	3	0.000	0.002	0.324	0.029	saprotrophic
<i>Sporidesmiella pini</i>	3	0.008	0.000	0.318	0.035	saprotrophic
<i>Tausonia pullulans</i>	3	0.037	0.144	0.533	0.041	saprotrophic

Discussion

Tree hollows filled with wood mold represent long lasting microhabitats shaped by gradual wood decay and the accumulation of organic material (Stokland et al. 2012). By examining fungal amplicon sequence variants (ASVs) across different degrees of wood mold decomposition, this study provides insight into how fungal communities develop during this process. Overall, our findings suggest that fungal community structure changes progressively with decomposition stage, primarily through shifts in species composition and the appearance of decomposition-stage-specific taxa, while overall diversity patterns remain comparatively stable.

Fungal diversity along the decomposition gradient

Although fungal richness increased from early to advanced stages of decomposition, the differences were not statistically significant. Nevertheless, the consistent upward trend suggests that more advanced wood mold stages offer a wider range of ecological niches that can support additional fungal taxa (Lepinay et al. 2021; Viotti et al. 2021). As wood decomposes, its physical structure becomes more

heterogeneous, and chemical composition shifts due to microbial activity and nutrient redistribution (Pioli et al. 2023; Shi et al. 2024; Hoppe et al. 2016). These changes likely create a broader spectrum of microhabitats, allowing coexistence of fungi with differing ecological strategies. In addition, this pattern may also reflect time-dependent colonization processes. Species accumulation in dead wood is often driven by successional dynamics, where fungal taxa require time to arrive and establish following dispersal. This effect may be particularly pronounced in substrates that are ephemeral or relatively rare, especially if fungal dispersal is limited or if species exhibit strong substrate specialization (Moor et al. 2021; Jönsson et al. 2008; Boddy und Heilmann-Clausen 2008).

Abundance-weighted diversity measures, including Shannon and Simpson diversity, showed more nuanced response to decomposition stage. While richness and Shannon diversity generally increased along the decomposition gradient, the clearest compositional shift occurred in the most advanced decay stage (DoD3), where the relative dominance of previously abundant taxa declined. This pattern may indicate increasing resource limitation and stronger competitive interactions in highly decomposed substrates. Under such conditions, some dominant early colonizers may lose part of their competitive advantage, consistent with dominance–tolerance trade-off hypotheses in which persistent taxa are stress tolerant but competitively weaker under stronger biotic interactions (Maynard et al. 2019). Rather than continuous replacement throughout succession, fungal community assembly therefore appears relatively similar across earlier decomposition stages, followed by a stronger restructuring in the latest stage of decay.

Successional shifts in fungal community composition

Indicator species analysis suggested taxonomic shifts along the decomposition gradient, consistent with a successional trajectory of fungal communities in wood mold, although these patterns should be interpreted with some caution. Multiple regression on matrices (MRM) did not reveal strong or clear-cut effects of decomposition stage on overall community dissimilarity, indicating that communities may not form sharply distinct clusters. The MRM analysis indicates that fungal communities from hollows with more distinct decomposition stages tended to be slightly more similar in community composition than communities from hollows at comparable stages of decomposition. One possible explanation is that a subset of fungal taxa occurs across a broad range of decomposition stages, thereby reducing species turnover between strongly contrasting hollows. Alternatively, decomposition stage alone may not be the primary driver of fungal community assembly, with other unmeasured factors such as microclimatic conditions, tree characteristics, substrate properties, or biotic interactions contributing more strongly to variation in community composition.

This interpretation was also consistent with the NMDS ordinations, which showed gradual shifts and overlapping community composition among decomposition stages rather than discrete clustering. Instead, the results point to more subtle, stage-associated changes that become apparent when examining indicator taxa. Early decomposition stages were associated with taxa such as *Penicillium brevicompactum*, *Allomusicillium domschii*, *Oxyporus corticola*, and *Pleurotus ostreatus*. *Pleurotus*

ostreatus for example is known as a rapid colonizer capable of exploiting relatively fresh woody substrates and high competitiveness (Lang et al. 1998). Their presence likely reflects the initial breakdown of cellulose, hemicellulose, and lignin and the utilization of easily accessible carbon compounds (Stokland et al. 2012).

At intermediate decomposition stages, the fungal community shifted toward taxa typically associated with ongoing saprotrophic activity. Species such as *Coprinopsis alnivora*, *Cephalotrichum stemonitis*, and *Fusarium avenaceum*. The strong association of *C. alnivora* with intermediate decomposition suggests that it may play an important role in transitional stages of wood mold formation.

Advanced decomposition stages supported the highest number of indicator taxa and showed greater taxonomic diversity overall. These communities included both saprotrophic and ectomycorrhizal fungi, such as *Rhizopogon vinicolor* and *Rhizopogon rudus*. The increasing occurrence of ectomycorrhizal fungi (ECM) at later decomposition stages may reflect growing connectivity between hollow substrates and surrounding soil and root systems, at least for tree hollows open to the ground. As wood mold becomes more humified and nutrient-rich, it likely resembles soil environments more closely (Mäkipää et al. 2017), allowing colonization by fungi that are not strictly wood specialists. In addition, late-stage substrates may accumulate nitrogen derived from microbial turnover, including the decomposition of fungal biomass. ECM fungi with strong proteolytic capacities may particularly benefit from such conditions, whereas contributions from bacterial nitrogen fixation may also play a role (Philpott et al. 2014; Tunlid et al. 2022; Chalot & Brun 1998). However, the extent to which hollow–soil connectivity occurs likely varies among trees, and in some cases may be limited, suggesting that both substrate transformation and local environmental conditions jointly influence ECM establishment.

Taken together, these findings emphasize a gradual successional progression—from pioneer saprotrophs to complex, soil-connected fungal communities—highlighting the dynamic transformation of tree hollow substrates and their progressive incorporation into increasingly soil-like environments resembling patterns described from deadwood decomposition (Mäkipää et al. 2017). As decomposition progresses, the accumulation of humified organic matter and the increasing occurrence of taxa typically associated with soil environments suggest a gradual functional convergence between late-stage wood mold and forest soil habitats.

The role of rare taxa in community turnover

Analyses of community dissimilarity require careful interpretation, as the patterns are not straightforward. NMDS ordinations based on Hill-number dissimilarities further supported this interpretation. Across all diversity orders ($q = 0, 1, 2$), fungal communities showed substantial overlap among decomposition stages rather than forming clearly separated clusters. Separation among stages was most apparent at $q = 0$, where rare taxa contribute more strongly to community dissimilarity, whereas ordinations based on abundance-weighted metrics ($q = 1$ and $q = 2$) showed increasingly overlapping and compact groupings. This pattern suggests that compositional turnover along the

decomposition gradient is driven primarily by rare and moderately abundant taxa, while dominant taxa remain taken relatively stable across stages. Decomposition stage did not result in clearly distinct fungal communities or strong turnover between stages, particularly when using abundance-weighted metrics. Instead, differences along the decomposition gradient appear to be driven mainly by rare and moderately abundant taxa, and even these effects are weak. The negative or weak correlations observed for rare taxa suggest that turnover occurs predominantly within decomposition stages rather than between them, likely reflecting fine-scale environmental heterogeneity such as variation in moisture, wood mold structure, or localized nutrient availability (Kubartová et al. 2012).

At the same time, the indicator species analysis identified consistent stage-associated taxa, indicating that decomposition stages are ecologically distinguishable. Rather than forming discrete clusters, fungal communities seem to change gradually along the gradient, with certain taxa showing preferences for specific stages. High variability within stages combined with subtle but consistent differences between them suggests that later decay stages support a wider range of ecological strategies, particularly among less abundant taxa. In addition, tree hollows typically increase in size with progressing decomposition, thereby expanding habitat volume and microhabitat availability. Further older hollows have also been exposed to colonization processes for longer periods of time (Stokland et al. 2012). Both factors may further contribute to the higher number of taxa detected in advanced decay stages. In contrast, dominant taxa likely represent generalist decomposers capable of persisting across a broad range of substrate conditions, contributing to the stability of abundance-weighted diversity patterns observed in this study.

Influence of spatial factors

Geographical distance did not significantly influence fungal community dissimilarity, suggesting that local environmental conditions and substrate characteristics play a more important role in structuring fungal assemblages than dispersal limitation at the spatial scale considered here. Fungal spores are typically highly dispersible and can disperse over several kilometers (Golan & Pringle 2017; Komonen & Müller 2018). Consequently, community assembly in wood mold appears to be driven mainly by habitat filtering linked to decomposition stage rather than by spatial separation among sampling sites.

Ecological significance of fungal succession in tree hollows

Fungal communities are central to the decomposition of woody material (Boddy 2001) and the formation of wood mold, which in turn serves as a critical resource for numerous saproxylic organisms (Micó 2018). Fungal communities inhabiting tree hollows tend to reach their highest diversity during the late stages of wood decay, when advanced decomposition has created a heterogeneous substrate rich in microhabitats and chemically diverse resources. Similar patterns have also been reported from decomposing spruce and beech logs, where fungal richness increased and peaked in the most decayed wood stages (Fukasawa & Matsukura 2021; Rajala et al. 2012), potentially because heavily degraded wood becomes increasingly colonized by soil-associated fungi (Buée et al. 2009). However,

interpretations of increasing diversity during late decomposition stages should be treated with caution in studies based on DNA metabarcoding, as sequence-based approaches may also detect inactive or dead fungal material that accumulates over time. Consequently, it remains unclear to what extent taxa detected by DNA metabarcoding represent metabolically active communities.

In contrast, beetle assemblages associated with tree hollows typically show peak diversity at intermediate decay stages, where partially decomposed wood still provides suitable structural conditions and resources for a wide range of saproxylic species (Schauer et al. 2018; Schauer et al. 2025). These contrasting diversity patterns across decay gradients highlight that different taxonomic groups respond to distinct phases of the decomposition process. Consequently, maintaining a continuous range of decay stages within forest stands is crucial for sustaining high multitaxon diversity in tree-hollow ecosystems, as it ensures the simultaneous availability of habitat conditions required by organisms specialized on different stages of wood decomposition. In practice, this may require the long-term retention of habitat trees and deadwood of different ages and decay states, as well as management strategies that allow natural aging and decomposition processes to continue within forest stands. Furthermore, changes in fungal community composition may influence the physical and nutritional properties of wood mold, potentially affecting habitat quality for hollow-dependent invertebrates, birds, and mammals and vice versa (Jacobsen et al. 2018). The successional dynamics documented here therefore contribute not only to understanding fungal ecology but also to broader forest ecosystem functioning.

Conclusions

Our findings point to gradual, rather than discrete, changes in fungal communities within tree hollow wood mold along the decomposition gradient. While richness increases and indicator species analyses reveal stage-associated taxa consistent with a successional trajectory, these shifts are subtle and do not translate into community clusters clearly separated by decomposition stage. Instead, variation in community composition appears to be driven largely by rare and moderately abundant taxa, with much of the turnover occurring within decomposition stages, likely reflecting fine-scale environmental heterogeneity. In contrast, dominant fungal species remain comparatively stable across stages or slightly drop in late stages of decay, contributing to the weak patterns observed in abundance-weighted metrics. Degree of decomposition shows a modest but stronger influence relative to geographical distance, but neither factor alone strongly structures community composition.

Together, these results highlight that tree hollows across all stages of decay function as dynamic and heterogeneous habitats, supporting diverse fungal assemblages. Rather than representing distinct community types, different stages contribute to overall diversity through subtle compositional shifts and the accumulation of specialized taxa, underscoring their importance for fungal biodiversity and forest ecosystem processes.

CRedit authorship contribution statement

Bastian Schauer: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Franz-Sebastian Krah:** Writing – review & editing, Formal analysis, Investigation. **Alfons Weig:** Writing – review & editing, Library preparation, NGS, reads processing, taxonomic classification. **Heike Feldhaar:** Writing – review & editing, Investigation. **Claus Baessler** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declarations

CRedit authorship contribution statement

Bastian Schauer: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Franz-Sebastian Krah:** Writing – review & editing, Formal analysis, Investigation. **Alfons Weig:** Writing – review & editing, Library preparation, NGS, reads processing, taxonomic classification. **Heike Feldhaar:** Writing – review & editing, Investigation. **Claus Baessler** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

All authors declare no conflict of competing interests.

Data Availability

All data used are included as electronical supplementary material.

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60. Supplement

Figures

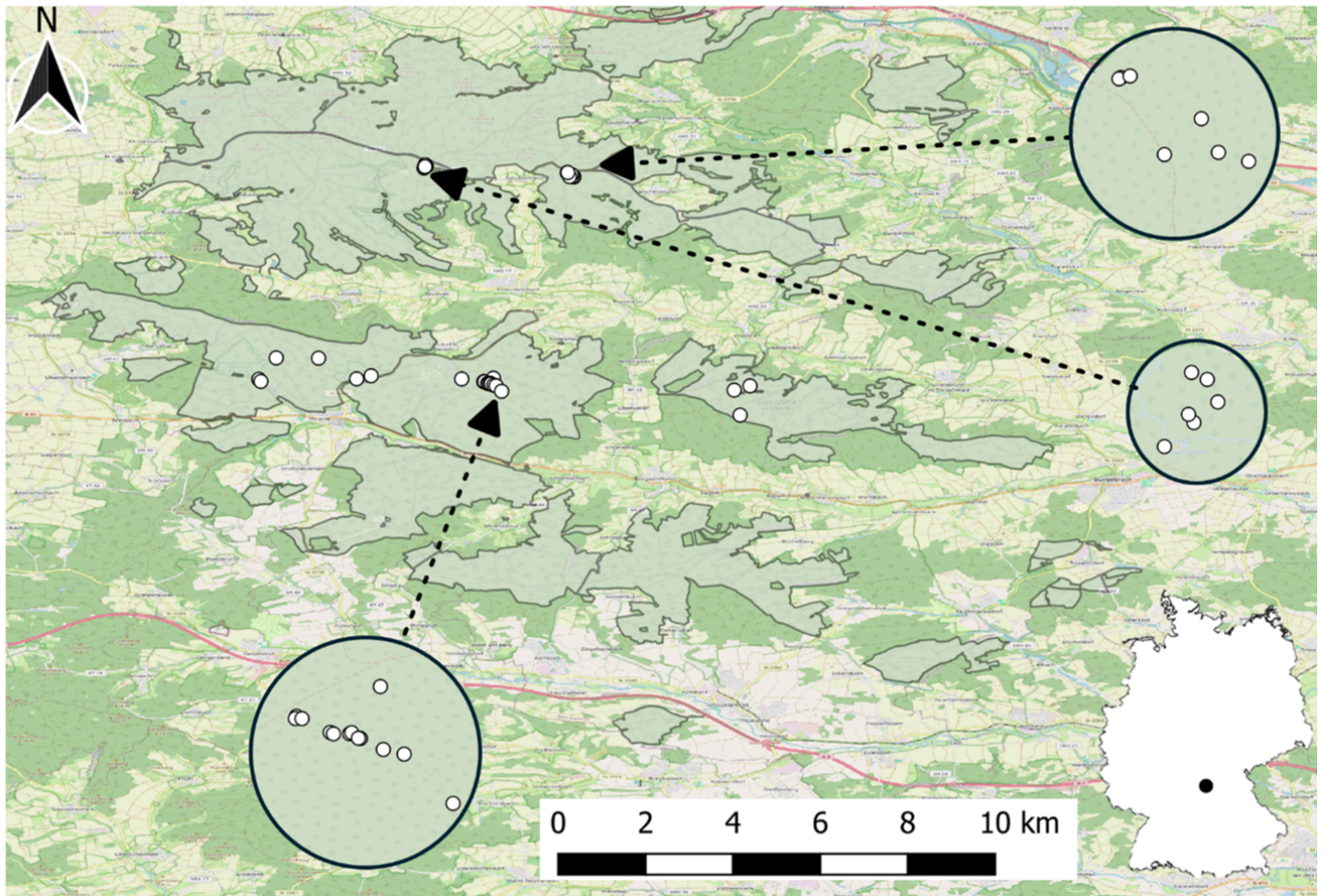


Figure 1

Location of the 36 sampled tree hollows (represented by circles) in the forestry department of Ebrach (Bavaria, Germany). Light green areas with gray boundaries indicate management areas of the forest district of Ebrach.

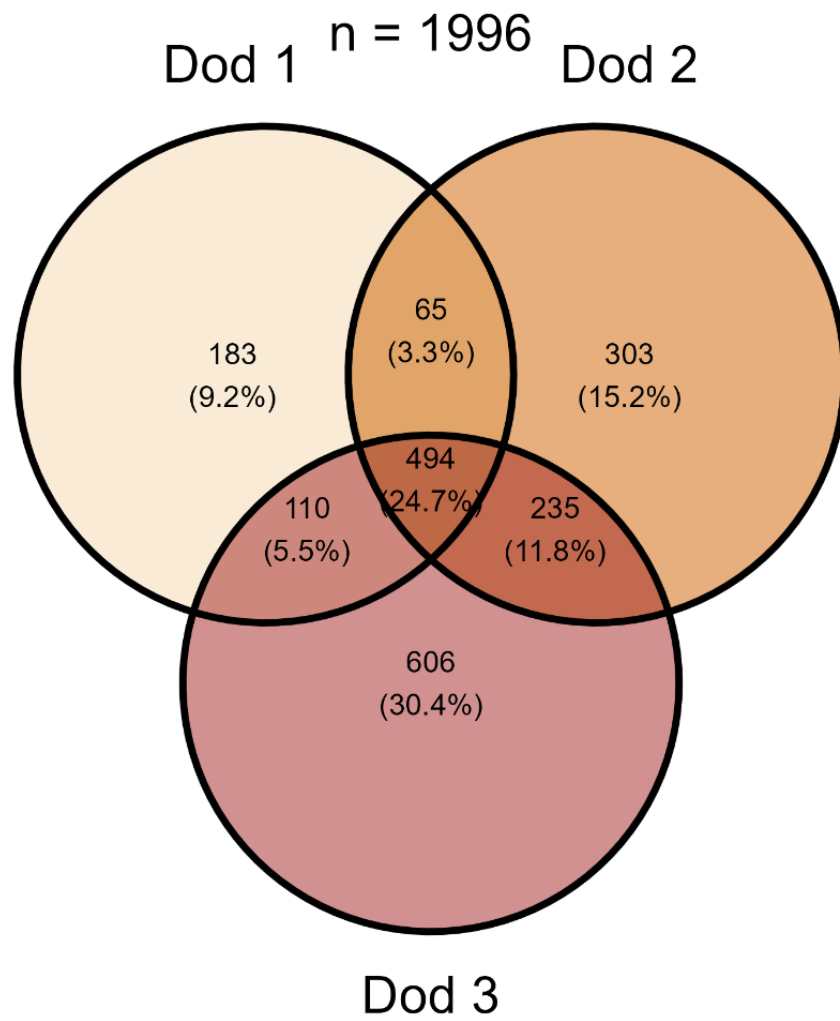


Figure 2

Number of fungal ASVs shared and unique for the degree of decomposition (Dod) of wood mold.

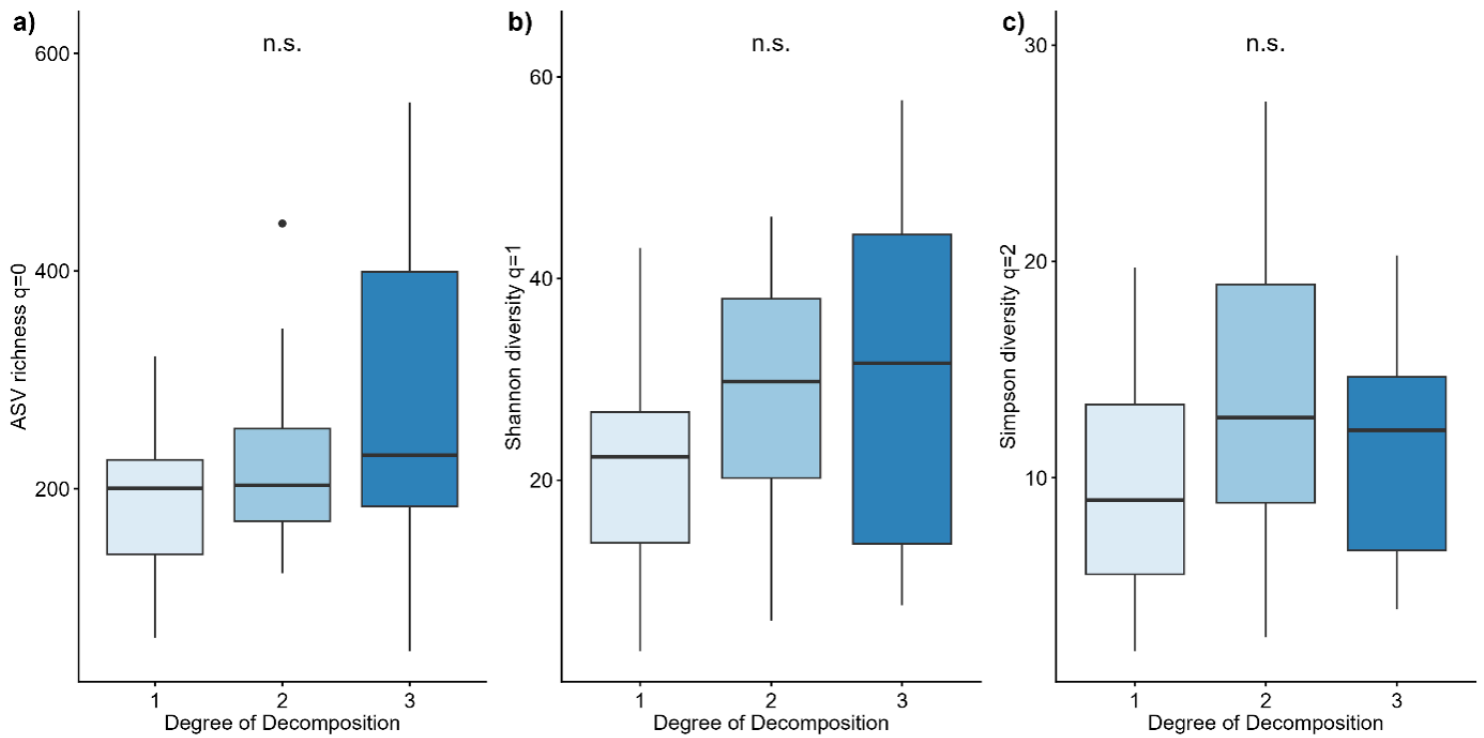


Figure 3

Amplicon sequence variant (ASV) richness ($q = 0$), (b) Shannon diversity ($q = 1$), and (c) Simpson diversity ($q = 2$) compared across three degrees of decomposition (1–3). No significant differences among decomposition degrees were detected for any diversity metric (n.s., not significant).

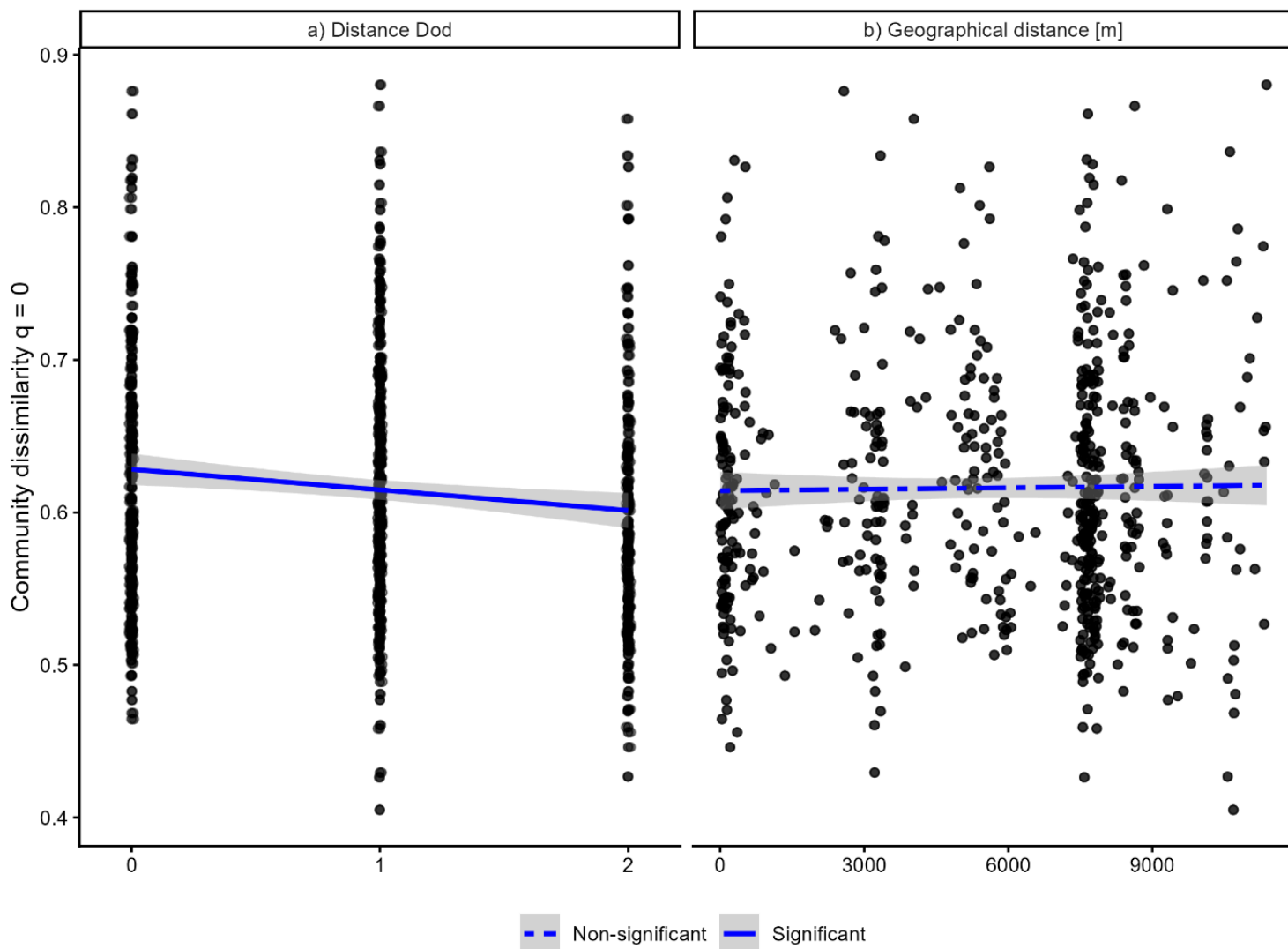


Figure 4

Pairwise dissimilarity for Hill number $q = 0$ plotted against a) distance of degree of decomposition (Dod) and b) geographical distance. Each point represents a pairwise comparison. The blue line shows the fitted linear regression with shaded 95% confidence interval.

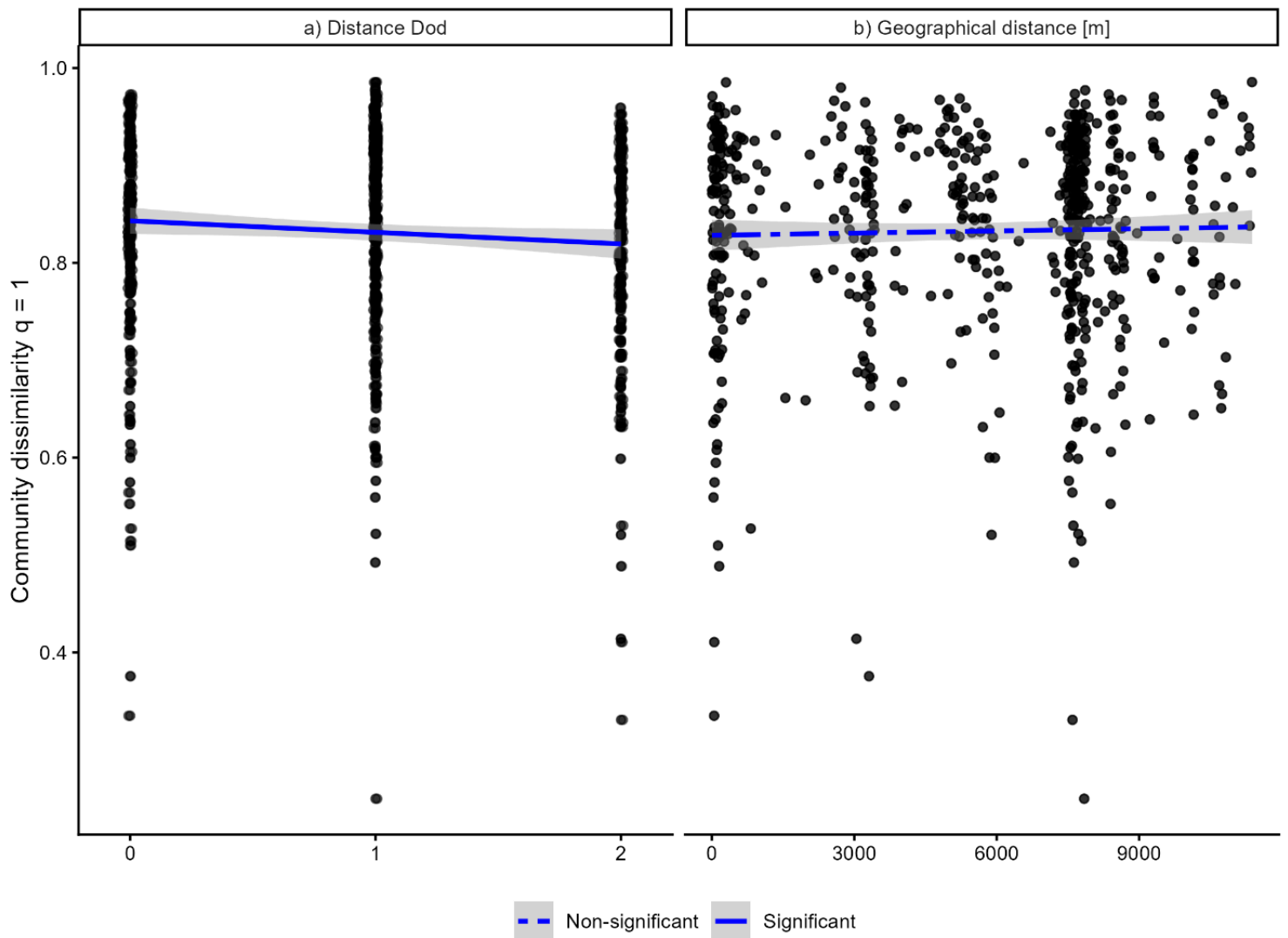


Figure 5

Pairwise dissimilarity for Hill number $q = 1$ plotted against a) distance of degree of decomposition (Dod) and b) geographical distance. Each point represents a pairwise comparison. The blue line shows the fitted linear regression with shaded 95% confidence interval.

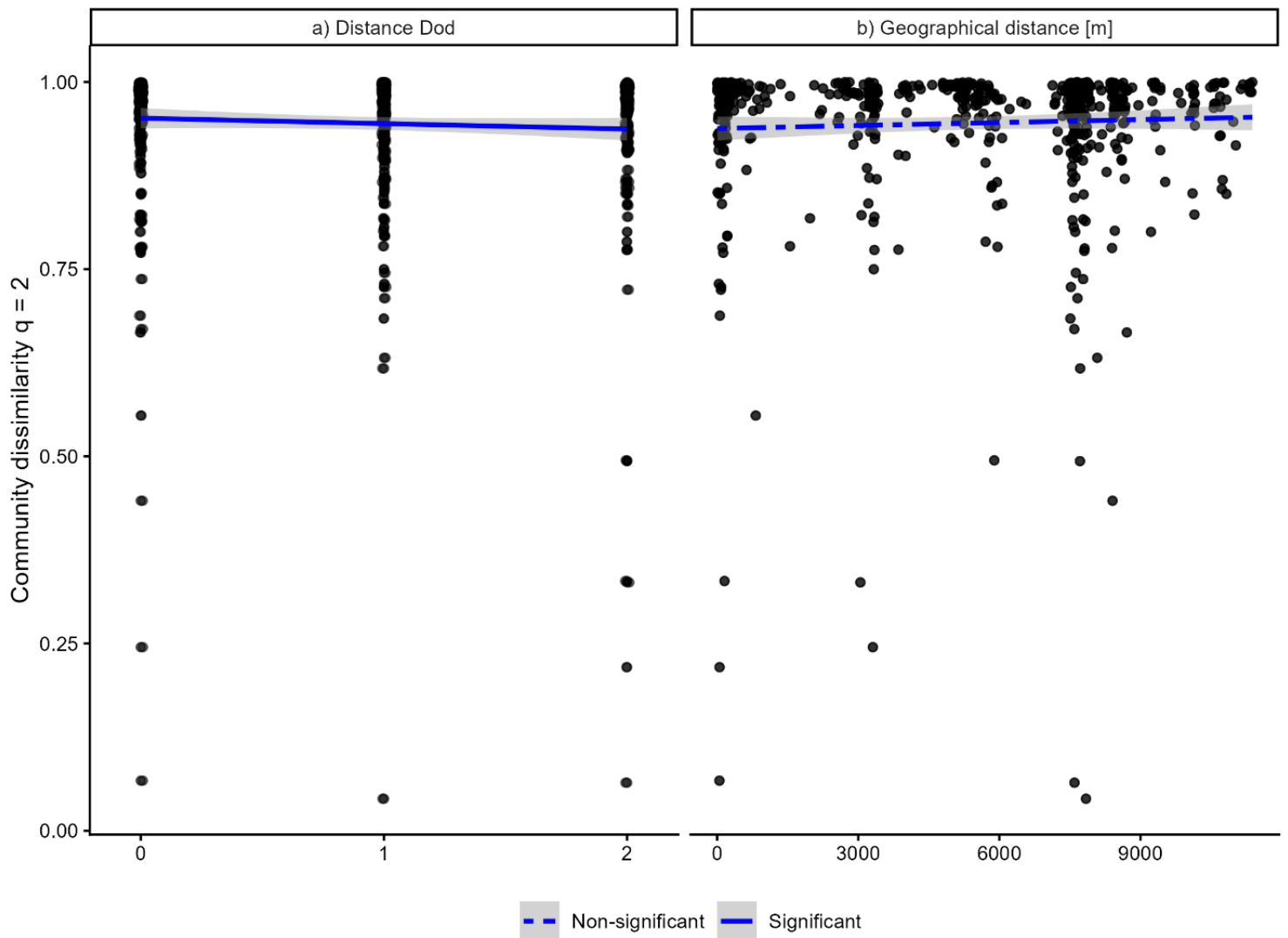


Figure 6

Pairwise dissimilarity for Hill number $q = 2$ plotted against a) distance of degree of decomposition (Dod) and b) geographical distance. Each point represents a pairwise comparison. The blue line shows the fitted linear regression with shaded 95% confidence interval.

Supplementary Files

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