RESEARCH ARTICLE

Functional convergence in the decomposition of fungal necromass in soil and wood

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One sentence summary: This study demonstrates that the decomposition dynamics of fungal mycelium in wood and soil are similar and that the initial chemical composition of mycelium is important in determining the structure of the microbial decomposer community.

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ABSTRACT

Understanding the post-senescent fate of fungal mycelium is critical to accurately quantifying forest carbon and nutrient cycling, but how this organic matter source decomposes in wood remains poorly studied. In this study, we compared the decomposition of dead fungal biomass (a.k.a. necromass) of two species, *Mortierella elongata* and *Meliniomyces bicolor*, in paired wood and soil plots in a boreal forest in northern Minnesota, USA. Mass loss was quantified at four time points over an 8-week incubation and the richness and composition of the fungal communities colonizing fungal necromass were characterized using high-throughput sequencing. We found that the structure of fungal decomposer communities in wood and soil differed, but, in both habitats, there was relatively rapid decay (~30% remaining after 56 days). Mass loss was significantly faster in soil and for high-quality (i.e. high nitrogen and low melanin) fungal necromass. In both habitats, there was a clear trajectory of early colonization by opportunistic fungal taxa followed by colonization of fungi with greater enzymatic capacities to degrade more recalcitrant compounds, including white-rot and ectomycorrhizal fungi. Collectively, our results indicate that patterns emerging regarding substrate quality effects on fungal necromass decomposition in soil and leaf litter can be largely extended to fungal necromass decomposition in wood.

INTRODUCTION

Forests cover approximately one-third of Earth's land surface and contain >800 petagrams of carbon (C), representing a significant component of the global C cycle (Pan *et al.* 2015). The vast majority of forest C is contained in soil, litter and wood, where the decomposition of organic matter (OM) is a critical determinant of both forest C stocks and nutrient availability (Schlesinger and Berhardt 2013). Historically, aboveground plant inputs were considered the primary determinants of soil OM accumulation in forest ecosystems (Swift, Heal and Anderson 1979), but recent work indicates that fungi represent a considerably larger proportion of forest soil OM pools than previously recognized (Hogberg and Hogberg 2002; Godbold *et al.* 2006; Soudzilovskaia *et al.* 2015; Zhang *et al.* 2018, 2019). For example, using bomb ^{14}C analyses, it was shown that between 50% and 70% of the C present in the top 20 cm of boreal forest soils originated from mycorrhizal fungi, as either extramatrical mycelium or colonized root tips (Clemmensen *et al.* 2013). Because of their relatively high

nutrient content relative to plant litters (Brabcová, Šturosová and Baldrian 2018), decomposing fungal mycelium also represents an important nutrient source in forest ecosystems. For example, the stimulation of nitrogen (N) cycling in soil has been estimated to be four times greater from mycorrhizal mycelial inputs than that from roots (Zhang *et al.* 2019). Taken together, these results indicate that understanding the post-senescent fate of fungal mycelium (hereafter referred to as fungal necromass) is critical to accurately quantifying forest C and nutrient cycling.

In addition to its role in forest C and nutrient dynamics, fungal necromass may also represent an important yet understudied resource for decomposer organisms in wood. The high C:N ratio of wood often requires the import and/or enrichment of nitrogen and other macronutrients by wood-rot fungi to facilitate decay (Philpott *et al.* 2014; Rinne *et al.* 2017). The much lower C:N ratio of fungal necromass likely makes it an attractive target for resource utilization, particularly in the later stages of wood decay when mycelium may be a relatively important source of N in comparison with wood (Bebber *et al.* 2011). Experimental support for fungal necromass as a nutrient resource in wood comes from a recent study of the ectomycorrhizal (ECM) fungus, *Paxillus involutus*, which has been shown to be an efficient degrader of OM via a mechanism similar to brown rot (Rineau *et al.* 2015). Using ^{13}C- and ^{15}N-labeled necromass of the wood-rot species *Postia placenta*, Akroume *et al.* (2019) demonstrated that *P. involutus* was able to mobilize both C and N from fungal necromass. The authors further demonstrated that a significant portion of the fungal necromass-derived N was then passed from *P. involutus* to its tree hosts. Since ECM fungi are commonly present in wood during the later stages of decay (Tedersoo *et al.* 2003; Rajala *et al.* 2015), fungal necromass likely represents a resource 'hotspot' (*sensu* Brabcová *et al.* 2016) in wood for both saprotrophic organisms accessing relatively labile C and other decomposer organisms targeting N.

There is also growing evidence that, like plant-derived OM, the chemical quality of fungal necromass has a significant effect on its rate of decomposition (Fernandez *et al.* 2016; Brabcová, Šturosová and Baldrian 2018). Field experiments using different fungal strains or species with varying N concentrations have shown that higher tissue N is strongly correlated with faster fungal necromass decomposition (Koide and Malcolm 2009; Fernandez and Koide 2012; Brabcová, Šturosová and Baldrian 2018; Certano *et al.* 2018; Fernandez *et al.* 2019). Conversely, increased melanin content, a structurally complex polymer present in the cell walls of a diverse range of fungi (Butler and Day 1998; Siletti, Zeiner and Bhatnagar 2017), has been consistently shown to retard fungal necromass decay, both within and among species (Fernandez and Koide 2014; Fernandez and Kennedy 2018). Collectively, these results indicate that fungal necromass should be considered as a resource gradient for decomposer organisms, ranging from low (low N and/or high melanin) to high (high N and/or low melanin) quality. The extent to which different groups of microbial decomposers show preferences for specific fungal necromass qualities, however, is still poorly understood. In temperate *Pinus*- and *Quercus*-dominated forest soils, Fernandez and Kennedy (2018) found that varying melanin content in fungal necromass differentially affected fungal guild abundances, and also that those patterns fluctuated over time. For example, pathotrophic fungi were more common on high-melanin fungal necromass, but showed declines in relative abundance over a three-month incubation. Alternatively, on low-melanin fungal necromass, ECM fungi increased in relative abundance over time while molds and yeasts decreased. While

these patterns hint at varying successional trajectories depending on fungal necromass quality, more studies working in different habitats are needed to better assess their generality.

The goal of this study was to examine the decomposition of fungal necromass in two contrasting habitats: soil and wood. To our knowledge, all the field-based experimental work on fungal necromass decomposition to date has focused on decay dynamics in either soil or leaf litter. Since wood represents a very different abiotic (e.g. pH, C:N, moisture content) environment, the decomposition of fungal necromass in this habitat may have different rates of mass loss than in other habitats. Similarly, because the decomposer communities present in wood and soil differ considerably (Makipaa *et al.* 2017; but see Purahong *et al.* 2019), the capacities and preferences for decomposing fungal necromass may be fundamentally different in these two habitats. We hypothesized that fungal necromass would decompose more rapidly in soil than wood due to the greater diversity of the microbial decomposer communities present. Due to the well-documented capacities of wood-rot fungi to decompose both lignin (e.g. Schilling *et al.* 2015) and melanin (e.g. Butler and Day 1998), however, we also hypothesized that differences in mass loss between low- and high-quality fungal necromass would be lower in wood than in soil. To test these hypotheses, we deployed litterbags containing fungal necromass of varying quality in paired soil and wood locations in a boreal forest in northern Minnesota, USA. Along with quantifying mass loss patterns at four times over an 8-week incubation, we also characterized the richness and composition of the fungal communities colonizing the fungal necromass using high-throughput sequencing.

METHODS

Necromass generation

Two species, *Meliniomyces bicolor* and *Mortierella elongata*, were chosen for generation of low- and high-quality fungal necromass, respectively (Table 1). *Meliniomyces bicolor* is an ericoid and ECM ascomycete fungus commonly encountered on understory plant and tree roots in boreal forests (Grelet *et al.* 2009).

Table 1. Analyses of initial necromass chemistry of *Mo. elongata* and *Me. bicolor*. Three independent replicates were analyzed where standard errors are reported, otherwise values are based on a single replicate. See the 'Methods' section for more details on each anaylsis.

	Mo. elongata (high quality)		*Me. bicolor* (low quality)	
	Mean	SE	Mean	SE
Elemental analysis (%)				
C	49.4	2.2	51.4	0.3
N	7.0	1.5	3.8	0.03
C/N	7.5	1.6	13.7	0.2
pyGCMS analysis (%)				
Aromatic	6.2	0.3	17.6	
Lipid	44.7	3.1	57.8	
Nitrogen containing	10.3	2.5	1.1	
Sterol	0.0	0.0	0.3	
Sugar	29.3	1.8	22.0	
Unspecified	9.5	0.3	1.3	
Melanin analysis (%)				
Melanin	4.3		17.5	

Mortierella elongata is a saprotrophic mucoromycete fungus frequently found in both forest and agricultural soils (Li *et al.* 2018). To generate fungal necromass, cultures of each species were first grown in Petri plates containing half strength potato dextrose agar (Difco, BD Products, Franklin Lakes, NJ) and maintained in the dark at room temperature (~25°C). Plugs from the actively growing edge of cultures were individually transferred to half strength potato dextrose broth. Due to differences in growth rates, *Mo. elongata* cultures were shaken at 50 rpm for 3 days at room temperature, while *Me. bicolor* cultures were shaken at 100 rpm for 30 days at room temperature. The faster shaker speed for *Me. bicolor* was applied to enhance oxygenation in the liquid medium, which we previously found stimulates melanization (Fernandez and Kennedy 2018). Once cultures had grown sufficiently, biomass was harvested by pouring cultures over sterilized mesh filters and rinsing with deionized water. Biomass was then transferred to a sterilized non-stick silicone mat (Chicochef, Morrisville, North Carolina, USA), placed in a drying oven at 75°C for up to 10 h and then stored in a zipseal plastic bags at room temperature. Litterbags ~6 × ~6 cm in size were constructed using 53-micron nylon mesh (Elko, Minneapolis, MN). Necromass was re-dried for 1 h and ~75 mg of tissue was placed in each litterbag.

Chemical analyses

To characterize the initial chemistry of both species, fungal necromass samples were analyzed for elemental composition, different compound classes and melanin content. Elemental analysis was run on a Costech elemental analyzer (Costech Analytical Technologies, Valencia, CA, USA). Samples were weighed in tin capsules and a set of standards was run every 10 samples. Standards used were acetanilide (71.09% C, 10.36% N), caffeine (49.48% C, 28.85% N), natural-abundance sorghum (41.58% C) and low-organic content soil (1.61% C, 0.13% N). Standard error was <0.6% for %C and 0.4% for %N. Samples were also analyzed by thermochemolysis-gas chromatography mass spectrometry (pyGCMS) (Çoban-Yıldız *et al.* 2000). Tetramethylammonium hydroxide was added to weighed, ground fungal necromass as a methylating agent. Samples were heated to 300°C at a rate of 720°C/min in a GERSTEL Thermal Desorption Unit (Gerstel, Mulheim an der Ruhr, Germany) and immediately introduced into the GC column (HP-5MS; 30 m × 0.250 mm, 0.25 μm film thickness). The GC oven 7890B (Agilent Technologies, Santa Clara, CA, USA) was heated from 50 to 320°C over 55 min and held at 320°C for 10 min. Molecules were ionized in an Agilent Technologies 5977A mass spectrometer by electron ionization with a voltage of 70 eV. Peaks were classified as aliphatics, aromatics, carbohydrates, nitrogen containing, sterols or compounds of unspecified origin by their mass spectra using Agilent ChemStation software (v. B.07.02), standard runs and the National Institute of Standards and Technology (NIST) library. Carbohydrate standards included mannan and glucan. Fatty acids and fatty alcohols were included in the aliphatic category. N-containing compounds included proteins and amino sugars; N-containing chitin compounds were included in the N-containing category, not carbohydrates. Single-ion monitoring was performed by Agilent MassHunter software (v. F.01.01). Relative abundance of each classification of compound was calculated using a MATLAB (Natick, MA, USA) script.

Melanin was isolated from ground necromass of both species based on the method of Prados-Rosales *et al.* (2015). Briefly, ~0.5 g freeze-dried necromass was suspended in a solution of phosphate-buffered saline (PBS), buffer (1 M sodium citrate,

0.1 M sorbitol, pH 5.5) and lysing enzyme (10 mg/mL) and incubated at 30°C for 24 h. The sample was centrifuged to collect the pellet and washed five times with PBS. A 4 M solution of guanidine thiocyanate was added and the sample was incubated at room temperature overnight with constant shaking. After centrifugation and PBS wash, buffer (10 mM Tris–HCl, 5 mM calcium chloride, 5% SDS, pH 8.0) and proteinase K (1 mg/mL) were added and it was incubated at 65°C for 4 h. The sample was centrifuged and the pellet was resuspended in PBS. A solution of methanol and chloroform (2:1 by volume) was added to achieve a final ratio of 4:2:1 (chloroform:methanol:water). The chloroform layer was extracted and disposed of. The remaining mixture was acid hydrolyzed with 6 M hydrochloric acid and heated for 1 h. The hydrolyzed product was dialyzed (MWCO 12 kDa) against carbon-free MilliQ water for 72 h at 4°C with constant stirring and daily water changes. The remaining mixture was freeze-dried to collect the melanin. Melanin content is presented as a percentage of initial bulk fungal necromass (g).

Field experiment and sampling

In July 2018, five plots were located within experimental research property of the University of Minnesota Itasca Biological Station & Laboratory, just north of Itasca State Park, MN, USA. Plots were set ~50 m apart around a central plot (N°47.25019, W°95.23028). Each plot was centered on a dead log that was at least 3 m long and located >2 m from any other downed logs. The decay class and species identity of logs were not determined, but appeared to represent a mixture of the overstory of both conifer (*Abies balsamea*, *Pinus resinosa*) and angiosperm (*Populus tremuloides, Betula papyrifera*) trees and a range of decay classes (our selection criteria attempted to broadly match the variation in both of these variables observed at the study site). A paired soil location was located 2 m away from the log and >2 m from other fallen logs when possible. For each plot, litterbags containing fungal necromass were arrayed in linear transects in wood and soil (Fig. 1A and B). For both wood and soil transects, litterbags were placed 0.25 m apart, with the wood transects starting >0.5 m from the end of each downed log. On 6 and 7 August 2018, all bags were inserted ~5 cm into the wood and soil using a sterilized chisel and hand trowel, respectively (Fig. 1C). Litterbags were then collected after 8, 15, 28 and 56 days of incubation corresponding to a total of 66 samples (see Table S1, Supporting Information, for complete details about the number of replicates for each combination of treatments). In the laboratory, samples were removed from each litterbag, placed in individual sterile weighing trays and dried overnight at 30°C (this lower temperature, which was sufficient for completely drying the fungal necromass, was chosen to minimize DNA degradation). Dry masses were measured and then samples were placed in sterile 2 mL tubes and stored at 0°C until molecular analyses.

Molecular analyses

Total genomic DNA was extracted from each sample using a DNEasy Powerlyzer PowerSoil Kit (Qiagen, Hilden, Germany) following manufacturer's instructions. Prior to the first step in the protocol, however, all samples were subjected to bead beating for 15 s (BioSpec Products, Bartlesville, OK) to facilitate sample homogenization. Fungal DNA from each sample was amplified for high-throughput sequencing using a two-step Polymerase Chain Reaction (PCR) process. For the first PCR, the ITS 5.8-Fun and ITS4-Fun primer pair (Taylor *et al.* 2017), which targets the ITS2 region, was used. Samples, including a synthetic mock
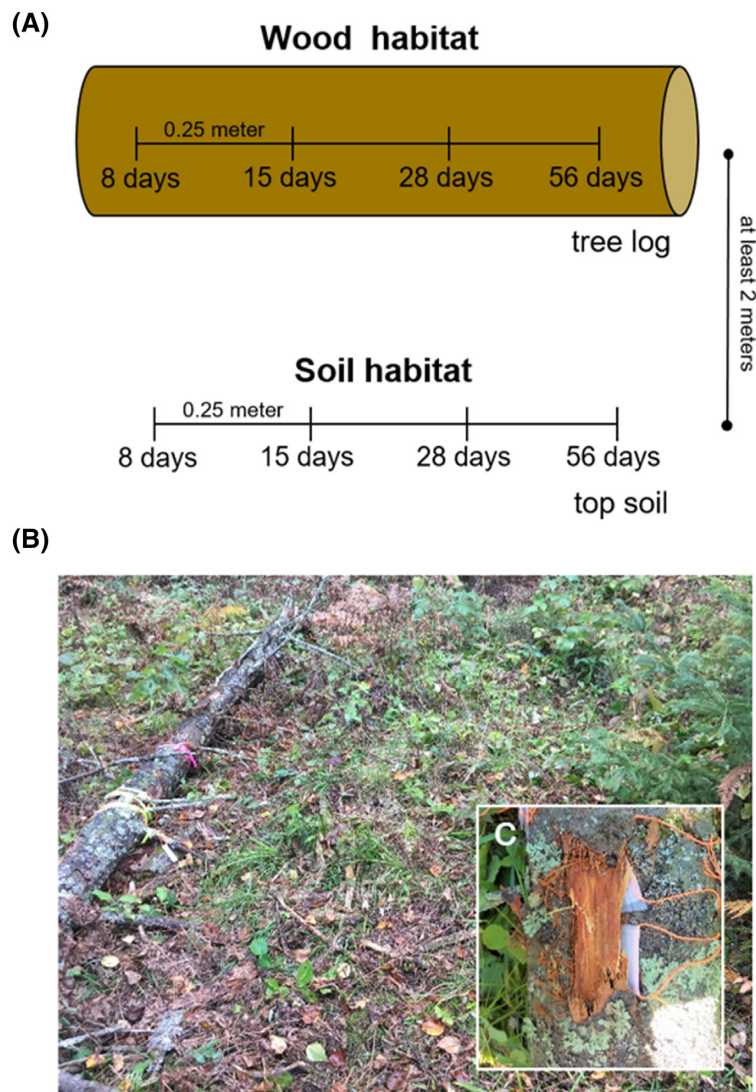
(A)

(B)

Figure 1. **(A)** Schematic representation of the experimental design. **(B)** One of the five paired wood and soil transects, with **(C)** a close-up of fungal necromass bags incubated in the wood habitat.

community developed by Palmer *et al.* (2018) and negative controls, were amplified in individual 20 μL reactions containing 10 μL of Phusion Hot Start II High-Fidelity PCR Master Mix (Thermo Scientific, Waltham, MA, USA), 0.5 μL of each 20 mM primer, 1 μL of DNA template and 8 μL of PCR-grade water. Thermocycling conditions were as follows: 1. 98°C for 30 s, 2. 98°C for 10 s, 3. 55°C for 30 s, 4. 72°C for 30 s, repeat steps 2–4 34 times, 5. 72°C for 10 min and 6. infinite hold at 4°C. For the second PCR, a second set of forward and reverse primers with unique Golay barcodes and Illumina adaptors were used. Reaction and thermocycling conditions were identical to the first PCR. Following the second PCR, all samples were cleaned and normalized using the Charm Just-a-Plate kit (Charm, San Diego, CA, USA) following manufacturer's instructions. Samples were then quantified on a Qubit fluorimeter (Thermo Scientific, Waltham, MA, USA), mixed at an equimolar concentration (3 nM) into a single sequencing library and sequenced using Illumina MiSeq 2 × 300 bp v3 chemistry at the University of Minnesota Genomics Center.

The raw demultiplexed .fastq files were processed using the 'amptk' pipeline outlined in Palmer *et al.* (2018). Briefly, primers were removed and sequences trimmed to 250 bp. Sequences were then denoised using UNOISE3 (Edgar 2016 and clustered into operational taxonomic units (OTUs) at 97% similarity. Read counts in the OTU × sample matrix were adjusted to account for index bleed between samples, using the amptk filter function '-t', which assesses the number of reads of mock community taxa present in environmental samples and then removes all reads under this threshold from each sample. Finally, taxonomy was assigned using a hybrid algorithm that integrates results from a USEARCH global alignment against the UNITE database (v8; Nilsson *et al.* 2018 and both UTAX and SINTAX classifiers. Based on the last common ancestor consensus taxonomic assignments, fungal OTUs were assigned to saprotrophic, pathotrophic and symbiotrophic trophic modes using FUNGuild (Nguyen *et al.* 2016). Saprotrophic fungi were further parsed between soft-rot and white-rot fungi, with the remaining OTUs classified as saprotroph. Symbiotrophic fungi were further parsed between ECM and endophyte fungi. Finally, any fungal OTUs belonging to Eurotiales, Hypocreales, Morteriellales, Mucorales, Saccharomycetales, Tremellales and Sporidiales as well as fungal OTUs defined by FUNGuild as microfungi, yeast and facultative yeast were classified into a single functional

group identified as molds and yeasts (Sterkenburg *et al.* 2015). No sequences belonging to the two fungal species used as the necromass sources (*Mo. elongata* and *Me. bicolor*) were recovered. Raw .fastq files for each sample were deposited in the NCBI Short Read Archive as accession PRJNA560400.

Decay-rate modeling

Because of the well-recognized non-linear nature of decomposition (Berg 2014), we assessed fungal necromass mass loss rates using non-linear exponential decay models. We analyzed the data either grouped by habitat or fungal necromass quality. For each grouping, we compared exponential decay fits using two and three parameters. In both cases, the three-parameter fit, which includes a decay constant (k), a scaling factor ($1 - A$) and an asymptote (A), was selected based on a lower Bayesian Information Criterion (BIC) score and higher r^2 value. Decay-rate modeling was done using JMP 14 Pro (Cary, NC, USA).

Statistical analyses

Statistical analyses and data visualization were performed on R software (R Core Team 2016) and considered significant at $P \leq 0.05$. Differences of mass remaining of fungal necromass samples by habitat (soil vs wood), incubation time (8, 15, 28, 56 days) and quality (low vs high) were assessed using a three-way factorial analysis of variance (ANOVA). Prior to running the ANOVA, variance homoscedasticity was confirmed using a Cochran's test. A *post-hoc* comparison among mass remaining means was done with a Tukey Honest Significant Differences (HSD) test. For the final fungal OTU dataset, some additional quality filtering was applied. Specifically, read counts of any OTUs present in PCR and DNA controls were summed and then subtracted from counts of those same OTUs in all other samples. After this step, one sample (FUN3) had only a single low-abundance OTU and was therefore removed. To account for uneven total read counts across samples, the fungal data were rarefied to 1385 counts/sample (Figure S1, Supporting Information). Seven samples, ranging from 0 to 761 read counts, were removed.

Fungal OTU richness (N0), Shannon diversity (*H*), effective richness (N1) and Shannon evenness (*E*) were calculated in vegan package (Oksanen *et al.* 2007). The effect of the habitat, incubation time and necromass quality on each of these four alpha

diversity metrics was tested using three-way ANOVA. Variance homoscedasticity was tested using Cochran's test and data were log-transformed if necessary. Differences in fungal OTU composition were visualized with non-metric multidimensional scaling (NMDS) plots based on Bray–Curtis dissimilarity matrix using the metaMDS function in vegan. Permutational multivariate analysis of variance (PERMANOVA) based on Bray–Curtis dissimilarity for fungal OTUs was applied to determine the effect of the habitat, incubation time and necromass quality. This analysis was paired with a betadisper test in vegan to assess heterogeneity in multivariate space for the aforementioned variables. Hierarchical clustering was performed using the 'hclust' function. To more fully assess the influence of habitat and necromass quality on fungal genera and guilds, we also calculated the log fold-change difference in read counts between each of these binary predictor variables. Finally, we used the heat tree representation in the Metacoder package (Foster, Sharpton and Grünwald 2017) to identify fungal genera significantly impacted by habitat and necromass quality.

RESULTS

After 8 weeks of incubation, fungal necromass samples had, on average, 29% mass remaining. Mass loss was significantly affected by habitat ($F_{1,60} = 5.613$, $P = 0.026$), time ($F_{3,60} = 28.210$, $P < 0.001$) and necromass quality ($F_{1,60} = 7.367$, $P = 0.009$). Although overall mass loss was significantly greater in soil than in wood [mass remaining: $42.1 \pm 2.3\%$ in soil, $50.6 \pm 3.5\%$ in wood (mean ± 1 s.e.) (note these values include all harvest time points, hence the higher mass remaining relative to the 8-week harvest values above)] and for high- versus low-quality fungal necromass (mass remaining: $42.4 \pm 2.4\%$ for high quality, $50.3 \pm 3.5\%$ for low quality), there was a significant interaction between necromass quality and time ($F_{3,60} = 4.286$, $P = 0.008$). This interaction was the result of significantly greater mass loss from high-quality fungal necromass compared to low-quality fungal necromass after 8 days in soil, but not in wood or between either habitat at later time points (Fig. 2). None of the other higher order interactions were significant.

The exponential decay models also showed significant differences in fungal necromass decay rate depending on habitat and necromass quality. The decay constant in soil ($k = -0.099$, lower 95% CI: -0.135, upper 95% CI: -0.065) was significantly
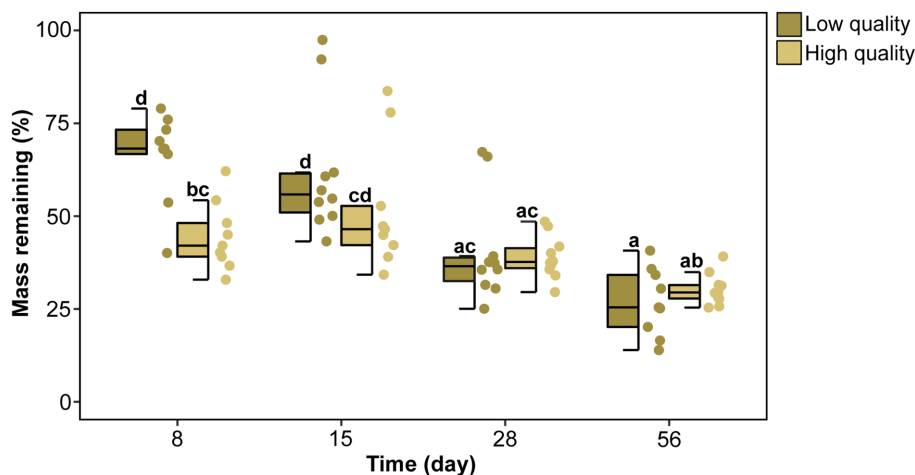

Figure 2. Mass remaining in low- (*Me. bicolor*) and high-quality (*Mo. elongata*) fungal necromass samples at each harvest day. Significant differences in masses based on a Tukey HSD test are denoted by different letters.
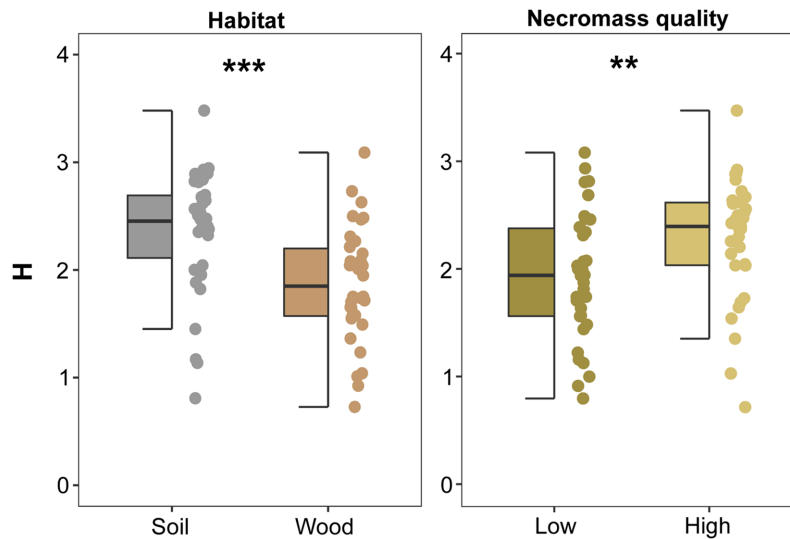
Figure 3. Fungal OTU diversity (*H*) of the necromass-colonizing fungal communities depending on habitat (soil and wood) or necromass quality (low and high). **$P \leq 0.01$; ***$P \leq 0.001$.

larger than the decay constant in wood ($k = -0.003$, lower 95% CI: -0.058, upper 95% CI: 0.051). Similarly, the decay constant of high-quality fungal necromass ($k = -0.199$, lower 95% CI: -0.297, upper 95% CI: -0.102) was significantly larger than that of low-quality fungal necromass ($k = -0.051$, lower 95% CI: -0.075, upper 95% CI: -0.027). The better fit of a three-parameter model in both cases also suggests that rates of necromass mass loss had significant non-zero plateaus after 8 weeks.

Fungal OTU diversity was significantly higher on fungal necromass in soil than in wood ($F_{1,60} = 15.156$, $P < 0.001$) and on high- than low-quality fungal necromass ($F_{1,60} = 7.561$, $P < 0.008$) (Fig. 3), but was not significantly different over time ($F_{3,60} = 0.993$, $P < 0.403$). These patterns appeared to be driven primarily by differences in fungal OTU richness rather OTU evenness (Figure S2, Supporting Information). In both habitats, the fungal necromass-associated community was dominated by fungi in the Ascomycota, particularly the classes Sordariomycetes and Eurotiomycetes (Fig. 4). Members of these two classes typically represented greater than 80% of the relative sequence reads per sample and were especially abundant on low-quality fungal necromass in wood. Members of the classes Mucoromycetes and Mortierellomycetes (i.e. fast-growing molds) were most abundant in samples harvested after 8 and 15 days, while members of the Agaricomycetes were absent from early harvests, but became more prominent at the later harvests (28 and 56 days), particularly in soil.

The overall composition of the fungal communities present on fungal necromass was most significantly influenced by habitat (soil vs wood), followed by time and necromass quality (Fig. 5A; Table S3, Supporting Information). There was also a significant interaction between habitat and necromass quality, which appeared to be driven by a lower heterogeneity in fungal community composition on low-quality fungal necromass in soil compared to in wood (Fig. 5B). When soil and wood samples were analyzed separately, the effects of time and necromass quality remained significant determinants of fungal community composition. In contrast, neither factor explained a significant proportion of the variance in necromass-associated fungal community composition in wood.

The composition of fungal guilds on decomposing fungal necromass was dominated by saprotrophs in both soil and wood, although the saprotroph guild had significant greater relative abundance in wood than in soil (Fig. 6; Table S4, Supporting Information). The relative abundance of pathotrophs was significantly higher in soil than wood, particularly in low-quality necromass. Guilds typical of the two habitats were also present on fungal necromass, with fungal communities having some colonization by white-rot fungi in wood and ECM fungi in soil. Both of these latter guilds had greater proportional colonization on the high-quality fungal necromass along fungi classified as soft-rot type, while saprotrophic and endophytic fungi had greater proportional abundances on low-quality fungal necromass.

A number of specific fungal genera displayed notable differences in relative abundance between both habitats and necromass qualities. For example, *Penicillium* and *Trichoderma* had relative read abundances that were 80× higher in wood than soil (Fig. 7). Conversely, *Fusarium* had relative read abundances that were 6× higher in soil than wood. Between necromass qualities, *Mortierella* and *Fusarium* were >60× greater on high-quality fungal necromass, while both *Penicillium* and *Trichoderma* were 6x more abundant on low-quality fungal necromass. In both comparisons, a number of unidentified fungal OTUs also showed large differences, further suggesting both habitat and necromass quality are important determinants of fungal relative abundances on decomposing fungal necromass.

DISCUSSION

In support of our first hypothesis, we found that the decomposition of fungal necromass was significantly faster in soil than wood. The overall trajectories in both cases, however, were similarly rapid, with <50% remaining after 28 days and only 30% remaining after 56 days, on average. Other studies of fungal necromass decomposition in soil have also reported rapid decay, with mass remaining being ~20% after 9 weeks of incubation in a temperate European forest (Brabcová, Štursová and Baldrian 2018) and ~30% after 8 weeks in a temperate US forest (Fernandez and Kennedy 2018). In contrast to the first hypothesis, we found no support for our second hypothesis that low-quality fungal necromass would degrade more quickly in wood than soil due to presence of decomposer organisms with enzymatic
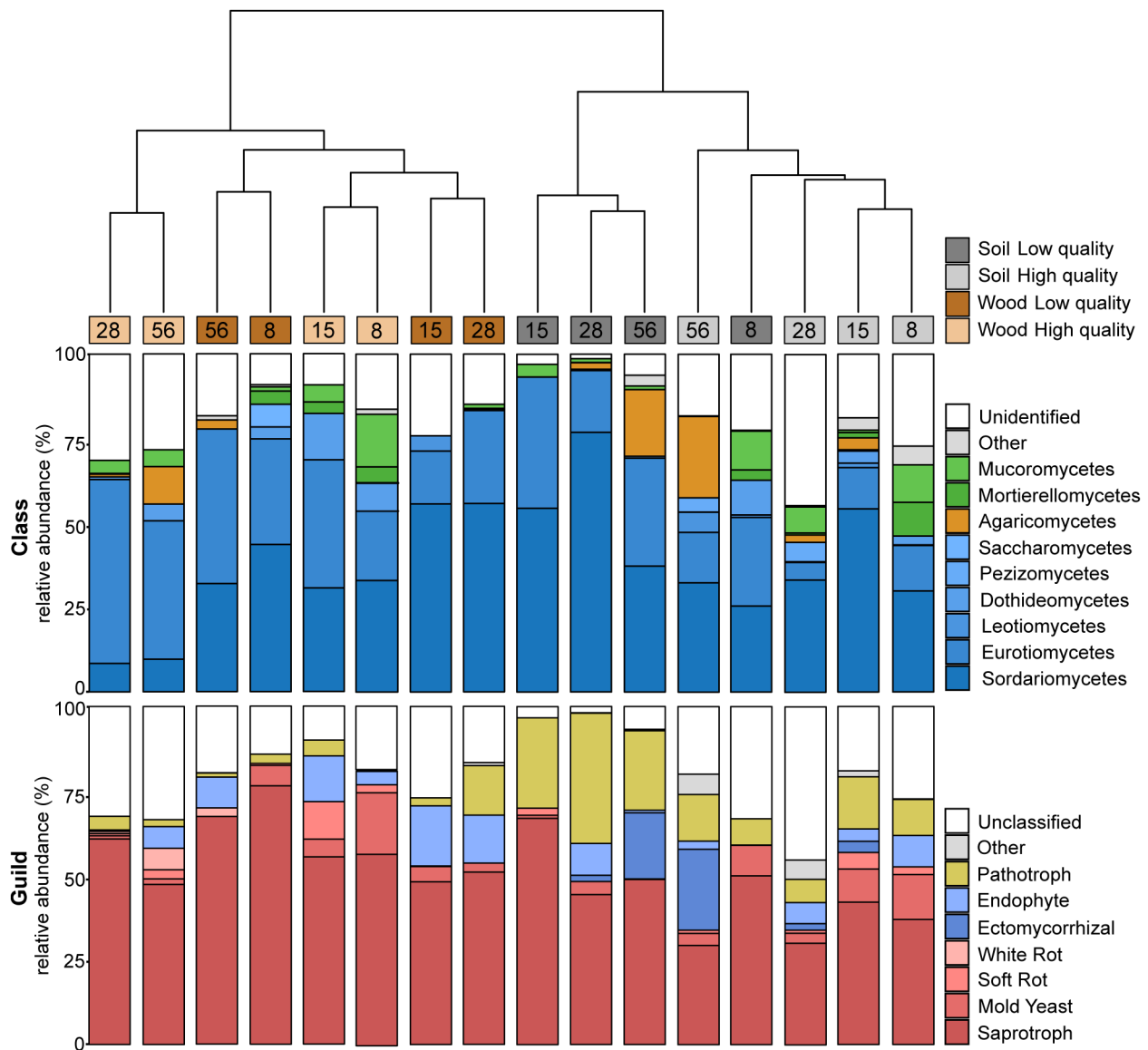
Figure 4. Hierarchical clustering of the necromass-colonizing fungal communities based on OTU composition depending on habitat (soil and wood), necromass quality (low and high) and the incubation period (8, 15, 28 or 56 days). Relative abundances of fungal classes and guilds depending on habitat, necromass quality and incubation period. Note that OTU abundances were summed for each combination of treatments before performing the hierarchical clustering and the class and guild relative abundance representations.

capacities well suited for recalcitrant substrates. Our molecular profiling of the fungal communities on necromass confirmed that white-rot fungi were able to colonize fungal necromass over the time frame of our experiment, but colonization by members of this guild was limited primarily to the final harvest point (56 days). We suspect that had the experiment been set up to run for a longer period, differences in mass loss by necromass quality and habitat would have become more pronounced, especially since the majority of the biomass remaining in relatively well-decomposed fungal necromass consists of aromatic compounds such as melanin (Fernandez *et al.* 2019).

We also found that the effect of necromass quality on mass loss varied over time, with the mass remaining being significantly higher in low- than high-quality fungal necromass at the first harvest (8 days), but not afterward. This pattern was similar to that observed in Fernandez and Kennedy (2018), where differences in mass loss between the low- and high-melanin

morphotypes of *Me. bicolor* also decreased (albeit not significantly) over time. Although we are not sure exactly why early mass loss differs by necromass quality, we speculated that it may be related, in part, to the physical reinforcement of fungal cell walls by melanin, which could result in less direct leaching of soluble materials during the early stages of fungal necromass decay. To test this possibility, we incubated two replicates of each necromass quality type in distilled water for 24 h and then compared their dry weights before and after incubation. We found that our high-quality fungal necromass type, *Mo. elongata*, lost nearly 50% of its mass (replicate 1 = 48.3%, replicate 2 = 47.4%) following a 24 h soaking, while our low-quality fungal necromass type, *Me. bicolor*, lost <25% of its mass (replicate 1 = 24.1%, replicate 2 = 22.3%) over the same time period. Although these leaching results are consistent with differences being related to physical differences between the two necromass types, they also suggest that a significant portion of
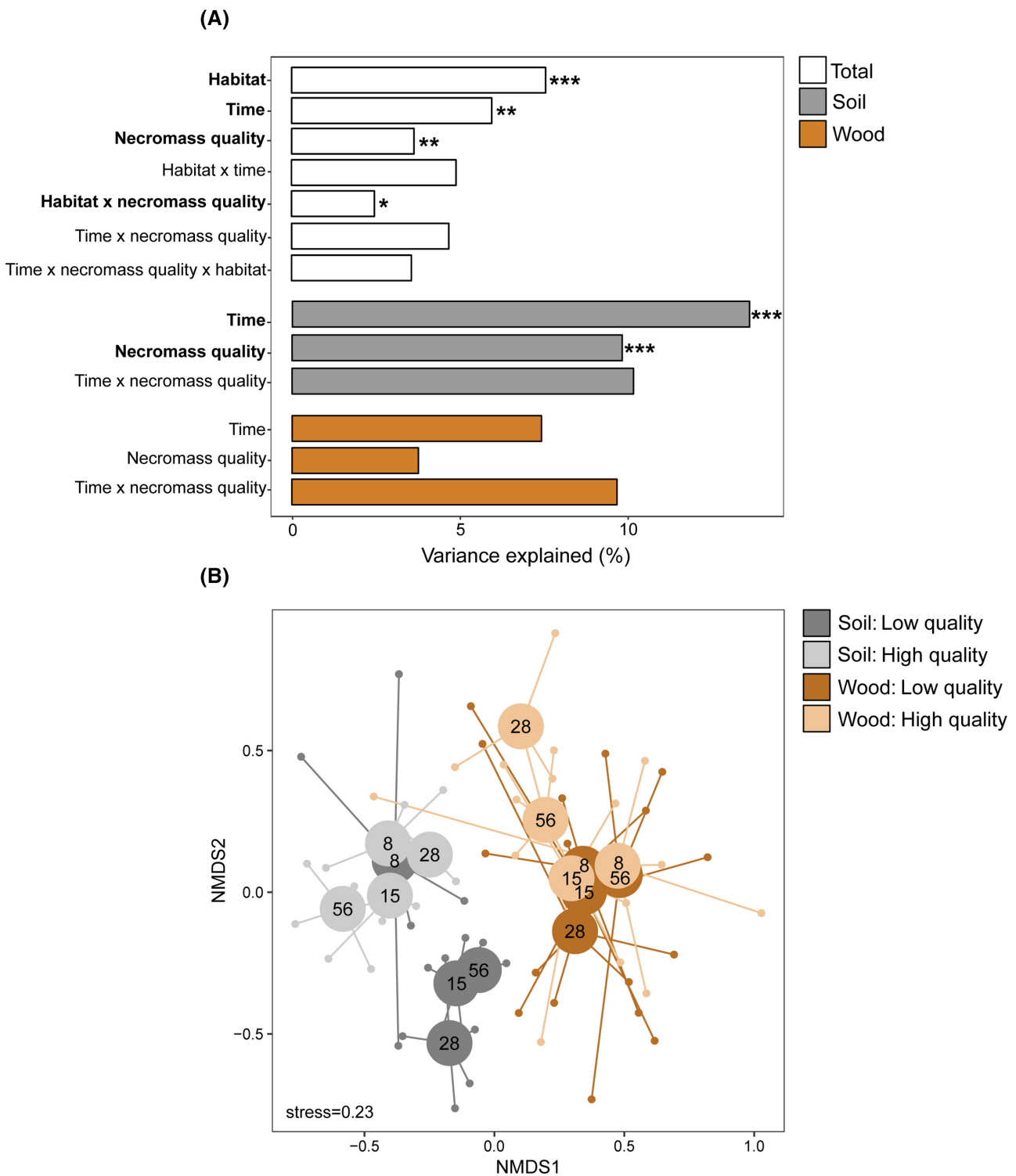
(A)

(B)

Figure 5. (A) Variance explained based on PERMANOVA depending on habitat (soil and wood), necromass quality (low and high) and incubation period (8, 15, 28 or 56 days). *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. **(B)** NMDS analysis of the necromass-colonizing fungal OTU communities depending on habitat, necromass quality and incubation period. Small circles represent individual samples and large circles represent the centroids.

the early mass loss from fungal necromass could be due to the leaching of soluble materials rather than direct biotically driven decay. In neither environment (soil or wood), however, were moisture levels ever at 100% during any harvest point, so the mass loss values obtained from these leaching tests should be interpreted as maximum possible estimates of abiotically driven decay. While the role of this leaching-associated mass loss on fungal necromass decay dynamics has not been regularly con-
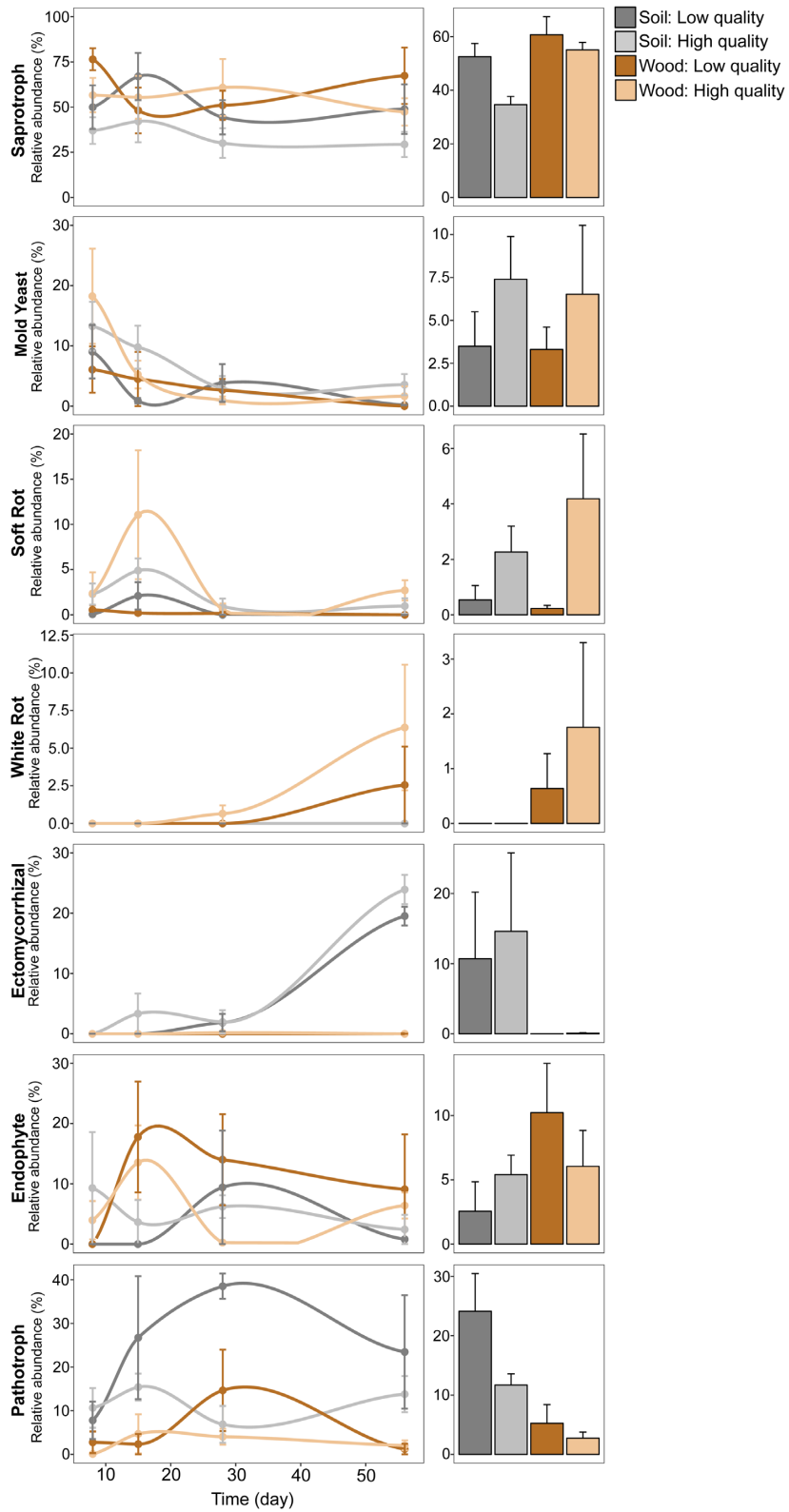
Figure 6. The relative abundance of various fungal guilds colonizing fungal necromass depending on habitat (soil and wood) and necromass quality (low and high) over the duration of the experiment. Bar plots (mean ± 1 s.e.) summarize the mean overall relative abundance of the fungal guilds depending on habitat and necromass quality.
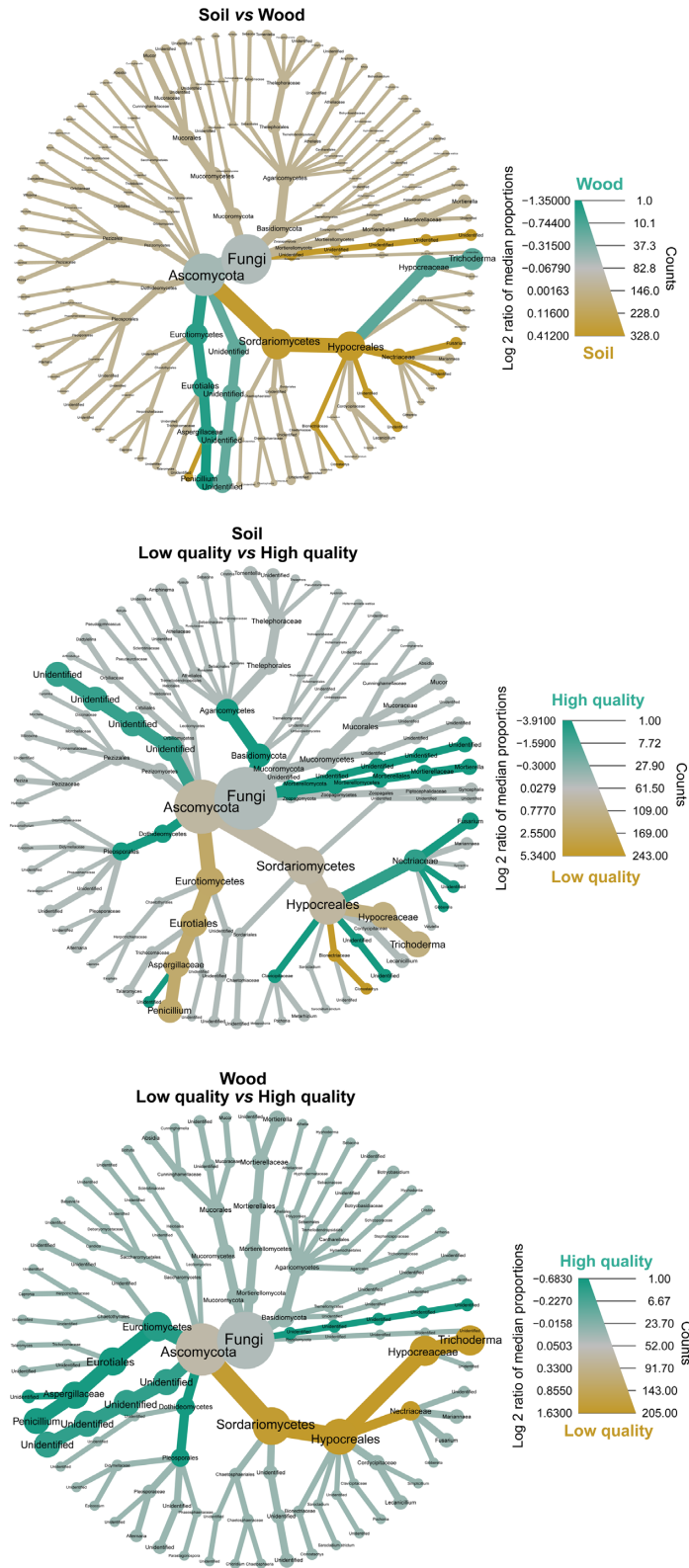
Figure 7. Heat tree differential analysis of necromass-colonizing fungal communities between habitats (soil vs wood) or necromass qualities (low vs high) within each habitat (soil and wood). Color indicates the sign and intensity of averaged changes of median proportions and the size of the nodes and edges indicates the relative abundance.

sidered [although see Akroume *et al.* (2019) for a discussion of why pre-washing of fungal necromass may be particularly relevant for analyses of chitin degradation], it could be ecologically important, particularly if it lowers the accumulation of humic substances, as has been demonstrated in leaf litter decomposition (Ni *et al.* 2018).

Consistent with our prediction regarding fungal community richness, there was significantly higher OTU richness on fungal necromass in soil than in wood regardless of necromass quality. We believe this result largely reflects differences in source pool sizes, as previous studies have shown that wood typically has significantly lower fungal richness than soil (Makipaa *et al.* 2017). Interestingly, the absence of significant time effect suggests that colonization of dead fungal necromass by living fungi is rapid, in both soil and wood. Averaging across the two necromass types, we found ~40 and ~20 fungal OTUs present in soil and wood after just 8 days, respectively. The communities in both habitats stayed at similar richness levels throughout the duration of the experiment and also maintained similarly high levels of OTU evenness. This pattern supports the assertion that fungal necromass represents a microbial decomposer 'hotspot' (Brabcová *et al.* 2016), in not only soil and litter but also wood. With regard to turnover in species composition, there was a trend toward less heterogeneity over time in both soil and wood habitats, although the stress value of the NMDS plot is relatively high. We hypothesize that a decrease in fungal species turnover (but not richness) may reflect an increasing selectivity of the fungal decomposer community for specific resources in fungal necromass during decomposition, but more in-depth analyses of changes in specific compound classes (e.g. polysaccharides, aliphatics, N-containing compounds) in decomposing fungal necromass are needed to test this possibility.

Much like previous analyses (Lindahl, De Boer and Finlay 2010; Brabcová, Štursová and Baldrian 2018; Fernandez *et al.* 2019), the dominant fungal taxa initially colonizing necromass were fast-growing molds, particularly members of the classes Mucoromycetes and Mortierellomycetes. The equivalently high relative abundance of these classes on both necromass types suggests this result is not an artifact on one of our necromass substrates being a member of this group (we also detected no reads matching the specific OTU of the *Mo. elongata* substrate). These molds appear to largely be generalists with regard to necromass quality, likely targeting easily to degrade compounds due to their limited enzymatic capacities (Baldrian 2017). A number of Ascomycete molds were also present on the decomposing fungal necromass, including the well-studied genera *Penicillium* and *Trichoderma*. Both of these genera had significantly greater relative abundance in wood than in soil, which is consistent with their known capacities for efficient cellulose degradation (Schuster and Schmoll 2010; Gusakov and Sinitsyn 2012). The stronger enzymatic potential of *Trichoderma* in particular, which includes enzymes enabled by carbohydrate-binding modules for substrate discovery and attachment (Tayagnacco *et al.* 2011), may explain why this genus was significantly more abundant on low-quality fungal necromass regardless of habitat. We also observed a notable increase in members of the Agaricomycetes over the course of the incubation. The increasing presence of white-rot fungi and ECM fungi in wood and soil, respectively, indicates that members of these guilds likely utilize the resources present in decomposing fungal necromass. Curiously, both of these guilds were, on aggregate, more abundant on high-quality than low-quality necromass. We speculate this may be due to their selective targeting of the more recalcitrant compounds that remain in high-quality fungal necromass after more

labile substrates are utilized by fast-growing molds (Floudas *et al.* 2012; Lindahl and Tunlid 2015), but additional studies using a combination of isotopic labeling and gene expression are required to better clarify the functional roles of these guilds in necromass decomposition. A final notable pattern involved the genus *Clonostachys*, which is a mycoparasite that has shown effectiveness as a fungal biocontrol of molds (Cota *et al.* 2008; Figure S3, Supporting Information). Pathotroph abundance was considerably higher in soil and on low-quality necromass, suggesting that the increased abundance of saprotrophic taxa such as *Trichoderma* is likely accompanied by pathogens specifically targeting fungal necromass decomposers as well.

Although we believe our results provide multiple new insights into the dynamics of fungal necromass decomposition, we recognize there are significant caveats to our findings as well. In particular, while our two necromass substrates differed significantly in multiple aspects of their initial chemistry, future tests of the effect of fungal necromass quality on mass loss and decomposer community structure including more than a single species per type will be key to assessing the generality of the patterns we observed. For example, using necromass from many different species, Fernandez and Koide (2014) and Brabcová, Štursová and Baldrian (2018) were able to show that necromass mass loss rates were strongly linked to initial melanin:N and C:N ratios, respectively. Similarly, our perspectives on shifts in the structure of fungal necromass-associated communities are only based on fungi, and recent work by Brabcová *et al.* (2016) and Brabcová, Štursová and Baldrian (2018) has shown that bacteria are able to quickly colonize decomposing fungal necromass. In fact, Lopez-Mondejar *et al.* (2018) found that bacteria were likely more prominent utilizers of fungal necromass C than fungi, although their results should be interpreted with some caution, as their laboratory soil microcosms excluded certain guilds such as ECM and wood-rot fungi. The specific functional roles of bacteria in wood decomposition are still relatively unclear (Johnston, Boddy and Weightman 2016), but it seems likely that there are significant cross-feeding or competitive scenarios between different groups of decomposers on fungal necromass in wood, much like the well-documented successional dynamics among different fungal guilds decomposing the wood itself (Rajala *et al.* 2015).

CONCLUSIONS

Our results indicate the initial ecological patterns emerging regarding fungal necromass decomposition in soil and leaf litter can be largely extended to fungal necromass decomposition in wood. While the specific structure of the fungal decomposer communities in wood and soil differed, we found that in both habitats, there was relatively rapid decay that was significantly influenced by initial fungal necromass quality. Similarly, in both habitats, there was a clear trajectory of early colonization by opportunistic fungal taxa followed by subsequent colonization of fungi with greater enzymatic capacities to degrade more recalcitrant compounds. Although the initial mass loss of fungal necromass has now been well demonstrated to be rapid (Koide and Malcolm 2009; Brabcová *et al.* 2016; Fernandez *et al.* 2016; Brabcová, Štursová and Baldrian 2018; Certano *et al.* 2018; Fernandez and Kennedy 2018; Lopez-Mondejar *et al.* 2018), Fernandez *et al.* (2019) recently demonstrated that the non-labile part of this substrate is highly stable in soils. Given the well-known degradation capacities of wood-rot fungi in particular, it seems possible that the longer term pattern of fungal necromass decomposition in wood may be different (i.e. there may be

more complete decomposition) than in soil. As such, we strongly encourage that further research on this topic include longer term incubations to better assess how fungal necromass moves through various ecosystem C and nutrient pools.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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