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MYCORRHIZAL FUNGI AS DRIVERS AND MODULATORS OF ECOSYSTEM PROCESSES

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Melanization of mycorrhizal fungal necromass structures microbial decomposer communities

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Abstract

1. Mycorrhizal fungal necromass is increasingly recognized as an important contributor to soil organic carbon pools, particularly in forest ecosystems. While its decomposition rate is primarily determined by biochemical composition, how traits such as melanin content affect the structure of necromass decomposer communities remains poorly understood.
2. To assess the role of biochemical traits on microbial decomposer community composition and functioning, we incubated melanized and non-melanized necromass of the mycorrhizal fungus *Meliniomyces bicolor* in *Pinus*- and *Quercus*-dominated forests in Minnesota, USA and then assessed the associated fungal and bacterial decomposer communities after 1, 2 and 3 months using high-throughput sequencing.
3. Melanized necromass decomposed significantly slower than non-melanized necromass in both forests. The structure of the microbial decomposer communities depended significantly on necromass melanin content, although the effect was stronger for fungi than bacteria. On non-melanized necromass, fungal communities were dominated by r-selected ascomycete and mucoromycete microfungi early and then replaced by basidiomycete ectomycorrhizal fungi, while on melanized necromass these groups were co-dominant throughout the incubation. Bacterial communities were dominated by both specialist mycophageous and generalist taxa.
4. *Synthesis.* Our results indicate that necromass biochemistry not only strongly affects rates of decomposition but also the structure of the associated decomposer communities. Furthermore, the observed colonization patterns suggest that fungi, and particularly ectomycorrhizal fungi, may play a more important role in necromass decomposition than previously recognized.

KEYWORDS
bacteria, carbon cycle, decomposition, fungi, melanin, mycorrhizal fungi, necromass, nitrogen cycle

1 | INTRODUCTION

In recent years, the turnover of mycorrhizal fungal biomass has become increasingly well recognized to be a significant input in soil carbon cycles, particularly in temperate and boreal forests (Ekblad et al., 2013; Fernandez, Langley, Chapman, McCormack, & Koide, 2016; Wallander, Johansson, Sterkenburg, Brandström Durling, & Lindahl, 2010). These residues, hereafter referred to as necromass,

represent significant precursors to the formation of soil organic matter (Godbold et al., 2006; Kögel-Knabner, 2002; Six, Frey, Thiet, & Batten, 2006) and because the biomass of mycorrhizal fungi is generated directly from carbon provided by their plant hosts, they may be a particularly efficient pathway of carbon movement from primary production into stabilized soil organic matter pools (Ekblad et al., 2013). Emerging isotopic evidence indicates that soil organic carbon can be disproportionately composed of root and mycorrhizal hyphal inputs, as opposed to those from above-ground litter (Clemmensen et al., 2013). Taken together, these findings point to the importance of understanding the factors controlling the decomposition and stabilization of the mycorrhizal fungal necromass (Fernandez & Kennedy, 2015; Fernandez et al., 2016).

Rates of fungal necromass decomposition appear to be largely controlled by biochemical composition, which is known to vary considerably across species (Crowther et al., 2015; Fernandez & Koide, 2012; Koide & Malcolm, 2009). Fernandez and Koide (2014) demonstrated that increased melanin content in particular significantly reduced the decomposability of fungal necromass. While melanin had large independent effects, nitrogen content was also important, and initial melanin-to-nitrogen content best explained the rate of necromass decomposition. The recalcitrance of melanin is largely a result of the large, complex and irregular chemical structure of these macromolecules, which are composed of phenolic (or indolic) monomers. Because of this structure, the catabolism of melanin requires non-specific but energetically expensive oxidative enzymes (Bull, 1970; Siletti, Zeiner, & Bhatnagar, 2017). Previous studies examining plant litter indicate that biochemistry and stoichiometry can have dramatic effects on decomposition, either slowing or speeding of carbon cycling depending on enzymatic capabilities of the microbes present (Allison et al., 2009; Liu, Keiblinger, Leitner, Mentler, & Zechmeister-Boltenstern, 2016; Schneider et al., 2012). Researchers have also noted strong successional patterns over the course of plant litter decomposition in both fungal and bacterial communities, which has been linked to their ability to degrade and acquire specific resources (Baldrian, 2017; Cline & Zak, 2015).

To date, knowledge of the microbial communities involved in fungal necromass decomposition has been limited by a lack of appreciation of these inputs into soil organic matter pools as well as a lack of high-throughput molecular tools to accurately identify the diversity of microbes involved. Previous work has indicated that the microbial communities present on decaying fungal necromass are dynamic and that carbon from that mycelium can be rapidly incorporated into soil carbon pools (Drigo, Anderson, Kannangara, Cairney, & Johnson, 2012). By experimentally severing connections between ectomycorrhizal fungi and their tree hosts in a Scandinavian boreal forest, Lindahl, De Boer, and Finlay (2010) showed a rapid increase in abundances of soil saprotrophic fungi (particularly yeasts and moulds) but not bacteria. They suggested that the rise in the fungal saprotroph populations was due to their utilization of dead ectomycorrhizal mycelium as a carbon source. More recently, Brabcová, Nováková, Davidová, and Baldrian (2016) examined the fungal and bacterial communities involved in the decomposition of sporocarps of the ectomycorrhizal fungus *Tylopilus felleus* in a European oak forest. Their findings indicated that the microbial communities associated with fungal necromass are distinct from those associated with plant litter and were composed of r-strategist microfungi (both ascomycetes and mucoromycetes) and bacteria that may specialize on degrading fungal compounds. It remains unclear, however, how biochemical traits of fungal necromass may alter the composition and functioning of microbial decomposer communities.

The goal of this study was to examine how fungal necromass biochemistry influenced both fungal and bacterial decomposer community structure and associated decomposition rates. We hypothesized that necromass with high concentrations of recalcitrant compounds, such as melanin, would select for fungal communities featuring basidiomycete fungi that possess high-oxidative potential at the expense of r-strategist microfungi in the phyla Ascomycota and Mucoromycota that only utilize soluble and hydrolysable compounds. Second, we hypothesized that compared to fungal communities, the composition of bacterial communities would be more consistent across changes in necromass melanin concentration, because they were likely relegated to the soluble and hydrolysable fractions. In order to focus on the effects of melanin in particular on decomposer community dynamics, we utilized mycelium of the mycorrhizal fungus *Meliniomyces bicolor*. The melanin concentration in the cell walls of this fungus can be naturally altered without using chemical inhibitors. This allows for direct comparisons of both melanized and non-melanized necromass of the same species, eliminating many of the covariates present in comparisons across species (Fernandez & Koide, 2014). We investigated both community shifts and mass loss differences between melanized and non-melanized necromass of *M. bicolor* during a 3-month field incubation. To additionally investigate how biochemical effects on necromass decomposition may vary in forests with differing tree species composition, we conducted our incubations in adjacent *Pinus*- and *Quercus*-dominated forests.

2 | MATERIALS AND METHODS

2.1 | Necromass generation

Meliniomyces bicolor is a mycorrhizal fungus that associates with both ericoid and ectomycorrhizal hosts (Grelet, Martino, Dickie, Tajuddin, & Artz, 2017). We manipulated the level of cell wall melanization in *M. bicolor* by growing identical starting mycelial plugs in 125 ml flasks with different levels of submersion (50 or 100 ml) of half-strength potato dextrose broth (PDB, Difco, BD Products, Franklin Lakes, New Jersey, USA). We conducted a set of preliminary experiments to test the possibility that reduced liquid media carbon content rather than colony submersion might have been the cause of this melanization response (Appendix S1). Those experiments clearly showed that level of submersion was the causal agent, likely due to differential exposure to oxygen, which is integral for the many oxidative reactions required to polymerize melanin precursors in melanin biosynthetic pathways (Bull, 1970; Siletti et al., 2017). Flasks were shaken on orbital shakers at 80 RPM at room temperature for 30 days. Fungal colonies were then harvested, rinsed in deionized water, and dried at 26°C in a drying oven for 24 hr. Nylon mesh litter bags (~3 × 3 cm, 53 μm nylon

mesh (Elko, Minneapolis, MN, USA)) containing either melanized or non-melanized *M. bicolor* were constructed. Approximately 25 mg of dry necromass was placed in each litter bag, which was then sealed closed via heat-sealing (American International Electric Inc., City of Industry, CA, USA).

2.2 | Decomposition incubation

The decomposition incubation was conducted at two forest sites at Cedar Creek Ecosystem Reserve in Central Minnesota, USA. The sites, located within 2 km, were dominated by mature northern pin oak (*Quercus ellipsoidalis*) or mixed age white pine (*Pinus strobus*) respectively. The climate of the region is classified as continental, with warm summers and cold winters (mean annual temperature = 8°C, mean annual precipitation = 810 mm). Soils at the sites are well-drained sandy soils that are characterized as mixed frigid Aquic Udipsamments (Soil Survey Staff, 1999). The soils at both forest stands are poorly developed and the O-horizon at each is approximately 3–5 cm in thickness. Within each site, six randomly located blocks were established approximately 10 m apart from one another. In July 2016, the litter bags were placed at the interface of mineral and O-horizon. They were incubated in the field for 1, 2 and 3 months, starting 29 July and concluding 27 October 2016. For each necromass type, six replicates per incubation period and per site were incubated (n = 72). Prior to deploying the litter bags, 5 × 10 cm soil cores were taken from the soil surface at each block, approximately 0.25 m from where the bags were incubated. At harvest, samples were placed on ice and immediately returned to the laboratory for processing. Incubated necromass was then placed in the drying oven set at 27°C overnight. Following drying, we measured the dry mass remaining for the necromass with weighing boats that had been sterilized with 6% sodium hypochlorite solution to prevent possible contamination. Necromass samples were then placed in 2 ml microcentrifuge tubes and stored at −20°C for molecular analyses.

2.3 | Fungal and bacterial identification

Total genomic DNA was extracted from the necromass and soil samples with PowerSoil kits (MoBio, Carlsbad, CA, USA). For the soil samples, all litter and root material was excluded from the extraction protocol. For each necromass sample, all remaining fungal material at each incubation time was added to the kit lysis tubes. Prior to starting the extraction, the necromass samples were bead beat (BioSpec Products, Bartlesville, OK, USA) for 5 s to ensure better homogenization of the necromass. The remainder of the extraction followed the manufacturer's instructions. Negative controls (unincubated litter bags without necromass and lysis tubes with no substrate added) were also extracted.

Both fungal and bacterial communities on the *M. bicolor* necromass and in surrounding soils were identified using high-throughput sequencing. For fungi, the ITS1 gene region was amplified using the primers and protocols of Smith and Peay (2014). For bacteria, the full-length 16S gene was amplified using the primers and protocols provided by Pacific Biosciences (http://www.pacb.com/wp-content/uploads/Unsupported-Full-Length-16S-Amplification-SMRTbell-LibraryPreparation-and-Sequencing.pdf). Based on preliminary tests, the forward 27F primer was modified by adding a thiol bond between the third and fourth base pairs on the 3′ end to prevent any primer editing activity of the KAPA polymerase (KAPA Biosystems, Sigma-Aldrich, St. Louis, MO, USA). After confirming PCR amplification via gel electrophoresis, fungal samples were cleaned using the Charm "Just-A-Plate" kit (Charm Biotech, San Diego, CA, USA), while bacterial samples were cleaned using the Agencourt AMPure XMP kit (Beckman Coulter Life Sciences, Indianapolis, IN, USA). Following cleaning, samples were added in equal concentration to a single fungal or bacterial library for sequencing at the University of Minnesota Genomic Center. The fungal library was sequenced using MiSeq 2 × 250 bp V2 Illumina chemistry. The bacterial library was sequenced using Magbead loading on the PacBio Sequel platform.

Fungal sequences were processed using the FAST pipeline version 1.102 (https://github.com/ZeweiSong/FAST). Briefly, primers were trimmed using cutadapt (Martin, 2011), forward and reverse reads were paired using PEAR (Zhang, Kobert, Flouri, & Stamatakis, 2013), and the SSU and 5.8S gene regions were removed. Low-quality sequences were removed (maximum expected error rate = 1), dereplicated, singletons discarded, and chimeras detected and eliminated using VSEARCH (https://github.com/torognes/vsearch) and the UNITE database (sh_general_release_dynamic_22.08.2016, Koljalg et al., 2013). Remaining sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using the greedy algorithm in VSEARCH. To remove possible non-fungal OTUs, only those matching the UNITE database at 75% match over 70% of their length were retained (as in Tedersoo et al., 2014). Taxonomy was assigned using VSEARCH.

The resulting sample × OTU matrix was further quality filtered by subtracting sequence read counts present in any negative controls from those in the experimental samples (as in Nguyen, Smith, Peay, & Kennedy, 2015). Following the recommendation of Lindahl et al. (2013), any cell containing less than four sequence reads was zeroed. In order to more precisely reflect the r-selected life histories of certain microfungi and yeasts, we re-assigned all OTUs belonging to Eurotiales, Hypocreales, Morteriellales, Mucorales, Saccharomycetales, Tremellales and Sporidiales as "moulds & yeasts" (Sterkenburg, Bahr, Brandström Durling, Clemmensen, & Lindahl, 2015). The remaining OTUs were assigned to saprotrophic, symbiotrophic, and pathotrophic trophic modes using FUNGuild (Nguyen et al., 2016). We further parsed symbiotrophic fungi into "ectomycorrhizal", "arbuscular mycorrhizal" and "lichenized" guilds. Finally, when possible, we manually assigned guilds to OTUs that were unassigned (due to missing genus taxonomy) as well as those OTUs that were assigned multiple trophic modes. Details of these assignments are discussed further in Appendix S2 and guild assignments for all genera are presented in Table S1.

Bacterial sequences were initially processed with the PacBio SMRTLink 4.0, where they were dereplicated and converted to .fastq files using the "CCS with barcoding" option (default parameters: minimum number of subreads = 3, minimum predicted accuracy = 0.9). In

Mothur v128 (Schloss et al., 2009), sequences without the universal tag and forward primer, as well as those with less than 1,400 bp or greater than 1,600 bp were removed. Sequences were then aligned against the SILVA database (Quast et al., 2012), trimmed to equivalent length and pre-clustered using the criteria of Schloss, Jenior, Koumpouras, Westcott, and Highlander (2016). Chimeric sequences were removed using UCHIME (Edgar, Haas, Clemente, Quince, & Knight, 2011). Possible archaeal, mitochondrial, and eukaryotic contaminants were screened by first classifying sequences with the RDP classifier and then removing any non-bacterial lineages. Finally, sequences were then clustered at 97% similarity to generate an OTU × sample matrix. As with the fungi, the matrix was further quality filtered by subtracting sequence read counts in the negative controls from all the experimental samples as well as all cells with less than two sequence reads were zeroed. Raw .fastq files for both the fungi and bacteria were deposited in the NCBI short-read archive (Accession: PRJNA400233 and PRJNA399749 respectively).

Because of the inherent differences in both sequencing platforms (Illumina vs. PacBio) and gene target lengths (ITS1 = ~200 bp vs. V1–V9 16S = ~1,500 bp), there was a ~20-fold difference in the total raw reads for the fungal (2,492,250) and bacterial (131,588) datasets respectively. These differences were maintained in the subsequent bioinformatic filtering steps, resulting in final sequence read counts per necromass sample being vastly different for fungi (M = 15,227, SE = 1,680) and bacteria (M = 252, SE = 31). As a result, we only focused on variation in community composition rather than absolute differences in either OTU richness or sequence read abundances across treatments. To account for differences in sequence reads among samples, both the fungal and bacterial datasets were normalized by making individual sequence read values proportional to each sample sum. The sample x OTU matrices for fungi and bacteria used in the final analyses are provided in Tables S2 and S3 respectively.

2.4 | Necromass biochemical quantification

Melanin content of *M. bicolor* necromass was assessed using a modified quantitative colorimetric assay (as in Fernandez & Koide, 2014). This assay utilizes Azure A dye, which has a strong binding affinity to melanins, allowing for the quantification of changes in absorbance once a solution has been in contact with melanin. An Azure A dye solution was made by dissolving the dye in 0.1 M HCl and filtering the solution through a Whatman 1 filter (ensuring no undissolved dye remained). The dye solution was diluted until an absorbance of 0.650 at 610 nm was achieved. Known amounts of melanin extracted from *Cenococcum geophilum* fungal mycelia by selective acid hydrolysis were used to generate a standard curve (Butler & Lachance, 1986; Frederick et al., 1999). Values ranging from 0 mg to 2 mg of that melanin were placed in 5 ml of Azure A solution and incubated overnight. The solutions were then filtered through a 0.22 μm syringe tip filter and absorbance of the filtrate was measured using a spectrophotometer at 610 nm (Implen P330, Westlake Village, CA, USA). A standard curve was generated using the change in absorbance after incubation with the *C. geophilum* melanin (melanin (g) = Δabs * 0.0091). Samples of dried *M. bicolor* necromass were run through the same steps and absorbance was used to calculate melanin content based on the standard curve. The carbon and nitrogen content of *M. bicolor* was determined using isotope ratio mass-spectrometery (vario PyroCube, Elementar, Mt. Laurel, NJ, USA) at the IRMS facility at the University of Minnesota.

2.5 | Statistical analyses

Differences in carbon and nitrogen content of melanized and non-melanized *M. bicolor* necromass were tested with independent two sample *t* tests. A three-way full factorial linear mixed model was constructed to test the fixed effects of incubation time, melanization and site on log-transformed mass remaining data. Block was also included as the random effect. To examine the effects of incubation time, melanization and site on necromass-associated fungal and bacterial community composition, we built a fully factorial generalized linear models (GLM) with the *manyglm* function using a negative binomial distribution for counts in "MVABUND" package (Wang, Naumann, Wright, & Warton, 2012) in R (R Core Team, 2014). We checked the mean–variance relationship of the OTU with the *meanvar.plot* function. To check the model fit and assumptions, we used the *plot* function to plot residuals vs. fitted values. The GLM models were conducted on fungal and bacterial OTUs as well as fungal guild data OTUs (in the latter, OTUs without guild classifications were removed). To satisfy the *manyglm* input parameters, the normalized proportional abundance data were multiplied by the arbitrary number 10,000 and rounded up to the nearest integer (to be input as count data). Significance was calculated using a Wald statistic and *p* values assigned following 999 PIT-trap resampling iterations to account for correlation in testing using the ANOVA function. Differences in fungal and bacterial community composition were visualized with the non-metric multi-dimensional scaling (NMDS) plots using the *metaMDS* function in R (R Core Team, 2014).

3 | RESULTS

3.1 | Effects of growth conditions on fungal biochemistry

Increasing the submersion level of the PDB medium did not appear to alter the growth rates or mass of the *M. bicolor* colonies. It did, however, dramatically reduce the melanin concentration of *M. bicolor* biomass (t = −7.74, df = 2.15, p = .0131), producing colonies that were off-white as opposed to black in colour (Figure 1). In addition, increased submersion significantly increased *M. bicolor* necromass nitrogen concentration (t = 14.55, df = 2.62, p = .0014), but had no significant effect on carbon concentration (Table 1). This change in nitrogen is likely related to the proportional inflation of nitrogenous cell wall components (e.g. chitin and glycoproteins) caused by the reduction of melanin concentration, which is devoid of nitrogen.

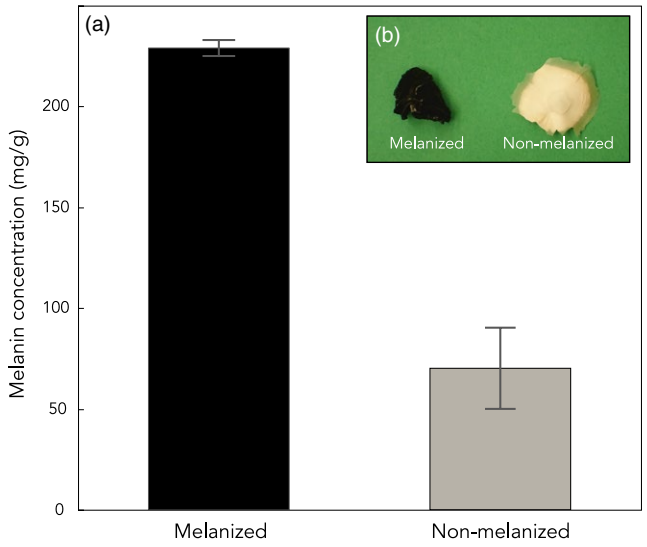

FIGURE 1 (a) Melanin concentrations (mg/g dry mass; $M \pm SE$) of the melanized and non-melanized *Meliniomyces bicolor* necromass ($n = 6$). (b) Photograph of representative melanized and non-melanized *M. bicolor* necromass [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | Effects of necromass melanization on decomposition

The decomposition of *M. bicolor* necromass was significantly influenced by incubation time and melanization (Table 2). Overall, mass loss from the non-melanized necromass was rapid, where an average of 73% was lost during the first month of the incubation compared to 51% for the melanized necromass (Figure 2). Over the full 3-month incubation, melanized necromass decomposed consistently more slowly than non-melanized necromass (Table 2). Between sites, decomposition rates were, on average, consistently faster at the pine than the oak site, although the magnitude of this difference (6%) was small and only marginally significant ($p = .081$).

3.3 | Soil vs. necromass microbial communities

Fungal communities colonizing *M. bicolor* necromass were significantly different from those found in adjacent soil (GLM: $Wald_{1,29} = 19.37$; $p = .003$; Figure S1, Table S4). At both sites, the soil fungal communities were dominated by ectomycorrhizal fungi in the phylum Basidiomycota, which accounted for more than 60% relative abundance of each community (Figure S2). In contrast, the fungal communities associated with necromass contained much higher proportions of moulds and yeasts in the phyla Ascomycota and Mucoromycota, particularly at the 1- and 2-month sampling times. This pattern, however, was less notable for melanized necromass at the pine site, which had fungal communities consistently dominated by the ectomycorrhizal fungal genus *Tomentella*. Due to read depths of the bacterial communities in the soil samples all being extremely low (median = 6 sequences per sample), an equivalent comparison between the bacterial communities colonizing necromass vs. those present in soil was not possible.

TABLE 1 Carbon (%), nitrogen (%) and carbon:nitrogen (C:N) ratio ($M \pm 1$ *SE*) for melanized and non-melanized *Meliniomyces bicolor* necromass

	Necromass type	
	Melanized	Non-melanized
%C (±*SE*)	51.3 (0.22) a	52.6 (1.85) a
%N (±*SE*)	3.8 (0.03) a	4.8 (0.07) b
C:N (±*SE*)	13.7 (0.13) a	11.0 (0.25) b

Different letters indicate significant differences as determined by *t* tests ($n = 6$).

3.4 | Fungal response to melanization of fungal necromass

Fungal OTU composition on necromass was significantly influenced by melanization ($Wald_{1,66} = 16.38$; $p = .004$) and site ($Wald_{1,65} = 20.20$; $p = .001$), but not by incubation time ($Wald_{2,63} = 25.89$; $p = .214$) or any higher order interactions (Figure 3, Table S4). The five most abundant fungal genera colonizing the fungal necromass were *Tomentella*, *Penicillium*, *Mortierella*, *Epicoccum* and *Russula* (Figure 4). Although incubation time was not a significant main effect at the fungal OTU level, there was a significant interaction between necromass incubation time and melanization on fungal guild composition ($Wald_{1,63} = 4.368$; $p = .026$). This interaction was primarily driven by differences in the temporal dynamics of the EM fungal guild and the mould and yeast guild. Specifically, melanized necromass was largely co-dominated by both EM fungi (Figure 3a) and moulds and yeasts (Figure 3c) throughout the 3-month incubation, whereas the non-melanized necromass was dominated initially by moulds and yeasts (Figure 3d) and then replaced with ectomycorrhizal fungi later in the incubation (Figure 3b). For both necromass types and across all harvests, both the general fungal saprotrophic and pathotrophic guilds had low relative abundance (Figure 3e–h).

3.5 | Bacterial response to melanization of fungal necromass

Bacterial OTU composition colonizing *M. bicolor* necromass was interactively affected by incubation time, melanization and site (three-way interaction; $Wald_{1,64} = 9.79$; $p = .013$, Table S4). The bacterial communities on both necromass types were dominated by Proteobacteria and Bacteroidetes, averaging 55% and 34% relative abundance across all samples respectively. The five bacterial genera with the highest average relative abundances were *Chitinophaga*, *Mucilaginibacter*, *Luteibacter*, *Burkholderia* and *Pseudomonas* (Figure 5). NMDS plots indicated that the necromass-colonizing bacterial communities separated most strongly by melanization in the pine site during the first month of incubation (Figure S3). Between forest types, the bacterial communities at the oak site generally showed less separation between melanized and non-melanized necromass, but were more dynamic through time relative to those at the pine site (Figure S3).

TABLE 2 Results from the three-way full factorial mixed model testing the effects of incubation time, melanization and site on fungal necromass decomposition (log-transformed % mass remaining)

Fixed effect tests						
Factors	***df***	**DFDen**	***F***	***p***		
Site	1	10	3.7699	.0809		
Melanization	1	50	66.6065	<.0001*		
Incubation time	2	50	23.5373	<.0001*		
Site × melanization	1	50	1.1883	.2809		
Site × incubation time	2	50	0.2179	.8049		
Melanization × incubation time	2	50	1.2228	.3031		
Site × melanization × incubation time	2	50	0.3936	.6767		
Random effect	**Variance ratio**	**Variance component**	***SE***	**95% Lower**	p_{Wald}	**Pct of total**
Block	0.174	0.011	0.010	−0.008	.264	14.830
Residual		0.063	0.013	0.044		85.170
Total		0.074	0.014	0.052		100.000

Block was also included as the random effect. Asterisks indicate significant *p* values for fixed effect tests (α < .05).

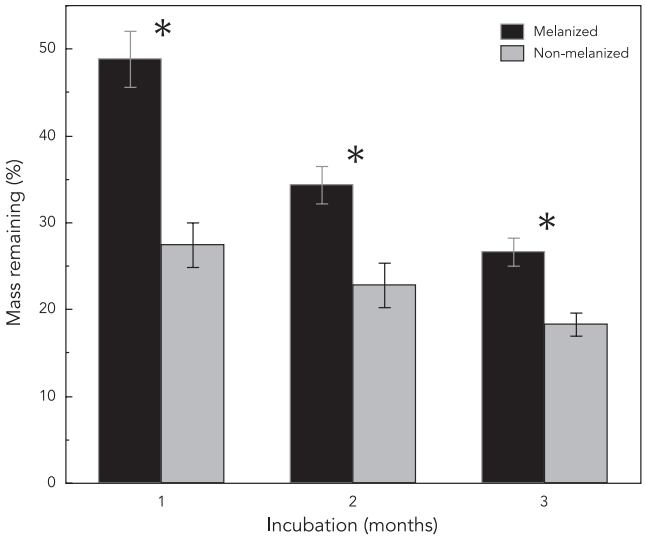

FIGURE 2 Mean per cent mass remaining (±*SE*) of melanized (black bars) and non-melanized (grey bars) *Meliniomyces bicolor* necromass after 1-, 2- and 3-month incubations (*N* = 72). Statistics were conducted using log-transformed data to meet the assumption of linearity of the mixed model. Asterisks represent significant differences (α < .05) between necromass types as determined by post hoc *t* tests for each time slice

4 | DISCUSSION

Consistent with previous findings, we found that variation in biochemical composition had highly significant effects on fungal necromass decomposition rates (Fernandez & Koide, 2014). The necromass of *M. bicolor* that we incubated varied in both melanin and nitrogen content, although the difference in the former was much larger. The positive relationship between nitrogen concentration and necromass decomposition rates has been well-established (Fernandez & Koide, 2014; Koide & Malcolm, 2009), and like lignin in plant litters, melanin dramatically increases necromass recalcitrance (Fernandez & Koide, 2014). Given the significant retardation in decomposition of the melanized compared to the non-melanized necromass, it appears that the changes in melanin concentration have much stronger effects on rates of decomposition than those of nitrogen content. As such, the further study of melanin as a functional effect trait (Koide, Fernandez, & Malcolm, 2014) and its interactions with both biotic (e.g. grazers; Schneider, Renker, & Maraun, 2005) and abiotic soil properties (e.g. mineral-melanin adsorption; Fomina & Gadd, 2003; Gadd & de Rome, 1988) will provide a deeper understanding of exactly how fungal necromass contributes to soil organic matter (Clemmensen et al., 2015; Siletti et al., 2017).

Fernandez et al. (2016) hypothesized that decomposer communities associated with fungal necromass may differ from those in the surrounding soil because fungal mycelia is generally constructed of relatively labile, sometimes unique (e.g. chitin), biochemical components and has narrower carbon-to-nitrogen ratios compared to plant litters. The results from this study support that hypothesis, as we found the fungal communities in soil and on necromass were significantly different in composition. Brabcová et al. (2016) also found that fungal and bacterial communities on decomposing fungal mycelium of *T. felleus* appeared to select for bacterial and fungal decomposers associated with r-selected life-history strategies, which were hypothesized to rapidly take advantage of the relatively large portion of soluble compounds and hydrolysable polysaccharides present in newly added fungal necromass. It is also possible that the necromass used here was devoid of competing microbial colonizers that would be present if naturally grown in the soil, as opportunistic microfungi are able to quickly colonize and take advantage of reduced competitive interactions (Lindahl et al., 2010). While our lack of successful sequencing of bacteria from soil prohibits a similar

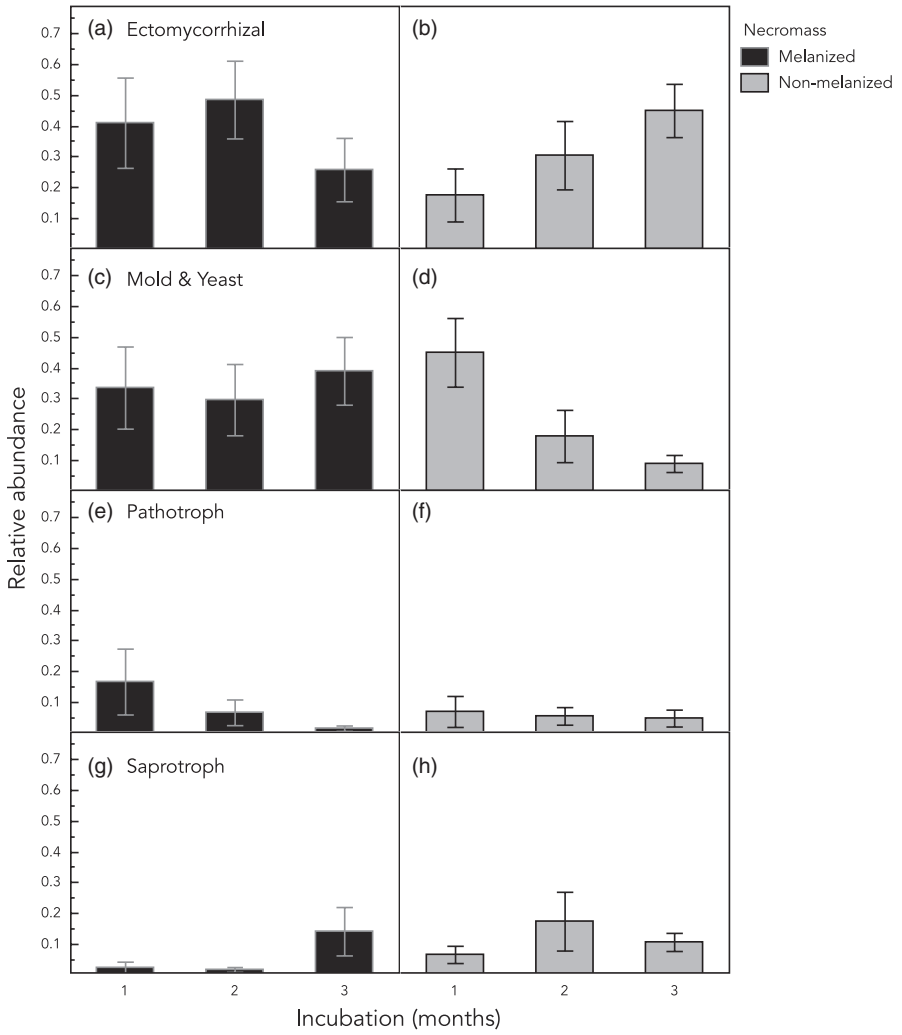

FIGURE 3 Relative abundance of fungal guilds associated with melanized (black bars; a, c, e, g) and non-melanized necromass (grey bars; b, d, f, h) at 1-, 2- and 3-month incubation times

comparison, our fungal results suggest that fungal necromass biochemistry also influences the life-history strategy dynamics of decomposers colonizing necromass. Specifically, the differences in the relative abundance patterns on the melanized and non-melanized necromass over the course of the incubation suggest that necromass chemistry may modify microbial interactions. On non-melanized necromass, we observed a shift from r-strategists to slower growing fungi that is consistent with previously observed successional dynamics in which fungal community structure reflects shifts in resource availability (Baldrian, 2017; Cline & Zak, 2015). Interestingly, the same patterns were not present on the melanized necromass, where co-dominance between r-strategists and slower growing fungi occurred throughout the incubation. We speculate that more recalcitrant nature of the melanized necromass may inhibit competitive dominance by any one fungal group and instead potentially favour cross-feeding between guilds. In future studies, more detailed chemical analyses of the necromass substrate over the course of decomposition will help resolve how the observed microbial community shifts are related to resource availability.

Of the fungal communities colonizing the *M. bicolor* necromass, it was surprising to observe such a high relative abundance of ectomycorrhizal fungi. This result, which was largely consistent between sites, suggests that this fungal guild may play a more central role in the turnover of fungal necromass than previously recognized (Baldrian, 2017; Brabcová et al., 2016; Drigo et al., 2012). There is growing consensus that some ectomycorrhizal fungi are proficient degraders of recalcitrant soil organic matter, due to their ability to mine energetically unfavourable substrates for bound organic nitrogen (Kuyper, 2016; Lindahl & Tunlid, 2015). Previous studies have focused on the enzymatic capacities of the ectomycorrhizal genus *Cortinarius* (Bödeker et al., 2014; Deslippe, Hartmann, Mohn, & Simard, 2011), but we found a particularly high abundance of the ectomycorrhizal genus *Tomentella* on decomposing necromass, suggesting this latter genus may also play an important role in soil organic matter turnover. Because this substrate is rich in nitrogen (Fernandez & Koide, 2014; Koide & Malcolm, 2009), the recycling of nutrients found in necromass may be an advantageous strategy to maximize nitrogen acquisition, which could in turn increase nitrogen immobilization and create stronger sinks for host carbon (Baskaran et al., 2017; Näsholm et al., 2013). In contrast to our expectations, we found that the relatively K-selected general "saprotroph" fungal guild (as opposed to the r-selected yeasts and moulds), which includes many leaf litter and soil saprotrophic fungi, showed relatively low colonization of both necromass types. Unlike more opportunistic saprotrophs, these fungi often specialize on distinct litter types

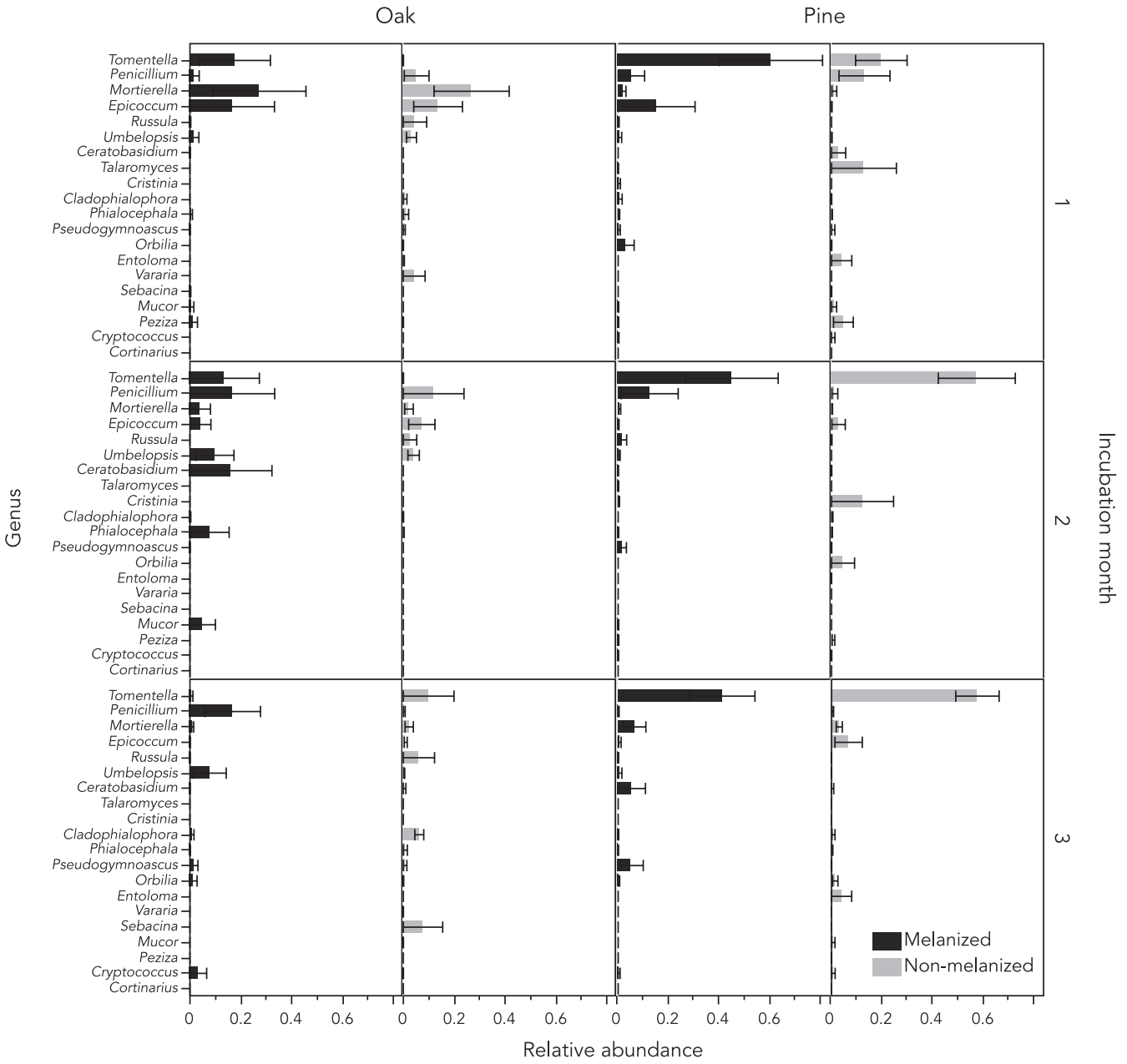

FIGURE 4 Mean relative abundances (±*SE*) of the 20 most abundant fungal genera associated with melanized (black bars) and non-melanized necromass (grey bars) at 1-, 2- and 3-month incubation times at oak and pine sites

at distinct successional stages of decomposition (Frankland, 1998; Osono, 2005; Voříšková & Baldrian, 2013) and/or in distinct soil horizons (Dickie, Xu, & Koide, 2002; Lindahl et al., 2010), which may partly explain their absence on the incubated necromass. An additional explanation may be that these fungal K-strategists become more dominant after 3 months when the remaining mass is composed of mostly recalcitrant fractions. Finally, it is also important to note that relative sequence abundance may not necessarily reflect the functional contribution of these fungi and their contribution to substrate decomposition may still be significant even at low relative abundance (Baldrian et al., 2012).

Of the bacterial communities colonizing the *M. bicolor* necromass, we observed considerable overlap in composition between those present in our study and those in Brabcová et al. (2016). This suggests that there is a widely distributed suite of bacterial necromass specialists present in forest soils (mykolytic bacteria sensu Baldrian, 2017), such as those in the genus *Chinophaga*. We also observed a higher relative abundance of a number of genera on the melanized relative to non-melanized necromass. Of these, the apparent affinities of *Cellvibrio* and *Massilia* towards melanized necromass were particularly interesting given that both genera have members that are well-known degraders of plant tissues. *Cellvibrio* species have been extensively studied for their ability to rapidly degrade many plant cell wall components (Wynne & Pemberton, 1986). Similarly, some *Masillia* species are capable of producing laccase enzymes that are involved in lignin depolymerization (Khalid, Arshad, & Crowley, 2008). We speculate

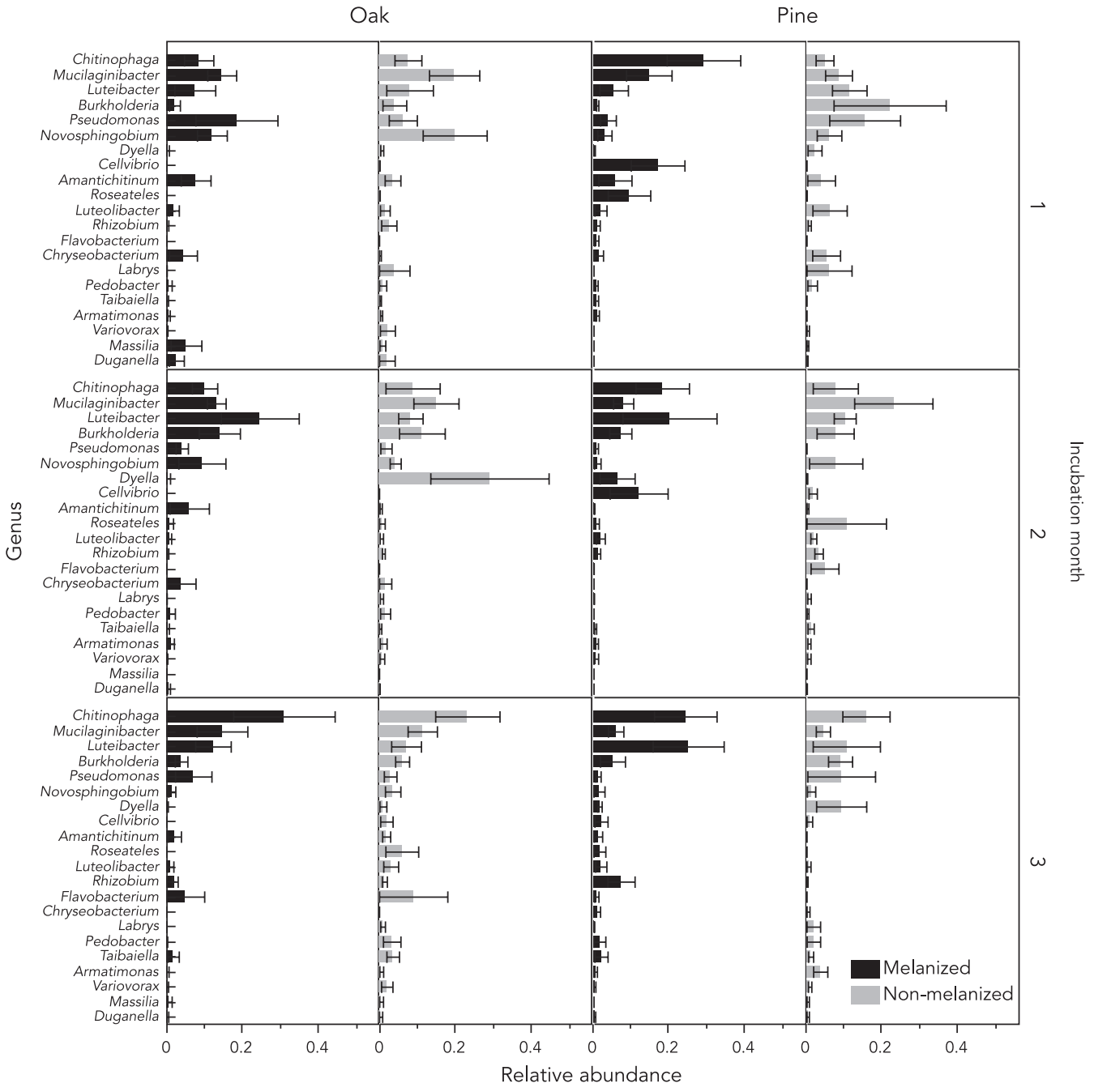

FIGURE 5 Relative abundances (*M* ± 1 *SE*) for the top 20 most abundant bacterial genera associated with melanized (black bars) and non-melanized necromass (grey bars) at 1-, 2- and 3-month incubation times at oak and pine sites

that perhaps the high concentrations of recalcitrant components may favour these bacteria, but it should be noted that these bacteria were in lower relative abundance compared to the most dominant genera that were present on both necromass types.

5 | CONCLUSIONS

Our experimental results are consistent with previous necromass decomposition studies (Fernandez & Koide, 2014) as well as recent observational studies indicating that melanin may serve as a key fungal trait strongly influencing soil carbon cycling (Clemmensen et al., 2015; Fernandez, McCormack, Hill, Pritchard, & Koide, 2013; Siletti et al., 2017). In addition, our results indicate that the biochemical traits of fungal necromass can significantly influence the composition and functioning of necromass-associated decomposer communities. Based on the Azure A assay, the melanin concentrations in the melanized *M. bicolor* necromass averaged more than 20% of the total dry mass. This is a considerable fraction that would be inaccessible to microbial decomposers that do not possess the necessary extracellular enzymatic suites to attack complex aromatic compounds to release carbon and nitrogen bound in the cell wall (Butler & Day, 1998). It has

been suggested that bacteria may play disproportionate role in fungal necromass decomposition (Baldrian, 2017), but given the generally richer suite of oxidative enzymes present in fungal than bacterial communities, our results suggest that the role of fungi, particularly EM basidiomycetes, may increase with necromass recalcitrance. While we did not specifically assess functional differences between these two decomposer groups, a growing number of techniques are currently available to more specifically characterize the relative contributions of different microbial groups (e.g. stable-isotope probing, meta-transcriptomics and proteomics). As such, future studies examining the decomposition of fungal necromasses with varying biochemical composition and quantifying resource use in a microbial group-specific manner will be key to more closely linking the structure and functioning of necromass-associated decomposer communities.

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AUTHORS' CONTRIBUTIONS

C.W.F. and P.G.K. conceived and designed the experiment. C.W.F. collected data. C.W.F. and P.G.K. analysed the data and wrote the manuscript.

DATA ACCESSIBILITY

Fungal and bacterial sequence data can be accessed through the NCBI short-read archive (Accession: PRJNA400233 and PRJNA399749 respectively). All other data associated with this study can be accessed through Dryad Digital Repository: https://doi.org/10.5061/dryad.5f5g8 (Fernandez & Kennedy, 2017).

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SUPPORTING INFORMATION

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