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Functional Ecology

RESEARCH ARTICLE

Bacterial and fungal growth on fungal necromass and its diverse components: Shared profiles and divergent constraints revealed by high-throughput phenotyping

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Abstract

1. While fungal necromass is increasingly recognized as a major source of persistent carbon (C) in soils, the relative functional roles of bacteria and fungi in decomposing necromass are not fully resolved, and the processes that select for necromass decomposer communities from the broader soil microbial community are an emerging area of interest.
2. In this study, we characterized the growth of 52 bacterial and 83 fungal strains isolated from necromass and soil on 22 C substrates, including different necromass phenotypes, fungal cell wall polymers, dimers and monomers.
3. We found that the isolation habitat of the strains used in this experiment (necromass vs. soil) had no effect on the substrates they were able to use. Isolates from both microbial domains were able to grow on different labile carbon substrates, polymers and necromass phenotypes. However, fungal growth was most limited by necromass melanin content, while bacterial growth was more limited by the abundance of cell wall polysaccharides. Additionally, overall differences in substrate use between bacteria and fungi were most pronounced on polymer substrates.
4. Collectively, our results suggest that there is substantial functional overlap in necromass substrate use across microbial domains, but some notable differences in bacterial and fungal utilization of cell wall polymers, which can function as a direct energy source or a means of accessing other compounds within necromass. Future studies assessing bacteria and fungi decomposing necromass together rather than in isolation will help to uncover potential physical and chemical

interactions within and between these two domains during the decay of this important source of persistent soil C.

KEYWORDS
dead fungal biomass, decomposition, functional diversity, phenotypes, soil microbes, substrate use

1 | INTRODUCTION

Soils are spatially and chemically complex environments that support highly diverse microbial communities (Fierer & Lennon, 2011; Torsvik & Øvreås, 2002). They are physically complex at both micro and macro scales, with a range of aggregate sizes, soil textures and porosities that vary key environmental conditions such as water holding capacity and aeration (Rawlins et al., 2016; Vogel, 2000; Young & Crawford, 2004; Young & Ritz, 2000). These wide-ranging physical properties create a variety of microhabitats that contain a diverse assortment of energy sources and nutrients available to microbes (Bach et al., 2018; Rabbi et al., 2016; Ruamps et al., 2011). The bioavailable constituents of soil are found in soil organic matter (SOM), the largest terrestrial carbon pool (Scharlemann et al., 2014). Soil organic matter is chemically diverse, containing larger molecules like polysaccharides and lignin, polypeptides and lipids and smaller aliphatic biopolymers that require different amounts of energy to break down (Kleber & Johnson, 2010; Lehmann et al., 2020; Six et al., 2001). The molecular and physical heterogeneity of SOM creates niches for millions of microbial species, which along with high spatial complexity, make soils one of the most diverse microbial environments on Earth (Papke & Ward, 2004; Rousk et al., 2010; Zhou et al., 2002).

Within SOM, dead microbial biomass (necromass) is a chemically varied resource pool of C- and N-based compounds that has received increased study due to the recognition that a significant portion of the most persistent soil C is microbial in origin (Angst et al., 2021; Liang et al., 2019; Wang et al., 2021). Soon after microbial death, necromass contains many labile substrates, including amino acids, nucleic acids and soluble sugars (Ryan et al., 2020; See et al., 2021). However, as necromass decomposes, many of its labile components are readily consumed by saprotrophs, leaving behind microbial residues enriched in the structural compounds of their cell walls (Ryan et al., 2020; See et al., 2021). These microbial cell wall components are primarily derived from mycelial residues, which account for ~65% of total soil microbial necromass C (Wang et al., 2021). As such, decomposed necromass includes fungal cell wall polymers such as chitin, glucan, mannan and melanin (Fontaine et al., 2000; Godbold et al., 2006). Many previous field and laboratory studies have found that the rate of fungal necromass decomposition is strongly influenced by initial substrate chemistry, with necromass higher in N-rich compounds (amino acids and sugars, nucleic acids, soluble proteins) decaying more rapidly (Brabcová et al., 2018; Koide & Malcolm, 2009; See et al., 2021) and necromass higher in melanin decaying much more slowly (Fernandez et al., 2019; Fernandez & Koide, 2014; Maillard, Michaud, et al., 2023; See et al., 2021). Overall, as necromass decays, microbes are exposed to compounds along a gradient of molecular complexity, beginning with undecayed necromass containing a variety of labile soluble monomers and dimers, and over time becoming enriched with more recalcitrant cell wall fragments and polymers.

Alongside necromass chemical properties, soil microbial communities have also been identified as strong predictors of fungal necromass decay rates (Beidler et al., 2025; Maillard, Beatty, et al., 2023). Decomposing necromass is populated by a compositionally distinct portion of the soil microbial community (Kennedy & Maillard, 2023) termed the necrobiome (Beidler et al., 2020). This community of bacteria and fungi colonizes necromass rapidly, with early decomposition dominated by copiotrophic bacteria such as *Pseudomonas*, *Chitinophaga* and *Pedobacter*, fast-growing fungi like *Trichoderma* and *Penicillium*, yeasts including *Apiotrichum* and moulds such as *Umbelopsis* and *Mortierella* (Beidler et al., 2020; Brabcová et al., 2016; Fernandez & Kennedy, 2018). The necrobiome community shifts notably over the course of decomposition as necromass is chemically transformed (Maillard et al., 2021; Ryan et al., 2020). In the later stages of decomposition (e.g. beginning ~1 month after senescence) there is an increase in oligotrophic bacteria as well as many saprotrophic, mycoparasitic and ectomycorrhizal fungi (Beidler et al., 2020; Fernandez & Kennedy, 2018; Maillard et al., 2021). This pattern of necrobiome community succession consistently occurs across a wide geographical range of temperate and boreal forests, indicating a common 'core' community of microbial taxa is associated with decaying necromass (Cantoran et al., 2023). Although the necrobiome is a compositionally distinct subset of the soil microbiome, the selective processes that determine necrobiome community assembly remain relatively unclear (Kennedy & Maillard, 2023). One possible explanation driving the differential assembly of microbes onto necromass is their greater ability to decompose necromass-derived compounds relative to bulk soil inhabiting communities. Support for this possibility comes from functional screenings of plant residues, which suggest that the microbial community adapts to decompose specific plant-derived compounds (Mieszkin et al., 2021). This study investigates whether the composition of the necrobiome similarly results from a functional shift of the soil microbial community to necromass-derived substrates.

Here, we measured the growth of an ecologically diverse set of bacteria and fungi on four necromass phenotypes and multiple soluble and insoluble chemical components of fungal cells. We chose

substrates to represent a range of molecular complexity, including monomers like glucose and mannose that are readily taken up by microbes and polymers such as chitin and glucan that require extracellular enzymes to decompose (Table 1). The necromass phenotypes included necromass with low- and high-melanin content to directly manipulate biochemistry. Additionally, each of these melanin phenotypes contained whole cell (unwashed) as well as cell wall-enriched (washed) versions to mirror early- and late-stage decayed necromass. By stripping away simple sugars, lipids and intracellular compound and leaving primarily cell wall fragments remaining, the washing resembles more decayed necromass (Ryan et al., 2020). Using microplate-based growth assays that included compounds unavailable in commercial microbial growth assays, we characterized the growth of 52 and 83 strains of bacteria and fungi, respectively, on 22 C substrates. The microbial strains were isolated from both necromass as well as surrounding soils, allowing for a comparison of how members of the necrobiome grew across substrates relative to microbes isolated from soil.

We tested the following four hypotheses: (1) bacterial and fungal strains isolated from necromass will grow better on all substrates than strains isolated from soil, given that they represent a distinct subset of the soil microbiome that may potentially be functionally adapted to necromass substrates, (2) bacteria and fungi will grow better on whole cell necromass compared to cell wall-enriched necromass, as the latter requires specific hydrolytic and oxidative enzymatic systems involved in cell wall polymer decomposition, (3) bacteria and fungi will grow better on low-melanin necromass compared to high-melanin necromass, due to the high biochemical recalcitrance of melanin and its ability to complex with other compounds and (4) bacterial and fungal growth on individual cell wall polymers will better predict growth on necromass than growth on monomers and dimers, due to polymers comprising a significant portion of necromass by mass.

1.1 | Replication statement

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Fungi or Bacteria	Strain	83 fungi, 52 bacteria

2 | MATERIALS AND METHODS

2.1 | Study design

We screened the growth of bacterial ($n=52$) and fungal strains ($n=83$) from a culture collection of microbes derived from necromass and soil on 22 substrates. The substrates were divided into three categories: (1) necromass phenotypes ($n=4$), (2) fungal polymers ($n=5$) and (3) monomers and dimers ($n=13$) (Table 1). Note that the 4 necromass phenotypes are denoted throughout as low melanin (LowMel) or high melanin (HighMel) and either whole cell versions (WC) or cell wall-enriched versions (CW). Finally, note that while glycogen was categorized as a fungal polymer, it is the sole soluble polymer in this study. The methods are presented below and can be visualized in Figure S1 in Supporting Information.

2.2 | Isolation of microbial strains from necromass and soil

The bacterial and fungal strains used in this study were isolated from *Hyaloscypha bicolor* necromass decomposed at the Cedar Creek Ecosystem Science Reserve in Minnesota, USA during Fall 2021. We aliquoted 100 mg of either high- or low-melanin *H. bicolor* necromass (see below for details on *H. bicolor* necromass production) into 53 μm polyethylene mesh bags (R510; ANKOM Technology, NY, USA) and then buried them in the top 5 cm of soil in 12 subplots at a *Pinus strobus* dominated forest stand (the same pine site as Fernandez & Kennedy, 2018; Maillard, Michaud, et al., 2023). Bags were harvested after 1 month of incubation, a duration that was previously demonstrated to be sufficient for considerable decomposition and microbial colonization (Fernandez & Kennedy, 2018; Maillard, Beatty, et al., 2023). Additionally, we collected a 5 cm depth × 2.5 cm width soil core directly adjacent to the bags at harvest. As such, strains were isolated from one of three habitats: (1) high-melanin *H. bicolor* necromass, (2) low-melanin *H. bicolor* necromass or (3) soils adjacent to both bags. The microbial communities were compositionally distinct in each of these habitats for bacteria and between necromass and soil for fungi (Figure S2).

For each habitat, we suspended 1 g of either necromass or soil in 20 mL of sterile water, vortexed the suspension for 2 min and then serially diluted it from 10^{-1} to 10^{-5}. For bacterial isolations, we used 10% Tryptic Soy Agar (pH 5) amended with 100 mg/L cycloheximide. For fungal isolations, we used a Modified (no malt extract) Melin-Norkans medium amended with 100 mg/L streptomycin sulphate. We inoculated each plate with 150 μL of each dilution, and after 5 days, randomly selected bacterial and fungal colonies to streak to isolation. Successful isolation and sequence identity were confirmed via Sanger sequencing using the 27F-1492R primer pair to amplify the 16S rRNA gene for bacteria (Weisburg et al., 1991), or the ITS1F-ITS4 primer pair to amplify the ITS rRNA gene region for fungi (Gardes & Bruns, 1993). The soil samples were also characterized with high-throughput sequencing (HTS) of the 16S and ITS2 regions for bacteria and fungi respectively. The HTS methods, including bioinformatic processing, followed exactly the methods detailed in Beidler et al. (2020). The raw sequencing files are available in the NCBI Short Read Archives under BioProjects PRJNA1231136 (16S) and PRJNA1233824 (ITS).

The strains obtained spanned a diverse set of lineages (Table 2), with the full list presented in Table S1. While they do not reflect the high diversity of communities present in field settings, about 10% of the bacterial and 15% of the fungal genera in our field communities were represented by strains we phenotyped (Table 2). Weighted by

TABLE 1 Carbon substrates screened for bacterial and fungal growth.

Substrate	Solubility	Chemical class	Monomer	Abundance in necromass
No C control	Soluble	Monosaccharide		10%
D-(+)-Glucose				
D-(+)-Mannose				
D-(+)-Galactose				
D-(+)-Xylose				
D-(+)-N-Acetyl-Glucosamine (NAG)				
D-(+)-Glucosamine				
D-(+)-Trehalose dihydrate		Disaccharide	Glucose	
Glycerol		Sugar alcohol		
D-Mannitol				
D-Xylitol				
D-(+)-Arabitol				
Meso-erythritol				
Myo-inositol				
Glycogen from bovine liver		Polysaccharide	Glucose	
ß-1,3-Glucan from *Eugenia gracilis*	Insoluble		Glucose	80%
α-Chitin from shrimp shells			NAG	
Chitosan			NAG+Glucosamine	
Melanin from *Sepia officinales*		Polymer	DHI	1%–10%
Low-melanin cell wall-enriched necromass		Necromass		
High-melanin cell wall-enriched necromass				
Low-melanin necromass	Mixed			
High-melanin necromass				

Note: Substrates are categorized by their solubility and chemical class, and their relative abundance in *H. bicolor* necromass approximated.
Abbreviations: DHI, 6-dihydroxyindole; L-DOPA, l-3,4-dihydroxyphenylalanine; NAG, N-acetyl-glucosamine.

TABLE 2 Comparison of the taxonomy and diversity of bacteria and fungi in culture used in this study relative to those present based on DNA sequencing of soil at the same field site.

	Bacteria		Fungi	
Taxonomy	Culture	Field	Culture	Field
Phylum	*n* = 3 *H*: 0.90	*n* = 20 *H*: 2.15	*n* = 3 *H*: 0.84	*n* = 11 *H*: 1.55
Class	*n* = 7 *H*: 1.80	*n* = 38 *H*: 2.76	*n* = 8 *H*: 1.80	*n* = 24 *H*: 2.36
Order	*n* = 8 *H*: 1.94	*n* = 42 *H*: 2.59	*n* = 11 *H*: 1.81	*n* = 51 *H*: 2.99
Genus	*n* = 19 *H*: 2.80	*n* = 198 *H*: 3.24	*n* = 19 *H*: 2.55	*n* = 115 *H*: 2.98
OTU	*n* = *52* *H*: 3.95	*n* = 1306 *H*: 7.17	*n* = 83 *H*: 4.42	*n* = 457 *H*: 6.12

Note: Both the total counts and diversity (calculated as the Shannon-Wiener Index, *H*) are shown at each taxonomic level.

relative abundance, 30% of the total bacterial sequence reads and 50% of the total fungal sequence reads in the field communities belong to genera in the culture collection (Table 2). Additionally, the top three most abundant bacterial phyla (Proteobacteria, Bacteroidetes and Actinobacteria) and fungal phyla (Ascomycota, Basidiomycota and Mucoromycota) in the field communities were phyla well represented in the culture collection.

2.3 | Necromass generation

We generated necromass of *Hyaloscypha bicolor* in the laboratory using methods described in Fernandez and Kennedy (2018). *H. bicolor* is a cosmopolitan mycorrhizal fungus found in forests (Grelet et al., 2009; Kohout et al., 2011; Martin, 2016) and has been utilized as a model substrate for several previous necromass studies due to its phenotypic plasticity in melanin content (Fernandez & Kennedy, 2018; Maillard, Michaud, et al., 2023). Manipulating growth conditions including oxygen availability and shaking speed generates an ~4-fold difference in melanin content in *H. bicolor* phenotypes (Fernandez & Kennedy, 2018; see Figure S1 for visual differences in melanization). Here, we first grew *H. bicolor* on cellulose membranes on 50% Potato Dextrose Agar plates. We inoculated 3 cm diameter plugs into 125 mL flasks filled with either 40 mL (low melanin) or 80 mL (high melanin) of Potato Dextrose

Broth and shook them for 1 month at 20°C at either 150 or 250 rpm, respectively. At harvest, we removed residual media by washing *H. bicolor* mycelia over sterile sieves with deionized water. The collected mycelia were then homogenized and killed by first freezing at −20°C for 24 h, followed by freeze-drying at −50°C for 72 h. After freeze-drying, the *H. bicolor* mycelia were ground to a fine powder using a mortar and pestle.

To create cell wall-enriched necromass, we followed the heating and alkali washing methods described in Fontaine et al. (2000). In short, necromass was flash-frozen in liquid nitrogen, treated with 1:30 (w:v) of 4M NaOH solution and then incubated for an hour at 100°C. Residual NaOH was rinsed away with sterilized deionized water until the necromass reached pH 7. This process was intended to enrich for cell wall polymers, removing nuclei, cytoplasmic elements and lipids from the necromass.

Three independent replicates of each necromass phenotype were analysed for total C and N using an Elemental Analyser (Costech Analytical Technologies Inc) and for biochemical composition through Fourier-transform infrared spectroscopy (FTIR; see Fernandez et al., 2019 for specific details regarding sample preparation). FTIR spectra for *H. bicolor* necromass were annotated for four specific functional groups (amide, aliphatic, aromatic and polysaccharides) based on Maillard, Pflender, et al. (2023).

2.4 | Bacterial growth assay

We dissolved 3.4 g/L of each soluble substrate into a modified M9 minimal medium (without glucose) amended with 0.5 g/L casein hydrolysate and dispensed 180 µL into 96-well flat-bottom plates (Caplugs Evergreen Labware Products, Buffalo, NY). We ground the insoluble substrates with a mortar and pestle and suspended them in M9 at double the concentration of the soluble substrates (at 6.8 g/L) to account for the heterogeneity of the substrate in liquid and the centre of the wells being removed. We added 25 g/L agar to the insoluble substrates so that the media would solidify firmly. We then filled 96-well plates with 150 µL of this solid media using wide-bore pipette tips while the agar was molten. Once the agar solidified, we removed the centre of each well using a flame-sterilized 4 mm cork-borer and filled it with 180 µL M9 so that bacterial cultures could be inoculated in the centre of each well and optical density read. Each plate accommodated four replicates per substrate and strain combination in fixed locations (Figure S3). Controls consisted of each bacterial strain inoculated into M9 control without an added C source to measure baseline microbial activity and non-inoculated wells filled with M9 plus each insoluble substrate to account for abiotic leaching of insoluble substrates into the centre of the wells. We inoculated the remainder of wells in plates with 20 µL of bacterial cells in mid-log phase, shook them at 150 rpm at 25°C for 4 days and then measured optical density (OD_{600}). Final growth was normalized by OD_{600} at time zero, abiotic uninoculated controls and inoculated no C controls.

2.5 | Fungal growth assay

The fungal growth assays consisted of two parts. First, we grew the fungi in 96-well plates using the same set of complex and simple substrates as in the bacterial screening (Figure S3). After the growth period, the biomass produced was assessed using a tetrazolium salt as an indicator of cell abundance, utilizing the WST-8 stain from the WST-8 Cell Counting Kit (Abcam, Cambridge, MA). This dye employs a colourimetric reaction to estimate cell counts, as tetrazolium is reduced to orange formazan by cellular dehydrogenases in active cells. While not an exact measure of cell counts, we used this reporter dye as a proxy for fungal growth. We chose this method for fungi because, unlike bacterial cultures that grew homogeneously in liquid culture (providing reliable direct proxies of growth using OD), fungal cultures generally exhibited aggregated mycelial growth, which did not allow for reliable direct measures of growth using OD.

Briefly, we dissolved 5 g/L of each substrate in modified M9 medium, and 180 µL was dispensed into round-bottom 96-well plates following Figure S3, with four technical replicates per substrate per fungal strain. Fungal cell suspensions were normalized to an OD_{600} of 0.2 and diluted 1:100 before being inoculated into the plates, and 20 µL was dispensed into each well. We grew fungi for 6 days, pelleting fungal biomass on Day 7 by centrifugation (5 min at 4000 g) and resuspending in 200 µL of M9 medium containing 5 g/L glucose as the C source and 20% WST-8. The 96-well plates were incubated in the dark at 22°C and read after 10 h of incubation with the WST-8 dye. All results were normalized based on the average growth across substrates (see below for the normalization approach). We centrifuged plates again for 5 min at 4000×*g*, and 100 µL of the supernatant was collected and transferred into flat-bottom 96-well plates for spectrophotometric reading at OD_{460}.

We normalized fungal growth data by subtracting the control treatment, which received an initial inoculum but no C source, as we observed some WST-8 activity likely due to the brief incubation in glucose to activate cell metabolism before WST-8 quantification. Further, WST-8 results were normalized by the average growth on all substrates for each fungal strain to account for differences in metabolic activity affecting WST-8 results, thereby allowing comparison between fungal strains. This was employed as an analogue to the average cell well colour development normalization technique commonly used on Biolog plates (Garland & Mills, 1991).

2.6 | Statistical analyses

All data were analysed and visualized in R 4.3.0 (R Core Team 2023). For the FTIR and total C and N data, we used an Analysis of Variance (ANOVA) to assess differences between necromass phenotypes in total C, N and FTIR functional group abundances. To analyse our substrate assays, we started by analysing the bacterial and fungal datasets separately. All data were transformed to fit assumptions of normality (log-transform for fungal data). We used linear mixed models, with strain identity as a random effect and either (i) substrate or

(ii) isolation habitat as fixed effects. For each of these analyses, we used ANOVAs to compare microbial growth across substrates and among substrate categories (i.e. necromass phenotypes, polymers and monomers and dimers). We used the *emmeans* package to conduct all post-hoc Tukey tests (Lenth, 2024). Finally, we merged bacterial and fungal datasets by applying the same normalization technique to the bacteria as the fungi (dividing each strain's growth on a given substrate by the average of its substrate growth across substrates and log-transforming). We then assessed how well microbial domain predicted substrate use across substrates and within each substrate category using NMDS and PERMANOVA, with the *vegan* package in R (Oksanen et al., 2001). Additionally, we calculated dissimilarity scores between domains versus points and assessed beta dispersion to determine the homogeneity of variance between domains.

3 | RESULTS

3.1 | Effect of isolation habitat on microbial growth

Isolation habitat did not predict substrate use for either bacteria or fungi, with microbes generally growing similarly regardless of whether they were isolated from soil, low-melanin necromass or high-melanin necromass (Figure 1). There were also no significant interactions between habitat and the three substrate categories (i.e. necromass phenotypes, fungal polymers, monomers and dimers), suggesting that the lack of habitat effect was robust to the type of substrate (Figure S5). As such, habitat was disbanded as a grouping variable, and strains across habitats were pooled for all subsequent analyses.

3.2 | Chemical analysis of necromass

Regarding the biochemical properties of the substrates used as primary C source, each of the 22 substrates assayed contained ca. 40% C except for melanin, which contained about 60% C. Among the four necromass phenotypes, the total amount of C did not differ significantly; however, cell wall-enriched necromass contained significantly less N than whole cell necromass ($F_{3,8}=868.20$, $p<0.05$) regardless of the melanization level (Table 3). Cell wall-enriched necromass was also significantly depleted in amides (Peak 1650) and aliphatics (Peaks 2850 and 2924) relative to whole cell necromass (Figure S4). In contrast, the polysaccharide: aliphatic ratios of cell wall-enriched necromass were significantly higher than whole cell necromass ($F_{1,10}=11.20$; $p<0.01$), but the ratios were not different between high- and low-melanin necromass (Figure S4). Aromatic peaks varied across necromass phenotypes, with some being higher in whole cell necromass (Peak 1250; C-O) and others being higher in cell wall-enriched necromass (Peak 1425; C=C).

3.3 | Evaluation of microbial growth across substrates

Across the 22 substrates, bacterial strains grew on 15±5 substrates (mean±S.E.), whereas fungal strains grew on 15±4 substrates. The substrates used were largely overlapping, with both bacteria and fungi growing most on glucose and over 80% of

TABLE 3 Total % C and N in each necromass phenotype.

Necromass phenotype	% C	% N
Low-melanin whole cell	44.60±1.98	3.74±0.19
High-melanin whole cell	42.88±1.71	3.13±0.04
Low-melanin cell wall-enriched	42.27±0.83	0.050±0.01
High-melanin cell wall-enriched	43.54±0.73	0.250±0.04

Note: Values represent the average of three replicates±standard deviation.

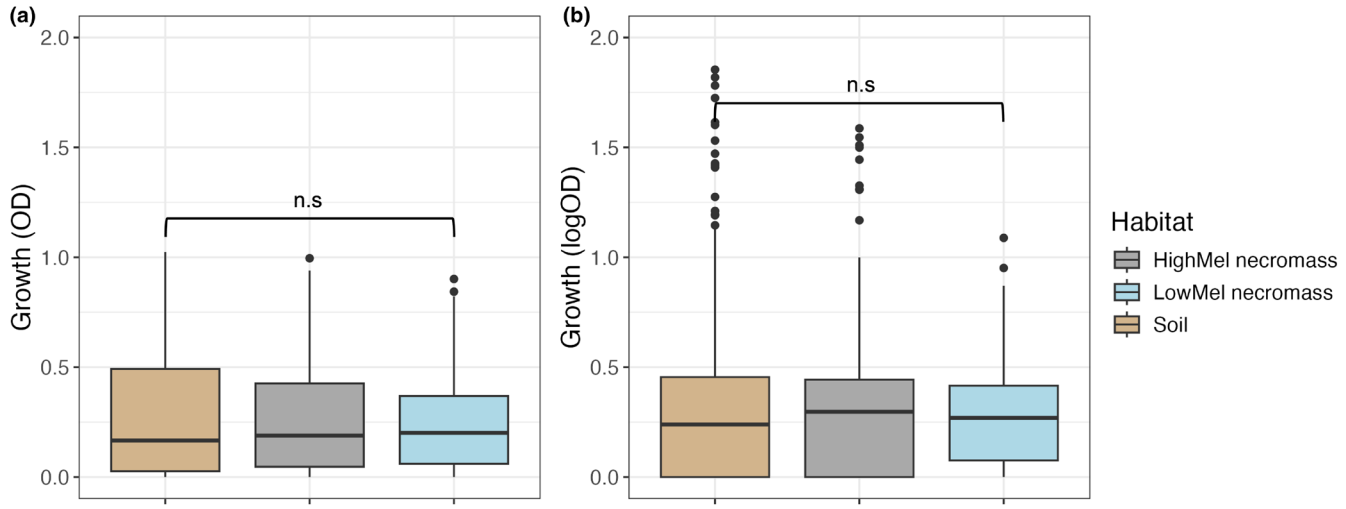

FIGURE 1 Growth of (a) bacterial and (b) fungal strains on all substrates by isolation habitat. n.s. indicates no significant difference based on a Tukey's HSD means comparison test on a linear mixed model of growth with strain as a random effect and habitat as fixed effect.

the microbial strains growing on at least one of the low-melanin necromass phenotypes (Figure 2). Microbial growth was most limited on select sugar alcohols and structural polymers such as chitin, melanin and glucan (Figure 2). Aggregating across strains, both bacteria and fungi grew on all four necromass phenotypes, though bacteria exhibited a significant preference for whole cell necromass ($F_{3,141} = 44.94$; $p < 0.01$) regardless of melanization level (Figure 3A), while fungal strains grew significantly better on low-melanin necromass ($F_{3,246} = 19.36$; $p < 0.01$) regardless of cell wall type (Figure 3B). Neither bacterial growth nor fungal growth varied significantly across the 4 insoluble polymers, though fungal growth was significantly higher on the soluble polymer (glycogen) than the rest ($F_{4,328} = 35.03$; $p < 0.01$) (Figure S6A,B). Microbial growth preferences did, however, vary among most monomers and dimers. Bacteria grew preferentially on simple sugars such as galactose, mannose, glucose, mannitol and N-acetyl-glucosamine (Figure S6C), while fungi grew most on N-acetyl-glucosamine, glucosamine, arabitol, glucose and trehalose (Figure S6D).

3.4 | Correlation analyses of growth between substrates

Growth on all fungal polymers (glucan, chitin, glycogen, chitosan and melanin) strongly predicted growth on all necromass phenotypes for bacteria (Figure 4a), and all but glycogen and glucan did for fungi as well, but only on whole cell necromass (Figure 4b). In contrast, growth on monomers and dimers did not correlate strongly with

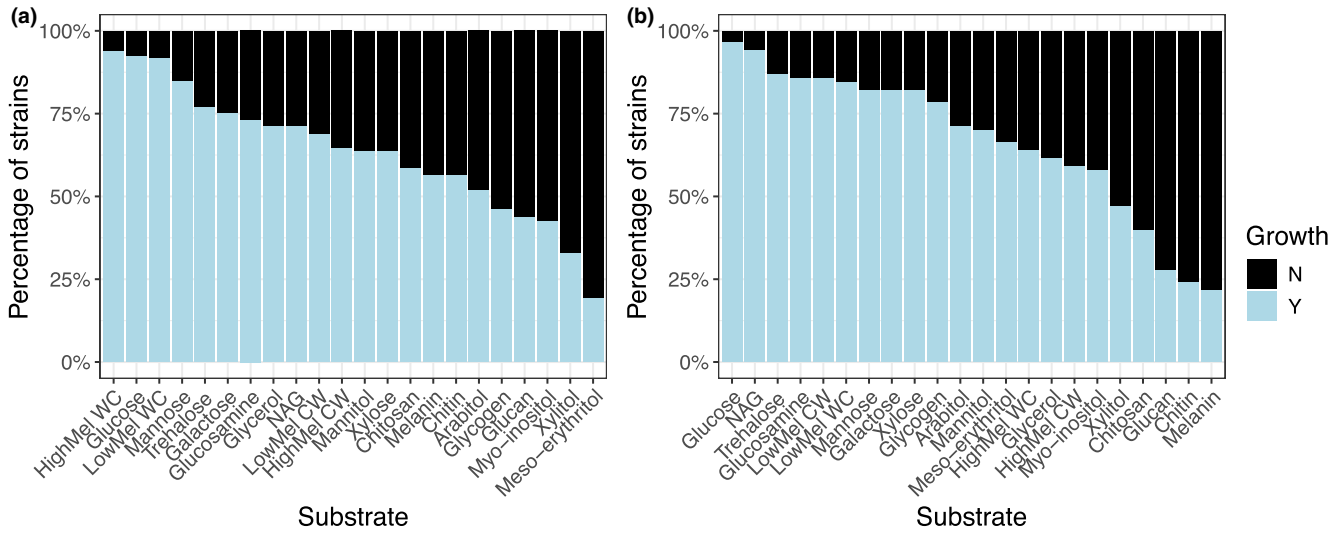

FIGURE 2 Percentage of (a) bacterial and (b) fungal strains that grew across 22 carbon substrates. N (in black) denotes no growth, while Y denotes growth occurred. A threshold of Δ OD > 0.1 was used to determine growth.

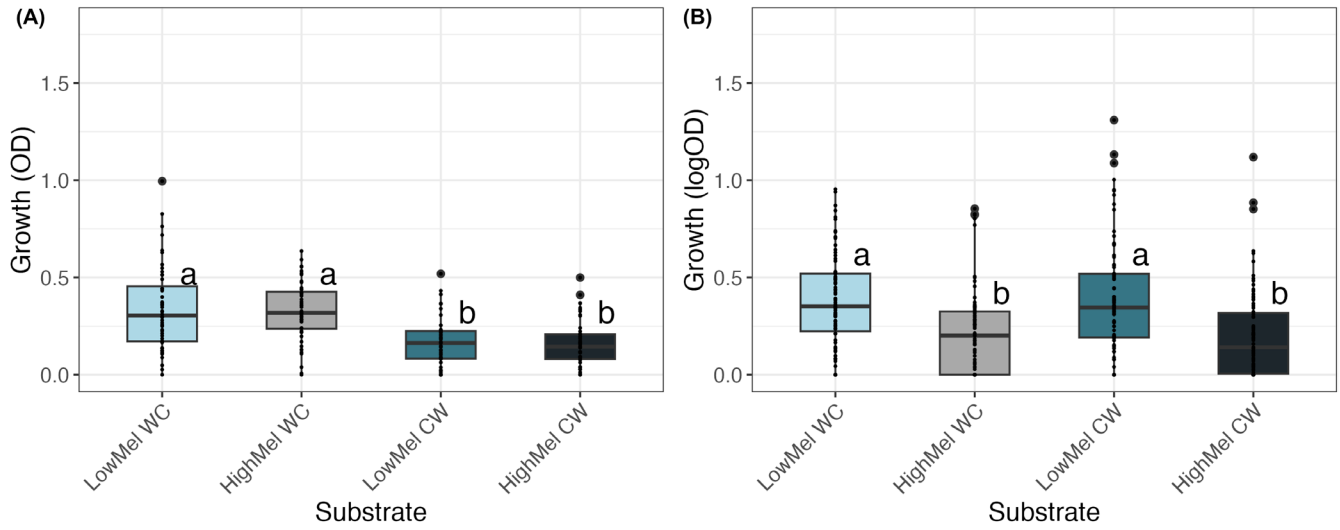

FIGURE 3 Growth of (A) bacterial and (B) fungal strains on necromass phenotypes. Letters indicate significant differences based on a Tukey's HSD means comparison test on a linear mixed model of growth with strain as a random effect and substrate as a fixed effect.

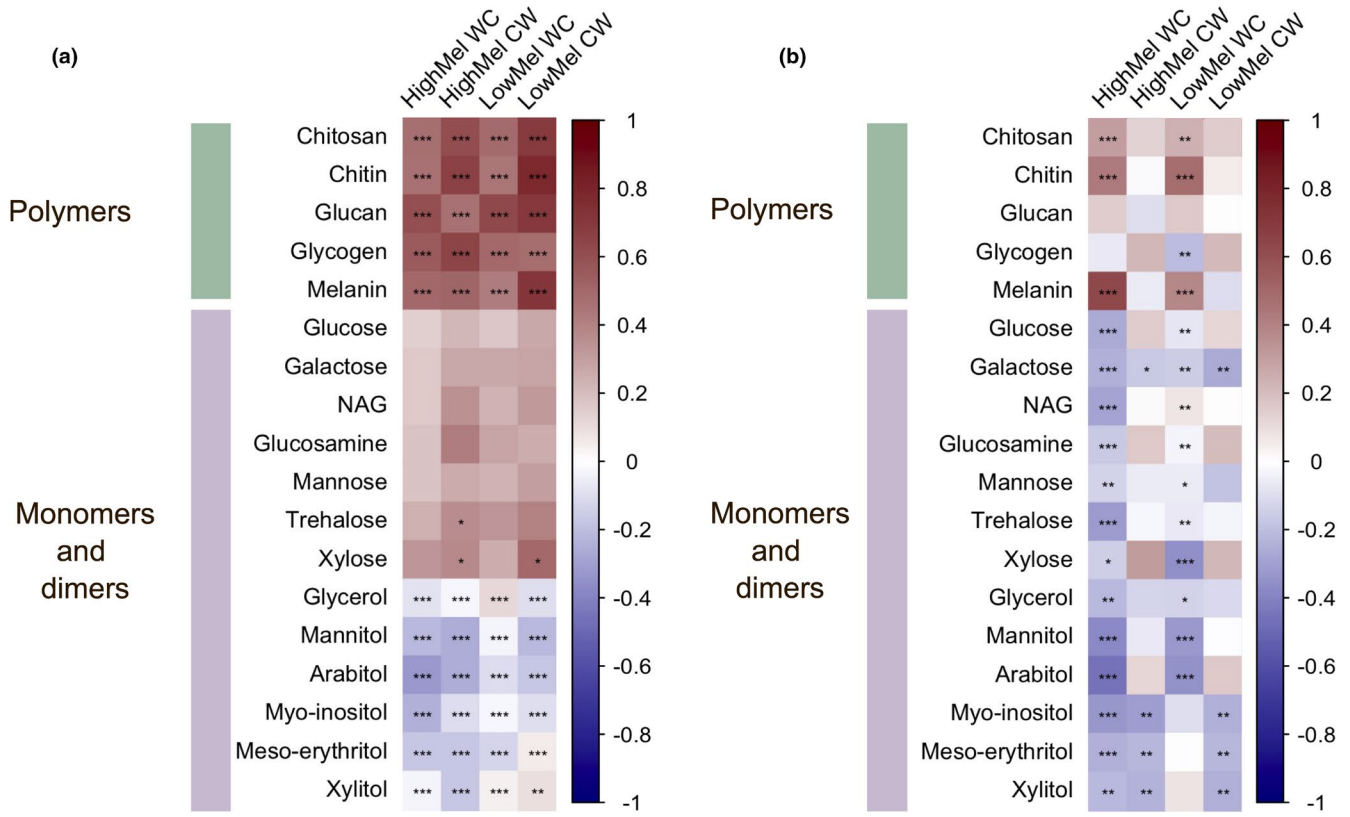

FIGURE 4 Correlations between (a) bacterial and (b) fungal growth on polymers, monomers and dimers with necromass phenotypes. Colours ranging from red to blue indicate the strength of the correlation (*R* from 1 to −1) based on a Pearson correlation coefficient, while asterisks indicate *p* values (**p* < 0.05, ***p* < 0.01, ****p* < 0.001).

growth on necromass for either bacteria or fungi. Additionally, there were consistent neutral or negative correlations between microbial growth on necromass and sugars/sugar alcohols.

3.5 | Evaluation of microbial growth across taxonomic groups

Bacterial growth varied across phyla on necromass phenotypes ($F_{2,45} = 9.87$; $p < 0.01$) and polymers ($F_{2,45} = 15.49$; $p < 0.01$), but not on soluble monomers or dimers. Growth on necromass phenotypes was highest in Bacteroidetes, with strains in the genera *Pedobacter* and *Flavobacteria* being most responsive (Figure 5A). There was, however, high intrageneric variation and few significant differences between genera, suggesting that genus was not a strong proxy for predicting bacterial substrate use across the substrates assayed. For fungi, there was significantly more growth by members of the Basidiomycota on polymers ($F_{2,79} = 6.23$; $p < 0.01$), but no difference on necromass phenotypes. Additionally, there was significantly more growth on monomers and dimers by strains of the Ascomycota ($F_{2,79} = 4.51$; $p = 0.01$). At the genus level, fungal growth on necromass was highest in *Pseudogymnoascus*, *Phacidium*, *Mucor* and *Apiotrichum* species (Figure 5B). On polymers, growth was highest in two basidiomycete yeasts, *Apiotrichum* and *Saitozyma* (Figure 5D), whereas growth on monomers and dimers was greatest in *Penicillium* and *Aspergillus* species as well as *Apiotrichum* (Figure 5F). Like bacteria, there was high variation in fungal growth within genus and few significant differences between genera.

3.6 | Comparison of substrate use profiles in bacteria and fungi

Multivariate analyses of the substrate use profiles using the combined bacterial and fungal datasets indicated that microbial domain did significantly predict substrate use on necromass phenotypes (Figure 6a; $F_{1,125} = 6.84$; $p < 0.01$) and on polymers (Figure 6b; $F_{1,122} = 27.84$; $p < 0.01$). Betadisper analyses suggested that variance for each domain was not different for polymers, but was for necromass phenotypes ($F_{1,125} = 11.24$; $p < 0.01$), with fungi having significantly greater variance than bacteria. Domain did not significantly predict growth on either soluble monomers or dimers, and betadisper was also not significant. Supporting these findings, the average dissimilarity score between centroids was lower than the dissimilarity among all points for necromass phenotypes (0.28 vs. 0.70), polymers (0.67 vs. 0.94) and monomer and dimer substrates (0.10 vs. 0.54).

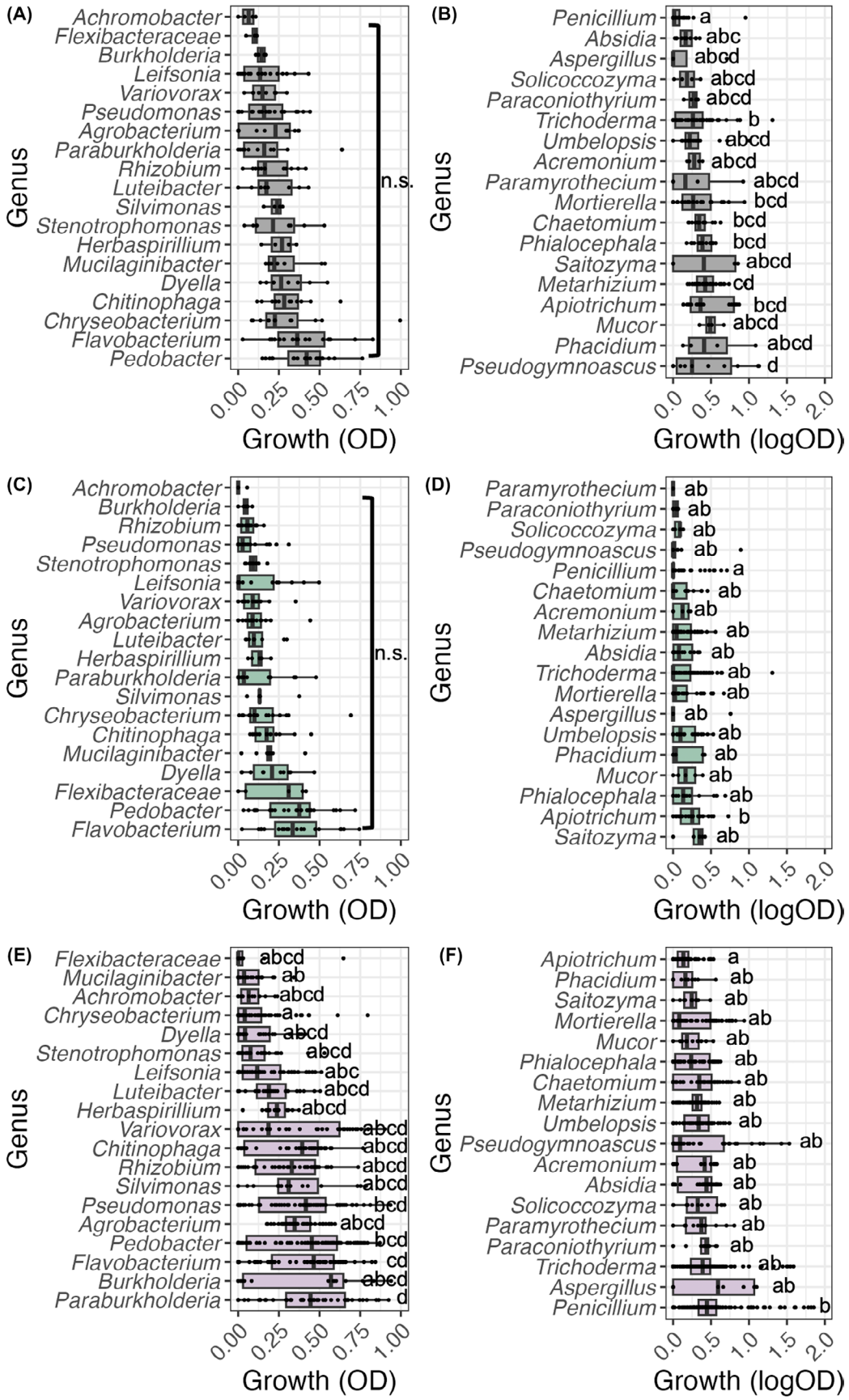

FIGURE 5 Growth of (A, C, E) bacterial and (B, D, F) fungal genera on necromass types (A, B), polymers (C, D) and monomers and dimers (E, F). Letters indicate significant differences based on a Tukey's HSD means comparison test on a linear mixed model of growth with strain as a random effect and genus as a fixed effect, while n.s. indicates no significant effect.

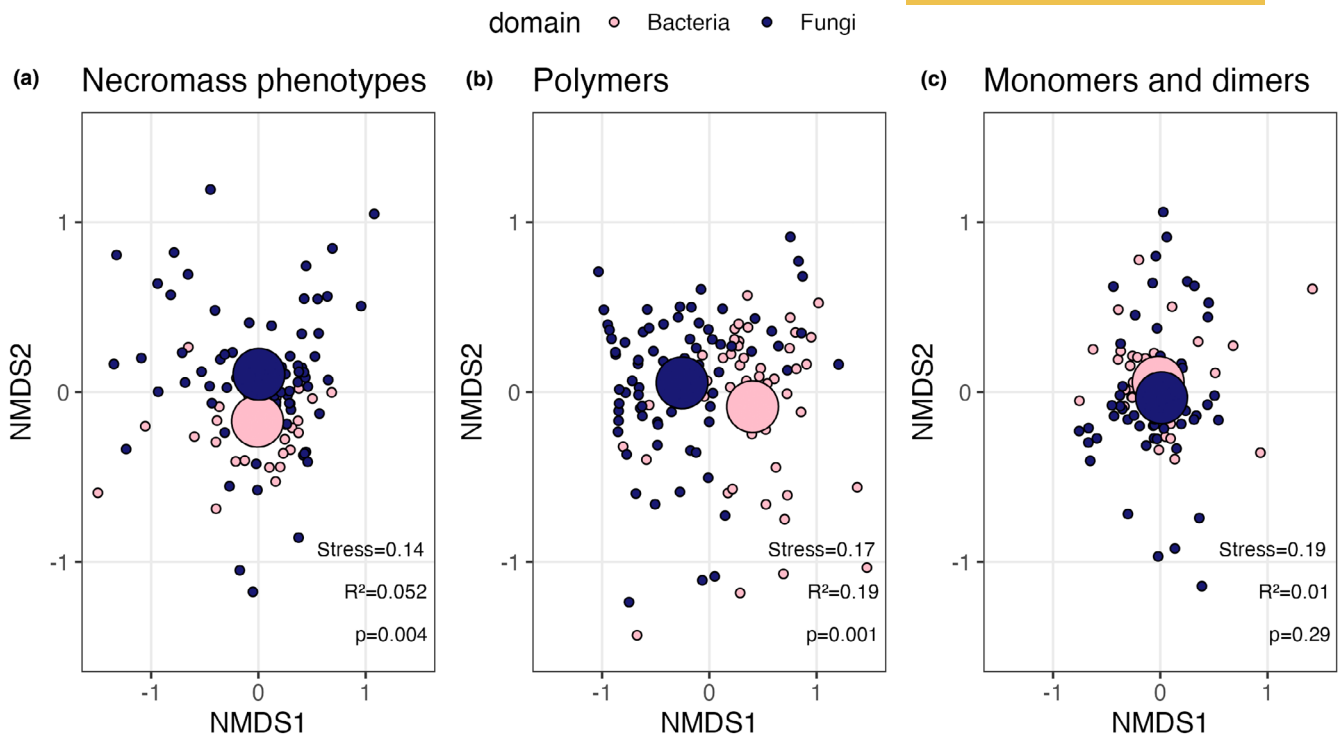

FIGURE 6 Microbial NMDS by domain for (a) necromass phenotypes (b) polymers and (c) soluble monomers and dimers. PERMANOVA results and stress values are displayed in the lower right of each plot.

4 | DISCUSSION

4.1 | Microbial substrate use did not vary across habitats

We found that across substrates and among substrate categories, microbes isolated from soil were functionally equivalent in their growth capabilities to microbes isolated from low- and high-melanin necromass; thus, hypothesis one was not supported. We had hypothesized that an ability to grow on necromass and fungal polymers would be an important factor shaping community assembly from the soil into the necrobiome, especially because litter, soil and necromass chemistry have all been shown to affect microbial community composition and explain the dominance of certain microbial taxa (Bhatnagar et al., 2018; Brabcová et al., 2016; Zhalnina et al., 2018). However, a host of other traits may explain the dominance of necrobiome taxa, including their growth rates, motility, stress tolerance, defence and extracellular enzyme production (Malik et al., 2020). For example, Biselli et al. (2020) found a trade-off between growth rate and maintenance cost, whereby decreased growth rates also decreased death rates and promoted long-term persistence of *E. coli* populations on their own necromass. There may also be a number of 'cheater' microbes in the necrobiome that benefit from extracellular products of co-inhabitants and dampen the signal of any necromass degrading specialists (Bruce et al., 2017; Butaitė et al., 2017). This possibility is consistent with the finding that bacteria release enzymes immediately before death (Gibson et al., 2025). Finally, because there is high functional redundancy among microbes in soils, the measured substrate use potential in this assay may not reflect real-time microbial activity (Louca et al., 2018), especially in dynamic environments.

4.2 | Bacteria and fungi were differentially affected by necromass phenotype chemistry

While our overall results suggested that bacteria and fungi shared similar resource use profiles on fungal necromass, we also found notable differences in microbial growth depending on both necromass cell wall abundance and melanin content. Partially consistent with our second hypothesis, growth on whole cell necromass was greater than on cell wall-enriched necromass for bacteria but not for fungi. These results parallel those of Starke et al. (2020), who found lower growth yields, enzyme activity and diversity of expressed extracellular proteins of bacteria growing on cell wall-enriched *Agaricus bisporus* necromass compared to that of *A. bisporus* whole cell necromass. Our chemical analyses indicated that the %C of cell wall-enriched and whole cell *H. bicolor* necromass was not different, but that the amount of N was significantly lower and there was a concomitant reduction of amides. We suspect that the reduced N availability and a wider C:N on cell wall-enriched necromass may have directly constrained bacterial growth compared with fungi, as bacteria have lower C:N ratios and at least double the N demand of fungi (Brabcová et al., 2018; Wang & Kuzyakov, 2024; Waring et al., 2013). Alternatively, it is possible that differences in C quality were the primary factor limiting bacterial growth on cell

wall-enriched necromass, which we found to contain significantly higher polysaccharide: aliphatic ratios. This possibility is consistent with bacteria having less extracellular enzyme capacity than fungi overall (Kirk & Farrell, 1987; Romaní et al., 2006). Additionally, enzyme cofactors, redox active components and P-containing energy molecules present in the cell cytosol may have been depleted in the cell wall-enriched necromass and further limited bacterial growth. These nutritional limitations are non-mutually exclusive and thus may all contribute to the lower growth of bacteria on cell wall-enriched necromass.

In contrast to cell wall abundance, we found that fungal growth was more limited by melanin content, with significantly less growth on high than low-melanin necromass. These results are consistent with lower fungal ^{13}C and ^{15}N uptake from high-melanin *H. bicolor* necromass incubated at the same field site as microbes were isolated in this study (Maillard, Michaud, et al., 2023). Melanin has been demonstrated to act similarly to lignin, as a recalcitrant compound limiting fungal decomposition (Fernandez & Koide, 2014). Fungal gene expression is strongly affected by melanin content in *H. bicolor*, particularly in protease and laccase encoding genes (Haq et al., 2024), so it was expected that fungal growth would vary with necromass melanin content. However, given that melanin can form complexes with proteins in fungal cell walls making N less available (Bull, 1970), it was surprising that bacterial growth was not more strongly affected by melanin content. We suspect that bacteria grew preferentially on the cell soluble components on both whole cell necromass phenotypes rather than the structural polymer matrix that included melanin, given that bacterial growth was limited on melanin as a sole polymer C substrate. Melanin may also more strongly inhibit fungal extracellular enzyme activity (Bull, 1970; Kuo & Alexander, 1967) relative to bacteria. Consistent with these possibilities is the recent study of Novak et al. (2024), who demonstrated no significant differences in CAZyme-encoding gene expression of bacteria grown on high- and low-melanin *H. bicolor* necromass. This suggests that bacteria are not consuming any additional carbohydrates on the high-melanin necromass phenotypes. However, Novak et al. (2024) did establish clear differences between *H. bicolor* phenotypes in gene expression related to N use. Given that the % N was not consistently higher in low-melanin necromass phenotypes, and was much more pronounced between whole cell and cell wall-enriched phenotypes, bacterial growth may have been less affected by the melanin phenotypes. Overall, these findings only partially support our third hypothesis that high-melanin necromass phenotypes would markedly limit both bacterial and fungal growth.

4.3 | Growth on polymers predicted growth on necromass phenotypes

The best predictor of growth on necromass phenotypes for both bacteria and fungi was growth on fungal cell wall polymers, with growth on soluble monomers and dimers either neutral or negatively correlated with growth on necromass. This result is consistent with our fourth hypothesis: because fungal cell wall polymers represent a significant portion of necromass, and particularly the carbon that can be acquired (Ryan et al., 2020, Table 1), it is reasonable that they served as strong predictors of growth on necromass. For bacteria, all 5 polymer substrates were strongly correlated with growth on necromass, while fungal growth was only predicted by chitin, chitosan and melanin, and not by glucan or glycogen. These latter two compounds were the only polymers without N, which may indicate active targeting of N by fungi during necromass decomposition. Additionally, polymers were only predictive of growth on whole cell necromass phenotypes for fungi, suggesting that growth on the surveyed polymers may not fully reflect fungal metabolic requirements for decomposing late-stage cell wall-enriched necromass. Growth on monomers did not predict growth on necromass in either domain, aligned with the fact that generalist, copiotroph microbes growing on labile compounds do not require oxidative and hydrolytic depolymerizing extracellular enzymes (Ramin & Allison, 2019). There were some negative correlations between growth on sugars and sugar alcohols and necromass phenotypes, which could reflect a potential trade-off between substrate use strategies among specialist microbial groups (Gralka et al., 2023), or of scavenger microbes that are only active after CAZyme-producing microbes degrade the substrate (McClure et al., 2022). For example, fungal polymers may be especially important if they act as spatial barriers to labile compounds, requiring chitinase and glucanase activity to penetrate the cell wall and provide access (Adams, 2004). Our finding that fungal polymers do predict bacterial and fungal growth on necromass aligns with other studies surveying biopolymers derived from plant and microbial biomass (Algora et al., 2022; Algora Gallardo et al., 2021; Martinović et al., 2022), and provides evidence that growth on biopolymers can serve as a proxy for growth on more heterogeneous substrates like necromass.

4.4 | Functional overlap across microbial domains varied with substrate complexity

Combining the bacterial and fungal datasets in a multivariate analysis allowed for a direct comparison of functional overlap between domains. We acknowledge that this analysis should be interpreted with caution, given that both datasets were collected using different techniques. To address this, we applied the same normalization technique to both datasets, attempting to eliminate strain- and domain-level bias. We found that microbial domain did not significantly predict substrate use for monomers and dimers, suggesting high niche overlap for these labile compounds, which has also been found in other studies (Kramer et al., 2016; Paterson et al., 2011; Strickland & Rousk, 2010; Waldrop & Firestone, 2004). There was, however, some degree of functional separation between bacterial and fungal domains on necromass phenotypes, consistent with our finding that bacteria and fungi have distinct chemical limitations on necromass. The highest degree of functional dissimilarity between domains was on polymer substrates. Other studies of microbial

substrate use preferences have also found the highest niche differentiation in complex substrates and polymers (Koranda et al., 2014; Nuccio et al., 2020; Starke et al., 2021; Wang & Kuzyakov, 2024). Given that fungi are regularly recycling their own cell wall polymers as they grow (Mitchell & Sabar, 1966; Perez-Leblic et al., 1982), and possess more hydrolytic and oxidative enzymes than bacteria (Baldrian et al., 2011; Gramss, 1997; Kellner & Vandenbol, 2010), it is reasonable to expect that functional differences across domains would be most pronounced on polymers. Overall, while there is a high degree of functional overlap between bacteria and fungi, the type and abundance of complex compounds may have the potential to predict domain interactions and resulting necromass decomposition dynamics.

4.5 | A wide range of bacterial and fungal genera grew on labile and complex substrates

In addition to our original hypotheses, we examined genus-specific patterns in substrate use. Overall, substrate use did not vary significantly across genera. Bacteria from the genera *Pedobacter* and *Flavobacteria* grew well on necromass. Many studies document a high presence and activity of these lineages on necromass (Brabcová et al., 2018; Starke et al., 2020) as well as chitinolytic activity in lab assays (Larsbrink et al., 2016; Someya et al., 2011). Several fungal genera grew well across substrates, including core necrobiome members *Metarhizium*, *Trichoderma* and *Mortierella* (Cantoran et al., 2023), but basidiomycete yeasts in the genera *Apiotrichum* and *Saitozyma* exhibited the most growth on fungal polymers. This may indicate a unicellular strategy is advantageous in the short-term over mycelial growth, or that yeast growth rates exceed those of filamentous fungi. Yeasts may also have benefitted from the liquid culture setting of this experiment, and a multicellular strategy may prevail more in a soil matrix. The latter interpretation would align with forest soil yeasts being primarily classified as decomposer opportunists, whose growth capacities are limited to polymer derivatives and monomers (Mašínová et al., 2018). An important caveat to phylum- and genus-level findings is that there was uneven representation within each taxonomic grouping, thus some patterns may result from uneven sample sizes.

4.6 | Study limitations and future directions

While our study design surveyed microbial growth on multiple necromass phenotypes and many associated C constituents using a wide range of ecologically relevant bacteria and fungi, we did not test growth on all C substrates that constitute necromass. For example, Maillard et al. (2022) found that remaining lipid content was a strong predictor of microbial community structure in a multi-month necromass field incubation, suggesting the capacity to utilize lipids may also be a significant driver of necrobiome composition. Further, this assay was only conducted with a single species as the source of necromass (*H. bicolor*) and thus may not reflect microbial strategies on diverse necromass phenotypes, including bacterial necromass (Buckeridge et al., 2020) and different fungal morphotypes (Certano et al., 2018). As such, future culture-based studies that directly impose substrate-level controls on N availability and C quality may help to better differentiate fungal versus bacterial requirements for growth on necromass. Finally, this assay did not directly measure decomposition or alteration of the substrate, but rather captured the ability of microbes to grow on necromass phenotypes, fungal polymers, dimers and monomers. Previous studies have shown that other proxies of microbial growth (i.e. microbial respiration) are significantly correlated with necromass mass loss (Pérez-Pazos et al., 2024), but more detailed studies assessing differences in carbon use efficiency (the ratio of microbial growth to C loss) across different bacteria and fungi will aid in better connecting necrobiome composition with rates of necromass decomposition.

The larger aim of this phenotyping screening was to determine whether two microbial groups that constitute a major part of the necrobiome, bacteria and fungi, differed fundamentally in the C compounds they were able to utilize. While we found these groups were differentially constrained by aspects of necromass chemistry and polymer content, species from both domains were able to grow on necromass phenotypes, polymers and soluble dimers and monomers. It is important to note, however, that this conclusion is based on growth in liquid culture, where access to the C substrates was less limited. The recent study of Pérez-Pazos et al. (2024), which used a subset of the fungal and bacterial strains profiled here, demonstrated that when growing in soil only fungi were able to actively decompose necromass. Further, they showed that bacteria benefitted from growing with fungi in necromass-infused liquid media. Those results, taken together with this study, suggest that the networks created by fungal hyphae may provide a physical scaffolding that bacteria need to better access necromass. Support for this scenario comes from other studies showing that bacteria can use fungal hyphal networks (fungal highways; Kohlmeier et al., 2005) to migrate and disperse (Banitz et al., 2011; Furuno et al., 2010), especially in heterogeneous, water-limited soil environments (Worrich et al., 2016). It is also possible that fungi may serve as initial critical degraders of recalcitrant fungal cell wall components that unlock more labile components for bacteria to consume, as is the case in leaf litter decomposition (Baldrian, 2017). Looking forward, we believe that experiments explicitly studying the relative importance of physical and chemical dependencies in bacterial-fungal interactions hold significant promise for better parsing the ecological controls on fungal necrobiome assembly as well as integrating microbial community attributes into predictive frameworks of soil C cycling.

AUTHOR CONTRIBUTIONS

Achala Narayanan, François Maillard and Peter G. Kennedy conceived the ideas and designed the methodology. Cultures used in this experiment were isolated by François Maillard and Briana H. Beatty. Data collection for fungal cultures was performed by François Maillard and Briana H. Beatty and for bacterial cultures by

Achala Narayanan and Jessica K. Novak. Achala Narayanan analysed the data, and Achala Narayanan and Peter G. Kennedy led the writing of the manuscript. All authors edited subsequent versions of the manuscript and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

All authors have no relevant financial or non-financial interests to disclose.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.x3ffbg7zc (Narayanan et al., 2025).

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REFERENCES

Adams, D. J. (2004). Fungal cell wall chitinases and glucanases. *Microbiology*, *150*(7), 2029–2035. https://doi.org/10.1099/mic.0.26980-0

Algora, C., Odriozola, I., Human, Z. R., Awokunle Hollá, S., Baldrian, P., & López-Mondéjar, R. (2022). Specific utilization of biopolymers of plant and fungal origin reveals the existence of substrate-specific guilds for bacteria in temperate forest soils. *Soil Biology and Biochemistry*, *171*, 108696. https://doi.org/10.1016/j.soilbio.2022.108696

Algora Gallardo, C., Baldrian, P., & López-Mondéjar, R. (2021). Litter-inhabiting fungi show high level of specialization towards biopolymers composing plant and fungal biomass. *Biology and Fertility of Soils*, *57*(1), 77–88. https://doi.org/10.1007/s00374-020-01507-3

Angst, G., Mueller, K. E., Nierop, K. G. J., & Simpson, M. J. (2021). Plant- or microbial-derived? A review on the molecular composition of stabilized soil organic matter. *Soil Biology and Biochemistry*, *156*, 108189. https://doi.org/10.1016/j.soilbio.2021.108189

Bach, E. M., Williams, R. J., Hargreaves, S. K., Yang, F., & Hofmockel, K. S. (2018). Greatest soil microbial diversity found in micro-habitats. *Soil Biology and Biochemistry*, *118*, 217–226. https://doi.org/10.1016/j.soilbio.2017.12.018

Baldrian, P. (2017). Microbial activity and the dynamics of ecosystem processes in forest soils. *Current Opinion in Microbiology*, *37*, 128–134. https://doi.org/10.1016/j.mib.2017.06.008

Baldrian, P., Voříšková, J., Dobiášová, P., Merhautová, V., Lisá, L., & Valášková, V. (2011). Production of extracellular enzymes and degradation of biopolymers by saprotrophic microfungi from the upper layers of forest soil. *Plant and Soil*, *338*(1–2), 111–125. https://doi.org/10.1007/s11104-010-0324-3

Banitz, T., Fetzer, I., Johst, K., Wick, L. Y., Harms, H., & Frank, K. (2011). Assessing biodegradation benefits from dispersal networks. *Ecological Modelling*, *222*(14), 2552–2560. https://doi.org/10.1016/j.ecolmodel.2010.07.005

Beidler, K. V., Huenupi, E., DeLancey, L. C., Maillard, F., Zhang, B., Persson, P., Kennedy, P. G., & Phillips, R. (2025). Minerals, microbes and melanin drive differential incorporation of fungal necromass carbon and nitrogen into mineral-associated organic matter. *Soil Biology and Biochemistry*, *208*, 109843. https://doi.org/10.1016/j.soilbio.2025.109843

Beidler, K. V., Phillips, R. P., Andrews, E., Maillard, F., Mushinski, R. M., & Kennedy, P. G. (2020). Substrate quality drives fungal necromass decay and decomposer community structure under contrasting vegetation types. *Journal of Ecology*, *108*(5), 1845–1859. https://doi.org/10.1111/1365-2745.13385

Bhatnagar, J. M., Peay, K. G., & Treseder, K. K. (2018). Litter chemistry influences decomposition through activity of specific microbial functional guilds. *Ecological Monographs*, *88*(3), 429–444. https://doi.org/10.1002/ecm.1303

Biselli, E., Schink, S. J., & Gerland, U. (2020). Slower growth of *Escherichia coli* leads to longer survival in carbon starvation due to a decrease in the maintenance rate. *Molecular Systems Biology*, *16*(6), e9478. https://doi.org/10.15252/msb.20209478

Brabcová, V., Nováková, M., Davidová, A., & Baldrian, P. (2016). Dead fungal mycelium in forest soil represents a decomposition hotspot and a habitat for a specific microbial community. *New Phytologist*, *210*(4), 1369–1381. https://doi.org/10.1111/nph.13849

Brabcová, V., Štursová, M., & Baldrian, P. (2018). Nutrient content affects the turnover of fungal biomass in forest topsoil and the composition of associated microbial communities. *Soil Biology and Biochemistry*, *118*, 187–198. https://doi.org/10.1016/j.soilbio.2017.12.012

Bruce, J. B., Cooper, G. A., Chabas, H., West, S. A., & Griffin, A. S. (2017). Cheating and resistance to cheating in natural populations of the bacterium *Pseudomonas fluorescens*: Cheating in natural bacterial populations. *Evolution*, *71*(10), 2484–2495. https://doi.org/10.1111/evo.13328

Buckeridge, K. M., La Rosa, A. F., Mason, K. E., Whitaker, J., McNamara, N. P., Grant, H. K., & Ostle, N. J. (2020). Sticky dead microbes: Rapid abiotic retention of microbial necromass in soil. *Soil Biology and Biochemistry*, *149*, 107929. https://doi.org/10.1016/j.soilbio.2020.107929

Bull, A. T. (1970). Inhibition of polysaccharases by melanin: Enzyme inhibition in relation to mycolysis. *Archives of Biochemistry and Biophysics*, *137*(2), 345–356. https://doi.org/10.1016/0003-9861(70)90448-0

Butaitė, E., Baumgartner, M., Wyder, S., & Kümmerli, R. (2017). Siderophore cheating and cheating resistance shape competition for iron in soil and freshwater *Pseudomonas* communities. *Nature Communications*, *8*(1), 414. https://doi.org/10.1038/s41467-017-00509-4

Cantoran, A., Maillard, F., Baldrian, P., & Kennedy, P. G. (2023). Defining a core microbial necrobiome associated with decomposing fungal necromass. *FEMS Microbiology Ecology*, *99*(9), fiad098. https://doi.org/10.1093/femsec/fiad098

Certano, A. K., Fernandez, C. W., Heckman, K. A., & Kennedy, P. G. (2018). The afterlife effects of fungal morphology: Contrasting decomposition rates between diffuse and rhizomorphic necromass. *Soil Biology and Biochemistry*, *126*, 76–81. https://doi.org/10.1016/j.soilbio.2018.08.002

Fernandez, C. W., Heckman, K., Kolka, R., & Kennedy, P. G. (2019). Melanin mitigates the accelerated decay of mycorrhizal necromass with peatland warming. *Ecology Letters*, *22*(3), 498–505. https://doi.org/10.1111/ele.13209

Fernandez, C. W., & Kennedy, P. G. (2018). Melanization of mycorrhizal fungal necromass structures microbial decomposer communities. *Journal of Ecology*, *106*(2), 468–479. https://doi.org/10.1111/1365-2745.12920

Fernandez, C. W., & Koide, R. T. (2014). Initial melanin and nitrogen concentrations control the decomposition of ectomycorrhizal fungal litter. *Soil Biology and Biochemistry*, *77*, 150–157. https://doi.org/10.1016/j.soilbio.2014.06.026

Fierer, N., & Lennon, J. T. (2011). The generation and maintenance of diversity in microbial communities. *American Journal of Botany*, *98*(3), 439–448. https://doi.org/10.3732/ajb.1000498

Fontaine, T., Simenel, C., Dubreucq, G., Adam, O., Delepierre, M., Lemoine, J., Vorgias, C. E., Diaquin, M., & Latgé, J.-P. (2000). Molecular organization of the alkali-insoluble fraction of *Aspergillus fumigatus* cell wall. *Journal of Biological Chemistry*, *275*(36), 27594–27607. https://doi.org/10.1074/jbc.M909975199

Furuno, S., Päzolt, K., Rabe, C., Neu, T. R., Harms, H., & Wick, L. Y. (2010). Fungal mycelia allow chemotactic dispersal of polycyclic aromatic hydrocarbon-degrading bacteria in water-unsaturated systems. *Environmental Microbiology*, *12*(6), 1391–1398. https://doi.org/10.1111/j.1462-2920.2009.02022.x

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Molecular Ecology*, *2*(2), 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Garland, J. L., & Mills, A. L. (1991). Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Applied and Environmental Microbiology*, *57*(8), 2351–2359. https://doi.org/10.1128/aem.57.8.2351-2359.1991

Gibson, S. E., Frost, I., Hierons, S. J., Moses, T., Poon, W. C., West, S. A., & Cann, M. J. (2025). Bacteria encode post-mortem protein catabolism that enables altruistic nutrient recycling. *Nature Communications*, *16*(1), 1400. https://doi.org/10.1038/s41467-025-56761-6

Godbold, D. L., Hoosbeek, M. R., Lukac, M., Cotrufo, M. F., Janssens, I. A., Ceulemans, R., Polle, A., Velthorst, E. J., Scarascia-Mugnozza, G., De Angelis, P., Miglietta, F., & Peressotti, A. (2006). Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter. *Plant and Soil*, *281*(1), 15–24. https://doi.org/10.1007/s11104-005-3701-6

Gralka, M., Pollak, S., & Cordero, O. X. (2023). Genome content predicts the carbon catabolic preferences of heterotrophic bacteria. *Nature Microbiology*, *8*(10), 1799–1808. https://doi.org/10.1038/s41564-023-01458-z

Gramss, G. (1997). Activity of oxidative enzymes in fungal mycelia from grassland and forest soils. *Journal of Basic Microbiology*, *37*(6), 407–423. https://doi.org/10.1002/jobm.3620370606

Grelet, G., Johnson, D., Paterson, E., Anderson, I. C., & Alexander, I. J. (2009). Reciprocal carbon and nitrogen transfer between an ericaceous dwarf shrub and fungi isolated from *Piceirhiza bicolorata* ectomycorrhizas. *New Phytologist*, *182*(2), 359–366. https://doi.org/10.1111/j.1469-8137.2009.02813.x

Haq, I. U., Kennedy, P., Schreiner, K. M., Agnich, J. C., & Schilling, J. S. (2024). Gene expression by a model fungus in the Ascomycota provides insight into the decay of fungal necromass. *Environmental Microbiology*, *26*(12), e70006. https://doi.org/10.1111/1462-2920.70006

Kellner, H., & Vandenbol, M. (2010). Fungi unearthed: Transcripts encoding lignocellulolytic and chitinolytic enzymes in forest soil. *PLoS One*, *5*(6), e10971. https://doi.org/10.1371/journal.pone.0010971

Kennedy, P. G., & Maillard, F. (2023). Knowns and unknowns of the soil fungal necrobiome. *Trends in Microbiology*, *31*(2), 173–180. https://doi.org/10.1016/j.tim.2022.08.011

Kirk, T. K., & Farrell, R. L. (1987). Enzymatic "combustion": The microbial degradation of lignin. *Annual Review of Microbiology*, *41*(1), 465–501. https://doi.org/10.1146/annurev.mi.41.100187.002341

Kleber, M., & Johnson, M. G. (2010). Advances in understanding the molecular structure of soil organic matter. *Advances in Agronomy*, *106*, 77–142. https://doi.org/10.1016/S0065-2113(10)06003-7

Kohlmeier, S., Smits, T. H. M., Ford, R. M., Keel, C., Harms, H., & Wick, L. Y. (2005). Taking the fungal highway: Mobilization of pollutant-degrading bacteria by fungi. *Environmental Science & Technology*, *39*(12), 4640–4646. https://doi.org/10.1021/es047979z

Kohout, P., Sýkorová, Z., Bahram, M., Hadincová, V., Albrechtová, J., Tedersoo, L., & Vohník, M. (2011). Ericaceous dwarf shrubs affect ectomycorrhizal fungal community of the invasive *Pinus strobus* and native *Pinus sylvestris* in a pot experiment. *Mycorrhiza*, *21*(5), 403–412. https://doi.org/10.1007/s00572-010-0350-2

Koide, R. T., & Malcolm, G. M. (2009). N concentration controls decomposition rates of different strains of ectomycorrhizal fungi. *Fungal Ecology*, *2*(4), 197–202. https://doi.org/10.1016/j.funeco.2009.06.001

Koranda, M., Kaiser, C., Fuchslueger, L., Kitzler, B., Sessitsch, A., Zechmeister-Boltenstern, S., & Richter, A. (2014). Fungal and bacterial utilization of organic substrates depends on substrate complexity and N availability. *FEMS Microbiology Ecology*, *87*(1), 142–152. https://doi.org/10.1111/1574-6941.12214

Kramer, S., Dibbern, D., Moll, J., Huenninghaus, M., Koller, R., Krueger, D., Marhan, S., Urich, T., Wubet, T., Bonkowski, M., Buscot, F., Lueders, T., & Kandeler, E. (2016). Resource partitioning between bacteria, fungi, and protists in the detritusphere of an agricultural soil. *Frontiers in Microbiology*, *7*, 1524. https://doi.org/10.3389/fmicb.2016.01524

Kuo, M. J., & Alexander, M. (1967). Inhibition of the lysis of fungi by melanins. *Journal of Bacteriology*, *94*(3), 624–629. https://doi.org/10.1128/jb.94.3.624-629.1967

Larsbrink, J., Zhu, Y., Kharade, S. S., Kwiatkowski, K. J., Eijsink, V. G. H., Koropatkin, N. M., McBride, M. J., & Pope, P. B. (2016). A polysaccharide utilization locus from *Flavobacterium johnsoniae* enables conversion of recalcitrant chitin. *Biotechnology for Biofuels*, *9*(1), 260. https://doi.org/10.1186/s13068-016-0674-z

Lehmann, J., Hansel, C. M., Kaiser, C., Kleber, M., Maher, K., Manzoni, S., Nunan, N., Reichstein, M., Schimel, J. P., Torn, M. S., Wieder, W. R., & Kögel-Knabner, I. (2020). Persistence of soil organic carbon caused by functional complexity. *Nature Geoscience*, *13*(8), 529–534. https://doi.org/10.1038/s41561-020-0612-3

Lenth, R. V. (2024). emmeans: Estimated marginal means, aka least-squares means. https://CRAN.R-project.org/package=emmeans

Liang, C., Amelung, W., Lehmann, J., & Kästner, M. (2019). Quantitative assessment of microbial necromass contribution to soil organic matter. *Global Change Biology*, *25*(11), 3578–3590. https://doi.org/10.1111/gcb.14781

Louca, S., Polz, M. F., Mazel, F., Albright, M. B. N., Huber, J. A., O'Connor, M. I., Ackermann, M., Hahn, A. S., Srivastava, D. S., Crowe, S. A., Doebeli, M., & Parfrey, L. W. (2018). Function and functional redundancy in microbial systems. *Nature Ecology & Evolution*, *2*(6), 936–943. https://doi.org/10.1038/s41559-018-0519-1

Maillard, F., Beatty, B., Park, M., Adamczyk, S., Adamczyk, B., See, C. R., Cavender-Bares, J., Hobbie, S. E., & Kennedy, P. G. (2023). Microbial community attributes supersede plant and soil parameters in predicting fungal necromass decomposition rates in a 12-tree species common garden experiment. *Soil Biology and Biochemistry*, *184*, 109124. https://doi.org/10.1016/j.soilbio.2023.109124

Maillard, F., Fernandez, C. W., Mundra, S., Heckman, K. A., Kolka, R. K., Kauserud, H., & Kennedy, P. G. (2022). Warming drives a 'hummockification' of microbial communities associated with decomposing mycorrhizal fungal necromass in peatlands. *New Phytologist*, *234*(6), 2032–2043. https://doi.org/10.1111/nph.17755

Maillard, F., Kennedy, P. G., Adamczyk, B., Heinonsalo, J., & Buée, M. (2021). Root presence modifies the long-term decomposition dynamics of fungal necromass and the associated microbial communities in a boreal forest. *Molecular Ecology*, *30*(8), 1921–1935. https://doi.org/10.1111/mec.15828

Maillard, F., Michaud, T. J., See, C. R., DeLancey, L. C., Blazewicz, S. J., Kimbrel, J. A., Pett-Ridge, J., & Kennedy, P. G. (2023). Melanization slows the rapid movement of fungal necromass carbon and nitrogen into both bacterial and fungal decomposer communities and soils. *MSystems*, *8*(4), e00390-23. https://doi.org/10.1128/msystems.00390-23

Maillard, F., Pflender, S., Heckman, K. A., Chalot, M., & Kennedy, P. G. (2023). Fungal necromass presents a high potential for Mercury immobilization in soil. *Chemosphere*, *311*, 136994. https://doi.org/10.1016/j.chemosphere.2022.136994

Malik, A. A., Martiny, J. B. H., Brodie, E. L., Martiny, A. C., Treseder, K. K., & Allison, S. D. (2020). Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. *The ISME Journal*, *14*(1), 1–9. https://doi.org/10.1038/s41396-019-0510-0

Martin, F. (Ed.). (2016). *Molecular mycorrhizal symbiosis*. John Wiley & Sons, Inc.

Martinović, T., Mašínová, T., López-Mondéjar, R., Jansa, J., Štursová, M., Starke, R., & Baldrian, P. (2022). Microbial utilization of simple and complex carbon compounds in a temperate forest soil. *Soil Biology and Biochemistry*, *173*, 108786. https://doi.org/10.1016/j.soilbio.2022.108786

Mašínová, T., Yurkov, A., & Baldrian, P. (2018). Forest soil yeasts: Decomposition potential and the utilization of carbon sources. *Fungal Ecology*, *34*, 10–19. https://doi.org/10.1016/j.funeco.2018.03.005

McClure, R., Farris, Y., Danczak, R., Nelson, W., Song, H.-S., Kessell, A., Lee, J.-Y., Couvillion, S., Henry, C., Jansson, J. K., & Hofmockel, K. S. (2022). Interaction networks are driven by community-responsive phenotypes in a chitin-degrading consortium of soil microbes. *MSystems*, *7*(5), e00372-22. https://doi.org/10.1128/msystems.00372-22

Mieszkin, S., Richet, P., Bach, C., Lambrot, C., Augusto, L., Buée, M., & Uroz, S. (2021). Oak decaying wood harbors taxonomically and functionally different bacterial communities in sapwood and heartwood. *Soil Biology and Biochemistry*, *155*, 108160. https://doi.org/10.1016/j.soilbio.2021.108160

Mitchell, R., & Sabar, N. (1966). Autolytic enzymes in fungal cell walls. *Journal of General Microbiology*, *42*(1), 39–42. https://doi.org/10.1099/00221287-42-1-39

Narayanan, A., Maillard, F., Beatty, B. H., Novak, J. K., Gardner, J. G., Schilling, J. S., Pett-Ridge, J., & Kennedy, P. G. (2025). Data from: Bacterial and fungal growth on fungal necromass and its diverse components: shared profiles and divergent constraints revealed by high-throughput phenotyping. *Dryad Digital Repository*. https://doi.org/10.5061/dryad.x3ffbg7zc

Novak, J. K., Kennedy, P. G., & Gardner, J. G. (2024). Transcriptomic analyses of bacterial growth on fungal necromass reveal different microbial community niches during degradation. *Applied and Environmental Microbiology*, *90*(10), e01062-24. https://doi.org/10.1128/aem.01062-24

Nuccio, E. E., Starr, E., Karaoz, U., Brodie, E. L., Zhou, J., Tringe, S. G., Malmstrom, R. R., Woyke, T., Banfield, J. F., Firestone, M. K., & Pett-Ridge, J. (2020). Niche differentiation is spatially and temporally regulated in the rhizosphere. *The ISME Journal*, *14*(4), 999–1014. https://doi.org/10.1038/s41396-019-0582-x

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., & Weedon, J. (2001). *vegan*: *Community ecology package*. https://doi.org/10.32614/CRAN.package.vegan

Papke, R. T., & Ward, D. M. (2004). The importance of physical isolation to microbial diversification. *FEMS Microbiology Ecology*, *48*(3), 293–303. https://doi.org/10.1016/j.femsec.2004.03.013

Paterson, E., Sim, A., Osborne, S. M., & Murray, P. J. (2011). Long-term exclusion of plant-inputs to soil reduces the functional capacity of microbial communities to mineralise recalcitrant root-derived carbon sources. *Soil Biology and Biochemistry*, *43*(9), 1873–1880. https://doi.org/10.1016/j.soilbio.2011.05.006

Perez-Leblic, M. I., Reyes, F., Martinez, M. J., & Lahoz, R. (1982). Cell wall degradation in the autolysis of filamentous fungi. *Mycopathologia*, *80*(3), 147–155. https://doi.org/10.1007/BF00437577

Pérez-Pazos, E., Beidler, K. V., Narayanan, A., Beatty, B. H., Maillard, F., Bancos, A., Heckman, K. A., & Kennedy, P. G. (2024). Fungi rather than bacteria drive early mass loss from fungal necromass regardless of particle size. *Environmental Microbiology Reports*, *16*(3), e13280. https://doi.org/10.1111/1758-2229.13280

R Core Team. (2023). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rabbi, S. M. F., Daniel, H., Lockwood, P. V., Macdonald, C., Pereg, L., Tighe, M., Wilson, B. R., & Young, I. M. (2016). Physical soil architectural traits are functionally linked to carbon decomposition and bacterial diversity. *Scientific Reports*, *6*(1), 33012. https://doi.org/10.1038/srep33012

Ramin, K. I., & Allison, S. D. (2019). Bacterial tradeoffs in growth rate and extracellular enzymes. *Frontiers in Microbiology*, *10*, 2956. https://doi.org/10.3389/fmicb.2019.02956

Rawlins, B. G., Wragg, J., Reinhard, C., Atwood, R. C., Houston, A., Lark, R. M., & Rudolph, S. (2016). Three-dimensional soil organic matter distribution, accessibility and microbial respiration in macroaggregates using osmium staining and synchrotron X-ray computed tomography. *The Soil*, *2*(4), 659–671. https://doi.org/10.5194/soil-2-659-2016

Romaní, A. M., Fischer, H., Mille-Lindblom, C., & Tranvik, L. J. (2006). Interactions of bacteria and fungi on decomposing litter: Differential extracellular enzyme activities. *Ecology*, *87*(10), 2559–2569. https://doi.org/10.1890/0012-9658(2006)87[2559:IOBAFO]2.0.CO;2

Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., Knight, R., & Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal*, *4*(10), 1340–1351. https://doi.org/10.1038/ismej.2010.58

Ruamps, L. S., Nunan, N., & Chenu, C. (2011). Microbial biogeography at the soil pore scale. *Soil Biology and Biochemistry*, *43*(2), 280–286. https://doi.org/10.1016/j.soilbio.2010.10.010

Ryan, M. E., Schreiner, K. M., Swenson, J. T., Gagne, J., & Kennedy, P. G. (2020). Rapid changes in the chemical composition of degrading ectomycorrhizal fungal necromass. *Fungal Ecology*, *45*, 100922. https://doi.org/10.1016/j.funeco.2020.100922

Scharlemann, J. P., Tanner, E. V., Hiederer, R., & Kapos, V. (2014). Global soil carbon: Understanding and managing the largest terrestrial carbon pool. *Carbon Management*, *5*(1), 81–91. https://doi.org/10.4155/cmt.13.77

See, C. R., Fernandez, C. W., Conley, A. M., DeLancey, L. C., Heckman, K. A., Kennedy, P. G., & Hobbie, S. E. (2021). Distinct carbon fractions drive a generalisable two-pool model of fungal necromass decomposition. *Functional Ecology*, *35*(3), 796–806. https://doi.org/10.1111/1365-2435.13728

Six, J., Guggenberger, G., Paustian, K., Haumaier, L., Elliott, E. T., & Zech, W. (2001). Sources and composition of soil organic matter fractions between and within soil aggregates. *European Journal of Soil Science*, *52*(4), 607–618. https://doi.org/10.1046/j.1365-2389.2001.00406.x

Someya, N., Ikeda, S., Morohoshi, T., Noguchi Tsujimoto, M., Yoshida, T., Sawada, H., Ikeda, T., & Tsuchiya, K. (2011). Diversity of culturable chitinolytic bacteria from rhizospheres of agronomic plants in Japan. *Microbes and Environments*, *26*(1), 7–14. https://doi.org/10.1264/jsme2.ME10149

Starke, R., Mondéjar, R. L., Human, Z. R., Navrátilová, D., Štursová, M., Větrovský, T., Olson, H. M., Orton, D. J., Callister, S. J., Lipton, M. S., Howe, A., McCue, L. A., Pennacchio, C., Grigoriev, I., & Baldrian, P. (2021). Niche differentiation of bacteria and fungi in carbon and nitrogen cycling of different habitats in a temperate coniferous forest: A metaproteomic approach. *Soil Biology and Biochemistry*, *155*, 108170. https://doi.org/10.1016/j.soilbio.2021.108170

Starke, R., Morais, D., Větrovský, T., López Mondéjar, R., Baldrian, P., & Brabcová, V. (2020). Feeding on fungi: Genomic and proteomic analysis of the enzymatic machinery of bacteria decomposing

fungal biomass. *Environmental Microbiology*, *22*(11), 4604–4619. https://doi.org/10.1111/1462-2920.15183

Strickland, M. S., & Rousk, J. (2010). Considering fungal:bacterial dominance in soils—Methods, controls, and ecosystem implications. *Soil Biology and Biochemistry*, *42*(9), 1385–1395. https://doi.org/10.1016/j.soilbio.2010.05.007

Torsvik, V., & Øvreås, L. (2002). Microbial diversity and function in soil: From genes to ecosystems. *Current Opinion in Microbiology*, *5*(3), 240–245. https://doi.org/10.1016/S1369-5274(02)00324-7

Vogel, H. J. (2000). A numerical experiment on pore size, pore connectivity, water retention, permeability, and solute transport using network models. *European Journal of Soil Science*, *51*(1), 99–105. https://doi.org/10.1046/j.1365-2389.2000.00275.x

Waldrop, M. P., & Firestone, M. K. (2004). Microbial community utilization of recalcitrant and simple carbon compounds: Impact of oak-woodland plant communities. *Oecologia*, *138*(2), 275–284. https://doi.org/10.1007/s00442-003-1419-9

Wang, B., An, S., Liang, C., Liu, Y., & Kuzyakov, Y. (2021). Microbial necromass as the source of soil organic carbon in global ecosystems. *Soil Biology and Biochemistry*, *162*, 108422. https://doi.org/10.1016/j.soilbio.2021.108422

Wang, C., & Kuzyakov, Y. (2024). Mechanisms and implications of bacterial–fungal competition for soil resources. *The ISME Journal*, *18*(1), wrae073. https://doi.org/10.1093/ismejo/wrae073

Waring, B. G., Averill, C., & Hawkes, C. V. (2013). Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: Insights from meta-analysis and theoretical models. *Ecology Letters*, *16*(7), 887–894. https://doi.org/10.1111/ele.12125

Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, *173*(2), 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991

Worrich, A., König, S., Miltner, A., Banitz, T., Centler, F., Frank, K., Thullner, M., Harms, H., Kästner, M., & Wick, L. Y. (2016). Mycelium-like networks increase bacterial dispersal, growth, and biodegradation in a model ecosystem at various water potentials. *Applied and Environmental Microbiology*, *82*(10), 2902–2908. https://doi.org/10.1128/AEM.03901-15

Young, I. M., & Crawford, J. W. (2004). Interactions and self-organization in the soil-microbe complex. *Science*, *304*(5677), 1634–1637. https://doi.org/10.1126/science.1097394

Young, I. M., & Ritz, K. (2000). Tillage, habitat space and function of soil microbes. *Soil and Tillage Research*, *53*(3–4), 201–213. https://doi.org/10.1016/S0167-1987(99)00106-3

Zhalnina, K., Louie, K. B., Hao, Z., Mansoori, N., Da Rocha, U. N., Shi, S., Cho, H., Karaoz, U., Loqué, D., Bowen, B. P., Firestone, M. K., Northen, T. R., & Brodie, E. L. (2018). Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nature Microbiology*, *3*(4), 470–480. https://doi.org/10.1038/s41564-018-0129-3

Zhou, J., Xia, B., Treves, D. S., Wu, L.-Y., Marsh, T. L., O'Neill, R. V., Palumbo, A. V., & Tiedje, J. M. (2002). Spatial and resource factors influencing high microbial diversity in soil. *Applied and Environmental Microbiology*, *68*(1), 326–334. https://doi.org/10.1128/AEM.68.1.326-334.2002

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Schematic describing study design.

Figure S2. Figure showing microbial community composition across different habitats.

Figure S3. Figure displaying example layout of substrates assayed.

Figure S4. Figure showing chemical profiles of four necromass phenotypes.

Figure S5. Figure showing growth across habitats for different substrate groups.

Figure S6. Figure showing growth across polymers, monomers and dimers.

Figure S7. Correlation matrix of growth between all substrates.

Table S1. Bacterial and fungal strains screened in this experiment.

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