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The afterlife effects of fungal morphology: Contrasting decomposition rates between diffuse and rhizomorphic necromass

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ABSTRACT

Microbial necromass is now recognized as an important input into stable soil organic matter pools in terrestrial ecosystems. While melanin and nitrogen content have been identified as factors that influence the decomposition rate of fungal necromass, the effects of mycelial morphology on necromass decomposition remain largely unknown. Using the fungus *Armillaria mellea,* which produces both diffuse and rhizomorphic biomass in pure culture, we assessed the effects of necromass morphology on decomposition in a 12 week field experiment in *Pinus* and *Quercus* dominated forests in Minnesota, USA. Diffuse and rhizomorphic necromass was incubated for 2, 4, 6, and 12 weeks to assess differences in decay rates and changes in residual necromass chemistry. Rhizomorphic necromass decomposed significantly slower than diffuse necromass in both forest types. This difference was correlated with initial necromass chemistry, particularly nitrogen content, but not with hydrophobicity. Over the course of the incubation, there was a greater change in the chemistry of diffuse versus rhizomorphic necromass, with both becoming more enriched in recalcitrant compounds. Given that many fungi with both saprotrophic and mycorrhizal ecologies produce rhizomorphs, these results suggest that mycelial morphology should be explicitly considered as an important functional trait influencing the rate of fungal necromass decomposition.

1. Introduction

The production and turnover of fungal biomass represents a significant, yet until recently, frequently overlooked contribution to carbon and nitrogen cycles in forest ecosystems (Wallander et al., 2001; Cairney, 2012; Clemmensen et al., 2013; Ekblad et al., 2013; Fernandez et al., 2016; Brabcová et al., 2016, 2018). Similar to plant litter (Melillo et al., 1982), dead fungal biomass (hereafter referred to as necromass) contains biochemical components that strongly influence its rate of decomposition (reviewed in Fernandez et al., 2016). Previous studies have found nitrogen (N) content and N-containing polymers, such as chitin, to be positively correlated with necromass decomposition rates (Koide and Malcolm, 2009; Fernandez & Koide 2012, 2014). Additionally, melanins, a group of complex aromatic polymers (Butler and Day, 1998), have been shown to negatively affect the carbon (C) quality of fungal necromass and consistently slow decomposition rates (Fernandez and Koide, 2014; Fernandez and Kennedy, 2018; Lenaers et al., 2018).

While the aforementioned studies have been instrumental in identifying biochemical factors involved in predicting fungal necromass decomposition, relatively little is known about the effects of mycelial morphology. Filamentous fungi have a range of growth forms, which can be broadly grouped into two categories: diffuse vs. rhizomorphic. Diffuse mycelial growth consists of even growth of individual hyphae across a substrate. This mycelial growth form is particularly efficient at exploiting local nutrient sources, however, resource availability in most environments is notably heterogeneous. As a result, many fungi produce cords, strands, or rhizomorphs to facilitate exploration for and acquisition of patchily distributed resources (Agerer, 2001; Unestam and Sun, 1995; Anderson and Cairney, 2007). Cairney et al. (1989) defined a rhizomorph as a linear mycelial organ composed of a cortex of fine hyphae surrounded by a medulla of larger diameter vessel-like hyphae. These mycelial aggregations are thought to function primarily as conduits for nutrient and water transport among local hotspots in resource availability (Boddy, 1999).

Using minirhizotron imaging, several studies have estimated persistence times for fungal rhizomorphs in soils, which ranged from several months to over one year (Treseder et al., 2005; Vargas and Allen,

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2008; McCormack et al., 2010). These studies contrast with persistence time estimates of diffuse mycelia in soils, which are on the order of days to weeks (Allen and Kitajima, 2013; Ekblad et al., 2013; Hendricks et al., 2016; Hagenbo et al., 2018). Taken together, these estimates suggest that mycelial morphology (rhizomorphic vs. diffuse) may play an important role in determining necromass decomposition rates (Brabcová et al., 2016; Fernandez et al., 2016). Unlike the many studies that have used diffuse mycelia (reviewed in Fernandez et al., 2016), to our knowledge, there has been only one study directly assessing rhizomorph decomposition rates in a field setting. Lamour et al. (2007) collected living *Armillaria* rhizomorphs and incubated them in soils at a 40-year-old *Pinus nigra* plantation in the Netherlands to determine if dead rhizomorphs were being included in their extensive mapping of *Armillaria* networks. Their results indicated that rhizomorphs decayed slowly in relation to other diffuse mycelial necromass experiments (Koide and Malcolm, 2009; Fernandez and Koide, 2014; Brabcová et al., 2016, 2018), with *ca.* 70% of rhizomorph necromass remaining at 10 weeks and *ca.* 50% remaining after 30 weeks. The slowed decomposition of rhizomorphic necromass may be due to a number of non-mutually exclusive factors, including high concentrations of recalcitrant compounds (e.g. melanin), low N content, accumulation of oxalate crystals (Crowther et al., 2015), and hydrophobic properties of rhizomorph surfaces. Although previous studies have provided a glimpse into rhizomorph decomposition, without a direct comparison of diffuse versus rhizomorphic mycelia, exactly how morphology affects necromass decomposition remains uncertain.

In this study, we examined the influence of mycelial morphology on fungal necromass decomposition. To do so, we used the same fungal isolate to generate both diffuse and rhizomorphic necromass, enabling us to directly compare the two necromass types without the confounding factors introduced by using multiple species. Based on the differences in persistence times between diffuse and rhizomorphic morphologies, we hypothesized that rhizomorphic necromass would have slower decomposition rates than diffuse necromass. We also expected that differences in decomposition rate would affect residual chemistry, with rhizomorphic necromass exhibiting less change in chemical composition compared to diffuse necromass. Finally, based on the recent findings of Fernandez and Kennedy. (2018) in the same study system, we predicted that these patterns of decay would be robust across forests differing in both biotic and abiotic conditions.

2. Materials and methods

2.1. Necromass generation

On September 27, 2016, we collected a single *Armillaria mellea* sporocarp (i.e. mushroom) from an oak-dominated forest at the Cedar Creek Ecosystem Science Reserve in East Bethel, MN, USA (45.421329, −93.196730). A sample was isolated from sterile material within the sporocarp, placed in a Petri dish containing half-strength potato dextrose agar (PDA; 12 g potato dextrose broth (Difco, BD Products, Franklin Lakes, New Jersey, USA) + 20 g agar per L), and subsequently subcultured to generate both diffuse and rhizomorphic necromass types. Diffuse biomass was generated by placing mycelial plugs into 125 mL Erlenmeyer flasks filled with 50 mL of half-strength potato dextrose broth (PDB, Difco, BD Products, Franklin Lakes, New Jersey, USA) adjusted to pH 7 with 10% HCl. The fungal cultures were grown at room temperature in ambient light on an orbital shaker at 80 RPM for 29 days. Rhizomorphic biomass was generated by growing the same starting plugs of *A. mellea* (hereafter referred to as *Armillaria*) in test tubes containing *ca.* 15 cm of 6 mm sterile glass beads submerged in 15 mL of liquid half-strength PDB at pH 7. After autoclaving, 150 μL of 100% ethanol was added before inoculation to encourage rhizomorph formation (Allermann and Sortkjær, 1973). Rhizomorphic cultures were grown at room temperature in ambient light for 34 days. At harvest, the initial agar plug was removed from all fungal biomass before rinsing in deionized water (to remove any adhering media) and drying for 48 h at 30 °C in a drying oven. Nylon mesh bags (3 × 3 cm, 53 μm nylon mesh (Elko, Minneapolis, MN, USA)) were filled with approximately 25 mg of either diffuse or rhizomorphic dried fungal necromass and then individually heat-sealed to close (American International Electric Inc., City of Industry, CA, USA).

2.2. Decomposition experiment

Our decomposition experiment was conducted in the same two sites used in Fernandez and Kennedy. (2018). The two sites are located within two km of each other, with one dominated by mixed age white pine (*Pinus strobus*) and the other dominated by mature northern pin oak (*Quercus ellipsoidalis*) (hereafter referred to as Pine and Oak, respectively). Climatic conditions at the sites are considered continental, with a mean annual temperature of 8°C and a mean annual precipitation of 810 mm. The soils are classified as frigid Udipsamments (Grigal et al., 1974), with a poorly developed 3–5 cm O-horizon. Within each site, five plots were established *ca.* 10 m apart (and within 2 m of those of Fernandez and Kennedy, 2018). On July 5, 2017, nylon mesh bags filled with either diffuse or rhizomorphic necromass were buried in each plot at the litter/soil interface and incubated for 2, 4, 6 or 12 weeks. For each necromass type, five replicates were collected per site (one per plot) at each incubation time point (n = 40). At collection, necromass bags were brought back to the laboratory on ice, where each bag was removed and the remaining necromass was dried for up to 48 h at 30 °C. Due to the limited starting material (*ca.* 25 mg per sample), necromass dried quickly and the low drying temperature was chosen to minimize any chemical transformations. Fully dried fungal necromass was weighed to determine dry mass remaining and then stored at room temperature ahead of further chemical analyses.

2.3. Soil characterization

To quantify soil moisture over the duration of the experiment, two soil cores of the top five cm of soil were taken for each plot at each time point (0, 2, 4, 6, 12 weeks) for both the Pine and Oak forest sites (n = 50 per site). Gravimetric soil moisture was estimated for plots in both sites by determining the percent mass loss after drying. Soil pH was measured at the beginning and end of the experiment (0 and 12 weeks) using a water-based method (Soil Survey Staff, 2004).

2.4. Necromass biochemical quantification

The carbon and nitrogen content of one sample of initial diffuse and rhizomorphic *Armillaria* necromass was determined using isotope ratio mass-spectrometry (vario 266 PyroCube, Elementar, Mt. Laurel, NJ, USA) at the IRMS facility at the University of Minnesota. Three technical replicates of each sample were run to assess machine-related variability.

2.5. Fourier transformed infrared spectroscopy (FTIR)

To quantify changes in chemical composition between the starting and ending necromass for both morphologies, FTIR was conducted on two initial samples (i.e. non-incubated) and two samples after 12 weeks of incubation (one diffuse and rhizomorphic necromass type at each time point). The diffuse and rhizomorphic 12 week incubated necromass samples came from the same plot in the Pine site in an effort to minimize effects of abiotic and biotic environmental variation. Samples were dried, ground, and mixed with KBr spectrograde powder (International Crystal Labs, Garfield, NJ, USA) in a sample-to-KBr ratio of 2:100 (wt:wt). After homogenization, samples were pressed into discs and transmission FTIR spectra were recorded using a Thermo Scientific Nicolet iS5 spectrometer with an iD1 Transmission accessory. Sixty-four scans were averaged across the 4000-400 cm^{-1} range at a

resolution of 4 cm^{-1}. Background subtraction was applied using a pure KBr spectrum and a baseline correction was applied to remove baseline distortions. Both background subtraction and baseline correction were done in OMNIC, version 9 (Thermo Fisher Scientific Inc., Waltham, MA, USA), while peak heights were normalized by calculating z-scores prior to final analysis.

2.6. Contact angle measurements

Samples of diffuse and rhizomorphic necromass, dried and stored as described above, were re-dried for an additional hour directly before measuring the contact angle of water droplets. To facilitate the accuracy of these measurements for rhizomorphs, rhizomorphic necromass was dried in a flattened position (via pressing between non-stick pads) to create the flattest surface possible. After drying, a 1 μL drop of distilled water was placed on the tissue surface of each necromass type. Pictures were taken from a 90° angle relative to the necromass tissue within the first 1–3 s of water droplet placement in order to negate the effects of evaporation. Contact angles were measured in ImageJ (v1.49) using the low-bond drop shape analysis (LB_ADSA) plug-in (http://bigwww.epfl.ch/demo/dropanalysis/). Contact angles were taken for six individual replicates for each diffuse and rhizomorphic necromass types. Necromass hydrophobicity was classified as > 90° contact angle = hydrophobic and < 90° contact angle = hydrophilic (Yuan and Lee, 2013).

2.7. Statistical analyses

To test for differences in soil moisture and soil pH across the two sites, we ran two two-way fully factorial analyses of variance (ANOVA), with forest type (Pine vs. Oak) and sampling time as the predictor variables. Prior to running the ANOVAs, variances were checked and, if non-homogenous (true for soil moisture but not soil pH), a log transformation was applied to reduce heterogeneity. Residual plots for both ANOVAs suggested no significant deviation from normality.

To assess differences in mass loss of diffuse and rhizomorphic necromass morphologies, we ran a three-way fully factorial ANOVA with incubation time (0, 2, 4, 6, 12 weeks), morphology (diffuse vs. rhizomorphic), and forest type (site) as the predictor variables, with plot included as a random effect and soil moisture as an effect nested within forest type. Soil pH was only measured at the beginning and end of the necromass incubation period (0 and 12 weeks) and such, was not included in this analysis. Prior to running the ANOVA, we determined there was no co-linearity between time and soil moisture and that the variances for forest type or time (excluding the time 0, which had no variance) were homogenous. The residual plot for the ANOVA model suggested no significant deviation from normality. Because of the well-recognized non-linear nature of decomposition (Berg, 2014), we also assessed mass loss rates of both necromass morphologies using a non-linear two-pool exponential decay model. We determined this type of model provided greater explanatory power than a single pool model based on a lower AIC score and a higher r^2 value. More highly parameterized models did not produce notably greater explanatory power. All of the ANOVAs and model assumption checking were run in JMP (v12) and considered significant at $P < 0.05$.

To examine shifts in the chemical composition from the beginning to the end of the experiment, we calculated two indices of recalcitrance for both initial and 12 week incubated samples for both necromass morphologies. The first index represents the ratio of aromatic to aliphatic bonds and the second index the ratio of C- to O- functional groups (Margenot et al., 2015). All main peaks were identified and normalized peak heights were used in the calculation of one or both indices, excluding both Amide I (1645 cm^{-1}) and Amide II (1535 cm^{-1}) peaks based on the confounding influence from multiple bond vibrations at these wavenumbers.

Table 1
Soil pH and moisture (%) at Oak and Pine forest sites (mean and 1 standard error). The carbon:nitrogen (C:N) ratio, percent carbon (C), percent nitrogen (N) and contact angle measurement (°) with hydrophobicity assignment for initial diffuse and rhizomorphic *Armillaria mellea*.

Forest type	N	Site factor	Mean	SE
Pine	20	pH	5.59	0.07
	50	soil moisture	8.98%	0.49
Oak	20	pH	4.31	0.08
	50	soil moisture	10.63%	0.52

Morphology	N	Element	Mean	SE	Hydrophobicity
Diffuse	3	C:N	11.35	0.20	hydrophobic
	3	C	45.51%	0.18	
	3	N	4.01%	0.06	
	3	6	111°	2.49	
Rhizomorphic	3	C:N	26.21	0.03	hydrophobic
	3	C	44.51%	0.06	
	3	N	1.70%	< 0.01	
	3	6	110°	2.97	

3. Results

Soils at the Pine and Oak sites differed in both moisture and pH, with the Oak soils being, on average, wetter and more acidic (Table 1). Both of these soil variables also fluctuated over the 12 week incubation (time × variable interaction; moisture: $F_{4,90} = 9.63$, $P < 0.001$, pH: $F_{1,16} = 16.18$, $P < 0.001$). The average C contents of the diffuse and rhizomorphic *Armillaria* necromass morphologies was very similar, while the amount of N in diffuse necromass was nearly two-fold higher. As a result, the C:N ratio of rhizomorphic necromass was more than double that of diffuse necromass (Table 1). The average contact angle for diffuse and rhizomorphic necromass only varied by a single degree, with both being classified as hydrophobic (Table 1).

The decomposition of *Armillaria* necromass was significantly influenced by both necromass morphology ($F_{1,54} = 518.08$, $P < 0.001$) and incubation time ($F_{1,56} = 19.22$, $P < 0.001$), but not forest type ($F_{1,9} = 4.51$, $P < 0.063$). Mass loss rates were consistently greater for the diffuse necromass with only 8% of initial mass remaining after 12 weeks, compared to 45% of initial mass remaining for the rhizomorphic necromass (Fig. 1). There was also a significant interaction between necromass morphology and time of incubation ($F_{4,90} = 5.66$, $P < 0.001$), with rhizomorphic necromass not differing significantly in mass remaining from the two week harvest to the end of the experiment, while diffuse necromass still lost significant amounts of mass over the same time period. No other higher-order interactions were significant (Table S1), although due to low sample sizes, these interactions may have remained undetected. The exponential decay model fit to the mass loss data also demonstrated consistent differences in the initial (first pool) and later (second pool) rates of decay between necromass types. Specifically, the decay rate (k) of the first pool was higher for diffuse than rhizomorphic necromass (0.11 ± 0.03 vs. 0.01 ± 0.007, mean ± 1 SE) and estimated percent mass remaining after 12 weeks was significantly lower (diffuse: 67.97 ± 6.16% vs. rhizomorphic: 43.97 ± 3.64%, mean ± 1 SE).

The FTIR absorbance profiles varied between initial and 12 week incubated samples (Fig. 2). The initial content of the rhizomorphic necromass was more recalcitrant in both indices than the diffuse necromass (Table 2). Following incubation, there was a notable reduction of aliphatic groups and accumulation of aromatic groups in the diffuse necromass, resulting in the increase of Index I values from initial to incubated diffuse samples, while incubation had no effect on the Index I values of the rhizomorphic necromass (Table 2). The increase for diffuse necromass compared to the unchanging Index I values for rhizomorphic necromass matched the greater amount of decomposition

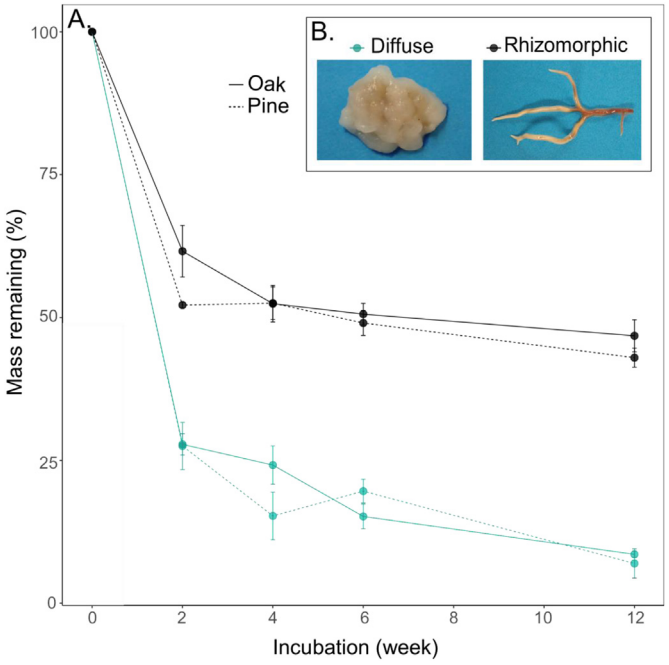

Fig. 1. A. Percent mass remaining (mean ± 1 SE) of diffuse (blue) and rhizomorphic (black) *Armillaria mellea* necromass in Oak (solid line) and Pine (dotted line) sites after 2, 4, 6, and 12 weeks of incubation (n = 20 for each necromass type in each forest type per time point, N = 40). B. Representative images of *A. mellea* diffuse and rhizomorphic mycelial morphologies in culture. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

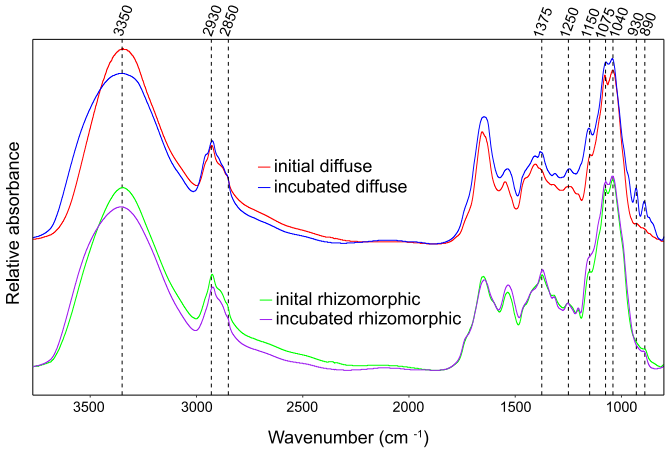

Fig. 2. FTIR absorbance profiles of initial and 12 week incubated diffuse and rhizomorphic *Armillaria mellea* necromass (N = 1). Diffuse spectra colors: initial = red, incubated (12 weeks) = blue. Rhizomorphic spectra: initial = green, incubated (12 weeks) = purple. Wavenumbers with peaks used to calculate recalcitrant indices are shown with dashed lines. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

experienced by diffuse necromass. Index II also increased with incubation for diffuse necromass, while, Index II dropped slightly with incubation for rhizomorphic necromass (Table 2).

4. Discussion

This study is the first to directly compare the effect of mycelial morphology (rhizomorphic vs. diffuse) on fungal necromass decomposition rates. We found that the decomposition of rhizomorphic necromass was significantly slower than that of diffuse necromass, which is consistent with previous findings of rhizomorphs having greater persistence times in soils (Treseder et al., 2005; Vargas and Allen, 2008; McCormack et al., 2010). Similar to previous studies comparing diffuse necromass (Koide and Malcolm, 2009), we saw that percent N content, which was more than two-fold higher in the diffuse than the rhizomorphic necromass, was positively associated with greater mass loss. Our analysis of both necromass types found very little difference in their hydrophobicity along with no detectable amount of melanin (Appendix), suggesting these factors did not contribute to the observed differences in decomposition. Taken together, these results indicate that mycelial morphology, likely via effects on initial N content, strongly influences the initial mass loss in decomposing fungal necromass.

To date, there have been few studies examining the chemical compounds lost or retained as necromass decomposes (Drigo et al., 2012; Fernandez and Koide, 2012). Our FTIR results, while only based on a single replicate per incubation and necromass type, revealed very little difference in absorbance profiles between the initial and 12 week incubated rhizomorphic necromass, while the initial and 12 week incubated diffuse necromass displayed differences over the entire spectra. Both morphologies showed reduced hydroxyl peaks at 3350 cm^{-1} in the incubated necromass compared to that of initial necromass, most likely due to the protonation of hydroxyl groups. The higher initial recalcitrance index values and the lower change following incubation suggest that in addition to having lower N content, rhizomorphic necromass also contains a larger proportion of recalcitrant compounds. We suspect these traits acted synergistically in retarding the decomposition for rhizomorphic compared to diffuse necromass. Given the very low sample size, however, our recalcitrance-related results should be considered preliminary and future research targeting a greater number of initial and incubated samples, both within and across species, is needed.

Many fungal species produce rhizomorphs in nature, but lab-grown diffuse mycelia or collected sporocarp tissue is most often used in fungal necromass decomposition studies (Koide and Malcolm, 2009; Fernandez & Koide 2012, 2014; Crowther et al., 2015; Brabcová et al., 2016, 2018; Fernandez and Kennedy, 2018). The preference for these latter necromass types appears to be due to the fact that most fungi have a diffuse growth form in pure culture and sporocarps can be easily collected. This is especially true for basidiomycete ectomycorrhizal fungi, which often produce rhizomorphs in soil (Agerer, 2001), but not in pure culture. Due to the challenges associated with cultivating rhizomorphic mycorrhizal necromass, targeting saprotrophic relatives that produce rhizomorphs in culture (such as in this study) represents a promising approach. Unlike saprotrophic fungal necromass, the production and turnover of mycorrhizal fungal biomass represents a direct carbon pathway between primary producer and stabilized organic matter (Ekblad et al., 2013). Given this, understanding the ecological factors influencing this pathway is critical to furthering knowledge of how fungal necromass influences C and N cycling within forest ecosystems.

With regard to placing our results in a larger forest soil C and N cycling context, a number of factors should be considered. The first is the relative contributions of these two necromass morphologies into forest soils. We are not aware of any studies specifically quantifying diffuse versus rhizomorphic mycelial abundances for soil saprotrophic fungi, but studies of ectomycorrhizal fungi often classify species by extramatrical mycelial exploration type (Agerer, 2001). The amounts of cord-forming 'long-distance' types versus 'short'- or 'medium-distance fringe' types might be used as a first approximation to estimate the inputs of different necromass morphologies, particularly in temperate and boreal forests where ectomycorrhizal fungi are dominant components of the microbial biomass (Högberg and Högberg, 2002). A second consideration is the turnover rates of fungal necromass in comparison to other soil organic matter inputs. It appears that the initial decomposition of fungal necromass is faster than that of plant litter (Koide

Table 2
Functional group assignments for the ten wavenumbers used to determine Index I and II. Index I: the ratio of aromatic to aliphatic functional groups (wavenumbers: 890 + 930 +/2850 + 2930 +3350). Index II: the ratio of C- to O- functional groups (wavenumbers: 890 + 930 + 1375 + 2850 + 2930/ 1040 + 1075 + 1150 + 1250 + 3350).

Wavenumber (cm^{-1})	Assignment	Functional group	References
890	**Index I & II: aromatic**	C-H	Galichet et al., 2001; Mohaček-Grošev et al., 2001; Šandula et al., 1999
930	**Index I & II: aromatic**	C-H	Margenot et al., 2015; Mohaček-Grošev et al., 2001
1040	**Index II**	C-O, C-C	Hribljan et al., 2017; Kosa et al., 2017; Mohaček-Grošev et al., 2001; Šandula et al., 1999
1075	**Index II**	C-O	Galichet et al., 2001; Margenot et al., 2015; Šandula et al., 1999
1150	**Index II**	C-O	Hribljan et al., 2017; Margenot et al., 2015
1250	**Index II**	C-O, O-H	Guo and Bustin, 1998; Kosa et al., 2017
1375	**Index II**	C-H	Kosa et al., 2017
2850	**Index I & II: aliphatic**	C-H	Guo and Bustin, 1998; Margenot et al., 2015; Michell and Sourfield, 1970; Mohaček-Grošev et al., 2001
2930	**Index I & II: aliphatic**	C-H	Guo and Bustin, 1998; Mohaček-Grošev et al., 2001; Šandula et al., 1999
3350	**Index I & II: aliphatic**	O-H, N-H	Guo and Bustin, 1998; Hribljan et al., 2017; Margenot et al., 2015
Morphology	**Aromatic**	**Aliphatic**	**Index I**
initial diffuse	0.67	6.87	0.10
incubated diffuse	1.88	6.48	0.29
initial rhizomorphic	0.97	6.67	0.15
incubated rhizomorphic	0.85	5.79	0.15
Morphology	**C-H**	**C-O**	**Index II**
initial diffuse	5.20	13.07	0.40
incubated diffuse	6.85	13.94	0.49
initial rhizomorphic	5.92	14.10	0.42
incubated rhizomorphic	5.39	14.21	0.38

et al., 2011), but it is important to recognize that highly melanized necromass such as *Armillaria* rhizomorphs in field settings (see Appendix 1) may be able to persist in soils for months to years (Lamour et al., 2007; Fernandez et al., 2013). In addition to these recalcitrant fractions that may directly contribute to carbon stabilization in forest soils, there is growing evidence that the residues of microbial decomposers can represent a large part of soil organic matter pools when they become mineral associated (Cotrufo et al., 2013). Since fungal necromass has been shown to be richer in N than plant litters (López-Mondéjar et al., 2018), the decomposition of labile components within fungal necromass may also fuel microbial decomposer growth and indirectly stimulate greater carbon soil stabilization.

While our study provides important evidence that mycelial morphology can influence fungal necromass decomposition, more research on additional fungal species with both diffuse and rhizomorphic growth types are needed to more confidently determine the effect of morphology and associated biochemistry on factors such as N content and hydrophobicity. One potentially important biochemical factor influencing necromass decomposition rates is the production of oxalate crystals by fungal tissues. Crowther et al. (2015) showed oxalate crystals can affect the recalcitrance of fungal necromass and therefore could have affected the decomposition rates found in this study. Another factor we did not measure was surface area:mass ratio, which may also play a role in necromass decomposition by influencing microbial access to the substrate. Interestingly, McCormack et al. (2010) found that rhizomorph persistence times were negatively related to rhizomorph diameter, meaning that smaller diameter rhizomorphs persisted in soil longer than larger diameter rhizomorphs. In the case of mycorrhizal fungi, this rhizomorph persistence pattern may be due to the ability of fungi to recycle their own biomass (Clemmensen et al., 2015). While it is clear that many aspects of fungal necromass decomposition need further study, the consistency of our results across two forest types differing in soil moisture, pH and canopy composition does suggest the effects we observed are likely to be broadly representative of how mycelial morphology influences necromass decomposition. Given the importance of biochemical traits such as melanin in driving microbial decomposer community structure (Fernandez and Kennedy, 2018), we suggest that future studies comparing the microbial communities on decomposing diffuse versus rhizomorphic necromass could also help in further understanding the observed differences in mass loss.

5. Conclusions

By quantifying the effect of mycelial morphology, this study contributes to the rapidly growing body of literature characterizing the dynamics of fungal necromass decomposition (Fernandez et al., 2016; Brabcová et al., 2016, 2018; Lenaers et al., 2018; López-Mondéjar et al., 2018). The large differences we observed in decomposition rates between diffuse and rhizomorphic necromass morphologies indicates that mycelial morphology and the associated changes in chemistry can strongly affect rates of mass loss. Given that many saprotrophic and mycorrhizal basidiomycete fungi produce rhizomorphs, our results suggest that mycelial morphology should be explicitly considered as a key functional trait influencing the rate of fungal necromass decomposition.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2018.08.002.

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