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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