

Chapter 16

Contribution of Fungal Macromolecules to Soil Carbon Sequestration

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Abstract Saprotrophic fungi are key moderators in the global carbon cycle because of their ability to degrade the three most abundant biopolymers: cellulose, lignin, and chitin. Fungi are a significant contributor to soil microbial biomass but little is known about the contributions of fungal biomass to diagenetically altered soil organic carbon. Here we show that a portion of fungal necromass is resistant to decay by a natural soil microbial community over a month-long degradation study. The results of FTIR analysis indicate that this resistant portion is likely composed mainly of fungal chitin.

Keywords Chitin • Soil organic matter • Soil fungi • FTIR

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Introduction

Saprotrophic fungi have the ability to enzymatically degrade the three most important biopolymers: cellulose, lignin, and chitin (Baldrian et al. 2011). A globally important flow of carbon is therefore processed by fungi, but little is known about how that carbon is transformed, metabolized, or perhaps stored in soil organic matter (SOM), defined here as the total belowground organic matter without plant pieces and roots. Additionally, the contributions of fungal biomass and necromass to SOM, and their impact on the potential carbon sequestration abilities of soil remain poorly understood.

Fungi have been shown to constitute a major portion of belowground biomass (Six et al. 2006) but the amount varies widely with land use and ecosystem function (Joergensen and Wichern 2008). Fungal hyphal tissue is more resistant to biodegradation than bacterial polymers (Guggenberger et al. 1999). Fungi have been shown to contribute to soil aggregate formation (Hu et al. 1995) and are likely important to soil carbon sequestration (Jastrow et al. 2007; Clemmensen et al. 2013). It is known that the ratio of fungi to bacteria in soils is affected by a variety of environmental factors including cultivation intensity, grazing intensity, and C–N ratios or substrate quality (Six et al. 2006).

It is likely that fungal production of a variety of biopolymers, including cell exudates and cell wall materials, contributes to diagenetically altered SOM. As fungal hyphae are known to be important to the formation of soil aggregates (Bossuyt et al. 2001; Tisdall and Oades 1982), fungi actively work to stabilize SOM originating from other sources, including plants and bacteria. Fungal biomass itself has a greater chance for stabilization compared to other microbial biomass because of their association with aggregates (Six et al. 2006).

Here we first briefly summarize the chemistry of the most important fungal biopolymers, and then describe the results of a degradation study of fungal cell wall material. The degradation of ectomycorrhizal fungal tissue has been described previously (Koide and Malcom 2009; Wilkinson et al. 2011; Drigo et al. 2012), but to the best of our knowledge this is the first time fungal necromass degradation has been studied in a saprotrophic fungal species. This study provides results and insights into the biochemical stabilization (or chemical recalcitrance) of fungal biopolymers, and in the last section we summarize questions that remain to be addressed.

Fungal Biopolymers

Fungal cell walls contain a variety of biopolymers, with chitin and glucan being the most abundant for most species. Chitin is a polymer composed of *N*-acetyl-*D*-glucosamine in β -(1–4)-glycosidic bonds. Glucans are matrix polysaccharides that are part of fungal cell walls (Kogel-Knabner 2002). In general, structural polysaccharides like chitin and β -glucan are non-water-soluble crystalline substances whereas matrix polysaccharides like α -glucan are amorphous and water

soluble. Fungal cell wall material is the main source of glucosamine to SOM (Parsons 1981), and amino sugar compounds like chitin in general contribute between 5 and 12 % of total soil organic nitrogen (Stevensen 1982).

Sporopollenin forms the outer wall of fungal spores as well as plant pollen and is another structural biopolymer synthesized by fungi. It is a polymer consisting predominantly of unbranched aliphatics and a varying amount of aromatics with a high degree of crosslinking, and is derived from the polymerization of unsaturated fatty acids. The complex polyether polymer contains no nitrogen and is composed of only carbon, oxygen, and hydrogen atoms (Bubert et al. 2002).

Melanin, another important fungal product, has long been hypothesized to be a precursor to humic materials in SOM (Linhares and Martin 1978; Six et al. 2006), but the exact mechanism of the formation of SOM from melanin is unclear as fungi contain melanin with many different structures (Knicker et al. 1995). However, fungal hyphae rich in melanin decay more slowly than hyphae low in melanin (Six et al. 2006), indicating that overall, melanin is relatively recalcitrant and may act to protect other hyphal organic matter from degradation.

Fungi have also been shown to contribute other, potentially recalcitrant, polymer-like materials to SOM through exudates. One example is glomalin-like soil protein (GLSP), which is thought to be a mucus-like substance produced by fungi, and has been estimated to contribute up to 5 % of soil C and N (Treseder and Turner 2007). However, because of the unspecific nature of the extraction procedure commonly used for GLSP analysis, it is likely that humic matter is extracted and analyzed along with GLSP and these numbers are an overestimate (Schindler et al. 2007). Soil fungi produce a range of extracellular polysaccharides that contribute to SOM and are important in soil aggregate formation, the mechanism for physical protection and slower decomposition of organic matter in soils (Wright and Upadhyaya 1998; Six et al. 2006).

The work described here is meant as a first estimate of the contribution of saprotrophic fungal necromass to diagenetically altered SOM. Only the contribution of the fungal cell wall material was studied, although fungal exudates are certainly also important contributors to SOM.

Degradation of *Fusarium avenaceum* Necromass

In order to determine the contributions of saprotrophic fungal necromass to diagenetically altered SOM, a degradation sequence of organic matter from the saprotrophic fungus *Fusarium avenaceum*, a member of the phylum Ascomycota, was studied. Fungal necromass was subjected to degradation by a natural soil microbial consortium over a period of 36 days. Fungal tissue was grown aseptically in liquid nutrient media for 14 days and at harvest was well-rinsed to remove traces of nutrient media and soluble organics from fungi, and oven-dried. It is assumed that after the rinsing steps the main source of organic carbon remaining was cell wall tissue. Fungal tissue was placed in mesh bags (100 μm pore size) and buried in the soil in pots containing a sterile 50:50 soil:sand mixture that was inoculated with a natural microbial community extracted from soil samples collected in a tallgrass

prairie site at the Chicago Botanic Garden. The pots were watered periodically and kept under relatively constant temperature and photosynthetically active radiation. *Eruca sativa* (arugala) seeds were planted in each microcosm at Day 0 and again just before the Day 24 harvest in an effort to replicate a natural environment. Four samples were harvested at each of four time points: 5, 12, 24, and 36 days. At each harvest, the mesh bags were opened, fungal tissue was identified and separated from soil particles via handpicking, and then oven-dried and weighed. Samples from each harvest were ground to powder with mortar and pestle and analyzed via Fourier Transform Infrared (FTIR) spectroscopy.

The majority of fungal biomass was found to turn over on a time period of days, but a portion appears to survive this initial phase of degradation. When compared to fungal packets not subjected to degradation, over 75 % of the original fungal mass was lost within the first 5 days of incubation (data not shown). Between Day 5 and Day 12 an additional 5 % of mass was lost, on average (though this change is not significant), and after Day 12 the amount of fungal tissue remaining in the mesh bags stayed relatively constant at approximately 10–15 % of the original biomass. This relatively recalcitrant portion, resistant to microbial degradation over a period of weeks, is hypothesized to be a contributor to diagenetically altered SOM.

FTIR (Fig. 16.1) analysis further indicates that the residual relatively recalcitrant fungal tissue is biochemically distinct from the original tissue. FTIR data specifically shows two areas of significant chemical change over the month-long experiment: the ester (C=O stretch), amide I (C=O stretch), and amide II (C–N bend) region (approximately 1840 to 1490 cm^{-1} , Fig. 16.1a Box 1) and the carbohydrate (C–O stretch) region (approximately 1190 to 950 cm^{-1} , Fig. 16.1a Box 2). The component absorption peaks were resolved in each region using the PeakFit software package (SyStat). Data are shown in Fig. 16.1b–d, showing curve fits from raw fungal material and Day 36 of degradation as an example. Chitin, a glucosamine polysaccharide synthesized by *Fusarium* and other fungi, should be reflected structurally in both of the ranges analyzed, though other fungal polymers, like β -1,4-glucan and melanins, are likely also represented in these spectral regions.

Striking differences are apparent between the raw fungal tissue and the Day 36 degraded tissue. First, ester functionality (represented by a peak at approximately 1745 cm^{-1} and shown as a red line in Fig. 16.1b) is present in raw fungal tissue (Fig. 16.1b) but is absent in degraded tissue (Fig. 16.1d). The carbohydrate region of the spectrum (between 1175 and 925 cm^{-1} , Fig. 16.1a Box 2, c, e) also shows significant changes after degradation. The raw fungal tissue has a mixture of absorbances in this region that is dominated by a doublet. By 36 days of degradation, however, the doublet is less pronounced as a result of the selective loss of at least one carbohydrate component. A similar phenomenon occurred in the carbonyl region between 1700 and 1580 cm^{-1} (Fig. 16.1a Box 1, b, d) that is dominated by amide I absorbances. These are likely from both protein and chitin. A loss of a non-amide carbonyl moiety at \sim 1715 cm^{-1} occurs. The relative intensities and absorption maxima of the various carbonyl stretches in the amide I range change over time. Curiously, the C–N absorbances in the amide II region are unchanged throughout the degradation sequence.

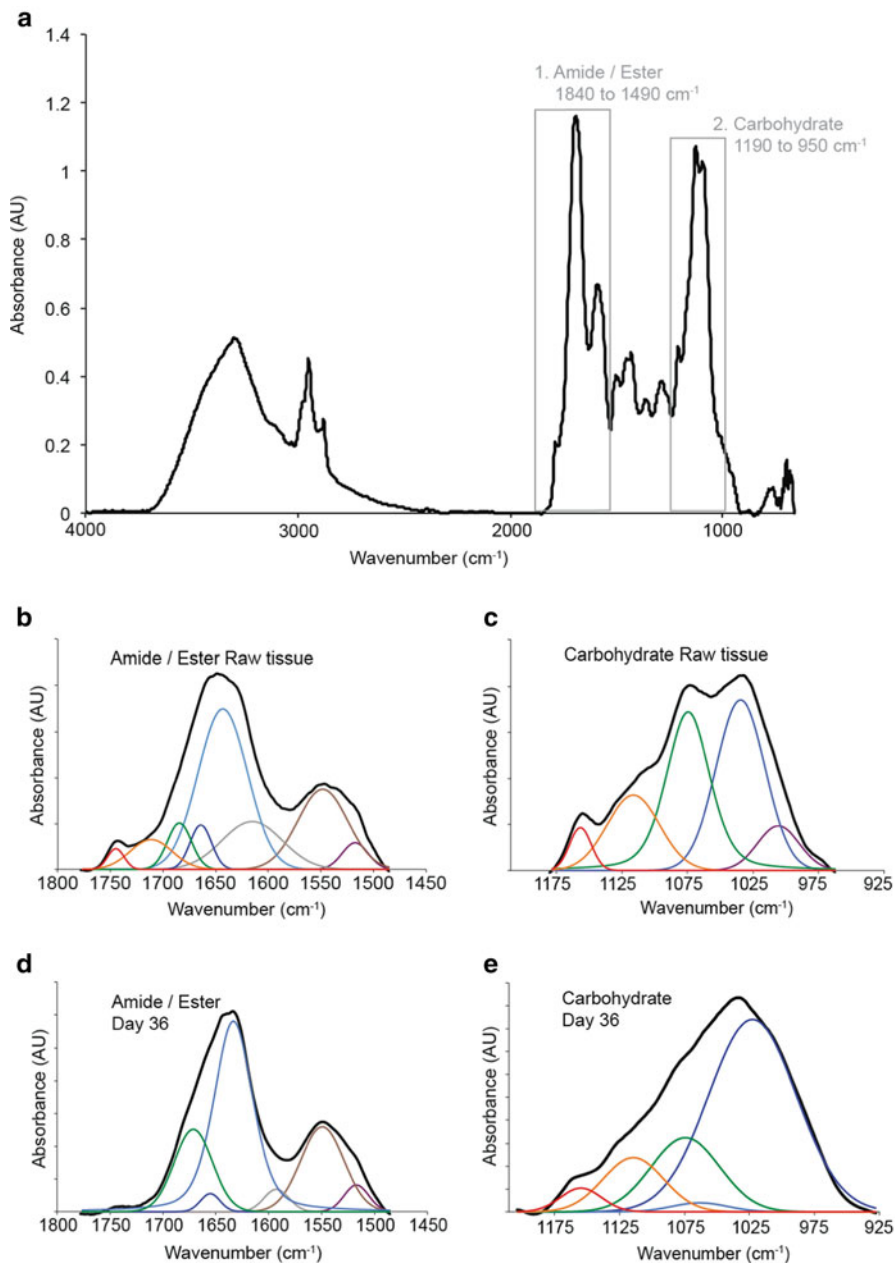


Fig. 16.1 FTIR spectra from fungal degradation experiment. Complete spectrum of raw fungal tissue with Amide/Ester and Carbohydrate regions indicated (a), Amide/Ester region from raw tissue (b), and Carbohydrate region from raw tissue (c), amide/ester region from day 36 tissue (d), and carbohydrate region from day 36 tissue (e). Colors correspond to the same peak in b and d and in c and e. *Thick black lines* in b–e correspond to the original spectrum

These data support the hypothesis that a portion of fungal biomass is relatively recalcitrant in these soils, as recently shown for arbuscular mycorrhizal fungi (Clemmensen et al. 2013) and ectomycorrhizal fungi (Drigo et al. 2012). The recalcitrant fraction contains polysaccharide and amide-linked functional groups, which is consistent with chitin. The breakdown of more labile organic carbon, including proteins (indicated by the changing amide I region), polysaccharides (indicated by the changing C–O stretch region assigned to carbohydrates), and esters (primarily associated with glycol- and phospholipids) occurs on a more rapid time scale than that of what we hypothesize to be fungal chitin. These results illustrate the potential contribution of fungal necromass to diagenetically altered SOM.

Future Research

The research summarized here sheds light on the role that fungal biomass plays in the formation and stabilization of diagenetically altered SOM. However, there is much that remains to be determined. The existence of fungal chitin after the degradation will be confirmed with additional analytical methods such as pyrolysis-gas chromatography analysis. Extending these analyses to the natural environment through *in situ* studies, examining the decomposition profiles of different species of fungi, and in different ecosystems are among our goals, as it is unknown whether the data summarized here can be broadly generalized to other soils and to fungal necromass from other species. Additionally, the microbial communities involved in the degradation of fungal tissue has been studied for only one species of ectomycorrhizal fungus (Drigo et al. 2012), and therefore are largely unknown. Physical protection of fungal biomass inside soil aggregates is likely also important for its stabilization in soils, and may actually be more important than the inherent biochemical stabilization of these biopolymers (Six et al. 2006), but this has yet to be systematically studied.

Further research may also focus on fungal extracellular polysaccharide exudates, of which there is even less information available than for fungal chitin. GLSP is still only operationally defined, and its exact function as well as the function of other fungal exudates, and how they may contribute to the protection and stabilization of SOM, is still not known.

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