Review paper

The decomposition of ectomycorrhizal fungal necromass

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A B S T R A C T

The turnover of ectomycorrhizal (EM) fungal biomass represents a significant input into forest carbon (C) and nutrient cycles. Given the size of these fluxes, understanding the factors that control the decomposition of this necromass will greatly improve understanding of C and nutrient cycling in ecosystems. Recent research has highlighted the considerable variation in the decomposition rates of EM fungal necromass, and patterns from this research are beginning to emerge. In this article we review the research that has examined both intrinsic and extrinsic factors that control the decomposition of EM fungal necromass and propose additional factors that may strongly influence EM fungal necromass decomposition and ecosystem properties. We argue that, as with most plant litter, the stoichiometry (C:N) of EM necromass is an important factor governing decomposition, but its role is modulated by the nature of the C and N in the tissue. In particular, melanin concentration appears to negatively influence the quality of EM fungal necromass much as lignin does in plant litters. Other intrinsic factors such as the morphology of the mycelium may also play a large role and suggest this as a focus for future research. Extrinsic factors, such as decomposer community activity and physical protection by soil, are also likely to be important in governing the decomposition of ectomycorrhizal necromass in situ. Finally, we highlight the potential importance of EM fungal necromass diversity and abundance in influencing terrestrial biogeochemical cycles. Understanding the factors that control the decomposition of EM necromass will then improve the predictive power of next-generation terrestrial biosphere models.

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1. Introduction

Ectomycorrhizal (EM) fungi play a critical role in carbon (C) and nutrient cycling in many terrestrial ecosystems. The influence of EM fungi on nutrient uptake has been well documented, and its implications for ecosystem processes are now generally appreciated (Read, 1991; Read and Perez-Moreno, 2003; Courty et al., 2010; Orwin et al., 2011). In addition, we are beginning to recognize the significance to biogeochemical cycles of inputs resulting from the death of EM fungal tissues (Fogel, 1980; Fogel and Hunt, 1983; Treseder and Allen, 2000; Langley and Hungate, 2003; Godbold et al., 2006; Cairney, 2012; Clemmensen et al., 2013; Ekblad et al., 2013). Until recently, little attention has been paid to EM fungal necromass inputs due to the fact that microbial necromass inputs have been considered to be relatively insignificant as standing pools of microbial biomass are often relatively small compared to those of standing plant biomass. However, due to the rapid turnover and replacement of microbial biomass, large quantities of C and nutrients flow through the microbial pool, making its contribution to soil organic matter (SOM) disproportionately large relative to standing microbial biomass (Grandy and Neff, 2008). The turnover of microbial residues is now recognized as a major pathway to SOM formation (Kögel-Knabner, 2002; Cotrufo et al., 2013; Schmidt et al., 2011). For instance, efforts are underway to explicitly represent microbial pools as key components in the next generation of coupled biosphere-climate models (Todd-Brown et al., 2012; Wieder et al., 2013).

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Mycorrhizal fungi, in particular, appear to have great influence on C cycles in many terrestrial ecosystems (Read, 1991; Read and Perez-Moreno, 2003; Godbold et al., 2006; Clemmensen et al., 2013; Averill et al., 2014; Clemmensen et al., 2015). Mycorrhizal fungi are unique among fungi in that the majority of their C is derived from living host plants (Hobbie et al., 2002). In terms of their C source, these fungi are analogous to fine roots. While there is still some uncertainty associated with estimates of total C allocation to EM fungi, evidence from multiple sites indicated that a considerable proportion of NPP is allocated belowground to these plant symbionts (Fig. 1). Using data from both lab and field studies, Hobbie (2006) found that C allocation to EM fungi ranged from 1 to 22% of annual net primary productivity. Ekblad et al. (2013) suggested that approximately 7% allocation of NPP to EM fungi is a reasonable estimate. More recently, Allen and Kitajima (2014) estimated that 27% of NPP allocated to EM fungi in a Californian mixed conifer-deciduous forest using minirhizotron techniques and 34% of NPP using an isotopic fractionation model proposed by Hobbie and Hobbie (2006). In any case, the turnover of EM fungal biomass results in large inputs into C and nutrient cycles in terrestrial ecosystems (Fig. 1) (Godbold et al., 2006). Furthermore, the intimate association of the EM fungi with fine roots alters root biochemistry and decomposition, which is another large litter input (Langley and Hungate, 2003). Recently, Clemmensen et al. (2013) provided evidence suggesting that a very large portion (i.e. >50%) of C stored in SOM in a boreal forest system was of fungal and root origin, supporting the hypothesis that belowground litter inputs are just as significant, if not more significant, than aboveground litter inputs into SOM pools in many ecosystems (Kätterer et al., 2011).

Given the large fluxes of C and nutrients entering the soil through fungal necromass and their substantial contribution to SOM, understanding the decomposition dynamics of these litter inputs has become an increasingly important line of research. In this review we focus on research that has examined the decomposition of EM fungal necromass with the goal of improving our understanding of the factors that control their decomposition. Specifically, we discuss the influence on the decomposition of EM fungal necromass of 1) chemistry, 2) the morphology of EM necromass, and 3) extrinsic factors (physical and physiochemical protection). We highlight the differences among EM species and the potential for this variation to influence biogeochemical cycles and argue that EM fungal community structure will likely play a significant role in how these cycles are affected. Finally we discuss future directions within this burgeoning research area. It is important to recognize that other major types of mycorrhizal necromass, such as those produced by arbuscular and ericoid mycorrhizal fungi, may be equally important in contributing to stable SOM in other ecosystems (e.g. grasslands; tropical forest; bogs; heath). In our discussion of these topics we draw from the general fungal literature and therefore many of the concepts can be applied to understanding necromass decomposition dynamics of other groups of fungi. That said, we urge careful consideration of the sometimes-vast dissimilarities in traits and life histories among groups of fungi.

2. How does biochemistry influence EM fungal necromass decomposition?

2.1. Necromass stoichiometry

A major factor determining the decomposition of litter is the initial litter “quality” as determined by chemical composition (Melillo et al., 1982; Berg, 1984). Quality is a function of both the concentration of growth-limiting nutrients (i.e. C, N, P) and the recalcitrance, or the resistance to decomposition, of the molecules comprising the litter. Decomposition is generally favored by relatively low litter C:N ratios (Cleveland and Liptzin, 2007), which presumably helps to maintain the low C:N ratio of microbial cells (Sinsabaugh et al., 2009; Manzoni et al., 2010), yet there is increasing evidence that additional aspects of quality beyond basic stoichiometry also can play a role determining decomposition rates.

Koide and Malcolm (2009) tested the role of C and N concentrations on the decomposition rate of EM fungal necromass in a litterbag study and found that initial N concentration was a good predictor of the decomposition rate of these tissues. However, Wilkinson et al. (2011) examined CO₂ efflux from soil microcosms amended with the EM fungal necromass and found no relationship between the C and N contents or the C:N ratio of the necromass with CO₂ emitted during decomposition. Inconsistencies between these studies may be the result of differences between in situ and in vitro approaches. For instance, the decomposer communities used in microcosm experiments may exclude key functional groups (i.e. live EM fungi) that are found in natural conditions and may result in different decomposition dynamics. Additionally, there may be other key biochemical factors influencing the quality of the fungal necromass. Litters with high concentrations of recalcitrant compounds tend to have slower decomposition rates due to their resistance to enzymatic breakdown (Meentemeyer, 1978; Melillo et al., 1982). Lignin is a compound found in plant tissues that is, relative to many other compounds, quite resistant to decomposition due to its complex and irregular molecular structure. The decomposition of lignin requires the production of extracellular oxidative enzymes by decomposers (Kirk and Farrell, 1987). As a result, lignin:N ratios are often good predictors of aboveground litter and root litter decomposition rates (Melillo et al., 1982; Berg, 1984; Silver and Miya, 2001).

2.2. Necromass chemical components

Chemical composition varies widely among fungal species and likely contributes to the wide variation in decomposition rates of EM fungal necromass (Table 1). Indeed, components of the fungal cell may control the decomposition of EM fungal necromass. In contrast, the cytoplasmic fraction of fungal tissue does not likely play a significant role in the decomposition of EM necromass for.
two reasons. First, in many cases little cytoplasm remains in senescing fungal tissues due to vacuolization accompanied by the movement of cytoplasm to vital regions of the mycelium (Saltarelly et al., 1998). Second, as with plant litters, the cytoplasmic fraction does not vary much in quality, is highly labile and appears to be rapidly taken up by decomposer organisms leaving mostly the cell wall fraction remaining (Nakas and Klein, 1979; Moucawi et al., 1981; Drigo et al., 2012). Therefore, it seems likely that the nature and composition of the cell wall fraction exerts the most control on the long-term decomposition of EM fungal necromass. Approximately 20–50% of fungal biomass is found in the cell wall (Ruíz-Herrera, 1992). Once viewed as a static structure, the cell wall is now known to be a highly dynamic structure with its composition varying depending on factors such as age, genotype, taxon, and environment (Bartnicki-Garcia, 1968; Wessels, 1994; Bowman and Free, 2006; Feoñova, 2010). EM fungi, which are primarily members of the Basidiomycota and Ascomycota, have cell walls that are composed of β-glucans, chitin, proteins and melanins, along with other minor components (Wessels, 1994; Bowman and Free, 2006; Feoñova, 2010). There is wide variation in the decomposition rates of fungal cells among species (Hurst and Free, 2006) suggested that chitin is a recalcitrant polymer in fungal necromass and may result in a large build up of fungal necromass in soil organic matter (SOM) (Treseder and Allen, 2000; Langley and Hungate, 2003; Godbold et al., 2006). One of the problems with decomposition studies is that recalcitrance is not explicitly defined in many cases (see Schmidt et al., 2011). Fernandez and Koide (2012) explicitly examined the recalcitrance of chitin relative to all other fungal cell components by measuring changes in chitin concentration over the course of decomposition of EM fungal necromass. For all the EM fungi isolates that were tested, a rapid decline in chitin concentration was found, suggesting that chitin was preferentially decomposed relative to other cell wall components. In addition, initial chitin concentrations were positively related to percent mass loss of the necromass. Supporting these findings, Drigo et al. (2012), used a microcosm experiment designed to examine the decay of cell wall components of the EM fungus *Psololithus microcarpus* utilizing stable isotope probing methods. They found a rapid decline (within 10 days of addition) in the chemical functional groups associated with the glucan–chitin complex. Zeqin et al. (2013) reported rapid assimilation of pure chitin and N-acetylglucosamine monomers into microbial biomass when added to soil microcosms containing fungal-mat communities from old-growth Douglas-fir forest. Similarly, Russell (2014) found rapid decomposition of chitin amendments to wet tropical forest soil. Both fungi and bacteria produce a variety of extracellular enzymes, classified as chitinases (e.g. NAGase), that hydrolyze the glycosidic bonds of chitin into simpler molecules making them

### Table 1

Major biochemical components of ectomycorrhizal fungal cells, the nutrients contained in those components, their function, and their relative lability during decomposition. Uncertainties are denoted with a (?)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Nutrients</th>
<th>Component function</th>
<th>Enzymatic attack</th>
<th>Lability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solubles</td>
<td>C, N, P</td>
<td>Various</td>
<td>N/A</td>
<td>High</td>
</tr>
<tr>
<td>Glucogen</td>
<td>C</td>
<td>Energy storage</td>
<td>Hydrolytic</td>
<td>High</td>
</tr>
<tr>
<td>Lipids</td>
<td>C</td>
<td>Energy storage &amp; Structural</td>
<td>Hydrolytic</td>
<td>High-Medium</td>
</tr>
<tr>
<td>Protein</td>
<td>C, N, P</td>
<td>Various</td>
<td>Hydrolytic</td>
<td>Medium</td>
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<tr>
<td>Cell Wall</td>
<td></td>
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<tr>
<td>Glucans</td>
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<tr>
<td>α-glucans</td>
<td>C</td>
<td>Matrix</td>
<td>Hydrolytic</td>
<td>High (?)</td>
</tr>
<tr>
<td>β-glucans</td>
<td>C</td>
<td>Structural</td>
<td>Hydrolytic</td>
<td>Medium (?)</td>
</tr>
<tr>
<td>Chitin</td>
<td>C &amp; N</td>
<td>Structural</td>
<td>Hydrolytic</td>
<td>Medium</td>
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<tr>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Glycoproteins</td>
<td>C &amp; N</td>
<td>Matrix, signaling</td>
<td>Hydrolytic</td>
<td>Medium (?)</td>
</tr>
<tr>
<td>Hydrophobins</td>
<td>C &amp; N</td>
<td>Water proofing</td>
<td>Hydrolytic(?)</td>
<td>Low (?)</td>
</tr>
<tr>
<td>Melanin</td>
<td>C</td>
<td>Structural &amp; Protectant</td>
<td>Oxidative</td>
<td>Low</td>
</tr>
</tbody>
</table>
available for uptake (Gray and Baxby, 1968). Furthermore, because chitin is a N-rich compound, its rapid degradation and incorporation may be driven by N limitations of decomposer fungi and bacteria (Fernandez and Koide, 2012). However, rapid degradation of chitin has also been found in relatively N-rich soils where decomposers were not likely limited by N (Russell, 2014). Taken together, these findings suggest that chitin, itself, is not resistant to decomposition relative to other compounds in fungal necromass, and may be an important source of both C and N to soil decomposer communities.

2.2.2. Proteins

While only a minor component (Table 1) relative to polysaccharides, the proteins found in fungal cell walls vary greatly in function, chemistry, and potential influence on decomposition dynamics of EM fungal necromass. Proteins are composed of amino acids that are linked with peptide bonds. Fungal cell protein concentrations can vary widely among fungal species (Christias et al., 1975). Despite being a relatively minor component (ca. 15–30% dry mass) in total biomass, approximately 60–70% of N in fungal cell walls occurs in proteins owing to their high N concentrations (Bowman and Free, 2006; Smiderle et al., 2012), mostly as glycoproteins, which have both structural and signaling functions (Bowman and Free, 2006). Proteins are generally thought to decompose rapidly (Kögel-Knabner, 2002), a result of proteins being hydrolysable compounds that are rich in growth-limiting N. However, glomalin-related soil proteins, a class of glycoprotein produced by arbuscular mycorrhizal (AM) fungi, is resistant to decomposition (Steinberg and Rillig, 2003) and may be involved in the stabilization of soil aggregates (Rillig, 2004). Glomalin-related soil proteins may be resistant to decomposition because of its hydrophobic nature (Rillig and Mummey, 2006). It is unclear if EM fungi synthesize similar glycoproteins because of its similarity to degradation, but hydrophobins (cysteine-rich hydrophobic proteins) produced by Basidiomycetes and Ascomycetes (Wessels, 1996; Wöstien and de Vocht, 2000; Rillig et al., 2007) may be similarly resistant to decomposition. These proteins are arranged as a film on the outside of the cell wall, making it unwettable (Wöstien and de Vocht, 2000). The unwettable nature of these tissues is likely to then impede their enzymatic decomposition (Rillig et al., 2007). Hydrophobins are also important in the formation of ectomycorrhizas and play a role in the retention and transportation of water in the extramatrical mycelium (Unestam and Sun, 1995).

2.2.3. Melanin

Melanins are complex, dark biopolymers that are produced by animals, bacteria and fungi. While the chemical structure of these compounds is poorly understood, they are known to be generally composed of phenolic and indolic monomers (Bull, 1970; Bell and Wheeler, 1986; Butler and Day, 1998a). Fungi produce four classes of melanin that vary in their precursors and biosynthetic pathways. These include γ-glutamyl-3,4-dihydroxybenzene (GDHB) melanin, dihydroxyphenylalanine (DOPA) melanin, dihydroxy-3-naphthalene (DHN) melanin and catechol melanin (Butler and Day, 1998a). Melanin type appears to be largely determined by phylogeny; Basidiomycetes produce GDHB and DOPA melanins, while Ascomycetes produce primarily DHN melanin but may also produce DOPA and catechol melanins (Bell and Wheeler, 1986; Butler and Day, 1998a). The production of melanin in fungi has been linked to the tolerance of various environmental stressors, including high temperature (Rosas and Casadevall, 1997), water stress (Rehnstrom and Free, 1996; Kogej et al., 2007; Fernandez and Koide, 2013; Kejzar et al., 2013), ultraviolet light (Wang and Casadevall, 1994). Melanin content also varies tremendously across taxa, with some fungi being completely hyaline (low melanin) and others heavily melanized (Hurst and Wagner, 1969; Butler and Day, 1998a, Table 1). As with lignin, melamins lack stereo-specific binding sites, which hydrolytic enzymes target, making these compounds unhydrolyzable (Butler and Day, 1998b). Two early studies using 14C labeled fungal material amendments to soil microcosms examined the potential recalcitrance of melanin. Hurst and Wagner (1969) contrasted the mineralization of cell wall amendments from melaninized and hyaline fungi and found that the cell walls from melanized fungi were mineralized relatively slowly when compared to those from hyaline fungi. These findings were later supported in a study by Malik and Haider (1982), who found that melanin fractions of fungal cells were mineralized at a slower rate than total cell wall and cytoplasm fractions in all fungal isolates tested.

Cenococcum geophilum, one of the most abundant and ubiquitous EM fungal species globally, produces a large amount of melanin in its cell walls (Pigott, 1982). Fernandez et al. (2013) demonstrated with minirhizotron imaging and vital staining that the ectomycorrhizas of C. geophilum were 4–10 times more persistent than ectomycorrhizas of other species, suggesting that their decomposition was significantly lower. The persistence of structures in soil can be the result of increased lifespan as opposed to resistance to decomposition. However, vitality staining of ectomycorrhizas revealed that a large proportion of C. geophilum ectomycorrhizas sampled were not metabolically active suggesting that longer persistence times associated with these ectomycorrhizas were likely the result of their resistance to decomposition. Later, Fernandez and Koide (2014) measured the decomposition rates of EM fungal necromass that varied in melanin concentration and found that there was a significant negative relationship between melanin concentration and decomposition rate. This finding was supported with a second, manipulative experiment in which melanin biosynthesis of C. geophilum isolates was inhibited with the melanin inhibitor, tricyclazole. Again, there was a negative relationship between melanin content and decomposition rate. Together these studies show that melanin itself is resistant to decomposition and reduces the overall decomposability of the fungal tissues in which it is found. Recently, Clemmensen et al. (2015) also provided support for the hypothesis that melanization contributes to the stabilization of fungal C in soil by showing significant correlations between the abundance of melanized fungi and C accumulation in SOM.

The relative recalcitrance of melamins is likely a result of the complex aromatic nature of these polymers that require oxidative enzymes to degrade. While Butler and Day (1998b) found that peroxidases produced by a lignin decomposer fungus effectively degraded fungal melanin, melanin has also been shown to inhibit common enzymes used to decompose other cell wall components including chitin and β-glucan (Kuo and Alexander, 1967). Thus, melanin may influence the overall decomposability of the fungal necromass by affecting the decomposition of other components. Because of its recalcitrance, its requirement for oxidative enzymes for degradation, and the large variation in concentrations across fungal species, melanin in fungal tissues may be analogous to lignin in plant tissues in biochemical control of necromass decomposition. Additionally, melanized hyphae also have higher sorption to soil mineral components compared to hyaline hyphae (Fomina and Gadd, 2003), which may result in physiochemical protection of necromass C, which would also result in increased incorporation into stable SOM (Fernandez and Kennedy, 2015).

2.2.4. Other secondary compounds

Fungi produce a myriad of other secondary compounds that likely influence the decomposition of their tissues (Keller et al., 2005). These compounds can range widely in function but a large
EM fungi exhibit a large degree of variation in the anatomy of hyphae, which can vary in cell wall thickness, branching, and cell diameter (Rillig and Mummey, 2006). This variation is compounded by the numerous mycelial morphologies produced by fungi. Most fungi will produce a diffuse mycelium when grown in culture in which resources are homogeneously distributed. Thus, the research examining the decomposition of fungal tissues has been biased towards lab-grown diffuse mycelium (see Koide and Malcolm, 2009; Koide et al., 2011; Wilkinson et al., 2011; Drigo et al., 2012; Fernandez and Koide, 2012). However, the physiochemical properties of hyphae, in turn, influence the decomposition of the resulting necromass. EM fungi allocate a great deal of resources to produce specialized structures that carry out certain functions in the life cycle of the fungus such as host resource exchange and interaction (ectomycorrhizas), soil resource acquisition (cords, rhizomorphs, hyphal mats), reproduction and dispersal (sporocarps), and dormancy (sclerotia) (Fig. 2), each of which may then have unique properties related to necromass decomposition.

**3. What effect does mycelial morphology have on ectomycorrhizal fungal necromass decomposition?**

The physiochemical properties of hyphae, in turn, influence the decomposition of the resulting necromass. EM fungi allocate a great deal of resources to produce specialized structures that carry out certain functions in the life cycle of the fungus such as host resource exchange and interaction (ectomycorrhizas), soil resource acquisition (cords, rhizomorphs, hyphal mats), reproduction and dispersal (sporocarps), and dormancy (sclerotia) (Fig. 2), each of which may then have unique properties related to necromass decomposition.

**Fig. 2.** A diagram illustrating the types of ectomycorrhizal fungal necromass resulting from the turnover of ectomycorrhizal fungal structures. Mycelium color (i.e. white, brown, black) represents tissues produced by different ectomycorrhizal fungal species. The ectomycorhiza is a composite organ consisting of both fungal and plant tissues. The physiochemical properties of the ectomycorhiza can vary dramatically among ectomycorrhizal fungal species. Extending from the ectomycorhiza is the extra-matrical mycelium which explores the soil for resources. The extra-matrical mycelium varies widely in morphology and length among ectomycorrhizal fungi but can be coarsely grouped as simple diffuse or long-distance mycelium (rhizomorphs; cords). Finally ectomycorrhizal fungi produce epigeous sporocarps or hypogeous sporocarps while others produce resting structures called sclerotia. B. A generalized schematic of the common components of fungal cell walls. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
3.1. The ectomycorrhiza

The ectomycorrhiza is a composite structure comprising both fungal and plant tissues. Because of the intimate association, the fungus significantly alters the chemistry of the root. Therefore, root decomposition is influenced by mycorrhizal colonization (Langley et al., 2006). Fine-root production represents a large annual C input into forest ecosystems, accounting for approximately one-quarter of global annual NPP (McCormack et al., 2015). Langley and Hungate (2003) highlighted the potential influence of EM fungal colonization on the decomposition of fine roots, hypothesizing that EM colonization would reduce the decomposition rate of fine roots. Later, Langley and McCormack (2006) found support for this hypothesis in an experiment comparing decomposition rates of non-mycorrhizal fine roots and EM fine roots of pinyon pine (Pinus edulis). With the goal of understanding potential species differences, Koide et al. (2011) examined the effects of colonization of different EM fungal species on the decomposition of red pine (Pinus resinosa) fine roots. Of the ectomycorrhizas examined in this study, only ectomycorrhizas from a Suillus sp. had significantly different decomposition rates from non-mycorrhizal fine roots, which actually decomposed faster than non-mycorrhizal fine roots. In a minirhizotron study, Fernandez et al. (2013) observed that ectomycorrhizas of C. geophilum persisted in the soil 4–10 times longer than other ectomycorrhizas of other species likely due to reduced decomposition rates from highly melanized tissues. Interestingly, the P. edulis site studied by Langley and McCormack (2006) is dominated by a single EM fungus, Geopora sp., that happens to be highly melanized (C. Gehring; Personal Communication) unlike the hyaline ectomycorrhizas examined in Koide et al. (2011), which may explain some of the discrepancy between the two studies. Together, these studies highlight the importance of fungal species identity to determine the effect of colonization on fine-root decomposition.

3.2. Rhizomorphs, cords, and mats

Ectomycorrhizal fungi vary widely in their morphology and physicochemical properties. We argue that these differences in morphology of extramatrical mycelium might lead to variation in decomposition. For instance, long distance exploration structures of EM fungi are often hydrophobic (Agger, 2001) and thus likely to be more resistant to decay than EM fungi with shorter exploration strategies that are often hydrophilic. Some Basidiomycete EM fungi produce structures thought to be important for efficient resource foraging across relatively long distances in the soil. Rhizomorphs are tube-like structures of linearly aligned hyphae differentiated into a medulla, composed of large diameter hyphae, upwards of 25 μm, encased by a cortex of smaller diameter, hydrophobic hyphae (Cairney et al., 1989). These structures grow apically and are able to conduct water and nutrients across meters of soil (Brownlee et al., 1983), which can be of particular advantage when resources are distributed heterogeneously. While similar in function, fungal cords are constructed from simple diffuse mycelium that subsequently aggregates after some time to form cord like structures. Rhizomorphs and cord structures may persist for months to years, indicating a possible resistance to decomposition (Treseder et al., 2005; Pritchard et al., 2008; McCormack et al., 2010). The resistance may be due to the hydrophobic surfaces, as well as the relatively low surface area-to-volume ratio compared to undifferentiated or diffuse hyphae. Interestingly, McCormack et al. (2010) found that larger diameter rhizomorphs had shorter persistence times than those with smaller diameters which is counterintuitive because smaller diameters would lead to more surface area exposed to decomposers and increased decomposition rates. These longer persistence times of the smaller diameter rhizomorphs could be the result of differences in lifespan and/or dormancy of these structures among functionally different species, which could not be identified in the study. In addition, the effective surface area of the larger diameter rhizomorphs could be greater if they have a large lumen, which would expose more internal tissue to decomposers and accelerate decomposition. Alternatively, Clemmensen et al. (2015) have suggested that rhizomorphic and cord-forming EM fungi may be efficient recyclers of their biomass and able to translocate resources to new growth. Recycling of biomass would thus reduce persistence times of these structures and potentially complicate the interpretation of minirhizotron data. In any case, because rhizomorphic strategies are common among EM fungi, future studies should be directed to better understand the factors that lead to the sometimes long, yet variable persistence of rhizomorphs and cords.

Ectomycorrhizal fungi can also form dense hyphal mats, which can account for ca. 50% of the dry mass of the organic layer in some forest soils (Griffiths et al., 1990). These fungal mats are common globally but are particularly well-studied in boreal forest systems dominated by Douglas fir species (Pseudotsuga sp.) (Griffiths et al., 1990, 1996). Fungal mats can be rhizomorphic and extremely hydrophobic depending on the species involved (Unestam and Sun, 1995). The community composition of mat soils was once thought to be dominated by single species of EM fungi but there is now evidence provided by molecular analyses that these communities are more diverse and may be formed by over 20 EM species (Dunham et al., 2007). Because of the large amount of aggregated biomass, EM fungal mats significantly alter the biogeochemistry of the area they inhabit (Kluber et al., 2010). The decomposition of these mat structures remain unexamined but are likely influenced by many of the chemical properties discussed above as well as unique physical aspects of the mat structures themselves.

3.3. Sporocarps and sclerotia

Perhaps the most underappreciated, yet most visible, EM fungal necromass results from sporocarp production. A great deal of C and N is allocated to the production of these structures in many EM fungal species (Vogt and Edmonds, 1980). The production of sporocarps is highly variable across species, time and ecosystems. Dahlberg et al. (1997) found the average sporocarp production in a spruce forest in southern Sweden to be 8.8 kg ha⁻¹ yr⁻¹, while Vogt et al. (1981) found levels of sporocarp production between 27 and 380 kg ha⁻¹ yr⁻¹ (dry weight) in spruce stands in western Washington, USA. The functional specialization of sporocarps is reflected in their chemistry. Protein and chitin concentrations of these structures are significantly different from the associated belowground mycelium; sporocarps are usually enriched in protein and depleted in chitin (Taylor et al., 1997). Thus, these structures may have different decomposition dynamics from the associated belowground mycelium, however this is pure speculation and to our knowledge these comparisons have not yet been made. Some EM fungi also produce sclerotia, essentially hardened resting structures composed of mycelia that can remain viable in the soil for at least 2 years (Miller et al., 1994). Cenococcum geophilum can produce sclerotia in great quantities, which may then build up to levels estimated to be as high as 3000 kg ha⁻¹ in some ecosystems (Vogt et al., 1981). Considering the extremely slow decomposition rate of structures produced by the heavily melanized Cenococcum geophilum, including ectomycorrhizas and sclerotia, may represent a large and incredibly stable C input into forest ecosystems (Watanabe et al., 2007). Many EM fungi have been shown to produce sclerotia with considerable variation in morphological traits across taxa (Smith et al., 2015), which
warrants further consideration and investigation into the production, turnover, and decomposition of these structures and the subsequent effects on forest C cycling.

4. Appreciating the diversity of ectomycorrhizal fungal necromass

Ectomycorrhizal fungal communities are often highly diverse, with species richness exceeding 100 in some ecosystems (Dickie, 2007). Given the variation in decomposability of EM fungal tissues, an understanding of the effects of mycorrhizal fungi on C and nutrient cycling therefore requires an understanding of fungal diversity. The importance of species differences in decomposition dynamics should not be underestimated for EM fungal necromass. Species of EM fungi span two Phyla: Basidiomycota and Ascomycota. As demonstrated above, differences in chemical and physical properties between two relatively related species can be quite large and lead to very different decomposition rates. Because a single tree may host dozens of EM fungal species, its fine roots (ectomycorrhizas) can then express different decomposition rates as a result and may have profound effects on SOM contribution to SOM of fungal necromass between studies may be unclear what traits are responsible for differential effects on aggregate formation and stabilization, but they may be the result of differences in cell wall components (i.e. glycoproteins and hydrophobins as discussed above in Section 2.2.2), exudation, and/or differences in mycelium morphology (i.e. diffuse vs. rhizomorphic). It stands to reason that cords and rhizomorphs are less likely to be protected in this manner because of their relatively large diameter though their hydrophobicity may have the potential to alter small-scale movement of water, which could reduce decomposition. Additionally, fine roots, which are of similar size are important in macroaggregate formation and stabilization so it may also be reasonable for rhizomorphs to serve a similar function.

EM fungi inhabit distinct vertical niches in soil, a likely consequence of resource niche partitioning (Dickie et al., 2002; Rosling et al., 2003). It is thought that EM fungi positioned higher in the profile rely more on recent aboveground litters containing more labile compounds, while EM fungi found deeper in the soil profile specialize on breaking down more recalcitrant compounds (Lindahl et al., 2007; Talbot et al., 2013). Position in the soil profile also dictates decomposer community structure as well as temperature, moisture, and other physical soil properties (Schmidt et al., 2011). Unlike aboveground litters, EM fungal necromass is produced and deposited at different positions in the soil profile. Thus, there is potential for a species’ litter decomposition to be controlled largely by location in the soil profile. For example, there are slower turnover and decomposition rates of root litter at deeper depths in the soil profile (Gill and Burke, 2002; Joslin et al., 2006; McCormack et al., 2010). Recently, Schweigert et al. (2015) traced the fate of 13C-labeled Laccaria bicolor biomass amendments in an in vitro soil bioreactor experiment. They found that a significant proportion of the 13C (52%) remained undecomposed in the form of stable SOM after 231 d. This is in contrast to prior studies that generally have found rapid decomposition of hyaline necromass (Koide and Malcolm, 2009; Wilkinson et al., 2011; Drigo et al., 2012; Fernandez and Koide, 2012). This discrepancy may be due to the fact that Schweigert et al. (2015) used only mineral soil and perhaps...
had higher protected fungal C via sorption to soil structures (see discussion below) while previous studies used amended organic layers (Koide and Malcolm, 2009; Drigo et al., 2012; Fernandez and Koide, 2012).

Because we see clear vertical niche patterns for many EM fungal species, there may be interesting interactions between necromass quality and factors related to soil profile (Fernandez and Kennedy, 2015). For instance, an EM fungal species can produce necromass of high quality, but the naturally rapid decomposition rate of that necromass may be counteracted by its occurrence in lower soil profiles. Depending on if sporocarps are epigeous (aboveground) or hypogeous (belowground) may also influence the rate at which they are decomposed. Finally, for some species that produce a relatively large number of sporocarps (Kikuchi and Futai, 2003), we may see a considerable amount of C and nutrients move above-ground from the mycelium belowground where it would presumably decompose at a faster rate. However, this may not necessarily hold true for spores produced by these structures, particularly those that are relatively persistent in the soil (Nguyen et al., 2012).

5.2. Decomposer communities and extracellular enzyme production

Once viewed as homogenous and functionally redundant, variation in the structure of soil microbial communities is now recognized as a major factor determining litter decomposition rates in soils (Zak et al., 1994; Strickland et al., 2009a,b). Our understanding of the decomposer communities that are responsible for the degradation of fungal necromass is poor when compared to that of plant litter. In particular, it remains uncertain whether microbes that specialize in decomposing fungal necromass are common in forest soils or if decomposition of fungal necromass is primarily carried out by generalist decomposers. Many of the substrates found in EM fungal necromass are degraded with extracellular enzymes that degrade substrates found in plant litter so specialization may be rare. For instance, some β-glucans share a similar chemical structure with cellulose and can be hydrolyzed with cellulase enzymes (e.g. β-glucosidase). Similarly, fungal melanins are degraded with the same oxidative enzymes involved in lignin decomposition (e.g. peroxidase), which are non-specific to substrate. Finally, proteins, regardless of origin, are hydrolyzed by protease enzymes (e.g. endoprotease; aminopeptidase). Chitin, however, is found only in fungal and arthropod tissues and is hydrolyzed with chitinases (e.g. endo-chitinase, N-acetylglucosaminidase). Early work by Gray and Baxby (1968) found that the ability of decomposer fungi and bacteria to utilize chitin as a substrate was common, but some taxa appeared to be more proficient than others. Presumably soils high in EM fungal necromass production have microbial decomposer communities specializing in the degradation of chitin and other fungal compounds. For example, in EM fungal mat communities, in which soils have large quantities of standing fungal biomass, chitin and N-acetyl-glucosamine (NAG) are cycled quickly compared to soils where mat-forming EM fungi are absent (Ze glin et al., 2013), perhaps due to decomposer communities specializing on EM fungal necromass in mat soils (Kluber et al., 2011). DNA-stable isotope probing (SIP) techniques coupled to molecular methods and bioinformatics might be an approach that will allow us to trace C and N that is found in EM fungal necromass into decomposer pools (Drigo et al., 2012). The extent to which C and nutrients in EM fungal necromass are recycled by live EM fungi is not clear but this represents a potentially significant line of future research, as this would reduce the amount of C entering both the decomposer and SOM pools (Kerley and Read, 1998; Lindahl et al., 2002; Clemmensen et al., 2015).

Extracellular enzyme activity is often linked to resource limitations in the soil (Burns, 1982; Sinsabaugh et al., 2002) and because EM fungal necromass are N-rich, substrates from these litters are likely to be in high demand under N-limited conditions. Inorganic N deposition alters decomposer (and EM fungal) communities and their enzymatic activity, which may have implications for the decomposition of EM fungal necromass. For instance, elevated concentrations of atmospheric CO2 in a loblolly pine forest were associated with increased NAGase activity, yet these increases were canceled out by N fertilization (Meier et al., 2015). More generally, depressions in NAGase activity have been associated with increased N availability (Olander and Vitousek, 2000), although this is not true in every ecosystem (Waldrop et al., 2004). Shifts away from oxidative to increased hydrolytic extracellular enzyme production have also been associated with elevated N deposition (Carreiro et al., 2000; Sinsabaugh et al., 2002; Frey et al., 2004). Because melanin is not hydrolysable and requires oxidative enzymes for its breakdown, we may then expect melanin decomposition to decline with elevated N, much like what has been found with lignin.

Climatic factors such as temperature and precipitation strongly influence the decomposition rates of litters (Aerts, 1997), and the effects of climate on litter decomposition and generation of SOM are of great interest in the light of climate change. Because the decomposition process is more sensitive to temperature shifts than primary productivity there is concern for positive feedbacks amplifying climatic warming (Davidson and Janssens, 2006). Generally speaking, the decomposition rate of litter increases with temperature. However, enzymes involved in the breakdown of complex, recalcitrant C substrates appear to be more sensitive to temperature than those involved in breakdown of labile substrates (O’Connell, 1990; Hobbie, 1996; Fierer et al., 2005). This sensitivity is due to the enzyme kinetics; more complex substrates require higher activation energies than less complex substrates (Fierer et al., 2005). It therefore stands to reason that the decomposition rates of chemically recalcitrant EM necromass would have similar temperature sensitivity to recalcitrant plant litters, but that is merely speculation and requires investigation.

Beyond fungal and bacterial decomposers, soil fauna play an important role in soil detrital food webs and can have a large influence on terrestrial biogeochemical cycles (Petersen and Luxton, 1982). Fungivorous nematodes, earthworms and arthropods may favor necromass of certain species of EM fungi over others and increase the speed at which the C and nutrients in those litters are cycled. Variation in grazing on various species of EM fungi may be due to variation in fungal mycelium defense and palatability. Bollmann et al. (2010) examined the effect of different defensive strategies of soil fungi on the grazing preference of Colembola and found that species producing chemical repellent metabolites (e.g. Clitocybe phyllophila, Lactarius pubescens, and Hebeloma sinapizans) deterred grazing the most while those producing crystalline structures (e.g. Piloderma croceum, Suillus flavus and Suillus luteus) also had some success at reducing grazing. Interestingly, heavily melanized fungal taxa, including C. geophilum, were preferred by the Colembola despite being lower in nutritional quality and harder to digest. Therefore, chemical repellents and crystalline structures likely have a stronger influence on grazer preference than nutritional quality and grazing defense strategies employed by EM fungi likely have an interactive effect with decomposer communities on the rate of EM necromass decomposition.

Important questions regarding the influence of soil decomposer communities on decomposition remain. How does the EM fungal community structure influence decomposer community structure? How does this indirectly influence EM fungal necromass decomposition and, as a consequence, SOM formation and N mineralization? Because EM fungi themselves produce extracellular enzymes that breakdown organic material in order to access nutrients, do EM fungi...
fungi directly compete with saprotrophic fungi and bacteria for resources found in their own tissues? Do EM fungi have an inherent advantage in accessing and processing these resources because they are biotrophic rather than saprotrophic?

6. Conclusions

Significant progress has been made in the past 10 years in our understanding and appreciation of the contribution of EM fungal necromass to forest biogeochemical cycles (Fernandez and Kennedy, 2015). In this review we highlight the importance of understanding EM fungal species differences in the decomposability of their tissues. Though this area of research is still relatively young, this synthesis of the existing literature allows us to draw some tentative conclusions about the factors controlling the decomposition of EM fungal necromass (Fig. 3). Recent studies have shown a great deal of variation in EM fungal necromass decomposability and suggest that differences in biochemistry play a large role. While once hypothesized to be a relatively recalcitrant fungal cell wall component, chitin concentration may actually contribute positively to the decomposability of EM fungal necromass. However, melanin concentration appears to govern the quality of EM fungal necromass much like lignin concentration does in plant litter. At the same time, decomposability of EM fungal necromass appears to be positively controlled by the N concentration. Together, melanin and N concentrations may explain a large fraction of the variation in decomposition rates observed among EM fungal necromass much like lignin and N concentrations do for plant litter decomposition.

Thus far, much of the research focused on fungal necromass decomposition has used lab grown fungal biomass and either used to inoculate soil microcosms (i.e. Wilkinson et al., 2011; Drigo et al., 2012) or used in a litterbag approach (i.e. Fernandez and Koide, 2012; Fernandez and Koide, 2014). While valuable information has been gained from these approaches, they do not necessarily reflect the production and decomposition of mycelia in situ. Furthermore, lab-grown fungal biomass may represent substrates that are artificially high in quality since resources are generally not limiting in culture conditions. Because the physiochemical properties of fungal tissue grown in cultures may differ from those grown under more natural conditions, we must begin to examine the turnover and decomposition of fungal tissues in situ to gain a better understanding of the differences in persistence of EM necromass (Fernandez et al., 2013). As the advancement of mini-rhizotron imaging technologies allows finer resolutions (Allen and Kitajima, 2013), investigators should be able to identify traits associated with fungi and relate them to persistence in the soil. These data will also allow for finer contrasts between EM fungal structures in situ. Finally, these imaging tools should be useful in elucidating extrinsic factors controlling decomposition rates of EM fungal necromass. A major goal of this area of research will be to explore the linkages between EM fungal community structure and biogeochemical cycling. For instance, do mono-dominant communities have distinctly different contributions to SOM formation than more diverse communities? It should be restated that mini-rhizotron techniques are only able to track the production and persistence of soil structures and cannot distinguish between the effects of lifespan and resistance to degradation; therefore care should be taken when generating conclusions about persistence. Ultimately, it will be important to link factors controlling the decomposition of necromass to SOM-formation. For instance, relatively labile compounds are often found to contribute to SOM-formation suggesting that biochemistry is not the only factor driving SOM accumulation and extrinsic factors such as physical and physiochemical protection from soil may be driving C sequestration in soils (Schmidt et al., 2011; Cotrufo et al., 2013). However, it is important to appreciate the likely possibility of multiple interacting factors, perhaps operating on different timescales, which ultimately determine the incorporation of EM fungal necromass into SOM. Litterbag experiments do not allow the investigator to trace the C in necromass into stable SOM pools and therefore are unable to examine these types of questions. Coupling next generation molecular, stable isotope probing and high-resolution chemistry analytical techniques is a promising approach to begin to address these questions. Exploring these linkages will be invaluable in understanding the role that EM fungal necromass plays in terrestrial biogeochemical cycles.

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