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Nutrient content affects the turnover of fungal biomass in forest topsoil and the composition of associated microbial communities

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ABSTRACT

Due to the standing biomass and turnover of fungal biomass in forest topsoils, decomposition of fungal biomass represents an important process. Within plant litter, dead fungal biomass represents a unique substrate that is typically nitrogen (N)-rich and is assumed to be subject to rapid decomposition. However, our current knowledge of mycelial decomposition has been largely derived from short-term studies, often limited to a single mycelia type, and the guilds of microbial mycelium decomposers have not yet been described. Furthermore, nutrient content may vary largely in fungal mycelia, and the consequences of this variation are unknown. Here, we followed the decomposition of dead biomass of 12 ectomycorrhizal (ECM) and saprotrophic fungi of a temperate forest using mycobags incubated in litter for 3 and 9 weeks. Loss of substrate dry mass, microbial biomass content and community composition as well as the activity of extracellular enzymes reflecting microbial activity on this substrate were followed. Decomposition rates of fungal biomass were typically high (0.13–0.30 week^{-1}), yet variable. The decomposition includes a rapid initial phase followed by a slower turnover of remaining biomass. The initial nitrogen content that ranged between 1.5% and 10% appeared to be the most important factor that affected colonization of dead mycelia and their decomposition. The relatively high content of N makes fungal mycelia an attractive resource in the N-poor habitat of plant litter. Decomposition of mycelia was performed by a guild of specialist decomposers that showed rather low abundance in surrounding litter and changed during decomposition. Bacteria were much more abundant on dead fungal biomass than in the surrounding litter and appeared to play an important role in decomposition. Fungi associated with dead mycelia were mainly represented by yeasts and moulds. Although the importance of fungal mycelia for the nutrient cycling in forests is not yet clear, the fact that they are turned over rapidly suggests that they may represent an important and dynamic pool of carbon and nitrogen.

1. Introduction

In temperate forest soils, fungi are fundamentally important for carbon dynamics and nutrient cycling through their involvement in the decomposition of dead plant biomass as well as the mycorrhizal mediation of carbon flow from primary producers into soil and their assistance in nitrogen and phosphorus acquisition by plants (Baldrian, 2017). The yearly mycelial production in the upper 10 cm of temperate forest soils was estimated to range between 100 and 300 kg ha^{-1}, with the production in deeper soil possibly reaching similar values (Bostrom et al., 2007; Ekblad et al., 2013; Majdi et al., 2008; Wallander et al., 2004). Moreover, the relative amount of fungal biomass in litter can be even higher (Baldrian et al., 2013b). Given this high production, fungal biomass represents an important pool of organic matter in temperate forest soil, regardless of the fact that only a minor part of mycelia may be retained in soil organic matter (Baldrian et al., 2013b; Hagenbo et al., 2017).

In contrast to plant biomass, fungal mycelia lack complex recalcitrant polymers such as lignin. Mycelia consist largely of various polysaccharides, representing up to 80–90% of the total cell wall mass, lipids and proteins (Baldrian et al., 2013b). Cell wall polysaccharides comprise chitin, various glucans, glucomannans, as well as other polysaccharides containing galactose, galactosamine, fucose and other components (Bartnicki-Garcia, 1968; Nilsson and Bjurman, 1998). Like cellulose in plants, fungal β-glucans interact with other cell wall components, such as chitin or melanin, which may alter the chemical properties and consequently the recalcitrance of fungal biomass (Treseder and Lennon, 2015).

Chitin and fungal cell wall proteins are rich in nitrogen, making the dead mycelium a valuable source of both carbon and nitrogen (Colpaert et al., 1996; Cooke and Whipps, 1993; Wallander et al., 2004; Zeglin and Myrold, 2013) and an attractive nutrient in the otherwise N limited

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soil and litter (Lindahl et al., 2007; Šnajdr et al., 2008).

The carbon to nitrogen ratio (C/N) in dead fungal biomass can be as low as 7, considerably lower than in fresh plant litter with C/N ratios typically above 30 (Koide and Malcolm, 2009; Mouginot et al., 2014; Nilsson et al., 2012; Šnajdr et al., 2013) and similar or higher than in bacterial biomass (Gunina et al., 2017). The low C/N ratio is generally considered to be favorable for fast decomposition (Fernandez et al., 2016; Drigo et al., 2012), and it is assumed to be the cause of fast decomposition of bacterial biomass (Gunina et al., 2017). Biomass decomposition rates are also affected by its chemical composition, such as the presence of recalcitrant or inhibitory compounds. For instance, melanin, a quantitatively important compound in the mycelia of certain fungi (Ekblad et al., 2013; Fernandez and Koide, 2014; Koide and Malcolm, 2009), and the polyphenolic lignin in plant biomass increase recalcitrance and slow down decomposition (Berg and Laskowski, 2005; Fernandez and Koide, 2014).

The turnover of dead fungal mycelia has been recently addressed for ectomycorrhizal fungi (ECM) (Ekblad et al., 2013; Hagenbo et al., 2017; Hendricks et al., 2015) and their contribution to soil organic matter (Clemmensen et al., 2013). However, the direct estimations of decomposition rates *in situ* were not performed, and the fate of mycelia of saprotrophic fungi was not addressed. Recently, we described the decomposition of mycelia of the ECM fungus *Tylopillus felleus*, where a rapid initial phase was followed by slow turnover of the recalcitrant biomass fractions (Brabcová et al., 2016). Similar biphasic kinetics was also observed during the decomposition of the ^{13}C-labeled mycelium of *Laccaria bicolor* (Schweigert et al., 2015). Although the variability in the rate of mycelia decomposition reflecting its mycelia chemical composition was first addressed more than 50 years ago (Hurst and Wagner, 1969), recent research has rather focused on particular questions, such as the effect of calcium oxalate (Keiluweit et al., 2015), melanin and chitin (Fernandez and Koide, 2014, 2012) on decomposition kinetics.

Most importantly, the function of the fungal biomass in the soil nutrient reservoir and the carbon flow remain largely unknown. Dead fungal mycelia represent a substrate with unique chemistry, different from the dominant plant-derived organic matter. Thus, it is likely that fungal biomass is decomposed by a specific guild of saprotrophic microbes that possess the necessary enzymatic apparatus. Indeed, the fungi and bacteria associated with decomposing *Tylopilus felleus* mycelia represent a distinct subset of the soil and litter microbiome (Brabcová et al., 2016), but generalizations from this single observation are impossible due to the variability of nutrient content and chemical composition among mycelia of different fungal species.

Here, we followed the decomposition of mycelia of 12 common taxa of saprotrophic and ectomycorrhizal fungi, representing a broad range of C/N values. We aimed to describe the decomposition kinetics and identify microorganisms associated with this substrate. To accomplish this, fungal biomass was incubated *in situ* and analyzed during the initial and advanced stages of decomposition after 3 and 9 weeks, respectively. Because the content of mycelia per g dry weight of substrate can be over 10-fold higher in litter than in soil (Baldrian et al., 2013b), the experiment was performed in litter. We hypothesized that the nitrogen content (or the C/N ratio) of fungal biomass affects the *in situ* decomposition rate. Due to similarities in chemical composition of cell wall polymers, we expect that mycelia of different fungi are decomposed by a common guild of decomposers. Due to the high N content, fungal biomass should be especially attractive for bacterial decomposers since they have higher demand for N.

2. Materials and methods

2.1. Study site, materials and experimental setup

The study site was a temperate oak (*Quercus petraea*) forest in the Xaverovský Háj Natural Reserve near Prague, Czech Republic (50°15′38″N, 14°36′48″E). The soil was an acidic cambisol with a developed litter and organic and mineral horizons (Baldrian et al., 2013a). The site has been extensively studied previously with respect to the activity of decomposition-related extracellular enzymes (Baldrian et al., 2013a, 2010; Šnajdr et al., 2008), the decomposition of leaf litter and associated changes in the community of fungal decomposers (Šnajdr et al., 2011; Tláskal et al., 2016; Voříšková and Baldrian, 2013). The microbial community composition in the forest topsoil and its changes across seasons have also been described (López-Mondéjar et al., 2015; Voříšková et al., 2014). Importantly, decomposition of fungal mycelia of *Tylopilus felleus* in forest topsoil was recently described, along with the activity of decomposing enzymes and associated microbial communities (Brabcová et al., 2016).

Fungal biomass was obtained by collecting the fruiting bodies of 12 ectomycorrhizal and saprotrophic fungi, namely, *Amanita* sp. (AM), *Cortinarius* sp. (CO), *Clitocybe nebularis* (CN), *Lactarius*, *helvus* (LH), *Trametes versicolor* (TV), *Mycena galericulata* (MG), *Agaricus campestris* (AC), *Hypholoma fasciculare* (HF), *Armillaria gallica* (AG), *Russula* sp. (RU), *Russula ochroleuca* (RO) and *Tylopilus felleus* (TF). Fresh fruiting bodies were cut into 4-mm pieces, freeze-dried and stored at room temperature. Mycobags — polyester mesh bags (10 × 20 cm, 1 mm mesh size) filled with 2 g of freeze-dried mycelia (Brabcová et al., 2016) — were sterilized by repeated gamma-irradiation and placed in the middle of the litter layer. The experiment started in the early spring and the mycobags were placed in the middle of litter layer approximately 3 cm at depth at the homogenous area of size 5 × 10 m under oak canopy in 12 rows. Four mycobags per treatment were randomly collected from each row after 3 and 9 weeks of incubation along with litter without immediate contact with mycobags that served as a control. The time points of collection were based on results from previous study, where week 3 and 9 represented end of early and beginning of late phase of mycelia decomposition (Brabcová et al., 2016). Materials were transferred to the laboratory, removed from mesh bags, cut if necessary, homogenized, freeze-dried and stored at −20 °C.

2.2. Sample chemistry and enzyme activity

The remaining dry mass of mycelia was measured after freeze-drying, and pH was measured after mixing with distilled water (1:10 w:vol). Oxidizable carbon (C) and total nitrogen (N) content was measured using an elemental analyzer in an external laboratory. C_{ox} was measured using sulfochromic oxidation, nitrogen content was estimated by the Kjeldahl method (Bremner, 1960).

The activities of extracellular enzymes were assayed in sample homogenates as described previously (Štursová and Baldrian, 2011). Briefly, the activities of β-glucosidase, α-glucosidase, β-xylosidase, N-acetylglucosaminidase, β-mannosidase, α-galactosidase, lipase and phosphomonoesterase (phosphatase) were measured at pH 5.0 in 1:100 (w/v) sample slurries using methylumbelliferol-based substrates, with an excitation wavelength of 355 nm and an emission wavelength of 460 nm. Product quantification was based on standard curves with a range of 4-methylumbelliferol concentrations in the same sample slurry.

2.3. DNA extraction, microbial biomass quantification and amplicon sequencing

Total DNA was extracted from 300 mg of sample material using a modified SK method (Ságová-Marečková et al., 2008) and cleaned with a GeneClean Turbo Kit (MP Biomedicals, Solon, OH, USA). Bacterial and fungal rRNA gene copies were quantified by qPCR using the 1108f and 1132r primers for bacteria (Amann et al., 1995; Wilmotte et al., 1993) and FR1 and FF390 primers for fungi (Prevost-Boure et al., 2011; Vainio and Hantula, 2000).

The eubacterial primers eub515F/eub806R (Caporaso et al., 2012) were used to amplify the V4 region of bacterial 16S rDNA, and the fungi-specific primers gITS7/ITS4 (Ihrmark et al., 2012) were used to