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Ectomycorrhizal mats alter forest soil biogeochemistry

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ABSTRACT

Dense hyphal mats formed by ectomycorrhizal (EcM) fungi are prominent features in Douglas-fir forest ecosystems, and have been estimated to cover up to 40% of the soil surface in some forest stands. Two morphotypes of EcM mats have been previously described: rhizomorphic mats, which have thick hyphal rhizomorphs and are found primarily in the organic horizon, and hydrophobic mats, which occur in the mineral horizon and have an ashy appearance. This study surveyed EcM mat and non-mat soils from eight early and late seral conifer forest stands at the H.J. Andrews Experimental Forest in western Oregon. EcM mats were classified by morphology and taxonomic identities were determined by DNA sequencing. A variety of chemical and biochemical properties, including enzymes involved in C, N, and P cycling were measured. Analysis was confined to a comparison of rhizomorphic mats colonizing the organic horizon with non-mat organic soils, and hydrophobic mats with non-mat mineral soils. Both the organic and mineral horizons showed differences between mat and non-mat enzyme profiles when compared on a dry weight basis. In the organic horizon, rhizomorphic mats had greater chitinase activity than non-mat soils; and in the mineral horizon, hydrophobic mats had increased chitinase, phosphatase, and phenoloxidase activity compared to the non-mat soil. The rhizomorphic mats had 2.7 times more oxalate than the non-mats and significantly lower pH. In the mineral horizon, hydrophobic mats had 40 times more oxalate and significantly lower pH than non-mat mineral soils. Microbial biomass C was not significantly different between the rhizomorphic mat and non-mat organic soils. In the mineral horizon, however, the hydrophobic mats had greater microbial biomass C than the non-mat soils. These data demonstrate that soils densely colonized by EcM fungi create a unique soil environment with distinct microbial activities when compared to non-mat forest soils.

1. Introduction

In exchange for photosynthate, mycorrhizal fungi provide their host plant with a range of benefits (Smith and Read, 2008), including increased nutrient uptake. Although forest ecosystems have an abundance of organic matter, nutrients (e.g., N and P) are often in complex organic forms that are unavailable for direct plant uptake. Ectomycorrhizal (EcM) fungi can access these otherwise recalcitrant pools and provide their host plant with nutrients (Allen et al., 2003). Various EcM fungi have been found to form dense aggregations of hyphae known as mats (Griffiths et al., 1990; Agerer, 2001), which are prominent features in Douglas-fir forest ecosystems and have been found to cover as much as 25–40% of the forest floor in a given stand (Cromack et al., 1979; Griffiths et al., 1996; Phillips, 2009).

There are several ways that soils colonized with EcM mats differ from non-mat forest soils. Not only can the fungal rhizomorphs account for up to half the dry weight of the mat-associated soil (Ingham et al., 1991), but mats typically have higher microbial biomass and organic matter content, and high levels of oxalate, which likely contributes to their lower pH (Malajczuk and Cromack, 1982; Griffiths et al., 1994). Previous studies in the Pacific Northwest have demonstrated the prevalence of EcM mats in Douglas-fir forests (Cromack et al., 1979; Griffiths et al., 1996) and shown their ability to have increased enzymatic activities and litter decay rates (Entry et al., 1991; Griffiths and Caldwell, 1992), accelerate mineral weathering (Cromack et al., 1979; Griffiths et al., 1994), provide habitat for soil animals (Cromack et al., 1988), and possibly enhance seedling survival (Griffiths et al., 1991b). In this earlier work, mat identification

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was limited to morphological descriptions and sporocarp identifications. EcM mats with rhizomorphic growth habits were often thought to be a *Hysterangium* species, whereas mats with an ashy, hydrophobic appearance were thought to be formed by *Gautieria* species (Cromack et al., 1979; Griffiths et al., 1996). More recently, a survey was done to determine the phylogenetic diversity of EcM mat-forming fungi in soils of Douglas-fir stands at the H.J. Andrews Experimental Forest (Dunham et al., 2007). By sequencing the fungal ITS region of EcM rhizomorphs and root tips, they found the diversity of mat-forming EcM fungi to be much greater than believed in the past. Previous studies in Douglas-fir forests focused on EcM mats in the mineral horizons; however, Dunham et al. (personal communication) found horizon-specific growth habits among the EcM mat-forming taxa and morphotypes. Although the majority of rhizomorphic mats are found only in the organic horizon, several taxa form rhizomorphic mats that colonize both the organic and mineral horizon. Hydrophobic mats, on the other hand, were found to colonize only the mineral horizon.

There has been increasing attention focused on the ability of ectomycorrhizal fungi to decompose organic material, enabling them to acquire N and P (Read and Perez-Moreno, 2003; Courty et al., 2010). Production of enzymes involved in nutrient cycling has been demonstrated for isolates of EcM fungi (Hutchison, 1990), EcM root tips (Pritsch et al., 2004; Buée et al., 2005; Courty et al., 2007; Cullings et al., 2008), and EcM mats (Griffiths and Caldwell, 1992). Because of their ability to ability to dominate soils locally, EcM mats have been hypothesized to be important players in forest nutrient cycling, and present a unique opportunity to examine the activities and properties associated with EcM hyphae in the soil. The survey of chemical and biochemical properties of EcM mat and non-mat soils presented herein provides an updated account of EcM mat properties and activities while accounting for the growth habits and taxonomy of EcM mat-forming fungi. Key extracellular enzymes involved in the C, N, and P cycles were analyzed in conjunction with soil chemical properties in EcM mat and non-mat samples from sites of varying stand ages throughout the H.J. Andrews Experimental Forest. The goals of this study were to: (1) determine whether the enzymatic activities and soil properties differ between EcM mat and non-mat soils, and (2) assess whether the taxonomic identity or morphotype of the mat-forming fungi corresponds to unique soil activities or properties.

2. Materials and methods

2.1. Site description

The study was conducted at the H.J. Andrews Experimental Forest located in the Western Cascade Mountains of Oregon (44°13′25′N, 122°15′30′W). Four old-growth and four second-growth stands dominated by Douglas-fir (*Pseudotsuga menziesii)* were selected to assess the diversity of EcM mats found at the HJA. A full description of the study location and soil properties can be found in Chaer et al. (2009).

2.2. Sampling

Eight sites (Chaer et al., 2009) were sampled, beginning on 22 June 2005 continuing through July, with one old-growth and 1 s-growth site sampled every other week. At each site, several people spread out over a 60 × 60 m area and gently raked away patches of the upper layers of moss, litter, and soils to expose the organic or mineral horizon. Four fungal mats were sampled from the organic horizon and four fungal mats were sampled from the mineral horizon. For the purposes of this study, fungal mats were defined as areas of densely aggregated fungal hyphae or rhizomorphs that covered an area with a minimum diameter of 20 cm, and non-mat soils are defined as patches of soil that were not heavily colonized with fungal hyphae or rhizomorphs. All mats were destructively sampled with the entire mat removed for analysis. Four organic and four mineral horizon samples were also taken from non-mat areas. Descriptive information on mat morphology, colonization, and horizon depth was recorded. All samples were transported on ice, sieved (4 mm for organic and 2 mm for mineral horizons) and stored at 4 °C until analysis (less than one week).

2.3. Molecular characterization of mats

Fungal mat samples were examined under a dissecting microscope to determine whether they possessed the characteristics necessary to be considered an EcM mat (Dunham et al., 2007; and personal communication). By examining the mats and EcM root tips under 10–40× magnification, we were able to determine whether the mat-forming rhizomorphs and hyphal material originated from the EcM roots found in the mat. If so, EcM root tip and rhizomorph material was selected for separate DNA extractions. If no EcM root tips were present in the fungal mats, the rhizomorph material alone was used for molecular identification of the mat-forming fungi. Selected root tips and mat-forming rhizomorphs were washed repeatedly with distilled water, then genomic DNA was extracted and the ITS region was amplified from each sample using the methods of Dunham et al. (2007). PCR products were purified using a QIAquick PCR purification Kit (Qiagen, Chatsworth, CA) and sequenced using direct dye-terminated, automated fluorescence methods performed at Oregon State University's Center for Genome Research and Biocomputing, using an ABI Prism 3730 genetic analyzer (Foster City, CA). Sequences were viewed, cleaned, and aligned using Bioedit (Hall, 1999) and taxa were assigned according to the best BLAST matches from the GenBank database using the Entrez query option of "all [filter] NOT uncultured" to reduce the number of hits from poorly identified uncultured clones. Although we were able to identify some EcM mats to the species level, mats were grouped at the genus level for statistical analysis.

2.4. Soil enzyme assays

Standard assay procedures, modified to work with 1-ml soil slurries (Chaer et al., 2009), were used to measure: phenoloxidase (phenoloxidase) using L-DOPA (Sinsabaugh et al., 1999), and β-glucosidase, N-acetyl-β-D-glucosaminidase (chitinase), and phosphatase using *p*-nitrophenol derivatives (Caldwell et al., 1999; Parham and Deng, 2000). Protease activity was determined by measuring casein degradation after a 24-h incubation with 1% sodium azide using the Folin–Ciocalteu phenol reagent method reported by Ladd and Butler (1972). Slurries for all enzyme assays were prepared by adding 60 ml deionized water to 6 g sieved soil in a 150-ml beaker. The mixture was vigorously mixed using a magnetic stir plate until the samples were homogenously suspended in solution. While mixing continued, 1 ml of slurry was removed with a large diameter pipette tip and placed in a 15 × 85 mm glass test tube. Three aliquots were removed per soil sample for each enzyme assay, allowing for a control and two laboratory replicates. Samples used in the protease assay were covered and frozen until analysis, while the other assays were performed immediately. Five, 1-ml aliquots from each sample were dried overnight to determine the dry weight of soil in each 1 ml of slurry.

2.5. Soil analyses

Gravimetric water content was determined by drying the soils for 90 h at 54 °C. Soil pH was measured with a pH meter after 30 ml