AN ABSTRACT OF THE DISSERTATION OF

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Dense hyphal mats formed by ectomycorrhizal (EcM) fungi are prominent features in Douglas-fir (*Pseudotsuga menziesii*) forest soils and have been estimated to cover up to 40% of the forest floor in some stands. Although previous studies have examined various aspects of EcM fungi, little is known about their associated microbial communities and activities. The objectives of my dissertation are 1) to provide a current account of chemical and biochemical properties associated with EcM fungi, and 2) to describe the communities and activities of microbes occupying EcM mat and non-mat forest soils. In the first phase of my research, I surveyed EcM mat and non-mat soils from early and late seral forest stands in the western Oregon Cascades. EcM mats were phylotyped and a variety of chemical and biochemical properties were measured. Results from this survey revealed distinct chemical and biochemical profiles for EcM mats in organic and mineral soils compared with their corresponding non-mat soil horizons. The second phase of my research followed up this work by focusing on

Piloderma mats in old-growth Douglas-fir stands. A combination of community fragment profiles, clone libraries, and quantitative PCR of bacterial 16S and fungal ITS rRNA genes, in conjunction with chitinase enzyme assays, were used to assess the microbial community composition, abundance, and activity in a year-long temporal study. I found that *Piloderma* mats harbor distinct fungal and bacterial communities compared with non-mat soils. Furthermore, although microbial populations and enzyme activity of both soil types fluctuated throughout the year, their community compositions remained relatively stable. The results presented in this dissertation demonstrate that EcM mats create a unique soil environment with distinct microbial communities and activities compared to non-mat forest soils. This work provides a significant contribution to the understanding of how EcM fungi impact the soil environment and microbial communities.

Microbial and Biochemical Dynamics of Ectomycorrhizal Mat and Non-mat Forest Soils

by
Laurel A. Kluber

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Approved:

Major Professor, representing Soil Science

Head of the Department of Crop and Soil Science

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Laurel A. Kluber, Author

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CONTRIBUTION OF AUTHORS

K.M. Tinnesand, B.A. Caldwell, and Drs. P.J. Bottomley, S.M. Dunham and R.R Yarwood were involved with study design and data collection for Chapter 2. Dr. J.E. Smith assisted in the interpretation of data for Chapter 3.

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Chapter One:

General Introduction

Laurel Kluber

Coniferous forests of the Pacific Northwest are a unique macro ecosystem (Waring and Franklin, 1979), and an impressive sight with towering trees reaching 80 m in height and 1.5-2 m diameter. However, the belowground biomass is equally impressive. In fact, belowground biomass accounts for approximately 15% of the total ecosystem biomass in Douglas-fir (*Pseudotsuga menziesii*) forests (Vogt, 1991). Roots represent an integral link between the above- and belowground ecosystems by directing carbon (C) produced through photosynthesis belowground. Many of these roots are associated with mycorrhizal fungi that provide their host plants with increased water and nutrient uptake in exchange for C (Smith and Read, 2008).

Ectomycorrhizal (EcM) fungi are a diverse subset of mycorrhizae with over 5000 species from all phyla of true fungi (Molina et al., 2002). Although a minority of tree species form EcM associations, the species that do are dominant in temperate coniferous forests creating one of the largest assemblages of EcM hosts in the world (Trappe et al., 2009). Douglas-fir, a dominant tree species in the western Cascades of Oregon, is estimated to serve as host to an estimated 2000 species of EcM fungi (Trappe, 1977) and the findings of Brant et al. (2006) suggested that EcM account for nearly half the fungal biomass in the soils of Douglas-fir forests. Not surprisingly, the western Cascades of Oregon represents a prime location to study EcM fungi; and indeed hosts a rich legacy of research examining the communities and ecological roles of EcM fungi (Molina et al., 2001; Trappe et al., 2009).

A subset of EcM fungi are known for their mat-like growth habit, with dense profusions of hyphae that aggregate the soil and alter its appearance and structure (Agerer, 2001). These EcM mats are prominent features in Douglas-fir forests and have been found to cover up to 40% of the forest floor in some stands (Cromack et al., 1979; Griffiths et al., 1996; Phillips et al., *in revision*). Forming in both the mineral and organic horizons of forest soils, EcM mats have been found to have a significant impact on the biological, chemical, and physical properties of the soils they occupy. Mats typically have higher microbial biomass and organic matter content than non-mat soils, in addition to elevated levels of oxalate and a lower pH (Cromack et al., 1979; Griffiths et al., 1994; Malajczuk and Cromack, 1982). Additionally, the respiration rates and enzymatic activities of EcM mats tend to be greater than seen in non-mat soils (Griffiths and Caldwell, 1992; Griffiths et al., 1991; Phillips et al., *in revision*). Although a considerable amount of research has been conducted on EcM mats, much of it focused on characterizing the mats thought to have been formed by two genera: *Hysterangium* mats that have rhizomorphic growth habits, and *Gautieria* mats that are hydrophobic and have an ashy appearance (Cromack et al., 1979; Griffiths et al., 1996; Griffiths et al., 1990; Griffiths et al., 1991). In these earlier studies, identification of the mat-forming fungi was limited to morphotype and sporocarp identification potentially leading to misidentification or underestimated diversity. Advances in molecular techniques have allowed for better identification of fungal species and aided Dunham et al. (2007) in their survey of the

phylogenetic diversity of EcM mat-forming fungi. The survey by Dunham et al. (2007) revealed not only a greater diversity of EcM mat formers than previously described, but also that *Hysterangium* and *Gautieria* were not the dominant mat-forming genera. Instead, the rhizomorphic mats formed by *Piloderma* species were found to be the dominant ones, accounting for nearly half the mats sampled from old and young forests. Furthermore, *Ramaria* species, rather than *Gautieria*, formed the majority of hydrophobic mats.

The objectives of my dissertation were: 1) to provide a current account of chemical and biochemical properties associated with EcM fungi, and 2) to describe the communities and activities of microbes occupying EcM mat and non-mat forest soils.

Chapter 2 presents a survey of EcM mat and non-mat soils from early and late seral Douglas-fir forests. A variety of physical and biochemical properties, including extracellular enzymes involved in the carbon, nitrogen, and phosphorus cycles, were examined for each of the soil types, and molecular methods were used to phylotype the mat-forming fungi. The findings of this survey provide an updated account of EcM mat properties and activities while accounting for the growth habits and true diversity of EcM mat-forming fungi.

Chapter 3 describes a study where I examined the temporal dynamics of microbial communities associated with the dominant genus of the EcM mat-forming fungus, *Piloderma*, and corresponding non-mat organic soils. A combination of community fragment profiles, clone libraries and quantitative

PCR of bacterial 16S and fungal ITS rRNA genes, in conjunction with chitinase enzyme assays, were used to assess the microbial community composition, abundance, and activity in a year-long temporal study. Through the assessment of organic horizon microbial communities, this study provides a rare look at an often-overlooked component of the soil ecosystem. Additionally, this study presents a significant contribution to current knowledge on how EcM fungi interact with other soil dwelling microbes and is the first to examine fungal communities associated with the ectomycorrhizosphere.

References

Agerer R. 2001. Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11, 107-114.

Brant J.B., Myrold D.D. and Sulzman E.W. 2006. Root controls on soil microbial community structure in forest soils. Oecologia 148, 650-659.

Cromack K., Jr, Sollins P., Graustein W.C., Speidel K., Todd A.W., Spycher G., Li C.Y. and Todd R.L. 1979. Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. Soil Biology and Biochemistry 11, 463-468.

Dunham S.M., Larsson K.-H. and Spatafora J.W. 2007. Species richness and community composition of mat-forming ectomycorrhizal fungi in old- and second-growth Douglas-fir forests of the HJ Andrews Experimental Forest, Oregon, USA. Mycorrhiza 17, 633-645.

Griffiths R.P. and Caldwell B.A. 1992 Mycorrhizal mat communities in forest soils. In Mycorrhizas in Ecosystems. Eds. D.J. Read, D.H. Lewis, A.H. Fitter and I.J. Alexander. Pp. 98-105. C.A.B. International, Wallingford, Oxon, UK.

Griffiths R.P., Baham J.E. and Caldwell B.A. 1994. Soil solution chemistry of ectomycorrhizal mats in forest soil. Soil Biology and Biochemistry 26, 331-337.

Griffiths R.P., Bradshaw G.A., Marks B. and Lienkaemper G.W. 1996. Spatial distribution of ectomycorrhizal mats in coniferous forests of the Pacific Northwest, USA. Plant and Soil 180, 147-158.

Griffiths R.P., Caldwell B.A., Cromack K., Jr., and Morita R.Y. 1990. Douglas-fir forest soils colonized by ectomycorrhizal mats. I. Seasonal variation in nitrogen chemistry and nitrogen cycle transformation rates. Canadian Journal of Forest Research 20, 211-218.

Griffiths R.P., Ingham E.R., Caldwell B.A., Castellano M.A. and Cromack K. Jr., 1991. Microbial characteristics of ectomycorrhizal mat communities in Oregon and California. Biology and Fertility of Soils 11, 196-202.

Malajczuk N. and Cromack K., Jr. 1982. Accumulation of calcium oxalate in the mantle of ectomycorrhizal roots of *Pinus radiata* and *Eucalyptus marginata*. New Phytologist 92, 527-531.

Molina R., Caldwell B.A., Castellano M.A., Horton T.R. and Smith J.E. 2002 Mycorrhizae: ectomycorrhizal fungi. In: Encyclopedia of Environmental Microbiology. Ed. G. Bitton. Pp. 2124-2132. Wiley, New York.

Phillips C.L., Kluber L., Pedersen J. and Bond B.J. *in revision*. Contributions of ectomycorrhizal fungal mats to forest soil respiration. New Phytologist.

Smith S. and Read D. 2008. Mycorrhizal Symbiosis. Academic Press, New York. 787 p.

Trappe J.M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Annual Review of Phytopathology 15, 203-222.

Trappe J.M., Molina R., Luoma D.L., Cázares E., Pilz D., Smith J.E., Castellano M.A., Miller S.L. and Trappe M.J. 2009. Diversity, ecology, and conservation of truffle fungi in forests of the Pacific Northwest. Gen. Tech. Rep. PNW-GTR-772. Department of Agriculture, Pacific Northwest Research Station. 194 p. Portland, OR.

Vogt K. 1991. Carbon budgets of temperate forests Ecosystems. Tree Physiology 9, 69-86.

Waring R.H. and Franklin J.F. 1979. Evergreen Coniferous Forests of the Pacific Northwest. Science 204, 1380-1386.

Chapter Two:

Ectomycorrhizal Mats: Biochemical Characterization and Comparison to Corresponding Non-mat Soils in a Douglas-fir Forest

Laurel A. Kluber, Kathryn M. Tinnesand, Bruce A. Caldwell, Susie M. Dunham, Rockie R. Yarwood, Peter J. Bottomley, David D. Myrold

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6277 Sea Harbor Drive

Orlando, FL 32887-4800 USA

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 generally are found 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 phylotyped by DNA sequencing. A variety of physical and biochemical properties, including chitinase, β-glucosidase, phosphatase, phenoloxidase and protease enzyme activities, were measured. Analysis was confined to a comparison of hydrophobic mats with non-mat mineral soils, and rhizomorphic mats to the non-mat organic soils. Both the organic and mineral horizons showed differences between mat and non-mat enzyme profiles. In the organic horizon, rhizomorphic mats had 1.7 times greater chitinase activity than non-mats; hydrophobic mats had a chitinase activity 2.5 times greater than mineral non-mats, in addition to increased phosphatase and phenoloxidase activity. The rhizomorphic mats had 2.7 times more oxalate than the non-mats and a significantly lower pH. A larger difference was seen in the mineral horizon with hydrophobic mats having 40 times more oxalate and a significantly lower pH than non-mat mineral soils. Hydrophobic mats had greater

microbial biomass C and soil organic matter, whereas the rhizomorphic mats showed no significant difference when compared to their corresponding non-mat soil horizons. These data demonstrated that EcM mats create a unique soil environment with distinct microbial activities when compared to non-mat forest soils.

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 to 40% of the forest floor in a given stand (Cromack et al., 1979; Griffiths et al., 1996; Phillips et al., in revision).

There are several ways that soils with EcM mats differ from the surrounding bulk soil. Not only do the mat-forming fungi account for up to half the biomass of the mat-associated soil (Ingham et al., 1991), but they also typically have higher microbial biomass and organic matter content, and high levels of oxalate which contribute to their lower pH and hydrophobic character

(Malajczuk and Cromack, 1982; Griffiths et al., 1994; Högberg and Högberg, 2002). There has been increasing attention focused on the ability of mycorrhizal fungi to decompose organic material, enabling them to acquire N and P (Read and Perez-Moreno, 2003; Talbot et al., 2008). Production of key nutrient cycling enzymes has been demonstrated for many EcM fungi (Hutchison, 1990; Pritsch et al., 2004; Buée et al., 2005; Courty et al., 2005) and for some EcM mats (Griffiths and Caldwell, 1992).

Although EcM mats in coniferous forest ecosystems have been studied previously, each paper focused on a set of chemical or biological properties (Ingham et al., 1991; Griffiths et al., 1994), or ecological roles (Griffiths et al., 1990; Griffiths et al., 1991b; Griffiths et al., 1995; Griffiths et al., 1996;) of specific mats, leading to a fragmented view of the overall EcM mat characteristics. Furthermore, mat identification 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., 1990; Griffiths et al., 1991a; 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 expected, based on the earlier studies. Additionally,

they found that the growth habits of specific mat-formers varied with rhizomorphic mats primarily forming in the organic horizon and the hydrophobic mats forming in the mineral horizon (S. Dunham, personal communication).

The survey of chemical and biochemical properties of EcM mat and non-mat soils presented herein was designed to provide an updated account of EcM mat properties and activities while accounting for the growth habits and true diversity of EcM mat-forming fungi. Key extracellular enzymes involved in the C, N, and P cycles were analyzed in conjunction with soil physical and biochemical properties in 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 biogeochemical properties differ between mat and non-mat soils, and (2) assess whether the phylotype or morphotype of the mat-forming fungi corresponds to unique soil activities or properties.

Materials and methods

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 represent the diversity of EcM mats found at the HJA. A full description of the study location and soils can be found in Chaer et al. (2009).

Sampling

Sampling began on 22 June, 2005 and continued through July, with one old-growth and one second-growth site sampled every other week. To identify EcM fungal mat and non-mat sampling locations at each site, several people spread out over a 60 x 60 m area and gently raked away patches of the upper layers of moss and litter to expose the organic or mineral horizon. Four rhizomorphic mats from the organic horizon, and four hydrophobic mats from the mineral horizon 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 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 (up to one week).

Phylotype determination

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 EcM root tips and hyphal material under 10 - 40 x magnification, we were able to determine whether the mat-forming rhizomorphs originated from the EcM roots found in the mat. If so, EcM root tip and rhizomorph material was selected and used for phylotyping. Selected root tips and associated rhizomorphs were washed repeatedly with distilled water, then genomic DNA was extracted using a modified CTAB extraction procedure, and DNA was purified using the

GENECLEAN extraction kit (Qbiogene, Irvine, CA). Successive 10-fold dilutions (10 to 1000-fold) of unquantified DNA extracts were amplified by PCR using primers ITS1-F and ITS4 that target the ITS region (Dunham et al. 2007). PCR products were purified using a QIAquick PCR purification Kit (Qiagen, Chatsworth, CA) and sequenced from both ends 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 aligned manually using Bioedit and identified using blastn BLAST searches of the GenBank database. Names were assigned to taxa according to the best BLAST matches. Although we were able to assign most mats to the level of species, phylotypes were grouped at the genus level for analysis.

Soil enzyme assays

Standard assay procedures, modified to work with 1-ml soil slurries, were used to measure: phenol oxidase (phenoloxidase) using L-DOPA (Sinsabaugh, 1999), and β-glucosidase, N-acetyl-β-D-glucosaminidase (chitinase), and phosphatase using *p*-nitrophenol derivatives (Caldwell, 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). 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 to achieve a homogenous

consistency. While mixing continued, 1 ml of slurry was removed with a large diameter pipette tip and placed in a 15 x 85 mm glass test tube. Three aliquots were removed per soil sample for each enzyme assay, allowing for a control and two duplicate samples for analysis. 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.

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 deionized water was mixed with 10 g field moist soil and allowed to equilibrate for 60 min. Oxalate content of an acid extract was measured by ion chromatography using a modified method of Griffiths et al. (1994): 10 g of soil was extracted with 20 ml of 30 mM HCl and centrifuged, the supernatant was transferred to a fresh tube, centrifuged, filtered (0.2 μm), and diluted in deionized water for analysis on a Dionex Ion Chromatograph 2000 (Sunnyvale, CA). Soil organic matter (SOM) was determined by loss on ignition (430 ˚C for 24 h) of oven-dry soil. Inorganic N was measured by extracting 5 g soil with 15 ml 2M KCl and analyzing the filtrate for NH_4^+ and NO_3^- on an Astoria-Pacific 300 series autoanalyzer (Portland, OR).

Microbial biomass C

Microbial biomass C (MBC) was measured by chloroform fumigation-extraction (Vance et al., 1987). Soil samples were fumigated for 24 h under

chloroform vapor, extracted using 30 ml 0.05 M K_2SO_4, and total C was measured by combustion. Control soil samples were extracted and analyzed without exposure to chloroform.

Statistical analyses

Separate multiple analysis of variance (MANOVA) analyses were used to determine effects of mat presence on soil enzymes (phenoloxidase, β-glucosidase, chitinase, phosphatase, and protease) for each EcM mat morphotype and its corresponding non-mat horizon (hydrophobic mat vs. mineral non-mat; and rhizomorphic mat vs. organic non-mat). The soil physical and chemical properties (SOM, MBC, NH_4^+, NO_3^-, oxalate, water content, and pH) were tested using individual ANOVA analysis. All MANOVA and ANOVA models were blocked by site to account for variation among sites and to avoid any potential bias caused by spatial variation or sampling date. All MANOVA and ANOVA analyses were performed using SAS 9.1 (SAS Institute, Inc., Cary, N.C.). Variables were log transformed as necessary to meet the normality assumptions of the statistical procedures. Therefore 95% confidence intervals, rather than standard errors were reported with the means allowing for consistency between back transformed and untransformed means. Results were considered significant at $p \leq 0.05$, with significant trends considered to be $p \leq 0.1$.

Non-metric multidimensional scaling (NMS) using PC-ORD Software (MjM Software Design, Gleneden Beach, OR) was carried out to further assess differences in enzyme activities. NMS was selected as the ordination tool

because, unlike principal component analysis, it does not make the assumption of linearity between variables; instead it attempts to find a stable solution by minimizing stress (measured as departure from monotonicity) from a series of random starting points (McCune and Grace, 2002). Two "species" matrices were constructed, using the enzyme activity per g of soil; one with the organic horizon enzyme activities, and the other with mineral horizon enzyme activities. Environmental matrices were constructed to account for the site, sampling date, sample type (mat/non-mat), phylotype, and morphotype, as well as the physical and chemical properties of each soil sample. The enzyme data columns were relativized by the standard deviate to normalize the scale of the different enzyme activities. The Euclidian distance measure was chosen to calculate distance matrices to account for the negative values that resulted from relativization. A Mantel test revealed no significant correlation between organic and mineral horizon activities, thus it was appropriate to analyze the horizons with separate ordinations. Multi-response permutation procedures (MRPP) were used to determine the statistical significance of observed differences in soil enzyme activities between preexisting groups (e.g., sample type, site, date). MRPP is a nonparametric procedure to test the hypothesis of no difference between groups and yields an A-statistic that describes the chance-corrected within-group agreement, as well as a *p*-value to evaluate how likely the observed difference is due to chance (McCune and Grace, 2002). A-statistic values range from zero to one, with higher values indicating greater within-group homogeneity. Generally,

for community ecology data an $A \geq 0.1$ is considered strong grouping but values < 0.1 are commonly observed in environmental data sets (McCune and Grace, 2002). By using MRPP, we were able to test for differences between enzyme activity profiles of EcM mat and non-mat soils as well as between the phylotypes of mat-forming fungi within a given horizon.

Results

EcM phylotypes

Visual inspection of EcM mat samples with a dissection microscope combined with the phylotype sequences revealed that not all samples collected as EcM mats met appropriate criteria to be considered an EcM mat (insufficient hyphal density, saprotrophic growth patterns, EcM root tips not present and rhizomorph material was not from known EcM phylotype). These samples were excluded from subsequent analysis, resulting in a total of 27 rhizomorphic and 11 hydrophobic mats to be used for statistical analysis. Definitive phylotypes were determined for 17 rhizomorphic and 5 hydrophobic EcM mats (Table 2.1). The additional 10 rhizomorphic and 6 hydrophobic mats listed in Table 2.1 exhibited all the proper characteristics of an EcM mat, yet the phylotype sample resulted in a poor sequencing read due to mixed templates, humic contamination, or low DNA concentration.

Organic horizon

The blocked MANOVA for the combined enzyme activities showed a significant difference between the enzyme profiles of rhizomorphic mats

compared to non-mat organic soils ($p = 0.007$). There was a consistent overall trend for mat enzyme activities to be greater than non-mat activities. However, chitinase was the only enzyme to have significantly greater activity in the mats, exhibiting 1.7 times the activity of non-mat organic soil ($p = 0.01$). The other four enzymes showed no significant differences between rhizomorphic mats colonizing the organic horizon and non-mat organic soils (Fig. 2.1). When the organic enzyme activities were normalized to MBC (Fig. 2.1), the significance of the results remained (MANOVA p-value = 0.03), probably because there was not a significant difference in the MBC between the two sample types (Table 2.2). Oxalate and pH were the only chemical properties that differed significantly between the rhizomorphic mats and the corresponding non-mat organic soils. Oxalate concentrations were 2.7 times greater in the mats while pH values were lower (Table 2.2).

MRPP results from the organic horizon enzyme activities agreed with the MANOVA results and confirmed differing enzyme activity profiles for rhizomorphic mat and non-mat samples ($p = 0.01$), but the effect size was low (A = 0.02), possibly because of high spatial/temporal variability. The NMS ordination of organic horizon enzyme activities (Fig. 2.2) visually reveals the separation of *Hysterangium* from the other samples along the first axis of the NMS. This is corroborated by MRPP results that indicate significant differences among the mat-forming genera (MRPP A = 0.05, $p < 0.01$). Phenoloxidase activity was strongly correlated with axis 2 (r = 0.96), protease correlated with

axis 1 (r = 0.85), and chitinase activity correlated with axis 3 (r = 0.80). No significant correlations were found between the soil physical and chemical characteristics and the NMS axes.

Mineral horizon

The comparison of hydrophobic mats colonizing the mineral horizon and mineral non-mat soils revealed a different pattern than seen in the organic horizon. The blocked MANOVA of the enzyme activities showed a significant difference in the enzyme profiles of the two sample types ($p = 0.03$). Hydrophobic mats expressed more than twice the chitinase ($p = 0.04$), phosphatase ($p = 0.01$), and phenoloxidase ($p = 0.02$) activity than the non-mat mineral soils (Fig. 2.1). Additionally, there was a consistent trend ($p \leq 0.1$) showing that nearly all the chemical soil properties differed between the two sample types (Table 2.2). Mineral horizon hydrophobic mats had strikingly higher oxalate content (40 times greater than the mineral non-mat soils), higher NH_4^+, and lower pH than non-mat mineral soils. SOM and MBC also differed, with hydrophobic mats tending to have greater SOM and MBC than non-mat soil ($p < 0.01$). Unlike the organic horizon, the enzyme profiles of hydrophobic mats were not significantly different than those for non-mat mineral soil (MANOVA $p = 0.44$) after normalizing for MBC (Fig. 2.1).

NMS ordination of the mineral horizon enzyme activities (Fig. 2.3) showed a significant separation between hydrophobic mat and non-mat soils, which was confirmed with the MRPP analysis (MRPP A = 0.12, $p < 0.01$).

However, there was not a significant difference among the hydrophobic mat phylotypes (MRPP A = -0.05, $p = 0.77$). Chitinase, β-glucosidase, and phenoloxidase activities are all strongly correlated with axis 2 (r = 0.84, 0.80, and 0.78 respectively), probably contributing to the separation of the mats from the non-mat soils seen on that axis.

Discussion

Previous studies in the Pacific Northwest have demonstrated the prevalence of EcM mats (Cromack et al., 1979; Griffiths et al., 1996) and shown their ability to have increased enzymatic activities (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). Our study followed up this previous work with a more comprehensive examination of C, N, and P cycling enzymatic capabilities combined with a variety of physical and chemical soil properties, while taking into account the horizon-specific growth habits and phylotypes of EcM mat-forming fungi.

Ectomycorrhizal mats have been described as distinct (Cromack et al., 1979; Griffiths et al., 1990); however, we found that determining EcM status and distinguishing morphotypes to be difficult in the field. Inspection under a dissecting microscope aided greatly in determining the status of fungal mats, by allowing us to visually determine whether the mat-forming rhizomorph material emanated from an EcM root tip. Several of the fungal mats sampled had no

obviously associated EcM roots and may have been formed by saprotrophic fungi. A number of variations of EcM mat growth habits were found with our initial sampling efforts, including “mixed mats” (presence of EcM tips and several colonizing EcM fungi), rhizomorphic mats directly above hydrophobic mats, and rhizomorphic mats that colonized both the O and A horizons, which was contrary to some previously published reports (Griffiths et al., 1996), although they also were encountered by Dunham et al. (2007). Furthermore, some EcM mat phylotypes were present at only one or two sites. These variations of EcM mat morphotype and phylotype distributions can complicate sampling and limit data analysis if not properly accounted for. Thus, it would be advantageous to phylotype mats prior to sampling and analysis and to assure proper replication between sites for statistical analysis of given phylotypes. To overcome these limitations, our study focused on examining the dominant EcM mat morphologies: rhizomorphic mats colonizing the organic horizon, and hydrophobic mats colonizing the mineral horizon.

Although our study was not designed to be a phylogenetic survey of EcM mat-forming fungi, the phylogenetic diversity of our samples was greater than described in the early EcM mat studies of Douglas-fir forests undertaken before molecular analysis was widely available (Cromack et al., 1979; Griffiths et al., 1991a); our work better compares with the more recent survey by Dunham et al. (2007). Nearly all of the EcM mats we identified were comprised of genera matching those found by Dunham et al. (2007) in this same ecosystem. It is not

surprising that exceptions were found (*Tricholoma* sp. and *Russula queletii*), given that the species accumulation curves constructed by Dunham et al. (2007) indicated that their sampling efforts were insufficient to capture the total richness of EcM mat-formers. Earlier surveys based on sporocarp collections were conducted in this same area (Luoma et al., 1991; Smith et al., 2002), and records were made of various mat-forming genera (Agerer, 2001). Phylotyping offers the significant advantage of identification without sporocarps. Increasing numbers of EcM mat-forming fungi are likely to be discovered with continued molecular work on this ecosystem.

Enzyme activities have been measured previously in EcM mats, although the number of samples and activities measured were limited (Griffiths and Caldwell, 1992). Our survey is the first to examine a suite of enzymes involved in C, N, and P cycling for a wide range of EcM mats. However, there have been studies on the enzymatic activities of EcM root tips, allowing for assessment of the enzymatic capabilities of EcM phylotypes (Conn and Dighton, 2000; Pritsch et al., 2004; Buée et al., 2005; Courty et al., 2005;Courty et al., 2007;). There also have been numerous studies of enzyme potentials from isolated cultures (Hutchison, 1990; Griffiths and Caldwell, 1992; Burke and Cairney, 2002), including some mat-forming genera. NMS and MRPP results from our study demonstrated that all hydrophobic mats exhibited similar enzyme profiles regardless of phylotype. The organic horizon mats appeared to show more variation between the phylotypes, with a significant grouping of the

Hysterangium mats. Although these results are notable, the confounding variables of uneven distribution of phylotypes among sites and the progressive sampling design make it difficult to determine whether the grouping is due to a *Hysterangium*-specific activity profile, or because all but two *Hysterangium* mats were found at the first two sites sampled in the spring (data not shown). Through blocking by site and analyzing the activity profiles for each morphotype, we were able to account for any spatial/temporal patterns resulting in more robust differences between the EcM mat and non-mat activity profiles for each horizon. The high chitinase activity that was found in the rhizomorphic mats is consistent with the work of López-Gutiérrez at the Holden Arboretum (personal communication), who found soil associated with *Piloderma* root tips to have increased chitinase activity. EcM mats formed by *Piloderma* species have been found to be prominent at the H.J. Andrews Experimental Forest (Smith et al., 2000; Dunham et al., 2007), and indeed, they accounted for nearly a third of the rhizomorphic EcM phylotypes identified in our study. Interestingly, the rhizomorphic mats were not found to have higher C- or protein-degrading enzyme activities as previously reported in some EcM mats (Griffiths and Caldwell, 1992). In the mineral horizon, the activities of chitinase, phenoloxidase, and phosphatase were significantly greater in the soils colonized by hydrophobic mats, which is consistent with previous work that showed elevated enzyme activities within hydrophobic mats formed thought to be *Gautieria monticola*

(Griffiths and Caldwell, 1992), although protease activity showed no increase in our study.

One would be prudent to resist concluding that the enzyme activities measured in the EcM mats reflect the activity of the EcM fungi alone. It has been well documented that there are a variety of organisms that live in conjunction with mycorrhizal fungi, ranging from the classic "helper bacteria" (Garbaye, 1994; Frey-Klett et al., 2007) to mycophagous bacteria (Leveau and Preston, 2008). In reality, many of the microorganisms associated with mats probably lie between these extremes (Bending et al., 2002). Several studies have examined bacterial communities associated with mycorrhizal roots and found that they significantly differ from the bulk soil or non-mycorrhizal rhizosphere environments (Mansfield-Giese et al., 2002; Marschner and Baumann, 2003), other research examined the bacterial communities associated with EcM root tips in particular (Timonen, 1998; Heinonsalo et al., 2001; Burke et al., 2008). Although our study did not examine the microbial community composition associated with EcM mats, we hypothesize that the communities associated with mat and non-mat soils are distinct and likely contribute to the differing enzyme activities. A follow-up study is underway to explore the dynamics of *Piloderma* mat and non-mat microbial communities and activities throughout the year.

Griffiths et al. (1991a) suggested that EcM mats perform different functions in mineral and organic forest soils. In our study, we were unable to make any direct comparisons between the organic and mineral horizon samples;

however, we did observe trends within each. For example, the most notable difference between the mineral and organic mats was the high oxalate values found in the mineral hydrophobic mats. Our results mirror earlier work that found significantly greater oxalate concentrations in EcM mats, with the hydrophobic mats having the greatest amount of oxalate (Cromack et al., 1979; Griffiths et al., 1994). The ability of some EcM to excrete oxalate has been well documented (Malajczuk and Cromack, 1982; Lapeyrie et al., 1987; Rineau et al., 2008; Tuason and Arocena, 2009). It has been hypothesized that the abundance of oxalate in the mineral horizon mats could be a mechanism to free and acquire phosphorus through mineral weathering (Griffiths and Caldwell, 1992; Griffiths et al., 1994). Another notable comparison between the rhizomorphic and hydrophobic mats in our study is that the enzymatic profiles of each were significantly different from their non-mat counterparts when analyzed on a dry weight basis. Once enzyme activity was normalized by MBC, however, the enzyme profiles of the hydrophobic mats were no longer significantly different than the non-mat mineral horizon, yet the rhizomorphic significance remained unchanged. This suggests that the increased enzyme activity of hydrophobic mats could simply be due to an increased number of microbes, whereas the microbial communities within rhizomorphic mats have distinct activities compared to the organic non-mat communities. In both horizons, we see that on a dry weight, or “per-area” basis, the EcM mats have greater enzyme activities than non-mats. Given that mats can cover 25 to 40% of the forest floor in some stands (Cromack et al., 1979; Phillips

et al., in revision) EcM mats may have a significant influence on the overall nutrient cycling of forest soils.

The findings of our survey lay the groundwork for a variety of future studies on EcM mat systems that will result in a better understanding of their functions and associated communities. We expect the knowledge gained from these unique "natural laboratories" will contribute to a better overall understanding of the mechanisms, functions and communities associated with EcM fungi, enabling researchers to take the next step in linking EcM fungi to ecosystem processes.

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this publication is dedicated to the memory of Kathryn M. Tinnesand. Katie was a dear friend and colleague who inspired us all to live life to the fullest.

References

Agerer R. 2001. Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11, 107-114.

Allen M.F., Swensom W., Querejeta J.I., Egerton-Warburton L.M. and Treseder K.K. 2003. Ecology of Mycorrhizae: A conceptual framework for complex interactions among plants and fungi. Annual Reviews of Phytopathology 41, 271-303.

Bending G.D., Poole E.J., Whipps J.M. and Read D.J. 2002. Characterisation of bacteria from *Pinus sylvestris-Suillus luteus* mycorrhizas and their effects on root-fungus interactions and plant growth. FEMS Microbiology Ecology 39 219-227.

Buée M., Vairelles D. and Garbaye J. 2005. Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (*Fagus silvatica*) forest subjected to two thinning regimes. Mycorrhiza 15, 235-245.

Burke D.J., Dunham S.M. and Kretzer A.M. 2008. Molecular analysis of bacterial communities associated with the roots of Douglas-fir (*Pseudotsuga menziesii*) colonized by different ectomycorrhizal fungi. FEMS Microbiology Ecology 65, 299-309.

Burke R. and Cairney J. 2002. Laccases and other polyphenol oxidases in ecto- and ericoid mycorrhizal fungi. Mycorrhiza 12, 105-116.

Caldwell B.A., Griffiths R.P., Sollins P. 1999. Soil enzyme response to vegetation disturbance in two lowland Coast Rica soils. Soil Biology and Biochemistry 31, 1603-1608.

Chaer G., Myrold D. and Bottomley P. 2009. A soil quality index based on the equilibrium between soil organic matter and biochemical properties of undisturbed coniferous forest soils of the Pacific Northwest. Soil Biology and Biochemistry 41, 822-830.

Conn C. and Dighton J. 2000. Litter quality influences on decomposition, ectomycorrhizal community structure and mycorrhizal root surface acid phosphatase activity. Soil Biology and Biochemistry 32, 489-496.

Courty P.E., Breda N. and Garbaye J. 2007. Relation between oak tree phenology and the secretion of organic matter degrading enzymes by *Lactarius quietus* ectomycorrhizas before and during bud break. Soil Biology and Biochemistry 39, 1655-1663.

Courty P.E., Pritsch K., Schloter M., Hartmann A. and Garbaye J. 2005. Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. New Phytologist 167, 309-319.

Cromack K., Jr, Fitcher B.L., Moldenke A.M., Entry J.A. and Ingham E.R. 1988. Interactions between soil animals and ectomycorrhizal fungal mats. Agriculture, Ecosystems and Environment 24, 161-168.

Cromack K., Jr, Sollins P., Graustein W.C., Speidel K., Todd A.W., Spycher G., Li C.Y. and Todd R.L. 1979. Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. Soil Biology and Biochemistry 11, 463-468.

Dunham S.M., Larsson K.-H. and Spatafora J.W. 2007. Species richness and community composition of mat-forming ectomycorrhizal fungi in old- and second-growth Douglas-fir forests of the HJ Andrews Experimental Forest, Oregon, USA. Mycorrhiza 17, 633-645.

Frey-Klett P., Garbaye J. and Tarkka M. 2007. The mycorrhiza helper bacteria revisited. New Phytologist 176, 22-36.

Garbaye J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytologist 128, 197-210.

Griffiths R.P. and Caldwell B.A. 1992 Mycorrhizal mat communities in forest soils. In: Mycorrhizas in Ecosystems. Ed. D.J. Read, D.H. Lewis, A.H. Fitter, and I.J. Alexander pp. 98-105. C.A.B. International, Wallingford, Oxon, UK.

Griffiths R.P., Baham J.E. and Caldwell B.A. 1994. Soil solution chemistry of ectomycorrhizal mats in forest soil. Soil Biology and Biochemistry 26, 331-337.

Griffiths R.P., Bradshaw G.A., Marks B. and Lienkaemper G.W. 1996. Spatial distribution of ectomycorrhizal mats in coniferous forests of the Pacific Northwest, USA. Plant and Soil 180, 147-158.

Griffiths R.P., Caldwell B.A., Cromack K., Jr and Morita R.Y. 1990. Douglas-fir forest soils colonized by ectomycorrhizal mats. I. Seasonal variation in nitrogen chemistry and nitrogen cycle transformation rates. Canadian Journal of Forest Research 20, 211-218.

Griffiths R.P., Caldwell B.A., Ingham E.R., Castellano M.A. and Cromack K. Jr. 1991a. Comparison of microbial activity in ectomycorrhizal mat communities in Oregon and California. Biology and Fertility of Soils 11, 196-202.

Griffiths R.P., Chadwick A.C., Robatzek M., Schauer K. and Schaffroth K.A. 1995. Association of ectomycorrhizal mats with Pacific yew and other understory trees in coniferous forests. Plant and Soil 173, 343-347.

Griffiths R.P., Castellano M.A. and Caldwell B.A. 1991b. Hyphal mats formed by two ectomycorrhizal fungi and their association with Douglas-fir seedlings: A case study. Plant and Soil 134, 255-259.

Heinonsalo J., Jørgensen K.S. and Sen R. 2001. Microcosm-based analyses of Scots pine seedling growth, ectomycorrhizal fungal community structure and bacterial carbon utilization profiles in boreal forest humus and underlying illuvial mineral horizons. FEMS Microbiology Ecology 36, 73-84.

Högberg M. and Högberg P. 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces together with associated roots, half the dissolved organic carbon in a forest soil. New Phytologist 154, 791-795.

Hutchison L.J. 1990. Studies on the systematics of ectomycorrhizal fungi in axenic culture. II. The enzymatic degradation of selected carbon and nitrogen compounds. Canadian Journal of Botany 68, 1522-1530.

Ingham E.R., Griffiths R.P., Cromack K. Jr. and Entry J.A. 1991. Comparison of direct vs. fumigation incubation microbial biomass estimates from ectomycorrhizal mat and non-mat soils. Soil Biology and Biochemistry 23, 465-471.

Ladd J.N. and Butler J.H. 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biology and Biochemistry 4, 19-30.

Lapeyrie F., Chilvers G.A. and Bhem C.A. 1987. Oxalic acid synthesis by the mycorrhizal fungus *Paxillus involutus.* New Phytologist 106, 139-146.

Leveau J.H.J. and Preston G.M. 2008. Bacterial mycophagy: definition and diagnosis of a unique bacterial-fungal interaction. New Phytologist 177, 859-876.

Luoma D., Frenkel R. and Trappe J. 1991. Fruiting of hypogeous fungi in Oregon Douglas-fir forests: seasonal and habitat variation. Mycologia 83, 335–353.

Malajczuk N. and Cromack K., Jr. 1982. Accumulation of calcium oxalate in the mantle of ectomycorrhizal roots of *Pinus radiata* and *Eucalyptus marginata*. New Phytologist 92, 527-531.

Mansfield-Giese K., Larson J. and Bodker L. 2002. Bacterial populations associated with mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices*. Microbial Ecology 41, 133-140.

Marschner P. and Baumann K. 2003. Changes in bacterial community structure induced by mycorrhizal colonisation in split-root maize. Plant and Soil 251, 279-289.

McCune B. and Grace J.B. 2002. Analysis of Ecological Communities. MjM Software Design, Gleneden Beach, Oregon.

Parham J.A. and Deng S.P. 2000. Detection, quantification and characterization of β-glucosaminidase activity in soil. Soil Biology and Biochemistry 32, 1183-1190.

Phillips C.L., Kluber L., Pedersen J. and Bond B.J. in revision. Contributions of ectomycorrhizal fungal mats to forest soil respiration. New Phytologist.

Pritsch K., Raidl S., Marksteiner E., Blaschke H., Agerer R., Schloter M. and Hartmann A. 2004. A rapid and highly sensitive method for measuring enzyme activities in single mycorrhizal tips using 4-methylumbelliferone-labelled fluorogenic substrates in a microplate system. Journal of Microbiological Methods 58, 233-241.

Read D.J. and Perez-Moreno J. 2003. Mycorrhizas and nutrient cycling in ecosystems: a journey towards relevance? New Phytologist 157, 475-492.

Rineau F., Courty P.E., Uroz S., Buée M. and Garbaye J. 2008. Simple microplate assays to measure iron mobilization and oxalate secretion by ectomycorrhizal tree roots. Soil Biology and Biochemistry 40, 2460-2463.

Smith J.E., Molina R., Huso M.M.P. and Larsen M.J. 2000. Occurrence of *Piloderma fallax* in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, U.S.A. Canadian Journal of Botany 78, 995-1001.

Smith J.E., Molina R., Huso M.M., Luoma D.L., McKay D., Castellano M.A., Lebel T. and Valachovic Y. 2002. Species richness, abundance, and composition of hypogeous and epigeous ectomycorrhizal fungal sporocarps in young, rotation-age, and old-growth stands of Douglas fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, USA. Canadian Journal of Botany 80, 186–204.

Smith S. and Read D. 2008. Mycorrhizal Symbiosis. Third edition. Academic Press, New York. 787 p.

Talbot J.M., Allison S.D. and Treseder K.K. 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Functional Ecology 22, 955-963.

Timonen S., Jorgensen K.S., Haahtela K. 1998. Bacterial community structure at defined locations of *Pinus sylvestris - Suillus bovinus* and *Pinus sylvestris - Paxillus involutus* mycorrhizospheres in dry pine forest humus and nursery peat. Canadian Journal of Microbiology 44, 499-513.

Tuason M.M.S. and Arocena J.M. 2009. Calcium oxalate biomineralization by *Piloderma fallax* in response to various levels of calcium and phosphorus. Applied and Environmental Microbiology 75, 7079-7085.

Vance E.D., Brookes P.C. and Jenkinson D.S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703-707.

Phylotype	Occurrence	Distribution
Rhizomorphic mat formers		
Hysterangium crassirhachis	4	2
Hysterangium setchellii	3	2
Hysterangium sp.	1	1
Piloderma fallax	6	5
Suillus sp.	1	1
Russula queletii	1	1
Tricholoma sp.	1	1
Unknown rhizomorphic	10	5
Hydrophobic mat formers		
Gomphus clavatus	2	2
Ramaria formosa	2	1
Ramaria cf. *gelatinosa*	1	1
Unknown hydrophobic	6	4

Table 2.1 Occurrence and distribution of EcM mat phylotypes. Occurrence is the number of times the phylotype was sampled throughout the survey. Distribution represents the number of sites where the phylotype was present.

	Oxalate (mg kg^{-1})	Water content (kg H_2O kg^{-1})	pH	NO_3^- (mg N kg^{-1})	NH_4^+ (mg N kg^{-1})	Soil organic matter (g kg^{-1})	Microbial biomass (g C kg^{-1})
Organic horizon							
Rhizomorphic mat	8.88 (5.65, 13.96)	1.32 (1.13, 1.50)	4.56 (4.46, 4.65)	2.88 (2.00, 3.76)	13.94 (10.22, 19.05)	629 (572, 691)	5.32 (4.41, 6.30)
Non-mat	3.28 (2.09, 5.16)	1.33 (1.15, 1.51)	4.87 (4.78, 4.97)	2.64 (1.76, 3.52)	13.42 (9.84, 18.34)	591 (538, 649)	4.90 (3.99, 5.82)
p-value	0.01	0.91	<0.01	0.65	0.83	0.29	0.45
Mineral horizon							
Hydrophobic mat	44.7 (13.9, 143.1)	0.39 (0.33, 0.44)	4.41 (4.09, 4.74)	1.31 (1.08, 1.54)	4.88 (3.61, 6.60)	224 (189, 266)	0.74 (0.42, 1.29)
Non-mat	1.10 (0.34, 3.51)	0.45 (0.39, 0.50)	5.02 (4.69, 5.35)	1.41 (1.18, 1.64)	2.78 (2.06, 3.76)	185 (156, 220)	0.40 (0.23, 0.70)
p-value	<0.01	0.09	0.02	0.43	0.02	0.09	0.09

Table 2.2 Soil physical and chemical properties of mat and non-mat soils in the organic and mineral horizons reported as means with 95% confidence intervals.

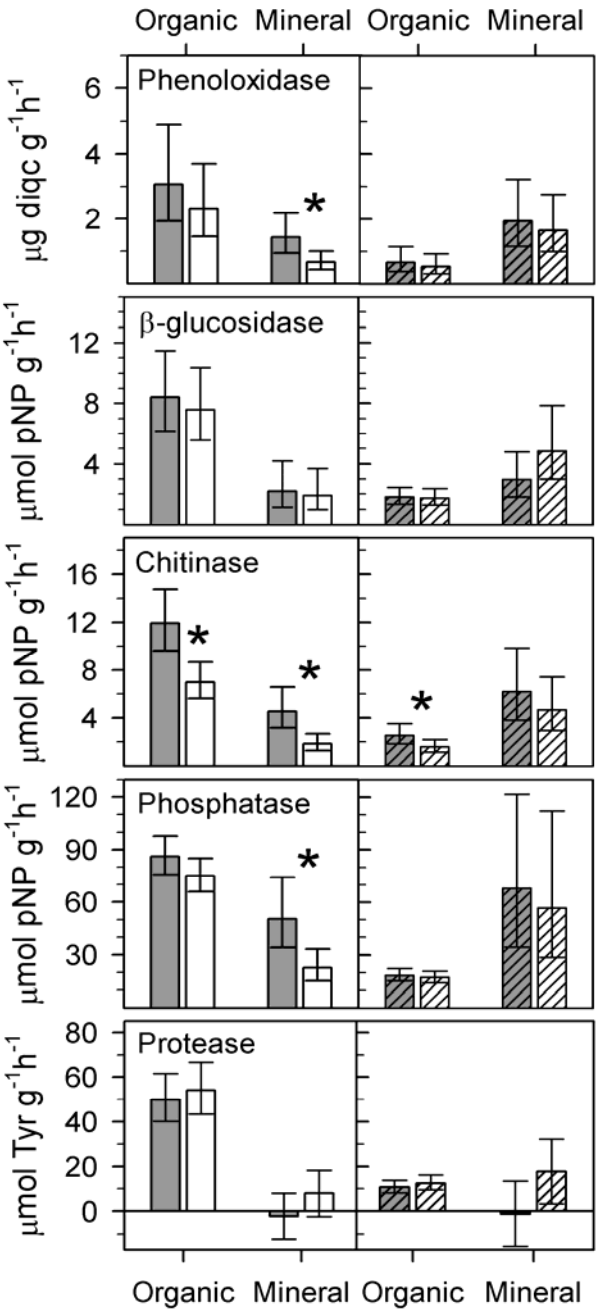

Fig. 2.1 Enzyme activities of mat (M, gray) and non-mat (N, white) soils in the organic and mineral horizons. Solid bars are activity per g soil; hatched bars are activity per g microbial biomass C. All data presented as means with 95% confidence intervals. Significant differences between mat and non-mat samples within a horizon are indicated with asterisks for $p \leq 0.05$.

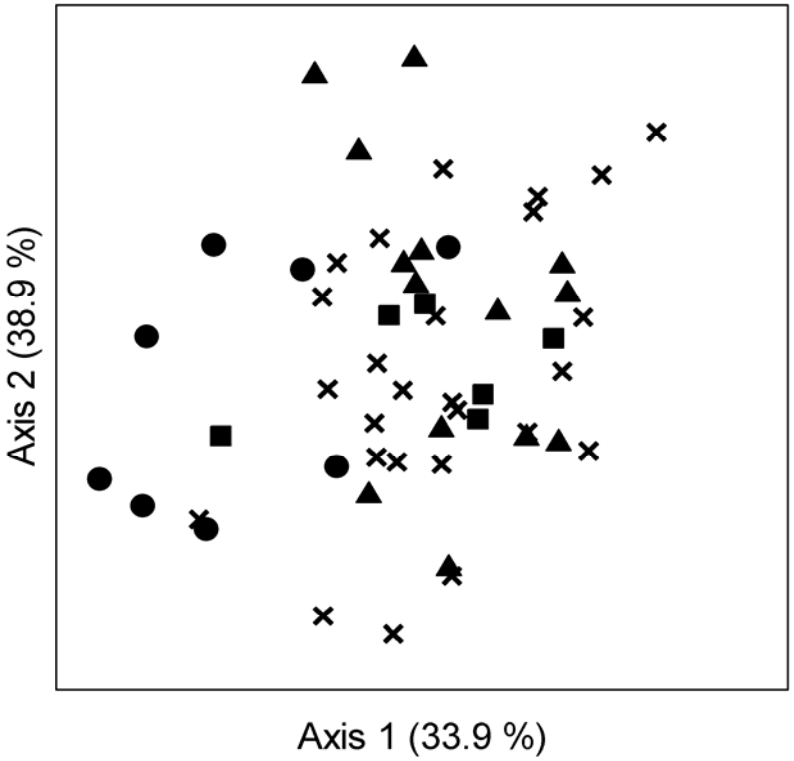

Fig. 2.2 First two dimensions of a three-dimensional NMS ordination of the organic horizon enzyme activities, stress = 10.60. Symbols represent the *Hysterangium* mats (circles), *Piloderma* mats (squares), other rhizomorphic mats (triangles), and non-mat soils (x's).

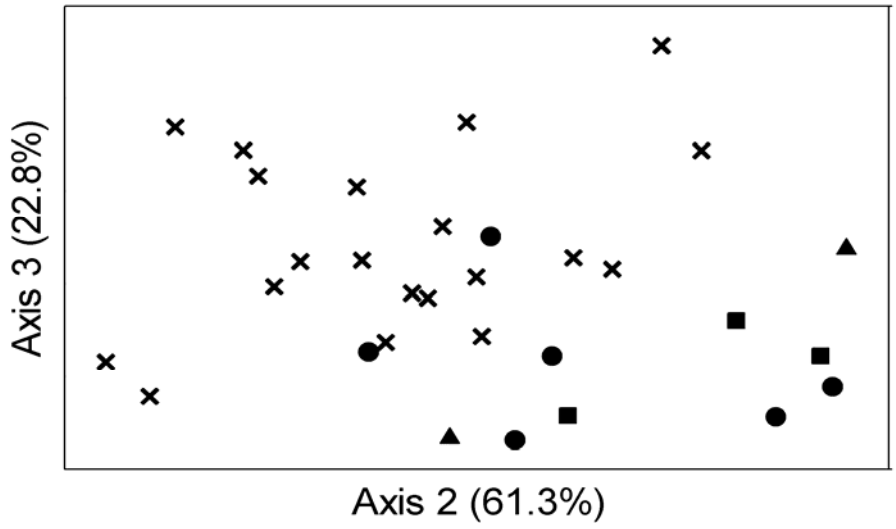

Fig. 2.3 Axis 2 and 3 of a 3-dimensional NMS ordination of mineral horizon enzyme activities (stress = 5.293). Symbols represent the *Ramaria* mats (squares), *Gomphus* mats (triangles), unknown hydrophobic mats (circles), and non-mat soils (x's).

Chapter Three:

Temporal Dynamics of Fungal and Bacterial Communities Associated with Ectomycorrhizal *Piloderma* Mat and Non-mat Soils

Laurel A. Kluber, Jane E. Smith, and David D. Myrold

To be submitted to:

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6277 Sea Harbor Drive

Orlando, FL 32887-4800 USA

Abstract

The distinct rhizomorphic mats formed by ectomycorrhizal *Piloderma* fungi are common features of the organic soil horizons of coniferous forests throughout the Pacific Northwest. These fungal mats have been found to cover over 40% of the forest floor in some Douglas-fir stands, and are associated with a variety of physical and biochemical properties that distinguish them from the surrounding non-mat soils. In this study, we examined the fungal and bacterial communities associated with paired *Piloderma* mat and non-mat soils. Each mat and non-mat area was repeatedly sampled at four times throughout the year. Characterization of the mat activity and community was achieved using a combination of N-acetylglucosaminidase (NAGase) enzyme assays, and molecular analysis of fungal and bacterial communities using T-RFLP profiles, clone libraries, and quantitative PCR. *Piloderma* mats had consistently greater NAGase activity across all dates, although the magnitude of the difference varied by season. Furthermore, we found distinct fungal and bacterial communities associated with the *Piloderma* mats, yet the size of the microbial populations differed little between the mat and non-mat soils. Significant temporal variation was seen in the NAGase activity and in the sizes of the fungal and bacterial populations, but the community composition remained stable through time. This work provides a significant contribution to the current knowledge on the fungal and bacterial communities associated extramatrical hyphae of ectomycorrhizal fungi.

Introduction

The coniferous forests of the Pacific Northwest (USA) are often thought of as nitrogen (N) limited because most of the N is tied up in complex organic forms that are unavailable for direct plant uptake (Chapin et al., 2002). Through their production of extracellular enzymes, fungi are especially adept at breaking down complex organic substrates with low N content that are ubiquitous in coniferous forest ecosystems (Dix and Webster, 1995). Although saprotrophic fungi play a primary role in the degradation of woody substrates, nearly all types of fungi participate in nutrient cycling within Pacific Northwest forest ecosystems (Molina et al., 2001).

Although a minority of tree species form ectomycorrhizal (EcM) associations, the species that do are dominant in temperate coniferous forests creating one of the largest assemblages of EcM hosts in the world (Trappe et al., 2009). EcM fungi play a critical role in forest ecosystems through their ability to provide host trees with enhanced nutrient uptake in exchange for photosynthate C (Allen et al., 2003; Smith and Read, 2008). A subset of EcM that form dense hyphal mats (Griffiths et al., 1990; Agerer, 2001) can be prominent features in Douglas-fir forests, covering 25-40% of the forest floor in some stands (Cromack et al., 1979; Griffiths et al., 1996; Phillips et al., in revision). Early work on EcM mats in Douglas-fir forests focused on characterizing mats formed by *Hysterangium* and *Gautieria* species (Cromack et al., 1979; Griffiths et al., 1990; Griffiths et al., 1991; Griffiths et al., 1996). However, at that time, identification

of the mat-forming fungi was limited to morphotype and sporocarp identification, potentially leading to misidentification or underestimated diversity. Not surprisingly, the findings of a recent molecular-based survey on the phlyogenetic diversity of EcM mat-forming fungi found much greater diversity than previously described (Dunham et al., 2007). Furthermore, Dunham et al. (2007) found mats formed by *Piloderma* species to be dominant, accounting for nearly 50% of the EcM mats sampled in old- and second-growth stands. These findings correlate with previous and more recent EcM studies that also found the rhizomorphic structures formed by *Piloderma* to be prominent in Douglas-fir forests (Smith et al., 2000; Phillips et al., in revision).

EcM mats formed by *Piloderma* species occur primarily in the organic horizon and have been associated with the presence of coarse woody debris (Smith et al., 2000; Kluber et al., submitted). In the coniferous forests of the Pacific Northwest, the organic horizon has been found to have nearly three times more N than the underlying mineral horizon (Chaer et al., 2009). Thus, *Piloderma* mats may play a significant ecological role by accessing the organically bound N from the organic horizon and providing it to the host plant. The findings of Kluber et al. (submitted) found rhizomorphic EcM mats, including those formed by *Piloderma*, to have enhanced enzymatic capabilities compared to the non-mat organic horizon. Most notably, N-acetylglucosaminidase, an enzyme involved in the degradation of chitin, activity of mats was 1.7 times greater than the non-mat soils. Early work on EcM mats reported increased respiration rates (Griffiths et

al., 1991) and more recently, Phillips et al. (in revision) determined that *Piloderma* mats were responsible for approximately 10% of the total soil respiration within an old-growth Douglas-fir stand.

Bacterial associations with mycorrhizal fungi have been documented for many years (Oswald and Ferchau, 1968). Bacteria associated with the mycorrhizosphere range from "helper bacteria" (Garbaye, 1994; Frey-Klett and Garbaye, 2005; Frey-Klett et al., 2007) to mycophagous bacteria (Leveau and Preston, 2008), although many of the mycorrhizal-associated microbes most likely lie between these extremes, as reported by Bending et al. (2002). Several studies have specifically examined the bacterial communities associated with EcM roots and hyphae (Knutson et al., 1980; Khetmalas et al., 2002; Kretzer et al., 2009). Culture dependent methods have shown that the physiological capabilities of bacteria from the ectomycorrhizosphere have distinct substrate use profiles when compared to bacteria from the bulk soil (Timonen et al., 1998; Heinonsalo et al., 2001). Using molecular techniques, Warmink et al. (2009) examined the bacteria associated with hyphal mats formed under fruiting bodies and found decreased overall bacterial diversity and increased *Pseudomonas* diversity compared to the bulk soil. Furthermore, they found that the bacteria associated with the EcM fungi *Lactarius hepaticus* and *Scleroderma citrina* were similar for samples collected a year apart, suggesting that the bacterial communities may be specific to EcM phylotypes. However, others have found that soil bacterial community composition appeared unrelated to EcM presence

(Burke et al., 2006) or the taxonomic identity of the EcM fungi (Burke et al., 2008).

A number of studies have documented seasonal trends in soil microbial communities and activities in a variety of ecosystems (Boerner et al., 2005; Høj et al., 2006; Allison and Treseder, 2008; Björk et al., 2008; Cruz-Martinez et al., 2009), including the coniferous forests of the Pacific Northwest (Brant et al., 2006; Moore-Kucera and Dick, 2008). Studies specifically examining EcM fungi have found that the community structure (Buée et al., 2005; Courty et al., 2008) and enzymatic and metabolic capabilities of EcM fungi exhibit considerable temporal variation (Buée et al., 2005; Courty et al., 2007). Additionally, ectomycorrhizas of *Lactarius quietus* have been found express some extracellular enzyme activity in correlation with the phenology of the host tree (Courty et al. 2007).

Because of their abundance and persistent nature, *Piloderma* mats offer a unique opportunity to examine microbial communities and activities associated with EcM fungi. Not only is it possible to sample hyphal material repeatedly from the same EcM fungal mat, but the wide distribution of *Piloderma* also allows for replication across forest stands. Although there have been studies on EcM diversity (Anderson and Cairney, 2007; Courty et al., 2010) and the diversity of bacteria associated with EcM root tips and hyphae (Burke et al., 2008; Warmink et al., 2009), little is known about the interactions between EcM and other soil fungi (Cairney and Meharg, 2002) beyond their ability to protect their host plant

from fungal pathogens (Fitter and Garbaye, 1994). Ascomycota root endophytes have been found in EcM root tips (Tedersoo et al., 2009), and microcosm studies have shown that EcM and saprotrophic fungi interact with varying outcomes (Leake et al., 2001; Lindahl, 2000). However, to our knowledge, this is the first study to examine the fungal and bacterial community associated with the ectomycorrhizosphere in natural soils. The objectives of this study were: 1) to assess and compare the community composition and activity associated with *Piloderma* mat and non-mat soils, and 2) to determine to what extent the community and activity vary seasonally with changes in moisture and temperature.

Materials and methods

Site description and sample collection

This study was conducted at the H.J. Andrews Experimental Forest (HJA) located in the western Cascade Mountains of Oregon (44˚13”25’N, 122˚15”30’W). The mean annual temperature is 8.7 °C and annual precipitation is ~2300 mm. Despite the relatively high annual precipitation, this region is classified as having a xeric climate with the majority of precipitation occurring between October and April. Soils at the HJA generally are weakly developed and andic in origin.

Five old-growth (350+ years) Douglas-fir (*Pseudotsuga menziesii*) stands ranging in elevation from 451 to 1,192 m were chosen for this study based on their previously high encounter rate for finding EcM mats formed by *Piloderma*

fungi (Dunham et al., 2007). During the summer of 2006, several rhizomorphic EcM mats and two non-mat areas (plots) were located and flagged at each of the five sites. EcM mats were phylotyped using the methods described in Kluber et al. (submitted). Phylotyping prior to sampling ensured that all EcM mats used in the study were indeed formed by *Piloderma* fungi.

Samples were taken at four times throughout the year: 10 September 2006 (hot and dry); 5 November 2006 (cool and wet); 5 May 2007 (post snowmelt); and 23 June 2006 (warm and moist). To provide a context for the environmental conditions surrounding each sampling date, average daily air temperature and precipitation data from the HJA primary meteorological station was provided for 15 July 2006 to 15 July 2007 (Fig. 3.1). Additionally, soil moisture content and temperature at the time of sampling were recorded (Fig. 3.2a). Because *Piloderma* mats colonize the organic soil horizon and this study's focus was on comparing mat and non-mat soil communities and activities, only the organic horizon (~3 to 6 cm deep, depending on location) was removed for analysis for each sample type. At each sampling date, three soil cores (3 cm diameter) from each plot were composited into a single sample, resulting in two mat and two non-mat samples per site per date. The core size was intentionally small to decrease the possibility that repeated sampling would negatively impact the mats over the course of the study. It should be noted that at the conclusion of the study, no mats showed obvious signs of death or decay. All samples were transported on ice, sieved to < 4 mm, and divided into aliquots for each type of analysis. Soils for water content

and enzymatic analysis were stored at 4 ˚C until analysis (up to 2 days), and soils used for molecular analysis were frozen at -20 ˚C.

Enzyme analysis

The standard *p*-nitrophenol assay procedure was used to measure N-acetyl-β-D-glucosaminidase (NAGase) activity (Parham and Deng, 2000) using modifications to work with 1-ml soil slurries (Caldwell et al., 1999; Kluber et al., submitted). NAGase activity was chosen as the sole enzyme assay for this study because elevated levels of NAGase have previously been reported in rhizomorphic mats, including those formed by *Piloderma*, whereas other enzymes involved in C, N, and P cycling did not differ significantly from the organic non-mat soils (Kluber et al., submitted).

DNA extraction and amplification

DNA was extracted from 0.5 g of each soil sample using a MOBio PowerSoil™ DNA isolation kit (MOBio Laboratories, Carlsbad, CA) using the manufacturer's protocol, except that the bead beating tubes were shaken for 45 s on a FastPrep instrument (Bio 101, Carlsbad, CA) to lyse cells. Because of the high humic content of these soils, it was occasionally necessary to carry out the final spin-column cleaning step a second time to avoid interference from humic contamination during downstream DNA manipulation and analysis. All extracts were quantified using a NanoDrop ND-1000 UV-visible spectrophotometer (NanoDrop Technologies, Wilmington, DE) and diluted to 25 ng μl^{-1}. Diluted DNA aliquots were stored at -80 ˚C awaiting downstream analysis.

Community fragment analysis and clone library construction

Amplification of DNA was carried out in 50-ml PCR reactions with 100 ng of template DNA and 5'-6-FAM (6-carboxyfluorescein) fluorescently labeled forward primers. The fungal ITS spacer region was amplified using ITS1f (Gardes and Bruns, 1993) and ITS4 (White et al., 1990) primers using the thermocycling procedure from (Manter and Vivanco, 2007). Bacterial 16S rRNA genes were amplified with Eub8F (Edwards et al., 1989) and Eub907R (Muyzer et al., 1995) using the thermocycling protocol from Hackl et al. (2004). PCR reactions were done in duplicate and pooled for each sample prior to cleaning with a Qiaquick™ PCR Purification kit (Qiagen Inc., Valencia, CA) and quantified. Fungal ITS PCR products were restricted with HinfI and bacterial 16S PCR products were digested using MspI and AluI restriction enzymes using the manufacturer's protocol (Promega Corp., Madison, WI). Restricted samples were submitted to the Oregon State University Center for Genome Research and Biocomputing for purification using Illustra SephadexTM columns (GE Healthcare, Piscataway, NJ) and analysis using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Unrestricted fungal ITS amplicons also were submitted for analysis using the same procedure to produce length heterogeneity (LH) profiles. GeneMapper v4.0 software (Applied Biosystems, Foster City, CA) was used to analyze LH and T-RF fragments using methods described by Avis and Feldheim (2005). Peak area data were exported for each dataset and normalized by total fluorescence of each individual sample as described by Boyle et al. (2006).

To construct clone libraries, 20 μl of each 25 ng μl^{-1} DNA extract from all dates within each site were pooled for each sample type, resulting in a single mat and non-mat sample per site. The pooled DNA samples then were amplified using the fungal ITS and bacterial 16S primers and protocols as described for the fragment analysis except that unlabeled primers were used. The resulting PCR product was cleaned, quantified, and diluted to 10 ng μl^{-1}. Equal volumes of diluted PCR product were pooled across sites to create mat and non-mat samples containing fungal or bacterial sequences from all sites and sampling dates. Four clone libraries were constructed with the final pooled PCR product using the TOPO TA® Cloning kit (Invitrogen, Carlsbad, CA) for sequencing by employing the manufacturer's protocol. Ligation products were shipped on ice to the Genome Center at Washington University (St. Louis, MO) where the ligation product was transformed into competent cells, and 96 colonies from each clone library were selected and sequenced using M13 forward and reverse primers. Priming from the plasmid, rather than the target DNA, was chosen to increase our ability to obtain complete sequence reads and improve our chances for matching sequences with LH and T-RF peaks seen in community profiles.

Sequences were screened for quality, trimmed, and contigs of forward and reverse reads were assembled using Geneious Pro (Drummond et al., 2009). Clone libraries were screened for chimeric sequences with Chimera_check (Maidak et al., 2000) and Bellerophon (Huber et al., 2004) programs. Fungal and bacterial clone libraries were grouped into Operational Taxonomic Units (OTUs)

of 97% sequence similarity using Geneious Pro. The 97% sequence similarity cutoff was chosen because it has previously been used to approximate species diversity in both fungi (Taylor et al., 2008) and bacteria (Schloss and Handelsman, 2005). Fungal sequence identities were determined by comparison to the GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) and UNITE (http://unite.ut.ee/) databases, and the bacterial sequences were identified using the Ribosomal Database Project Classifier (Wang et al., 2007). Sequences generated from our study are available in GenBank. In silico digests of full-length sequences from the clone libraries were carried out in Geneious Pro using the Hinf1 for fungal sequences and Msp1 and Alu1 for bacterial sequences.

Quantitative PCR

Relative population size of bulk soil fungal and bacterial communities was determined with quantitative PCR. This method provides an index of microbial biomass by measuring the abundance of fungal and bacterial rRNA genes. Bacterial 16S gene and fungal ITS copy numbers were measured following the method of Fierer et al. (2005) as modified by Boyle et al. (2008) using general fungal (5.8S and ITS1f) and bacterial (Eub 338 and Eub518) primers. DNA template from each soil sample was diluted to 5 ng μl^{-1}, and 2 µl were then amplified in triplicate on an ABI 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) using the Brilliant II QPCR Master Mix with ROX (Stratagene, Jolla, CA). Amplification conditions, clone standards, and quality control measures were as described in Boyle et al. (2008).

Statistics

Prior to each statistical analysis, data from within-site mat and non-mat pseudoreplicates were averaged, resulting in one mat and one non-mat data point per date. Repeated measures ANOVAs were used to test whether mat presence or date had a significant effect on the NAGase activity, fungal population size, or bacterial population size. Variables were log transformed to meet normality assumptions as needed, and analysis was carried out using a PROC MIXED model in SAS 9.2 (SAS Institute, Inc., Cary, NC) with treatment and date as fixed effects and site (block) as a random effect. AICC, similar to AIC but corrected for small sample size, was used to determine the best covariance structure for each data set, and the SLICES option was used to test for the effect of mat presence within each date. Results were considered significant at $p \leq 0.05$.

The structure of the fungal and bacterial communities was investigated with non-metric multidimensional scaling (NMS) (McCune and Grace, 2002) using PC-ORD v5 (MjM Software Design, Gleneden Beach, OR). Two data matrices were constructed: the fungal community matrix containing the combined LH and HinfI fragment profiles, and the bacterial community matrix containing the MspI and AluI T-RF profiles. Ordinations were constructed in autopilot mode using the "slow and thorough" setting with the Sørensen distance measure. PC-ORD was used also to run indicator species analysis on the fungal and bacterial communities to determine which fragments were most influential in determining treatment differences (McCune and Grace, 2002). Indicator values (IV) were

calculated for each species, or fragment, and ranged from 0 (no indication) to 100 (perfect indication); therefore, a species with a high IV is more likely to be associated with a given treatment. Multivariate analysis of variance (MANOVA) PROC GLM code in SAS 9.2 tested the effects of sample type and date on NMS axes scores while blocking for site. This method was chosen to examine clustering of samples on the NMS ordination over the more commonly used multi-response permutation procedures (MRPP) because MANOVA is able to account for more complicated study designs, thus allowing us to test for the effects of mat presence or date while accounting for site-to-site variation. MANOVA has been used previously to test for treatment differences using the axes scores resulting from other ordination methods (Brenner, 2000; Kourtev et al., 2002; Yi et al., 2009) and has been shown to be a robust, quantitative method for examining treatment and seasonal patterns in community ordinations. EstimateS (Colwell and Coddington, 1994) was used to create rarefaction curves for the community fragment profiles and clone libraries.

Results

NAGase activity

A significant difference between mat and non-mat NAGase activity was detected. On average, the *Piloderma* mat activity was 1.4 times greater than non-mat activity ($p < 0.01$), and both sample types had significant variation in NAGase activity among the sampling dates ($p < 0.01$). We found that during the summer sampling there was no significant difference between the NAGase

activity of *Piloderma* mat and non-mat soils, whereas mats had greater activity than non-mats at all other dates (Fig. 3.2b), with the fall sampling date showing the greatest difference between the two sample types.

Fungal and bacterial communities

NMS ordinations of fungal and bacterial fragment profiles show significant grouping of the mat communities (Fig. 3.3). MANOVAs on the NMS axes scores for each ordination confirmed that fungal and bacterial communities differed between *Piloderma* mat and non-mat samples ($p < 0.01$ for each ordination). Furthermore, there were significantly different fungal and bacterial communities among sites ($p < 0.01$ for each ordination). However, neither the fungal ($p = 0.12$) nor the bacterial ($p = 0.82$) community showed significant temporal variation. The microbial communities represented by the fragment profiles were likely representative of the total soil diversity because the rarefaction curves constructed from these data reach an asymptote (Fig. 3.4a). Although T-RFLP and LH-PCR allow us to examine differences in overall community structure, these techniques provide no information on the identities of the community members. For this reason, clone libraries were constructed to identify dominant members of the fungal and bacterial communities and to potentially determine which organisms were associated with indicator fragments from the community analysis.

A total of 384 clones were sequenced for the four clone libraries. After chimeras and sequences of poor quality were excluded, 149 fungal (78 mat and 71

non-mat) and 138 bacterial (67 mat and 71 non-mat) sequences remained. At a 97% similarity level the fungal ITS sequences grouped into 57 OTUs representing >12 orders (Fig. 3.5a). *Atheliales* sequences dominate the ITS clone library from mats and are much more abundant in the mats than the non-mats; not surprisingly, the majority of the *Atheliales* sequences are from the genus *Piloderma*. The non-mat clone library is more evenly distributed among taxa, and tends to have more *Russulales, Sebacinales,* and *Agaricales* than the mat library. The mat and non-mat ITS libraries had roughly equal abundance of *Cenococcum geophilum* sequences and percentage of *Ascomycetes* and *Basidiomycetes*.

Bacterial 16S sequences grouped into 77 OTUs representing 10 bacterial classes (Fig. 3.5b). *Acidobacteria* and *α-Proteobacteria* clones were abundant in both the mat and non-mat 16S libraries, although the total number of *Acidobacteria* clones was greater in the non-mat library. Interestingly, *γ-Proteobacteria* was the third most abundant class in the mat library, yet had only a few representatives in the non-mat library. The rarefaction curves constructed from clone library data (Fig. 3.4b) show fewer fungal and bacterial OTUs encountered in the mat samples than the non-mat samples, suggesting that microbial communities in *Piloderma* mats are less diverse. However, these differences were not statistically significant, as the 95% confidence intervals overlap (data not shown to improve figure clarity). Furthermore, because these rarefaction curves do not reach an asymptote, the clone libraries do not represent

the total diversity of the microbial communities and caution is needed when comparing sample diversity.

Indicator species analysis on the fungal fragment profiles revealed three significant indicator fragments for the mat samples and five for the non-mat samples. Not surprisingly, the three mat indicator fragments (IVs = 64, 80, and 84) matched *Piloderma* sequences from the clones and phylotype samples. Of the four non-mat indicator fragments, two could not be matched to clone libraries, however the remaining two (IV's = 77 and 60) matched ascomycete sequences representing the family *Myxotrichaceae*.

Overall, the bacterial indicator values were not as high as the fungal indicators. Nonetheless, there were three significant bacterial indicator fragments for the bacterial mat community that matched clone sequences representing *α-Proteobacteria* (IV = 58), *β-Proteobacteria* (IV = 57), and *Actinobacteria* (IV= 56). Five bacterial fragments were found to be significant non-mat indicators; however, only three matched digested sequences from the clone library, including *Sphingobacteria* (IV = 60), *Acidobacteria* and *Actinobacteria* (IV = 59) and *γ-Proteobacteria* and *β-Proteobacteria* (IV = 58). It should be noted that the sequences associated with each indicator fragment represent only a subset of sequences from the listed taxon, and other sequences from each taxon may be represented by additional fragments. Thus it is possible for sequences from the same taxon to be indicators for mat associated soils while others are indicators for non-mat soils.

Quantitative PCR

The fungal population size, measured by ITS copy number, was not significantly different between the *Piloderma* mat and non-mat soils ($p = 0.47$) although there was significant seasonal variation ($p < 0.01$; Fig. 3.2c). Similarly, the bacterial population size, measured by 16S gene copies, did not differ between mat and non-mat soils when compared across all dates ($p = 0.07$), although bacterial populations were significantly greater in the non-mat soils at the fall sampling ($p < 0.01$). There were significant seasonal fluctuations in bacterial population size ($p < 0.01$; Fig. 3.2d). Both the fungal and bacterial populations showed similar seasonal trends with lower copy numbers in the summer when soils were hot and dry, and greater gene copy numbers in the early spring when soils were cool and moist.

Discussion

Comparison of Piloderma mats to non-mat organic soils

EcM mats have previously been associated with elevated microbial activity compared to their non-mat counterparts (Griffiths et al., 1991; Griffiths and Caldwell, 1992; Kluber et al., submitted). In our study, we found greater NAGase activity in the *Piloderma* mats than in the non-mat soils. These results are consistent with earlier work, although the overall magnitude of the difference is not as large as previously reported for rhizomorphic EcM mats (Kluber et al., submitted). This could be due to the single phylotype being examined in our study or to the fact that previous work did not sample over multiple seasons. The matter

of "who" is responsible for producing the NAGase activity remains a question, however. A number of EcM fungi, including several *Piloderma* species, have been found to possess the genetic potential to produce these chitinolytic enzymes and it has been hypothesized that EcM fungi can use their capability to degrade chitin as a means of obtaining N for themselves and host plants (Lindahl and Taylor, 2004). Such a capability would allow EcM potentially to prey on saprotrophic fungi as a means of obtaining N (Buée et al. 2007). Although it is tempting to conclude that the enhanced NAGase activity of *Piloderma* mats is a product of the mat-forming fungi, one must remember that the mats harbor a diversity of organisms that could potentially be producing NAGase to access the chitin-rich fungal hyphae of the mat-former. Accordingly, N-acetylglucosamine has been found to be a preferred substrate of mycorrhizosphere bacteria (Timonen et al., 1998). In all likelihood, a variety of organisms contribute to the enhanced NAGase activity seen in EcM mats.

In our study, a significantly different fungal community occupied the *Piloderma* mats than the non-mat soils, and no significant community shifts were observed among sampling dates. From the NMS ordination, it was apparent that the non-mat fungal communities tended to group with other samples from the same site, showing little temporal variation, and the mat samples had a distinct community regardless of site or sampling date. NMS ordinations were constructed using peak area as a surrogate for "species" abundance, thus it is not surprising that the *Piloderma* mats group together due to of the abundance of *Piloderma* in

these samples. To examine the community structure without the overwhelming influence of the mat-former, we removed the fragments associated with *Piloderma* from the species matrix, repeated the analysis, and found similar results (data not shown). This finding suggests that the difference between the fungal communities goes beyond the dominance of the mat-former.

Atheliales sequences were abundant in the mat clone library and, although the majority of these sequences were *Piloderma*, we also found another non-mycorrhizal genus from *Atheliales*, *Leptosporomyces* (K-H Larsson, personal communication) to be associated with the mats. The second most abundant sequence in the mat clone library was that of *Cenococcum geophilum*, an EcM fungus commonly found on a wide range of hosts. Beyond these were a number of orders with only a few representative sequences. Although not statistically significant, the rarefaction curve tended to be lower for mats, indicating less species-level diversity. However, there were a greater number of orders found in the mat clone library. Conversely, although the non-mat community was not as diverse across orders, the number of species represented by the orders was greater than in the mat soils. Fungi that form mycorrhizal associations with the ericoid plants, common in the understory of this ecosystem, seemed to play a role in structuring the non-mat community. The *Myxotrichaceae* sequences that were associated with the non-mat indicator peaks are from the genus *Oidiodendron*, which forms ericoid mycorrhizas (Dalpe, 1989). Furthermore, *Sebacina*, a fungus known to form mycorrhizal symbioses with salal (*Gaultheria shallon)* a common

understory plant at these sites, was found in much greater abundance in the non-mat clone library. The non-mat library also contains clones from common EcM genera, such as *Cortinarius*, *Russula*, and even *Piloderma,* in addition to a number of sequences from the ascomycete class, *Leotiomycetes*.

Piloderma mats are one of the most visually striking EcM mats due to their tendency to heavily colonize the soil with thick, cord-like rhizomorphs. Despite the strong visual delineation between *Piloderma* mats and adjacent non-mat soil, the mats did not have significantly larger fungal populations. Although this result was initially surprising, a potential explanation is that the rhizomorphs formed by *Piloderma* could merely be pipe-like structures lacking cytoplasm (Smith and Read, 2008) that are used primarily for nutrient and water transport (Agerer, 2001). In such a case, the total biomass of the mat-former could be underrepresented with our method of quantifying fungal populations using ITS copy number, since our results represent only the active, or nucleated, biomass. Despite this, the results from our community analysis demonstrated that *Piloderma* sequences were dominant in the mat samples, indicating that much of the *Piloderma* biomass was probably detected with our methods. In this case, one may argue that although the non-mat soils do not contain rhizomorphs, they are certainly not devoid of fungi. Indeed, a variety of saprotrophic (Robinson et al., 2005) and EcM (Dickie et al., 2002; Genney et al., 2006) fungi are known to colonize the organic horizon soils. Perhaps the microscopic hyphae from these

fungi are indeed equal in abundance to the hyphal biomass in mats, yet not visible to the naked eye.

Trends seen in the bacterial community were similar to those of the fungi, with significant differences between mat and non-mat soils, and among sites, but showing little temporal variation. It should be noted, however, that the bacterial communities had a greater tendency to be site specific, even within the *Piloderma* mats, which differs from the findings in the fungal community, where the mats had similar community structure regardless of site. Because of this site-to-site variation, the indicator values generally were weaker than for the fungal community. Although α-*Proteobacteria*, β-*Proteobacteria*, and *Actinobacteria* sequences were associated with indicator fragments for the mats, other sequences from these classes were associated with indicator fragments for the non-mat community as well. Consequently no bacterial class stood out as a primary indicator for mats. Somewhat unexpectedly, our study did not find significantly greater numbers of actinomycetes, or other known chitin degraders, in the mat clone library; however, indicator species analysis showed that T-RFLP peaks from a subset of *Actinobacteria* sequences commonly were associated with the mats. The most notable difference between the bacterial clone libraries was the increased abundance of *γ-Proteobacteria* in the mat library. Members of the *γ-Proteobacteria*, such as *Pseudomonas*, have been previously associated with EcM fungi (Frey-Klett et al., 2007; Warmink et al., 2009). However, due to our limited ability to identify these sequences beyond the class level, we will not conjecture

about the role of these organisms within EcM mats. The overall structures of the mat and non-mat clone libraries were similar to other studies of forest soils where α-*Proteobacteria* and *Acidobacteria* were abundant (Fierer et al., 2005; Lauber et al., 2008; Yarwood et al., accepted). Interestingly, the majority of α-*Proteobacteria* sequences were from the order *Rhizobiales*, comprising 22% of the mat and 29% of the non-mat clone libraries. Several potentially N_2-fixing families within *Rhizobiales* were present in the clone libraries, although *Bradyrhizobiaceae* sequences were dominant. These bacteria have previously been reported from clone libraries of Douglas-fir forest mineral soils (Yarwood et al., accepted), although potential N_2-fixing plant hosts are not common in old-growth Douglas-fir forests. Several studies have associated *Rhizobiales* with EcM fungi (Izumi et al., 2006; Burke et al., 2008; Kretzer et al., 2009), which could account for their presence in this fungal-dominated forest ecosystem.

The mycosphere has been referred to as a "nutritional hotspot" for soil bacteria (Nazir et al., 2010) and the presence of hyphal mats below EcM fruiting bodies has been shown to have an influence on bacterial community structure (Warmink and van Elsas, 2008; Warmink et al., 2009). Although the bacterial community associated with *Piloderma* mats was distinct from the non-mat soils, we did not see a significant increase in EcM-associated bacteria, as reported in a microcosm study (Heinonsalo et al., 2001). We hypothesize that because our soils are from the organic horizon, the bacteria in our non-mat soils were not as C limited as bacterial communities from the mineral horizon; and thus, any

additional C inputs from the mycorrhizosphere had little effect on their population. November was the only sampling time when the mat and non-mat bacterial communities differed, and the non-mat community was greater. This could in part be due to the flush of nutrient availability and microbial activity that is commonly associated with soils "wetting up" (Fierer and Schimel, 2002); however, it is interesting that the mat soils experienced no such effect.

Temporal effect on microbial communities and activities

In examining the temporal dynamics of the microbial communities and activities, we saw significant changes in NAGase activity throughout the year and fluctuations in the size of the fungal and bacterial populations, with no temporal effect on the community composition. The temporal variation in NAGase activity is not surprising, as others have described seasonal variation in enzyme activities for bulk soil (Boerner et al., 2005) and EcM root tips (Courty et al., 2007). Although mats had an overall increase in activity across the time points, the summer was the only date that did not show a significant difference in activity between the two sample types. It is likely that the low water content of the soils was limiting microbial activity (Skopp et al., 1990). Using direct count methods, Ingham et al. (1991) found that EcM mats had considerably greater hyphal length per g soil compared to non-mat soils and that rhizomorphic structures accounted for up to 50% of the dry weight of EcM mat soils. In our study, rhizomorphic structures were present in the mats at all sampling dates, yet the active fungal biomass, as measured by QPCR, was lowest during the summer. Suggesting that

some of the rhizomorphic material senesced over the summer. With the return of moisture in the fall, this material would have provided a chitin-rich substrate available for reassimilation by the mat-former, or for degradation by mat-associated organisms. Accordingly, NAGase activity in mat soils increased dramatically with the fall moisture, whereas the non-mat soils showed a slower response, despite increases in fungal and bacterial populations. A number of studies have shown temporal variation in soil microbial communities in this ecosystem (Brant et al., 2006; Moore-Kucera and Dick, 2008) and others (Burke et al., 2009; Cruz-Martinez et al., 2009) ecosystems, but no such trend was detected in our study. Although our ordinations showed slight changes in community composition among the four sampling dates for each site, there was no significant effect of date on the microbial communities, and the effects of sample type and site largely overshadowed any effect of sampling date. Microbial populations, on the other hand, did show significant temporal changes, with both the fungal and bacterial populations following the same trend as the NAGase with lower populations in the late summer and the highest populations in the early spring during snowmelt. Our results are more similar to findings from other ecosystems showing greater microbial biomass when soils were cold (Lipson et al., 1999; Schadt et al., 2003), than from previous results from this ecosystem showing the greatest biomass in the late spring (Brant et al., 2006; Moore-Kucera and Dick, 2008).

Our findings present a significant contribution to the current knowledge on the fungal and bacterial communities associated with EcM extramatrical hyphae. By studying *Piloderma* mats, we were able to examine microbial communities associated with the ectomycorrhizosphere at multiple time points and demonstrate that the dense rhizomorphic mats formed *Piloderma* fungi are associated with distinct fungal and bacterial communities. Furthermore, although there was temporal variation in NAGase activity and the size of the microbial populations, *Piloderma* colonization had a greater effect on the overall community structure than sampling date. Future work is necessary to determine the nature of the interactions between EcM fungi and their associated microbial communities.

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References

Agerer R. 2001. Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11, 107-114.

Allen M.F., Swensom W., Querejeta J.I., Egerton-Warburton L.M. and Treseder K.K. 2003. Ecology of Mycorrhizae: A conceptual framework for complex interactions among plants and fungi. Annual Reviews in Phytopathology 41, 271-303.

Allison S.D. and Treseder K.K. 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Global Change Biology 14, 2898-2909.

Anderson I.C. and Cairney J.W.G. 2007. Ectomycorrhizal fungi: exploring the mycelial frontier. FEMS Microbiology Reviews 31, 388-406.

Avis P.G. and Feldheim K.A. 2005. A method to size DNA fragments from 50 to 800 bp on a DNA analyser. Molecular Ecology Notes 5, 969-970.

Bending G.D., Poole E.J., Whipps J.M. and Read D.J. 2002. Characterisation of bacteria from *Pinus sylvestris-Suillus luteus* mycorrhizas and their effects on root-fungus interactions and plant growth. FEMS Microbiology Ecology 39 219-227.

Björk R.G., Björkman M.P., Andersson M.X. and Klemedtsson L. 2008. Temporal variation in soil microbial communities in Alpine tundra. Soil Biology and Biochemistry 40, 266-268.

Boerner R.E.J., Brinkman J.A. and Smith A. 2005. Seasonal variations in enzyme activity and organic carbon in soil of a burned and unburned hardwood forest. Soil Biology and Biochemistry 37, 1419-1426.

Boyle S.A., Yarwood R.R., Bottomley P.J. and Myrold D.D. 2008. Bacterial and fungal contributions to soil nitrogen cycling under Douglas-fir and red alder at two sites in Oregon. Soil Biology and Biochemistry 40, 443-451.

Boyle S.A., Rich J.J., Bottomley P.J., Cromack K. Jr. and Myrold D.D. 2006. Reciprocal transfer effects on denitrifying community composition and activity at forest and meadow sites in the Cascade Mountains of Oregon. Soil Biology and Biochemistry 38, 870-878.

Brant J.B., Myrold D.D. and Sulzman E.W. 2006. Root controls on soil microbial community structure in forest soils. Oecologia 148, 650-659.

Brenner G.J. 2000. Riparian and adjacent upslope beetle communities along a third order stream in the western Cascade Mountain Range, Oregon 341 p. Ph.D. Dissertation, Oregon State University, Corvallis, OR.

Buée M., Vairelles D. and Garbaye J. 2005. Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (*Fagus silvatica*) forest subjected to two thinning regimes. Mycorrhiza 15, 235-245.

Buée M., Courty P.E., Mignot D. and Garbaye J. 2007. Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community. Soil Biology and Biochemistry 39, 1947-1955.

Burke D.J., Dunham S.M. and Kretzer A.M. 2008. Molecular analysis of bacterial communities associated with the roots of Douglas fir (*Pseudotsuga menziesii*) colonized by different ectomycorrhizal fungi. FEMS Microbiology Ecology 65, 299-309.

Burke D.J., Kretzer A.M., Rygiewicz P.T. and Topa M.A. 2006. Soil bacterial diversity in a loblolly pine plantation: influence of ectomycorrhizas and fertilization. FEMS Microbiology Ecology 57, 409-419.

Burke D.J., Lopez-Gutierrez J.C., Smemo K.A. and Chan C.R. 2009. Vegetation and soil environment influence the spatial distribution of root-associated fungi in a mature beech-maple forest. Applied and Environmental Microbiology 75, 7639-7648.

Cairney J.W.G. and Meharg A.A. 2002. Interactions between ectomycorrhizal fungi and soil saprotrophs: implications for decomposition of organic matter in soils and degradation of organic pollutants in the rhizosphere. Canadian Journal of Botany 80, 803-809

Caldwell B.A., Griffiths R.P. and Sollins P. 1999. Soil enzyme response to vegetation disturbance in two lowland Coast Rica soils. Soil Biology and Biochemistry 31, 1603-1608.

Chaer G., Myrold D. and Bottomley P. 2009. A soil quality index based on the equilibrium between soil organic matter and biochemical properties of undisturbed coniferous forest soils of the Pacific Northwest. Soil Biology and Biochemistry 41, 822-830.

Chapin F.S., Matson P.A. and Mooney H.A. 2002. Principles of Terrestrial Ecosystem Ecology. Springer-Verlag, New York.

Colwell R. and Coddington J. 1994. Estimating terrestrial biodiversity through extrapolation. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 345, 101-118.

Courty P.E., Breda N. and Garbaye J. 2007. Relation between oak tree phenology and the secretion of organic matter degrading enzymes by *Lactarius quietus* ectomycorrhizas before and during bud break. Soil Biology and Biochemistry 39, 1655-1663.

Courty P.E., Franc A., Pierrat J.-C. and Garbaye J. 2008. Temporal changes of the ectomycorrhizal community in two soil horizons of a temperate oak forest. Applied and Environmental Microbiology 74, 5792-5801.

Courty P.E., Buée M., Diedhiou A.G., Frey-Klett P., Le Tacon F., Rineau F., Turpault M.-P., Uroz S. and Garbaye J. 2010. The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. Soil Biology and Biochemistry 42, 679-698.

Cromack K. Jr., Sollins P., Graustein W.C., Speidel K., Todd A.W., Spycher G., Li C.Y. and Todd R.L. 1979. Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. Soil Biology and Biochemistry 11, 463-468.

Cruz-Martinez K., Suttle K.B., Brodie E.L., Power M.E., Andersen G.L. and Banfield J.F. 2009. Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. The ISME Journal 3, 738-744.

Dalpe Y. 1989. Ericoid mycorrhizal fungi in the *Myxotrichaceae* and *Gymnoascaceae*. New Phytologist 113.

Daly C. and McKee A.W. 2009. Meteorological data from benchmark stations at the Andrews Experimental Forest. Long-Term Ecological Research.

Forest Science Data Bank, Corvallis, OR. [Database]. Available: http://andrewsforest.oregonstate.edu/data/abstract.cfm?dbcode=MS001 (25 March 2010).

Dickie I.A., Xu B. and Koide R. 2002. Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. New Phytologist 156, 527–535.

Dix N.J. and Webster J. 1995. Fungal Ecology. University Press, Cambrige, Great Britain.

Drummond A., Ashton B., Cheung M., Heled J., Kearse M., Moir R., Stones-Havas S., Thierer T. and Wilson A. 2009. Geneious v4.7. Available from http://www.geneious.com/.

Dunham S.M., Larsson K.-H. and Spatafora J.W. 2007. Species richness and community composition of mat-forming ectomycorrhizal fungi in old- and second-growth Douglas-fir forests of the HJ Andrews Experimental Forest, Oregon, USA. Mycorrhiza 17, 633-645.

Edwards U., Rogall T., Blöcker E.M. and Böttger E.C. 1989. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding 16S ribosomal RNA. Nucleic Acids Research 17, 7843-7852.

Fierer N. and Schimel J.P. 2002. Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry 34, 777-787.

Fierer N., Jackson J.A., Vilgalys R. and Jackson R.B. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71, 4117-4120.

Fitter A.H. and Garbaye J. 1994. Interactions between mycorrhizal fungi and other soil organisms. Plant and Soil 159, 123-132.

Frey-Klett P. and Garbaye J. 2005. Mycorrhiza helper bacteria: a promising model for the genomic analysis of fungal-bacterial interactions. New Phytologist 168, 4-8.

Frey-Klett P., Garbaye J. and Tarkka M. 2007. The mycorrhiza helper bacteria revisited. New Phytologist 176, 22-36.

Garbaye J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytologist 128, 197-210.

Gardes M. and Bruns T.D. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology Notes 2, 113-118.

Genney D.R., Anderson I.C. and Alexander I.J. 2006. Fine-scale distribution of pine ectomycorrhizas and their extramatrical mycelium. New Phytologist 170, 381-390.

Griffiths R.P. and Caldwell B.A. 1992 Mycorrhizal mat communities in forest soils. In: Mycorrhizas in Ecosystems. Eds. D.J. Read, D.H. Lewis, A.H. Fitter and I.J. Alexander. Pp. 98-105. C.A.B. International, Wallingford, Oxon, UK.

Griffiths R.P., Bradshaw G.A., Marks B. and Lienkaemper G.W. 1996. Spatial distribution of ectomycorrhizal mats in coniferous forests of the Pacific Northwest, USA. Plant and Soil 180, 147-158.

Griffiths R.P., Caldwell B.A., Cromack K. Jr. and Morita R.Y. 1990. Douglas-fir forest soils colonized by ectomycorrhizal mats. I. Seasonal variation in nitrogen chemistry and nitrogen cycle transformation rates. Canadian Journal of Forest Research 20, 211-218.

Griffiths R.P., Ingham E.R., Caldwell B.A., Castellano M.A. and Cromack K. Jr. 1991. Microbial characterists of ectomycorrhizal mat communities in Oregon and California. Biology and Fertility of Soils 11, 196-202.

Hackl E., Zechmeister-Boltenstern S., Bodrossy L. and Sessitsch A. 2004. Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. Applied and Environmental Microbiology 70.

Heinonsalo J., Jørgensen K.S. and Sen R. 2001. Microcosm-based analyses of Scots pine seedling growth, ectomycorrhizal fungal community structure and bacterial carbon utilization profiles in boreal forest humus and underlying illuvial mineral horizons. FEMS Microbiology Ecology 36, 73-84.

Høj L., Rusten M., Haugen L.E., Olsen R.A. and Torsvik V.L. 2006. Effects of water regime on archaeal community composition in Arctic soils. Environmental Microbiology 8, 984-996.

Huber T., Faulkner G. and Hugenholtz P. 2004. Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. Bioinformatics 20, 2317-2319.

Ingham E.R., Griffiths R.P., Cromack K. Jr. and Entry J.A. 1991. Comparison of direct vs. fumigation incubation microbial biomass estimates from ectomycorrhizal mat and non-mat soils. Soil Biology and Biochemistry 23, 465-471.

Izumi H., Anderson I.C., Alexander I.J., Killham K. and Moore E.R.B. 2006. Diversity and expression of nitrogenase genes (nifH) from ectomycorrhizas of Corsican pine (*Pinus nigra*). Environmental Microbiology 8, 2224-2230.

Khetmalas M.B., Egger K., Massicotte H.B., Tackaberry L.E. and Clappertion M.J. 2002. Bacterial diversity associated with subalpine fir (*Abies lasiocarpa*) ectomycorrhizae following wildfire and salvage-logging in central British Columbia. Canadian Journal of Microbiology 48, 611-625.

Kluber L.A., Tinnesand K.M., Caldwell B.A., Dunham S.M., Yarwood R.R., Bottomley P.J. and Myrold D.D. Submitted. Ectomycorrhizal mats: biochemical characterization and comparison to corresponding non-mat soils in a Douglas-fir forest. Soil Biology and Biochemistry.

Knutson D.M., Hutchins A.S. and Cromack K. Jr. 1980. The association of calcium oxalate-degrading *Streptomyces* with conifer ectomycorrhizae. Antonie Van Leeuwenhoek 46, 611-619.

Kourtev P.S., Ehrenfeld J.G. and Haggblom M. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83, 3152-3166.

Kretzer A., King Z. and Bai S. 2009. Bacterial communities associated with tuberculate ectomycorrhizae of *Rhizopogon* spp. Mycorrhiza 19, 277-282.

Lauber C.L., Strickland M.S., Bradford M.A. and Fierer N. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology and Biochemistry 40, 2407-2415.

Leake J.R., Donnelly D.P., Saunders E.M., Boddy L. and Read D.J. 2001. Rates and quantities of carbon flux to ectomycorrhizal mycelium following ^{14}C pulse labeling of *Pinus sylvestris* seedlings: effects of litter patches and interaction with a wood-decomposer fungus. Tree Physiology 21, 71-82.

Leveau J.H.J. and Preston G.M. 2008. Bacterial mycophagy: definition and diagnosis of a unique bacterial-fungal interaction. New Phytologist 177, 859-876.

Lindahl B.D. 2000. Ectomycorrhizal fungi raid saprotrophic ones. Mycological Research 104, 385-387.

Lindahl B.D. and Taylor A.F.S. 2004. Occurrence of N-acetylhexosaminidase-encoding genes in ectomycorrhizal basidiomycetes. New Phytologist 164, 193-199.

Lipson D.A., Schmidt S.K. and Monson R.K. 1999. Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. Ecology 80, 1623-1631.

Maidak B.L., Cole J.R., Lilburn T.G., Parker C.T., Jr, Saxman P.R., Stredwick J.M., Garrity G.M., Li B., Olsen G.J., Pramanik S., Schmidt T.M. and Tiedje J.M. 2000. The RDP (Ribosomal Database Project) continues. Nucleic Acids Research 28, 173-174.

Manter D.K. and Vivanco J.M. 2007. Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. Journal of Microbiological Methods 71, 7-14.

McCune B. and Grace J.B. 2002. Analysis of Ecological Communities. MjM Software Design, Glendale Beach, Oregon.

Molina R., Pilz D., Smith J., Dunham S., Dreisbach T., O'Dell T. and Castellano M. 2001 Conservation and management of forest fungi in the Pacific Northwestern United States: an integrated ecosystem approach. In Fungal conservation issues and solutions. Eds. D. Moore, N.N. Nauta, S.E. Evans and M. Rotheroe. Pp. 19-63. Cambridge University Press, Cambridge, UK.

Moore-Kucera J. and Dick R.P. 2008. PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. Microbial Ecology 55, 500-511.

Muyzer G., Teske G., Wirsen C.O. and Jannasch H.W. 1995. Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. Archives of Microbiology 164, 165-172.

Nazir R., Warmink J.A., Boersma H. and van Elsas J.D. 2010. Mechanisms that promote bacterial fitness in fungal-affected soil microhabitats. FEMS Microbiology Ecology 71, 169-185.

Oswald E.T. and Ferchau H.A. 1968. Bacterial associations of coniferous mycorrhizae. Plant and Soil 28, 187-192.

Parham J.A. and Deng S.P. 2000. Detection, quantification and characterization of b-glucosaminidase activity in soil. Soil Biology and Biochemistry 32, 1183-1190.

Phillips C.L., Kluber L., Pedersen J. and Bond B.J. in revision. Contributions of ectomycorrhizal fungal mats to forest soil respiration. New Phytologist.

Robinson C.H., Miller E.J.P. and Deacon L.J. 2005 Biodiversity of saprotrophic fungi in relation to their function: do fungi obey the rules? In Biological diversity and function in soils. Eds. R.D. Bardgett, D.W. Hopkins and M.B. Usher. pp. 189-215. Cambridge University Press, New York, NY.

Schadt C.W., Martin A.P., Lipson D.A. and Schmidt S.K. 2003. Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301, 1359-1361.

Schloss P.D. and Handelsman J. 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Applied and Environmental Microbiology 71, 1501-1506.

Skopp J., Jawson M.D. and Doran J.W. 1990. Steady-state aerobic microbial activity as a function of soil water content. Soil Science Society of America Journal 54, 1619-1625.

Smith J.E., Molina R., Huso M.M.P. and Larsen M.J. 2000. Occurrence of *Piloderma fallax* in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, U.S.A. Canadian Journal of Botany 78, 995-1001.

Smith S. and Read D. 2008. Mycorrhizal Symbiosis. Academic Press, New York. 787 p.

Taylor D.L., Booth M.G., McFarland J.W., Herriott I.C., Lennon N.J., Nusbaum C. and Marr T.G. 2008. Increasing ecological inference from high throughput sequencing of fungi in the environment through a tagging approach. Molecular Ecology Resources 8, 742-752.

Tedersoo L., Partel K., Jairus T., Gates G., Poldmaa K. and Tamm H. 2009. Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the *Helotiales*. Environmental Microbiology 11, 3166-3178.

Timonen S., Jørgensen K.S. and Haahtela K. 1998. Bacterial community structure at defined locations of *Pinus sylvestris - Suillus bovinus* and *Pinus sylvestris - Paxillus involutus* mycorrhizospheres in dry pine forest humus and nursery peat. Canadian Journal Microbiology 44, 499-513.

Trappe J.M., Molina R., Luoma D.L., Cázares E., Pilz D., Smith J.E., Castellano M.A., Miller S.L. and Trappe M.J. 2009. Diversity, ecology, and conservation of truffle fungi in forests of the Pacific Northwest. Gen. Tech. Rep. PNW-GTR-772. Department of Agriculture, Pacific Northwest Research Station. 194 p. Portland, OR.

Wang Q., Garrity G.M., Tiedje J.M. and Cole. J.R. 2007. Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73, 5261-5267.

Warmink J.A. and van Elsas J.D. 2008. Selection of bacterial populations in the mycosphere of *Laccaria proxima*: is type III secretion involved? ISME J 2, 887-900.

Warmink J.A., Nazir R. and van Elsas J.D. 2009. Universal and species-specific bacterial 'fungiphiles' in the mycospheres of different basidiomycetous fungi. Environmental Microbiology 11, 300-312.

White T.J., Bruns T.D., Lee S.B. and Taylor J.W. 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols — a Guide to Methods and Applications. Eds. M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White. Pp. 315-322. Academic Press, San Diego, CA

Yarwood S.A., Bottomley P.J. and Myrold D.D. accepted. Soil microbial communities associated with Douglas-fir and red alder stands at high- and low-productivity forest sites in Oregon, USA. Microbial Ecology.

Yi H., Kim H.-J., Kim C.-G., Harn C., Kim H. and Park S. 2009. Using T-RFLP to assess the impact on soil microbial communities by transgenic lines of watermelon rootstock resistant to cucumber green mottle mosaic virus (CGMMV). Journal of Plant Biology 52, 577-584.

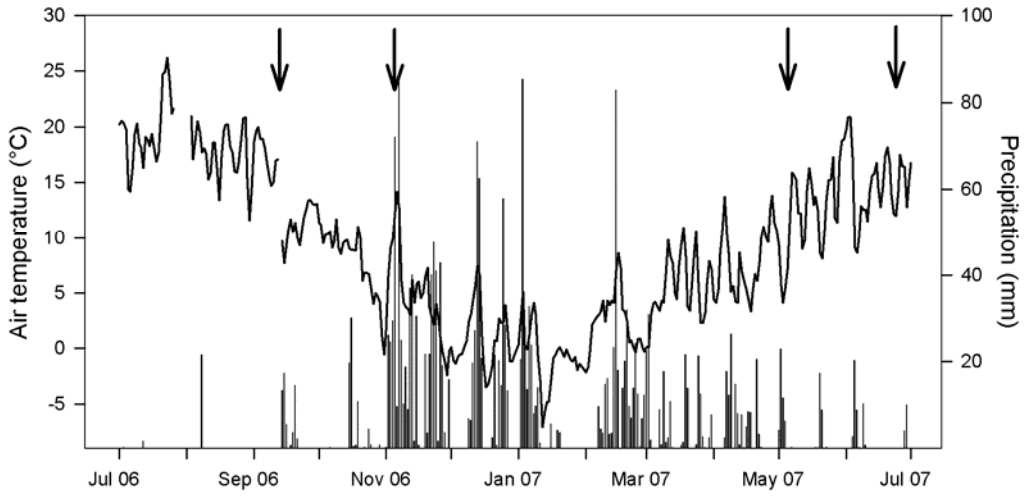

Fig. 3.1 Time series of air temperature (black line) and precipitation (black bars) from the H.J. Andrews headquarters weather station (430 m above sea level) from July 2006 through July 2007. Black arrows indicate our four sampling dates. Data from Daly et al. (2009).

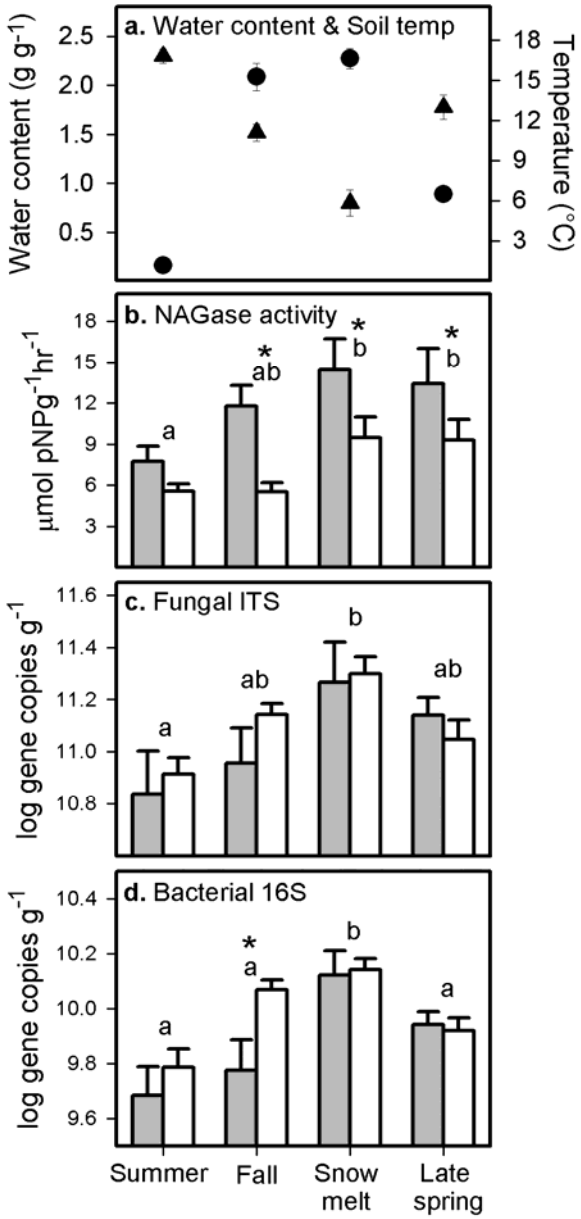

Fig. 3.2 Means and standard errors for soil water content and temperature (**a**), NAGase activity (**b**), fungal ITS copy number (**c**), and bacterial 16S copy number (**d**) at each of the four sampling dates. In panel **a**, soil water content (circles) and soil temperature (triangles) are presented to demonstrate seasonal variation in soil conditions. In panels **b-d**, *Piloderma* mat (gray bars) and non-mat (white bars) data are shown for each sampling date. Letters denote statistical differences between sampling dates and asterisks indicate statistical differences between mat and non-mat samples within a given date.

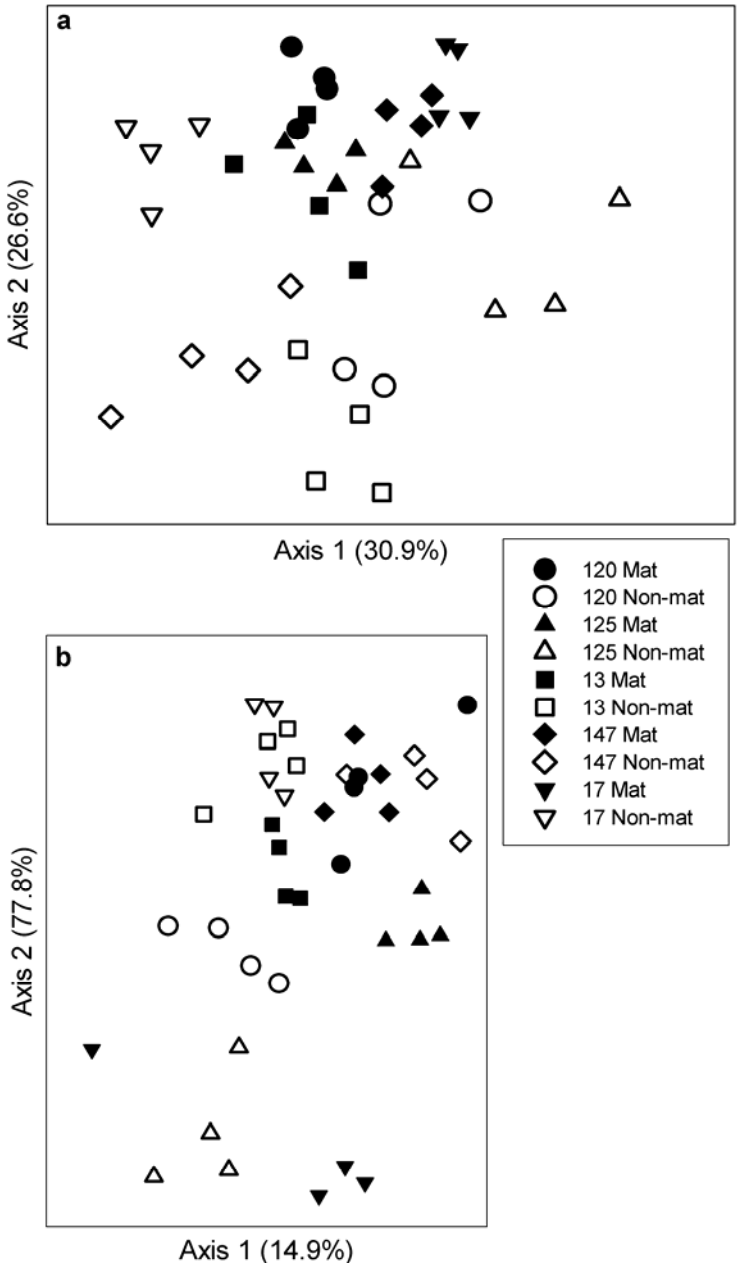

Fig. 3.3 Composition of fungal and bacterial communities in *Piloderma* mat (solid symbols) and non-mat (open symbols) samples over the four sampling dates. The five sites are represented by different symbols to demonstrate the spatial and temporal variation. Panel **a** shows the first two axes of a 3D NMS ordination of the fungal HinfI TRFLP and LH-PCR profiles (stress = 13.99) and panel **b** shows the 2D NMS ordination of the bacterial AluI and MspI TRFLP profiles (stress = 12.24).

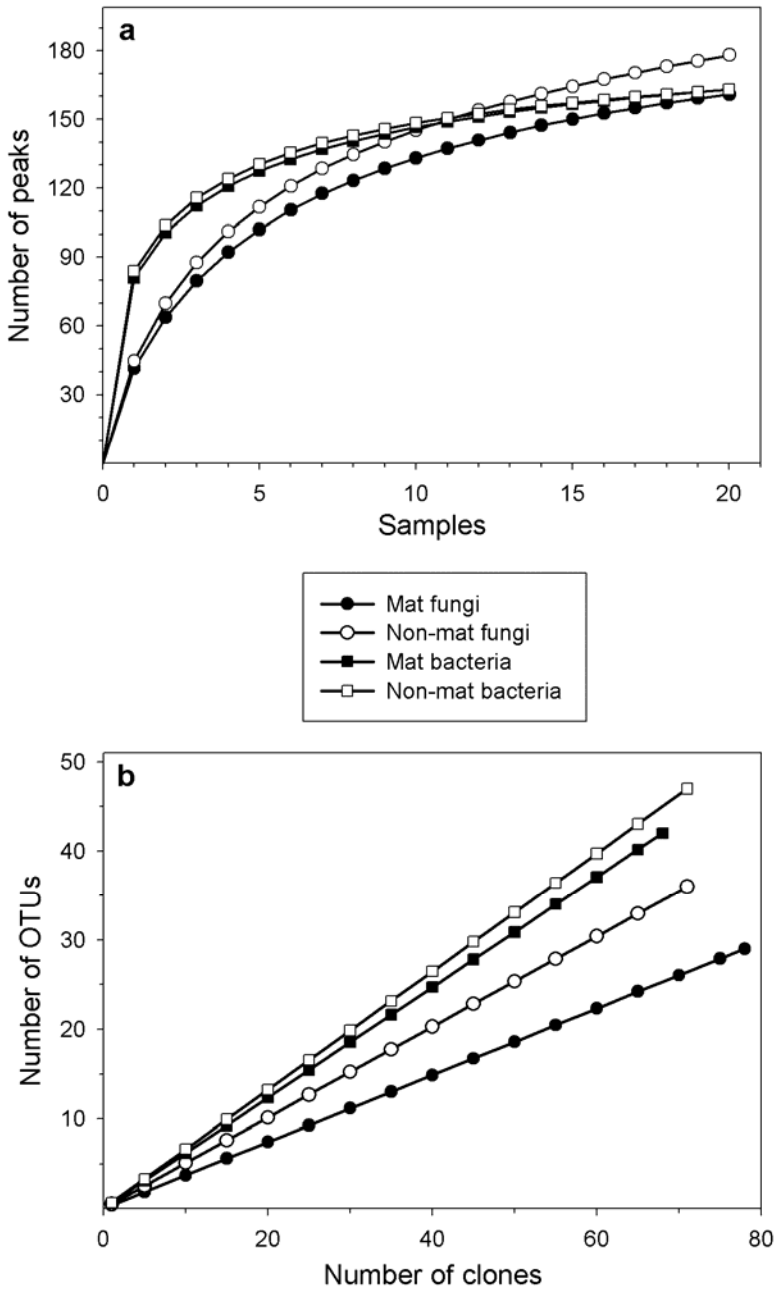

Fig. 3.4 Rarefaction curves for fungal and bacterial communities. Panel **a** shows the curves constructed with community fragment profiles; the curves in panel **b** were constructed using clone library data. In both panels, *Piloderma* mats are represented with solid symbols and non-mats with open symbols; fungal communities are shown by circles and bacterial communities by squares.

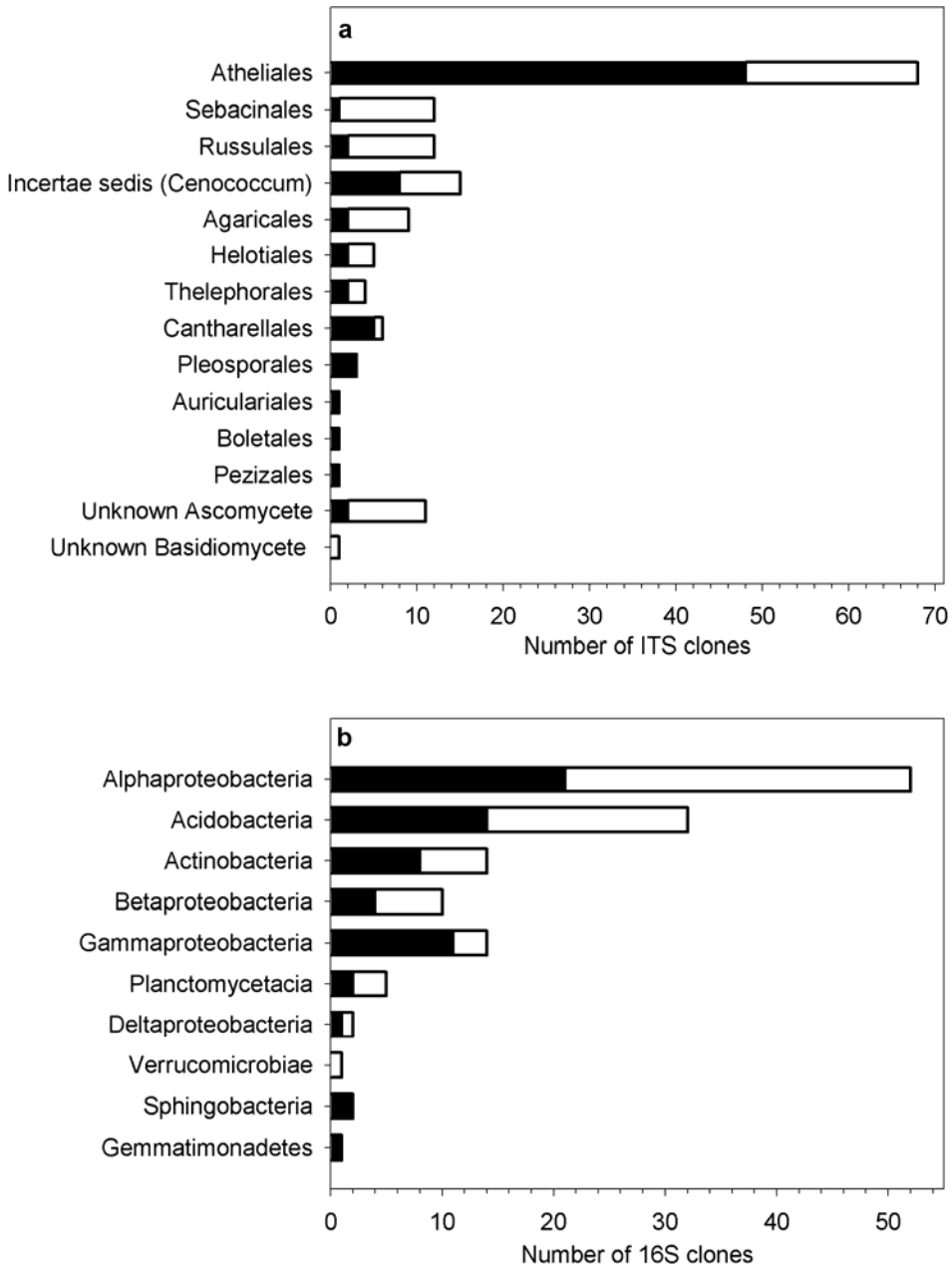

Fig. 3.5 Abundance of taxa present in *Piloderma* mat (black) and non-mat (white) clone libraries. Fungal orders are shown in panel **a**, and bacterial classes are shown in panel **b**.

Chapter Four:

General Conclusions

Laurel Kluber

Forest ecosystems are often considered N limited because much of the N is contained in organic matter, and thus unavailable for plant uptake (Chapin et al., 2002). Through the production of extracellular enzymes, fungi are adept at breaking down complex organic matter (Dix and Webster, 1995). Although saprotrophic fungi play a primary role in the degradation of woody substrates, nearly all types of fungi participate in nutrient cycling (Molina et al., 2001). Ectomycorrhizal (EcM) fungi play a critical role in forest ecosystems through their ability to provide host plants with N acquired from organic matter (Lindahl et al., 2007; Smith and Read, 2008). The findings of Brant et al. (2006) showed a decrease in fungal biomass after root trenching, suggesting that mycorrhizal fungi are responsible for as much as half the fungal biomass of Douglas-fir forest soils. These estimates are on par with findings from other forested ecosystems where mycorrhizae have been found to make significant contributions to the soil microbial biomass (Wallander et al., 2001; Högberg and Högberg, 2002).

Numerous studies have assessed EcM diversity (Goodman and Trofymow, 1998; Smith et al., 2002; Courty et al., 2008; Trappe et al., 2009) and distribution in the soil profile (Dickie et al., 2002; Genney et al., 2006; Koide et al., 2007). Furthermore, enzymatic activities have been determined for many EcM cultures and root tips (Hutchison, 1990; Conn and Dighton, 2000; Buée et al., 2007; Courty et al., 2007;), and bacterial communities associated with EcM root tips have been described for a number of EcM fungi (Khtemalas et al., 2002; Frey-Klett et al., 2005; Burke et al., 2008). Despite this immense body of knowledge,

little is known about the activities and communities associated with the mycelial systems of EcM fungi in forest soils (Anderson and Cairney, 2007). Recent attempts to examine bacterial communities and activities associated with the mycorrhizasphere have examined hyphal aggregations that form under the fruiting bodies of some fungi (Warmink and van Elsas, 2008; Warmink et al., 2009). However, because fruiting bodies generally are seasonal and temporary structures, this approach has limited utility for examining temporal dynamics of EcM activity.

Similar to the approach used by Warmink et al. (2008; 2009), EcM mats offer an opportunity to examine the concentrated effects of EcM hyphae, with the distinct advantage of having a soil that is continually occupied by the EcM fungus. Previous studies have demonstrated the prevalence of EcM mats (Cromack et al., 1979; Griffiths et al., 1996) and shown their tendency to exhibit elevated microbial activities (Griffiths et al., 1991b; 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., 1991a). These studies focused on mats formed by *Hysterangium* and *Gautieria* species; however, Dunham et al. (2007) revealed that these genera were not dominant mat-formers in all PNW forest soils.

The objectives of my dissertation were to: 1) provide a current account of chemical and biochemical properties associated with EcM fungi, and 2) describe

the communities and activities of microbes occupying EcM mat and non-mat forest soils.

In Chapter 2, chemical and biochemical properties were assessed on mat and non-mat soils from both the organic and mineral soil horizons. Additionally, phylotypes were determined on the majority of EcM mats used for this study. EcM mats from both horizons had chemical, and enzymatic profiles that were distinct from their non-mat counterparts. When enzyme activity was normalized by microbial biomass, rhizomorphic mats colonizing the organic horizon had a significantly different enzymatic profile suggesting that the organic horizon mat communities have a unique physiological potential. However, in the mineral horizon, enzyme activities of hydrophobic mats were not significantly different than the non-mat soils after normalization, thus suggesting that the elevated activity in hydrophobic mats colonizing the mineral horizon is primarily due to increased biomass rather than a specialized microbial community. Additionally, phylotype-specific profiles were detected in the organic horizon rhizomorphic mats, whereas the mineral horizon hydrophobic mats exhibited similar enzymatic profiles regardless of phylotype.

In Chapter 3, the dominance and extensive coverage of *Piloderma* mats (Smith et al., 2000; Dunham et al., 2007), allowed me to assess the microbial community dynamics associated with the hyphae of this prolific mat-former. *Piloderma* mats can cover 40% of the forest floor and is responsible for approximately 10% of the total soil respiration from a given Douglas-fir stand

(Phillips et al., in revision). Using molecular techniques, I assessed the fungal and bacterial communities of *Piloderma* mat and non-mat organic soils. *Piloderma* mats showed elevated N-acetylglucosaminidase activity in addition to unique fungal and bacterial communities that were stable throughout time. However, the fungal and bacterial populations and N-acetylglucosaminidase activity of mat and non-mat soils exhibited significant temporal variation.

The results presented herein demonstrate that EcM mats create unique soil conditions with distinct microbial communities and activities in contrast to non-mat forest soils. This work furthers our understanding of the influence of EcM fungi on the soil environment and microbial communities, and how forest soil microbial communities respond to seasonal changes in temperature and moisture. Additionally, the assessment of organic horizon microbial communities provides a rare look at an often-overlooked component of the soil ecosystem. Future work is necessary to better elucidate the mechanisms and biological processes leading to the unique microbial communities and activities associated with EcM mats, as well as the interactions between EcM and their associated microbial communities.

References

Anderson I.C. and Cairney J.W.G. 2007. Ectomycorrhizal fungi: exploring the mycelial frontier. FEMS Microbiology Reviews 31, 388-406.

Brant J.B., Myrold D.D. and Sulzman E.W. 2006. Root controls on soil microbial community structure in forest soils. Oecologia 148, 650-659.

Buée M., Courty P.E., Mignot D. and Garbaye J. 2007. Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community. Soil Biology and Biochemistry 39, 1947-1955.

Burke D.J., Dunham S.M. and Kretzer A.M. 2008. Molecular analysis of bacterial communities associated with the roots of Douglas-fir (*Pseudotsuga menziesii*) colonized by different ectomycorrhizal fungi. FEMS Microbiology Ecology 65, 299-309.

Chapin F.S., Matson P.A. and Mooney H.A. 2002. Principles of Terrestrial Ecosystem Ecology. Springer-Verlag, New York.

Conn C. and Dighton J. 2000. Litter quality influences on decomposition, ectomycorrhizal community structure and mycorrhizal root surface acid phosphatase activity. Soil Biology and Biochemistry 32, 489-496.

Courty P.E., Breda N. and Garbaye J. 2007. Relation between oak tree phenology and the secretion of organic matter degrading enzymes by *Lactarius quietus* ectomycorrhizas before and during bud break. Soil Biology and Biochemistry 39, 1655-1663.

Cromack K., Jr, Fitcher B.L., Moldenke A.M., Entry J.A. and Ingham E.R. 1988. Interactions between soil animals and ectomycorrhizal fungal mats. Agriculture Ecosystems and Environment 24, 161-168.

Cromack K., Jr, Sollins P., Graustein W.C., Speidel K., Todd A.W., Spycher G., Li C.Y. and Todd R.L. 1979. Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. Soil Biology and Biochemistry 11, 463-468.

Dix N.J. and Webster J. 1995. Fungal Ecology. University Press, Cambrige, Great Britain.

Dunham S.M., Larsson K.-H. and Spatafora J.W. 2007. Species richness and community composition of mat-forming ectomycorrhizal fungi in old- and second-growth Douglas-fir forests of the HJ Andrews Experimental Forest, Oregon, USA. Mycorrhiza 17, 633-645.

Frey-Klett P., Chavatte M., Clausse M.-L., Courrier S., Roux C.L., Raaijmakers J., Martinotti M.G., Pierrat J.-C. and Garbaye J. 2005. Ectomycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent pseudomonads. New Phytologist 165, 317-328.

Griffiths R.P. and Caldwell B.A. 1992 Mycorrhizal mat communities in forest soils. In: Mycorrhizas in Ecosystems. D.J. Read, D.H. Lewis, A.H. Fitter and I.J. Alexander. pp. 98-105. C.A.B. International, Wallingford, Oxon, UK.

Griffiths R.P., Baham J.E. and Caldwell B.A. 1994. Soil solution chemistry of ectomycorrhizal mats in forest soil. Soil Biology and Biochemistry 26, 331-337.

Griffiths R.P., Castellano M.A. and Caldwell B.A. 1991a. Hyphal mats formed by two ectomycorrhizal fungi and their association with Douglas-fir seedlings: A case study. Plant and Soil 134, 255-259.

Griffiths R.P., Bradshaw G.A., Marks B. and Lienkaemper G.W. 1996. Spatial distribution of ectomycorrhizal mats in coniferous forests of the Pacific Northwest, USA. Plant and Soil 180, 147-158.

Griffiths R.P., Ingham E.R., Caldwell B.A., Castellano M.A. and Cromack K. Jr. 1991b. Microbial characterists of ectomycorrhizal mat communities in Oregon and California. Biology and Fertility of Soils 11, 196-202.

Högberg M. and Högberg P. 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces together with associated roots, half the dissolved organic carbon in a forest soil. New Phytologist 154, 791-795.

Hutchison L.J. 1990. Studies on the systematics of ectomycorrhizal fungi in axenic culture. II. The enzymatic degradation of selected carbon and nitrogen compounds. Canadian Journal of Botany 68, 1522-1530.

Khetmalas M.B., Egger K., Massicotte H.B., Tackaberry L.E. and Clappertion M.J. 2002. Bacterial diversity associated with subalpine fir (*Abies lasiocarpa*) ectomycorrhizae following wildfire and salvage-logging in central British Columbia. Canadian Journal of Microbiology 48, 611-625.

Lindahl B.D., Ihrmark K., Boberg J., Trumbore S.E., Högberg P., Stenlid J. and Finlay R.D. 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytologist 173, 611-620.

Molina R., Pilz D., Smith J., Dunham S., Dreisbach T., O'Dell T. and Castellano M. 2001 Conservation and management of forest fungi in the Pacific Northwestern United States: an integrated ecosystem approach. In Fungal conservation issues and solutions. Eds. D. Moore, N.N. Nauta, S.E. Evans and M. Rotheroe. pp. 19-63. Cambridge University Press, Canbridge, UK.

Phillips C.L., Kluber L.A., Pedersen J. and Bond B.J. in revision. Contributions of ectomycorrhizal fungal mats to forest soil respiration. New Phytologist.

Smith J.E., Molina R., Huso M.M.P. and Larsen M.J. 2000. Occurrence of *Piloderma fallax* in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, U.S.A. Canadian Journal of Botany 78, 995-1001.

Smith S. and Read D. 2008. Mycorrhizal Symbiosis. Academic Press, New York. pp. 787.

Wallander H., Nilsson L.O., Hagerberg D. and Bääth E. 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. New Phytologist 151, 753-760.

Warmink J.A. and van Elsas J.D. 2008. Selection of bacterial populations in the mycosphere of *Laccaria proxima*: is type III secretion involved? ISME Journal 2, 887-900.

Warmink J.A., Nazir R. and van Elsas J.D. 2009. Universal and species-specific bacterial 'fungiphiles' in the mycospheres of different basidiomycetous fungi. Environmental Microbiology 11, 300-312.

Bibliography

Agerer R. 2001. Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11, 107-114.

Allen M.F., Swensom W., Querejeta J.I., Egerton-Warburton L.M. and Treseder K.K. 2003. Ecology of mycorrhizae: A conceptual framework for complex interactions among plants and fungi. Annual Reviews in Phytopathology 41, 271-303.

Allison S.D. and Treseder K.K. 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Global Change Biology 14, 2898-2909.

Anderson I.C. and Cairney J.W.G. 2007. Ectomycorrhizal fungi: exploring the mycelial frontier. FEMS Microbiology Reviews 31, 388-406.

Avis P.G. and Feldheim K.A. 2005. A method to size DNA fragments from 50 to 800 bp on a DNA analyser. Molecular Ecology Notes 5, 969-970.

Bending G.D., Poole E.J., Whipps J.M. and Read D.J. 2002. Characterisation of bacteria from *Pinus sylvestris-Suillus luteus* mycorrhizas and their effects on root-fungus interactions and plant growth. FEMS Microbiology Ecology 39, 219-227.

Björk R.G., Björkman M.P., Andersson M.X. and Klemedtsson L. 2008. Temporal variation in soil microbial communities in alpine tundra. Soil Biology and Biochemistry 40, 266-268.

Boerner R.E.J., Brinkman J.A. and Smith A. 2005. Seasonal variations in enzyme activity and organic carbon in soil of a burned and unburned hardwood forest. Soil Biology and Biochemistry 37, 1419-1426.

Boyle S.A., Rich J.J., Bottomley P.J., Cromack K. Jr. and Myrold D.D. 2006. Reciprocal transfer effects on denitrifying community composition and activity at forest and meadow sites in the Cascade Mountains of Oregon. Soil Biology and Biochemistry 38, 870-878.

Boyle S.A., Yarwood R.R., Bottomley P.J. and Myrold D.D. 2008. Bacterial and fungal contributions to soil nitrogen cycling under Douglas-fir and red alder at two sites in Oregon. Soil Biology and Biochemistry 40, 443-451.

Brant J.B., Myrold D.D. and Sulzman E.W. 2006. Root controls on soil microbial community structure in forest soils. Oecologia 148, 650-659.

Brenner G.J. 2000. Riparian and adjacent upslope beetle communities along a third order stream in the western Cascade Mountain Range, Oregon 341 p. Ph.D. Dissertation, Oregon State University, Corvallis, OR.

Buée M., Vairelles D. and Garbaye J. 2005. Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (*Fagus silvatica*) forest subjected to two thinning regimes. Mycorrhiza 15, 235-245.

Buée M., Courty P.E., Mignot D. and Garbaye J. 2007. Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community. Soil Biology and Biochemistry 39, 1947-1955.

Burke D.J., Dunham S.M. and Kretzer A.M. 2008. Molecular analysis of bacterial communities associated with the roots of Douglas-fir (*Pseudotsuga menziesii*) colonized by different ectomycorrhizal fungi. FEMS Microbiology Ecology 65, 299-309.

Burke D.J., Kretzer A.M., Rygiewicz P.T. and Topa M.A. 2006. Soil bacterial diversity in a loblolly pine plantation: influence of ectomycorrhizas and fertilization. FEMS Microbiology Ecology 57, 409-419.

Burke D.J., Lopez-Gutierrez J.C., Smemo K.A. and Chan C.R. 2009. Vegetation and soil environment influence the spatial distribution of root-associated fungi in a mature beech-maple forest. Applied and Environmental Microbiology 75, 7639-7648.

Burke R. and Cairney J. 2002. Laccases and other polyphenol oxidases in ecto- and ericoid mycorrhizal fungi. Mycorrhiza 12, 105-116.

Cairney J.W.G. and Meharg A.A. 2002. Interactions between ectomycorrhizal fungi and soil saprotrophs: implications for decomposition of organic matter in soils and degradation of organic pollutants in the rhizosphere. Canadian Journal of Botany 80, 803-809

Caldwell B.A., Griffiths R.P. and Sollins P. 1999. Soil enzyme response to vegetation disturbance in two lowland Coast Rica soils. Soil Biology and Biochemistry 31, 1603-1608.

Chaer G., Myrold D. and Bottomley P. 2009. A soil quality index based on the equilibrium between soil organic matter and biochemical properties of undisturbed coniferous forest soils of the Pacific Northwest. Soil Biology and Biochemistry 41, 822-830.

Chapin F.S., Matson P.A. and Mooney H.A. 2002. Principles of Terrestrial Ecosystem Ecology. Springer-Verlag, New York.

Colwell R. and Coddington J. 1994. Estimating terrestrial biodiversity through extrapolation. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 345, 101-118.

Conn C. and Dighton J. 2000. Litter quality influences on decomposition, ectomycorrhizal community structure and mycorrhizal root surface acid phosphatase activity. Soil Biology and Biochemistry 32, 489-496.

Courty P.E., Buée M., Diedhiou A.G., Frey-Klett P., Le Tacon F., Rineau F., Turpault M.-P., Uroz S. and Garbaye J. 2010. The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. Soil Biology and Biochemistry 42, 679-698.

Courty P.E., Franc A., Pierrat J.-C. and Garbaye J. 2008. Temporal changes of the ectomycorrhizal community in two soil horizons of a temperate oak forest. Applied and Environmental Microbiology 74, 5792-5801.

Courty P.E., Pritsch K., Schloter M., Hartmann A. and Garbaye J. 2005. Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. New Phytologist 167, 309-319.

Courty P.E., Breda N. and Garbaye J. 2007. Relation between oak tree phenology and the secretion of organic matter degrading enzymes by *Lactarius quietus* ectomycorrhizas before and during bud break. Soil Biology and Biochemistry 39, 1655-1663.

Cromack K., Jr, Fitcher B.L., Moldenke A.M., Entry J.A. and Ingham E.R. 1988. Interactions between soil animals and ectomycorrhizal fungal mats. Agriculture, Ecosystems and Environment 24, 161-168.

Cromack K., Jr, Sollins P., Graustein W.C., Speidel K., Todd A.W., Spycher G., Li C.Y. and Todd R.L. 1979. Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. Soil Biology and Biochemistry 11, 463-468.

Cruz-Martinez K., Suttle K.B., Brodie E.L., Power M.E., Andersen G.L. and Banfield J.F. 2009. Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. The ISME Journal 3, 738-744.

Dalpe Y. 1989. Ericoid mycorrhizal fungi in the *Myxotrichaceae* and *Gymnoascaceae*. New Phytologist 113.

Daly C. and McKee W. 2009. Meteorological data from benchmark stations at the Andrews Experimental Forest. Long-Term Ecological Research. Forest Science Data Bank, Corvallis, OR. [Database]. Available: http://andrewsforest.oregonstate.edu/data/abstract.cfm?dbcode=MS001 (25 March 2010).

Dickie I.A., Xu B. and Koide R. 2002. Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. New Phytologist 156, 527–535.

Dix N.J. and Webster J. 1995. Fungal Ecology. University Press, Cambrige, Great Britain.

Drummond A., Ashton B., Cheung M., Heled J., Kearse M., Moir R., Stones-Havas S., Thierer T. and Wilson A. 2009. Geneious v4.7. Available from http://www.geneious.com/.

Dunham S.M., Larsson K.-H. and Spatafora J.W. 2007. Species richness and community composition of mat-forming ectomycorrhizal fungi in old- and second-growth Douglas-fir forests of the HJ Andrews Experimental Forest, Oregon, USA. Mycorrhiza 17, 633-645.

Edwards U., Rogall T., Blöcker E.M. and Böttger E.C. 1989. Isolations and direct complete nucleotide determination of entire genes. Characterization of a gene coding 16S ribosomal RNA. Nucleic Acids Research 17, 7843-7852.

Fierer N. and Schimel J.P. 2002. Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry 34, 777-787.

Fierer N., Jackson J.A., Vilgalys R. and Jackson R.B. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71, 4117-4120.

Fitter A.H. and Garbaye J. 1994. Interactions between mycorrhizal fungi and other soil organisms. Plant and Soil 159, 123-132.

Frey-Klett P. and Garbaye J. 2005. Mycorrhiza helper bacteria: a promising model for the genomic analysis of fungal-bacterial interactions. New Phytologist 168, 4-8.

Frey-Klett P., Garbaye J. and Tarkka M. 2007. The mycorrhiza helper bacteria revisited. New Phytologist 176, 22-36.

Frey-Klett P., Chavatte M., Clausse M.-L., Courrier S., Roux C.L., Raaijmakers J., Martinotti M.G., Pierrat J.-C. and Garbaye J. 2005. Ectomycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent pseudomonads. New Phytologist 165, 317-328.

Garbaye J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytologist 128, 197-210.

Gardes M. and Bruns T.D. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology Notes 2, 113-118.

Genney D.R., Anderson I.C. and Alexander I.J. 2006. Fine-scale distribution of pine ectomycorrhizas and their extramatrical mycelium. New Phytologist 170, 381-390.

Griffiths R.P. and Caldwell B.A. 1992 Mycorrhizal mat communities in forest soils. In: Mycorrhizas in Ecosystems. Eds. D.J. Read, D.H. Lewis, A.H. Fitter and I.J. Alexander. pp. 98-105. C.A.B. International, Wallingford, Oxon, UK.

Griffiths R.P., Baham J.E. and Caldwell B.A. 1994. Soil solution chemistry of ectomycorrhizal mats in forest soil. Soil Biology and Biochemistry 26, 331-337.

Griffiths R.P., Castellano M.A. and Caldwell B.A. 1991. Hyphal mats formed by two ectomycorrhizal fungi and their association with Douglas-fir seedlings: A case study. Plant and Soil 134, 255-259.

Griffiths R.P., Bradshaw G.A., Marks B. and Lienkaemper G.W. 1996. Spatial distribution of ectomycorrhizal mats in coniferous forests of the Pacific Northwest, USA. Plant and Soil 180, 147-158.

Griffiths R.P., Caldwell B.A., Cromack K., Jr and Morita R.Y. 1990. Douglas-fir forest soils colonized by ectomycorrhizal mats. I. Seasonal variation in nitrogen chemistry and nitrogen cycle transformation rates. Canadian Journal of Forest Research 20, 211-218.

Griffiths R.P., Caldwell B.A., Ingham E.R., Castellano M.A. and Cromack K. Jr. 1991. Comparison of microbial activity in ectomycorrhizal mat communities in Oregon and California. Biology and Fertility of Soils 11, 196-202.

Griffiths R.P., Chadwick A.C., Robatzek M., Schauer K. and Schaffroth K.A. 1995. Association of ectomycorrhizal mats with Pacific yew and other understory trees in coniferous forests. Plant and Soil 173, 343-347.

Griffiths R.P., Ingham E.R., Caldwell B.A., Castellano M.A. and Cromack K. Jr. 1991. Microbial characteristics of ectomycorrhizal mat communities in Oregon and California. Biology and Fertility of Soils 11, 196-202.

Hackl E., Zechmeister-Boltenstern S., Bodrossy L. and Sessitsch A. 2004. Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. Applied and Environmental Microbiology 70.

Heinonsalo J., Jørgensen K.S. and Sen R. 2001. Microcosm-based analyses of Scots pine seedling growth, ectomycorrhizal fungal community structure and bacterial carbon utilization profiles in boreal forest humus and underlying illuvial mineral horizons. FEMS Microbiology Ecology 36, 73-84.

Högberg M. and Högberg P. 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces together with associated roots, half the dissolved organic carbon in a forest soil. New Phytologist 154, 791-795.

Høj L., Rusten M., Haugen L.E., Olsen R.A. and Torsvik V.L. 2006. Effects of water regime on archaeal community composition in Arctic soils. Environmental Microbiology 8, 984-996.

Huber T., Faulkner G. and Hugenholtz P. 2004. Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. Bioinformatics 20, 2317-2319.

Hutchison L.J. 1990. Studies on the systematics of ectomycorrhizal fungi in axenic culture. II. The enzymatic degradation of selected carbon and nitrogen compounds. Canadian Journal of Botany 68, 1522-1530.

Ingham E.R., Griffiths R.P., Cromack K. Jr. and Entry J.A. 1991. Comparison of direct vs. fumigation incubation microbial biomass estimates from ectomycorrhizal mat and non-mat soils. Soil Biology and Biochemistry 23, 465-471.

Izumi H., Anderson I.C., Alexander I.J., Killham K. and Moore E.R.B. 2006. Diversity and expression of nitrogenase genes (nifH) from ectomycorrhizas of Corsican pine (*Pinus nigra*). Environmental Microbiology 8, 2224-2230.

Khetmalas M.B., Egger K., Massicotte H.B., Tackaberry L.E. and Clappertion M.J. 2002. Bacterial diversity associated with subalpine fir (*Abies lasiocarpa*) ectomycorrhizae following wildfire and salvage-logging in central British Columbia. Canadian Journal of Microbiology 48, 611-625.

Kluber L.A., Tinnesand K.M., Caldwell B.A., Dunham S.M., Yarwood R.R., Bottomley P.J. and Myrold D.D. Submitted. Ectomycorrhizal mats: biochemical characterization and comparison to corresponding non-mat soils in a Douglas-fir forest. Soil Biology and Biochemistry.

Knutson D.M., Hutchins A.S. and Cromack K. Jr. 1980. The association of calcium oxalate-degrading *Streptomyces* with conifer ectomycorrhizae. Antonie Van Leeuwenhoek 46, 611-619.

Kourtev P.S., Ehrenfeld J.G. and Haggblom M. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83, 3152-3166.

Kretzer A., King Z. and Bai S. 2009. Bacterial communities associated with tuberculate ectomycorrhizae of *Rhizopogon* spp. Mycorrhiza 19, 277-282.

Ladd J.N. and Butler J.H. 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biology and Biochemistry 4, 19-30.

Lapeyrie F., Chilvers G.A. and Bhem C.A. 1987. Oxalic acid synthesis by the mycorrhizal fungus *Paxillus involutus.* New Phytologist 106, 139-146.

Lauber C.L., Strickland M.S., Bradford M.A. and Fierer N. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology and Biochemistry 40, 2407-2415.

Leake J.R., Donnelly D.P., Saunders E.M., Boddy L. and Read D.J. 2001. Rates and quantities of carbon flux to ectomycorrhizal mycelium following 14C pulse labeling of *Pinus sylvestris* seedlings: effects of litter patches and interaction with a wood-decomposer fungus. Tree Physiology 21, 71-82.

Leveau J.H.J. and Preston G.M. 2008. Bacterial mycophagy: definition and diagnosis of a unique bacterial-fungal interaction. New Phytologist 177, 859-876.

Lindahl B.D. 2000. Ectomycorrhizal fungi raid saprotrophic ones. Mycological Research 104, 385-387.

Lindahl B.D. and Taylor A.F.S. 2004. Occurrence of N-acetylhexosaminidase-encoding genes in ectomycorrhizal basidiomycetes. New Phytologist 164, 193-199.

Lindahl B.D., Ihrmark K., Boberg J., Trumbore S.E., Högberg P., Stenlid J. and Finlay R.D. 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytologist 173, 611-620.

Lipson D.A., Schmidt S.K. and Monson R.K. 1999. Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. Ecology 80, 1623-1631.

Luoma D., Frenkel R. and Trappe J. 1991. Fruiting of hypogeous fungi in Oregon Douglas-fir forests: seasonal and habitat variation. Mycologia 83, 335–353.

Maidak B.L., Cole J.R., Lilburn T.G., Parker C.T., Jr, Saxman P.R., Stredwick J.M., Garrity G.M., Li B., Olsen G.J., Pramanik S., Schmidt T.M. and Tiedje J.M. 2000. The RDP (Ribosomal Database Project) continues. Nucleic Acids Research 28, 173-174.

Malajczuk N. and Cromack K., Jr. 1982. Accumulation of calcium oxalate in the mantle of ectomycorrhizal roots of *Pinus radiata* and *Eucalyptus marginata.* New Phytologist 92, 527-531.

Mansfield-Giese K., Larson J. and Bodker L. 2002. Bacterial populations associated with mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices*. Microbial Ecology 41, 133-140.

Manter D.K. and Vivanco J.M. 2007. Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. Journal of Microbiological Methods 71, 7-14.

Marschner P. and Baumann K. 2003. Changes in bacterial community structure induced by mycorrhizal colonisation in split-root maize. Plant and Soil 251, 279-289.

McCune B. and Grace J.B. 2002. Analysis of Ecological Communities. MjM Software Design, Gleneden Beach, Oregon.

Molina R., Caldwell B.A., Castellano M.A., Horton T.R. and Smith J.E. 2002 Mycorrhizae: ectomycorrhizal fungi. In: Encyclopedia of Environmental Microbiology. Ed. G. Bitton. pp. 2124-2132. Wiley, New York.

Molina R., Pilz D., Smith J., Dunham S., Dreisbach T., O'Dell T. and Castellano M. 2001 Conservation and management of forest fungi in the Pacific Northwestern United States: an integrated ecosystem approach. In: Fungal conservation issues and solutions. Eds. D. Moore, N.N. Nauta, S.E. Evans and M. Rotheroe. pp. 19-63. Cambridge University Press, Canbridge, UK.

Moore-Kucera J. and Dick R.P. 2008. PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. Microbial Ecology 55, 500-511.

Muyzer G., Teske G., Wirsen C.O. and Jannasch H.W. 1995. Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. Archives of Microbiology 164, 165-172.

Nazir R., Warmink J.A., Boersma H. and van Elsas J.D. 2010. Mechanisms that promote bacterial fitness in fungal-affected soil microhabitats. FEMS Microbiology Ecology 71, 169-185.

Oswald E.T. and Ferchau H.A. 1968. Bacterial associations of coniferous mycorrhizae. Plant and Soil 28, 187-192.

Parham J.A. and Deng S.P. 2000. Detection, quantification and characterization of β-glucosaminidase activity in soil. Soil Biology and Biochemistry 32, 1183-1190.

Phillips C.L., Kluber L., Pedersen J. and Bond B.J. in revision. Contributions of ectomycorrhizal fungal mats to forest soil respiration. New Phytologist.

Pritsch K., Raidl S., Marksteiner E., Blaschke H., Agerer R., Schloter M. and Hartmann A. 2004. A rapid and highly sensitive method for measuring enzyme activities in single mycorrhizal tips using 4-methylumbelliferone-labelled fluorogenic substrates in a microplate system. Journal of Microbiological Methods 58, 233-241.

Read D.J. and Perez-Moreno J. 2003. Mycorrhizas and nutrient cycling in ecosystems a journey towards relevance? New Phytologist 157, 475-492.

Rineau F., Courty P.E., Uroz S., Buée M. and Garbaye J. 2008. Simple microplate assays to measure iron mobilization and oxalate secretion by ectomycorrhizal tree roots. Soil Biology and Biochemistry 40, 2460-2463.

Robinson C.H., Miller E.J.P. and Deacon L.J. 2005 Biodiversity of saprotrophic fungi in relation to their function: do fungi obey the rules? In Biological diversity and function in soils. Eds. R.D. Bardgett, D.W. Hopkins and M.B. Usher. pp 189-215. Cambridge University Press, New York, NY.

Schadt C.W., Martin A.P., Lipson D.A. and Schmidt S.K. 2003. Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301, 1359-1361.

Schloss P.D. and Handelsman J. 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Applied and Environmental Microbiology 71, 1501-1506.

Skopp J., Jawson M.D. and Doran J.W. 1990. Steady-state aerobic microbial activity as a function of soil water content. Soil Science Society of America Journal 54, 1619-1625.

Smith J.E., Molina R., Huso M.M.P. and Larsen M.J. 2000. Occurrence of *Piloderma fallax* in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, U.S.A. Canadian Journal of Botany 78, 995-1001.

Smith J.E., Molina R., Huso M.M., Luoma D.L., McKay D., Castellano M.A., Lebel T. and Valachovic Y. 2002. Species richness, abundance, and composition of hypogeous and epigeous ectomycorrhizal fungal sporocarps in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, USA. Canadian Journal of Botany 80, 186–204.

Smith S. and Read D. 2008. Mycorrhizal Symbiosis. Academic Press, New York. 787 p.

Talbot J.M., Allison S.D. and Treseder K.K. 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Functional Ecology 22, 955-963.

Taylor D.L., Booth M.G., McFarland J.W., Herriott I.C., Lennon N.J., Nusbaum C. and Marr T.G. 2008. Increasing ecological inference from high throughput sequencing of fungi in the environment through a tagging approach. Molecular Ecology Resources 8, 742-752.

Tedersoo L., Partel K., Jairus T., Gates G., Poldmaa K. and Tamm H. 2009. Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the *Helotiales*. Environmental Microbiology 11, 3166-3178.

Timonen S., Jørgensen K.S. and Haahtela K. 1998. Bacterial community structure at defined locations of *Pinus sylvestris - Suillus bovinus* and *Pinus sylvestris - Paxillus involutus* mycorrhizospheres in dry pine forest humus and nursery peat. Can. J. Microbiol. 44, 499-513.

Trappe J.M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Annual Review of Phytopathology 15, 203-222.

Trappe J.M., Molina R., Luoma D.L., Cázares E., Pilz D., Smith J.E., Castellano M.A., Miller S.L. and Trappe M.J. 2009. Diversity, ecology, and conservation of truffle fungi in forests of the Pacific Northwest. Gen. Tech. Rep. PNW-GTR-772. Department of Agriculture, Pacific Northwest Research Station. 194 p. Portland, OR.

Tuason M.M.S. and Arocena J.M. 2009. Calcium oxalate biomineralization by *Piloderma fallax* in response to various levels of calcium and phosphorus. Applied and Environmental Microbiology 75, 7079-7085.

Vance E.D., Brookes P.C. and Jenkinson D.S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703-707.

Vogt K. 1991. Carbon budgets of temperate forest ecosystems. Tree Physiology 9, 69-86.

Wallander H., Nilsson L.O., Hagerberg D. and Bääth E. 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. New Phytologist 151, 753-760.

Wang Q., Garrity G.M., Tiedje J.M. and Cole. J.R. 2007. Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73, 5261-5267.

Waring R.H. and Franklin J.F. 1979. Evergreen coniferous forests of the Pacific Northwest. Science 204, 1380-1386.

Warmink J.A., Nazir R. and van Elsas J.D. 2009. Universal and species-specific bacterial 'fungiphiles' in the mycospheres of different basidiomycetous fungi. Environmental Microbiology 11, 300-312.

Warmink J.A. and van Elsas J.D. 2008. Selection of bacterial populations in the mycosphere of *Laccaria proxima*: is type III secretion involved? ISME Journal 2, 887-900.

White T.J., Bruns T.D., Lee S.B. and Taylor J.W. 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols- a Guide to Methods and Applications. Eds. M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White. pp. 315-322. Academic Press, San Diego, CA.

Wu T., Kabir Z. and Koide R.T. 2005. A possible role for saprotrophic microfungi in the N nutrition of ectomycorrhizal *Pinus resinosa*. Soil Biology and Biochemistry 37, 965-975.

Yarwood S.A., Bottomley P.J. and Myrold D.D. Accepted. Soil microbial communities associated with Douglas-fir and red alder stands at high- and low-productivity forest sites in Oregon, USA. Microbial Ecology

Yi H., Kim H.-J., Kim C.-G., Harn C., Kim H. and Park S. 2009. Using T-RFLP to assess the impact on soil microbial communities by transgenic lines of watermelon rootstock resistant to cucumber green mottle mosaic virus (CGMMV). Journal of Plant Biology 52, 577-584.