AN ABSTRACT OF THE THESIS OF

Jed E. Cappellazzi for the degree of Master of Science in Soil Science and Forest Ecosystems and Society presented on March 17, 2014.

Title: The Development and Decline Dynamics of Two Ectomycorrhizal Fungal Mat Soil Microbial Communities in Response to a Reciprocal Soil Transfer Experiment in Old-growth Douglas-fir Forests.

Abstract approved:

David D. Myrold Jane E. Smith

Ectomycorrhizal fungi (EmF) form symbioses with trees. These symbioses profoundly influence forest ecology. Certain EmF form specialized profusions of hyphae, known as ectomycorrhizal fungal mats (mats) which are visible to the naked eye, alter forest soil biogeochemistry, substantially contribute to soil microbial biomass/respiration and support unique microbial communities. *Piloderma* and *Ramaria* mats stratify in organic and upper mineral soil, respectively, and are the dominant mat-forming fungi of old-growth Douglas-fir forests of the Pacific Northwest, USA. The importance of *Piloderma* and *Ramaria* mats to forest ecosystem processes has driven the need to better understand their associated microbial communities, particularly development (birth) and decline (death) dynamics. To explore these dynamics, a reciprocal soil transplant experiment was established at seven old-growth Douglas-fir sites in the H.J. Andrews Experimental Forest. At each site *Piloderma*, *Ramaria* and non-mat soils underwent birth (non-mat into mat enclosed in 2-mm mesh), death (mat into non-mat enclosed in PVC pipe), disturbance control (core non-mat soil, replace in 2-mm mesh) and background (no manipulation) treatments. After 51 months, treatments were harvested and three microbial community components were assessed through molecular analyses: active EmF root-tips using Sanger sequencing and soil fungi and bacteria using 454-pyrosequencing. Results from this study revealed differential persistence of mats

formed by *Piloderma* and *Ramaria*. In the mineral horizon, we found few microbial community differences. Originally unique *Ramaria* mat microbiota were no different from non-mat soils after 51 months, and the mat-forming genus, *Ramaria*, was notably missing from fungal sequences; these data support the ephemeral nature of *Ramaria* mats where their hydrophobic powdery structure may, at times, be a visual legacy of mat presence, a remnant of physical alteration of the soil environment. In the organic horizon, *Piloderma* mat fungal communities persisted for 51 months and remained distinct from non-mat soils; this permitted birth and death treatment analysis. Our data indicate strong development of *Piloderma* mat fungal communities in birth treatments, beyond colonization by *Piloderma*, making them indistinguishable from *Piloderma* mats; mat development can take many years. Death treatments were dissimilar to *Piloderma* mats and contained similar fungal communities to non-mat soils. Enclosure in PVC pipe, thereby removing roots and EmF from the system, significantly shifted the soil fungal community toward saprotrophic dominance. To compliment *Piloderma*, the EmF genus *Russula* was a robust indicator of non-mat organic soils; there was strong evidence for the competitive exclusion of *Russula* in *Piloderma* mats, though it may take many years for exclusion to occur. For organic horizon bacterial communities, only death treatments differed from others. Strong similarities were found between overall *Piloderma* mat and non-mat bacterial communities; however, *Piloderma* mat and non-mat soils impose selection pressure on a small subset of bacterial taxa masked when the community is considered as a whole. This work contributes to the body of knowledge regarding complex microbial community dynamics of EmF mats. The occurrence and distinct microbial taxa of *Piloderma* mats in these forests suggests large-scale spatial differences in ecological function. The extent of functional differences is currently unknown, but *Piloderma* mats present a unique microbial system, supported by over 30 years of research, to test difficult microbial ecology questions.

The Development and Decline Dynamics of Two Ectomycorrhizal Fungal Mat Soil Microbial Communities in Response to a Reciprocal Soil Transfer Experiment in Old-growth Douglas-fir Forests

by
Jed E. Cappellazzi

A THESIS

Submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented March 17, 2014
Commencement June 2014

Master of Science thesis of Jed E. Cappellazzi presented on March 17, 2014.

APPROVED:

_____

Co-Major Professor, representing Soil Science

_____

Co-Major Professor, representing Forest Ecosystems and Society

_____

Head of the Department of Crop and Soil Science

_____

Head of the Department of Forest Ecosystems and Society

_____

Dean of the Graduate School

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

_____

Jed E. Cappellazzi, Author

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my co-major advisors, Dave Myrold and Jane Smith for their support, guidance and wisdom throughout this process. I am particularly thankful to Dave Myrold for providing me with the opportunity to attend Graduate School at Oregon State University, a longstanding dream of mine. To Jane Smith, I sincerely thank you for the opportunity to complete a dual major degree in Forest Ecosystems and Society. I would also like to thank my committee members Peter Bottomley and Dan Luoma, for advice and ideas. Countless others helped me along the way and I would like to recognize Carl Evans, Doni McKay, Lydia Zeglin, Tara Jennings, Chris Sullivan, Cedar Hesse, Tom Wanzek and Mark Dasenko for assistance in the field, laboratory and/or with high throughput sequencing, Joey Spatafora, Kermit Cromack and Bruce Caldwell for their thoughtful advice and Bruce McCune for his statistical support. Thank you to Joe Cacka and my co-workers at Crop Production Services, for giving me a shot in Agriculture and working with me during the completion of this document. I express my deepest gratitude to my wonderful mother, without whom I would have never made it to this point in my life. Beyond all else, I thank my lovely wife Margot Mercer for always being there and helping me in every sense of the word, as well as all of my friends near and far. This research was supported by a combination of a graduate research assistantship in the laboratory of Dave Myrold, Soil Biology, and a graduate teaching assistantship with Oregon State University.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

Page

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF APPENDICES

The Development and Decline Dynamics of Two Ectomycorrhizal Fungal Mat Soil Microbial Communities in Response to a Reciprocal Soil Transfer Experiment in Old-growth Douglas-fir Forests

1. Introduction and Literature Review:

1.1. General Introduction

The mycorrhizal association is diverse in both phylogeny and form. It is pervasive in land plants, occurring in over 80% of extant taxa (Wang & Qui, 2006), includes members in most major fungal and plant lineages (Cairney, 2000) and assumes various morphologies (Smith & Read, 2008). Trappe (1996) defined mycorrhizas as dual absorptive organs formed when symbiotic hyphal fungi inhabit healthy underground plant tissue, typically roots. The term mycorrhiza originates from the Greek words 'mykes' (fungus) and 'rhiza' (root), introduced by Albert Bernhard Frank in 1885 (Frank, 1885). Mycorrhizas, not roots, are regarded as the primary vectors by which plants obtain nutrients and water (Smith & Read, 2008), and in return the fungal partner receives plant photosynthate to facilitate growth, reproduction and soil exploration. Mycorrhizal associations should not be pigeonholed into distinct functional categories. For instance, at the species level, our understanding of mycorrhizal C acquisition has recently expanded from traditional biotrophy to a biotrophy-saprotrophy continuum (Koide *et al.*, 2008). Additionally, mycorrhizal associations are not necessarily beneficial to both partners; rather they exist along a mutualism-parasitism (cost/benefit) continuum (Johnson *et al.*, 1997; Jones & Smith, 2004) and, in temperate forests, may depend on fungal-plant species interactions, host age, forest successional stage, carbon/nutrient availability and reproductive strategy to name a few. It is an ancient and ubiquitous symbiosis between plant and fungus that links above and below-ground biogeochemical processes, and substantial support exists for the theory that this inter-kingdom coevolution may be responsible for the radiation of life onto land ~480 mya (Pirozynski & Malloch, 1975; Wang & Qiu, 2006; Cappellazzi *et al.*, 2007; Bidartondo *et al.*, 2011).

During the ensuing epochs, numerous mycorrhizal types evolved and were defined on the basis of plant/fungal structures. The four major recognized classifications include ectomycorrhizal fungi (EmF), arbuscular mycorrhizal fungi (AmF), ericoid mycorrhizal fungi (ErM) and orchid mycorrhizal fungi (Brundrett, 2004; Smith & Read, 2008).

Arbuscular mycorrhizas, the most ancient type of mycorrhizal fungi (Wang & Qiu, 2006), are obligate biotrophs of the phylum *Glomeromycota* (Schüßler et al., 2001). These fungi form intracellular nutrient and carbon exchange organs (arbuscules), as well as storage structures (vesicles) that also function as asexual propagules. Aseptate hyphae from these organisms breach plant cell walls yet remain separated from the cell cytoplasm by the cell membrane (Peterson & Massicotte, 2004). AmF associate with phylogenetically and ecologically diverse plant species, from trees to herbs; however, they are less abundant in temperate coniferous forests than other ecosystem types.

Ericoid and Orchid mycorrhizal fungi exhibit greater host specificity and functional plasticity than AmF. ErM form hyphal coils that penetrate thin Ericaceous plant roots, while orchid mycorrhizas form intracellular pelotons in Orchidaceae roots (Brundrett, 2004). These fungi are capable of associating with EmF plants (Smith & Read, 2008) and can survive in the soil when disconnected from a host, reducing their obligate status (Brundrett, 2004). Of the major mycorrhizal types, ErM are most capable of decomposing soil organic matter and may retain more saprotrophic capabilities of ancestral lineages than other mycorrhizal types (Hibbett & Matheney, 2009).

Ectomycorrhizas, the most frequent and widespread mycorrhizal type in temperate and boreal forests (Alexander, 2006), are formed when fungal hyphae ensheath the fine root-tips of host plants, forming a mantle, and establish an extracellular hyphal network around root cortical cells, known as the Hartig-net (Smith & Read, 2008). The EmF structure can, in effect, exclude host plant absorptive tissues from direct interaction with the soil environment. This is accomplished with near 100% fine root colonization as well as the hydrophobic nature and tight regulation of solute movement through the mantle (Taylor & Alexander, 2005), phenomena that may indeed leave host plants completely reliant on the fungal uptake pathway. It is at this root-hyphal interface where

the plant exchanges photosynthate in return for nutrients and water acquired by fungal associates in the soil matrix.

EmF have diverse methods of soil exploration, which Agerer (2001) classified into five strategies: (1) contact exploration; (2) short-distance exploration; (3) medium-distance exploration; (4) long-distance exploration; and (5) pick-a-back exploration. Within the medium-distance exploration category is the mat subtype, defined by hyphal occupancy of large areas with individual mycorrhizas having limited exploratory range through undifferentiated to slightly differentiated rhizomorphs. Further discussion of this type will be considered later.

The ecological functions of EmF have been thoroughly investigated and reported in the literature for decades (Marks & Kozlowski, 1973; Kottke & Oberwinkler, 1986; Simard *et al*., 1997; Allen *et al.*, 2003; Smith & Read, 2008; Courty *et al*., 2010; Koide *et al*., 2014), with modern techniques greatly expanding the depth and breadth of our understanding of this critical forest ecosystem component. To summarize, EmF confer a suite of benefits to host plants that include, but are not limited to: (1) enhanced mobilization and uptake of inorganic and organic nutrients, from both simple and complex substrates; (2) drought tolerance through hyphal and rhizomorphic uptake and translocation of water; (3) protection from root pathogens and nematodes; (4) increased host vigor to protect against aboveground pathogens; (5) enhanced soil structural stability for improved growth and erosion control; (6) alteration of the competitive dynamics among plants of the same and different species; and (7) facilitation of below-ground carbon and nutrient transfer among plants of the same and different species. Functional differences at the species level may be attributable to the EmF habit evolving from saprotrophic ancestors many times in diverse fungal lineages; in the Basidiomycota alone there were at least eight independent derivations of the EmF habit (Hibbett & Matheney, 2009), leading to the idea of differential saprotrophic retention among EmF taxa.

The AmF association, though ubiquitous among vascular plants globally, is less abundant in the forests of the Pacific Northwest United States (PNW) where the EmF association predominates. The United States Forest Service classifies the PNW as region

6, which occupies 440,000 km^2 in the states of Oregon and Washington and contains 17 national forests, two national scenic areas, one national grassland and two national volcanic monuments. The natural evergreen coniferous forest type for this region is overwhelmingly dominated by the ectomycorrhizal Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*) association west of the Cascade Mountain crest, particularly at lower to middle elevations, where timberland occupies more than half of total forested land (Smith *et al.* 2009), conferring high ecological and economic value to these species. Douglas-fir is an early seral species whereas western hemlock is a climax species; the former aggressively colonizes open sites until canopy closure when its relative shade intolerance gives way to a co-dominant western hemlock in the old-growth understory. Common plant associates in these forests include the arbuscular mycorrhizal species western red cedar (*Thuja plicata*), maples (*Acer* spp.), dwarf Oregon grape *(Mahonia nervosa)* and numerous understory herbs, a suite of ericoid mycorrhizal plants including salal (*Gaultheria shallon*), Pacific rhododendron (*Rhododendron macrophyllum*) and red huckleberry (*Vaccinium parvifolium*), as well as high orchid diversity (Cappellazzi *et al.*, 2007). Douglas-fir, which the Oregon Department of Forestry estimates to be $4/5^{th}$ of the total standing tree crop in region 6, is of primary importance to regional public/private landowners and ecosystem management is critical to healthy productive forests.

It is estimated that 7,000-10,000 fungal species and over 8,000 plant species are capable of forming ectomycorrhizal symbioses (Taylor & Alexander, 2005; Smith & Read, 2008). The fungi represent a large diversity across the Basidiomycota and Ascomycota, as well as a few species in the Zygomycota (Molina *et al.*, 2002). The functional redundancy of these fungi is less prominent than many think, a fact supported by the high diversity of fungal species at small spatial scales (Horton *et al.*, 2005). EmF communities tend to be hyperdiverse, patchy, and composed of a few common species and many rare species (Horton *et al.*, 2005). Ectomycorrhizal host plants include a wide variety of woody trees and shrubs in ecologically and economically important families: Betulaceae, Ericaceae, Fagaceae, Pinaceae, Rosaceae and Salicaceae. Although the

number of plant species forming EmF associations is relatively small (~3% of known species), the Pinaceae, which is estimated to be almost entirely ectomycorrhizal (Newman & Reddell, 1987; Cairney, 2000; Smith & Read, 2008), extensively dominates temperate forest ecosystems such as the Cascade mountains of the PNW. Even a single species, Douglas-fir, can associate with over 2,000 different EmF (Molina *et al.*, 1992), each capable of affecting microbial community composition within the respective ectomycorrhizosphere; these levels of community structure can vastly increase overall soil microbial diversity in PNW forests. Uniquely, Douglas-fir can simultaneously host a broad range of specialist and generalist EmF. For example, ~72% of Douglas-fir EmF can associate with multiple hosts (Molina *et al.*, 1992), whereas ~205 are specific to Douglas-fir throughout its range (Smith & Read, 2008). Horton *et al.* (2005) examined EmF in Douglas-fir/western hemlock stands. They confirmed an earlier hypothesis proposed by Kropp and Trappe (1982) where fungal host selection pressure is less specific in later successional stages as trees are born into established EmF networks. This supports the idea that old-growth EmF trees are generalist EmF hosts, a fact that can increase physiological diversity of their microbial organs and broaden the availability of soil nutrient pools (Smith & Read, 2008). In the PNW, a subset of EmF are capable of forming uniquely prominent structures on the forest floor known as ectomycorrhizal fungal mats (mats) and will be discussed in detail below.

1.2. Ectomycorrhizal Fungal Mats

In 1999, with funding from the National Science Foundation, Oregon State University established the microbial observatory (MO) at the HJ Andrews LTER (HJA) in Western Oregon, devoted to the study of fungal and bacterial biogeochemical processes in coniferous forest ecosystems ("Microbial Observatory at the HJ Andrews LTER"). Initial research focused on the functional diversity of microbes integral to nitrogen cycling processes, as nitrogen is thought to be the most limiting nutrient in these systems. The grant was renewed in 2004 when research shifted to the microbial communities associated with mats. Over the course of 10 years, the original MO

hypotheses ─ (1) to identify microorganisms associated with mat and non-mat communities and (2) to determine microbial functional roles in the differentiation of mat from non-mat soils ─ have evolved. Concurrently, with the development of next-generation sequencing technologies, the analytical depth expanded from phospholipid fatty acid (PLFA) fingerprinting, cloning and Sanger sequencing to the deep sequencing of entire microbial communities via 454-pyrosequencing. The evolution of these hypotheses led to the development of a novel experiment examined here, which aimed to study the development and decline of microbial communities associated with mat and adjacent non-mat soils (described later).

The phenomenon of ectomycorrhizal mats is globally widespread in forested regions, tropical to temperate (Högberg, 1982; Alexander & Högberg, 1986; Castellano, 1988; Dung, 2012), and there is no scarcity of descriptions and definitions for mats (Trappe *et al.*, 2012 and references therein). Common characterizations include copious and interwoven mycelia having broad coverage in organic and mineral soil; entwined roots and aggregated soil particulates; and homogenous appearance with a distinct boundary and rhizomorphic features. Recent research from the HJA MO has coalesced around the definition proposed by Dunham *et al.* (2007): "dense profusions of rhizomorphs associated with obvious ectomycorrhizal root-tips that aggregate soil and alter its appearance and are uniform in structure and appearance for an area at least 0.5 m in diameter." The current work will define mats in this manner. To date, literature suggests approximately 25 EmF genera contain species capable of forming mats with varying morphologies and soil profile distributions. These include *Alpova, Arcangeliella, Austrogauteria, Bankera, Boletopsis, Chondrogaster, Cortinarius, Geastrum, Gautieria, Gomphus, Hebeloma, Hydnellum, Hysterangium, Lactarius, Mycoamaranthus, Phellodon, Piloderma, Ramaria, Rhizopogon, Russula, Sarcodon, Sistotrema, Suillus, Trechispora* and *Tricholoma* (Agerer, 2001 and references therein; Dunham *et al.*, 2007; Trappe *et al.*, 2012 and references therein). Moreover, Trappe *et al.* (2012) identified saprotrophic genera (*Flavoscypha, Gastropila, Lepiota* and *Xenasmatella*) capable of

forming fungal mats, an ecological phenomenon distinct from ectomycorrhizal mats with uniquely important ecological significance. The focus here will be on mat-forming EmF.

In the PNW, mat research has been particularly thorough; work from the Oregon Coast and Cascade Range temperate conifer forests dates back over three decades to the first regionally published work specifically addressing this phenomenon (Cromack *et al.,* 1979). Since 1979, PNW research has approached the topic by comparing soil inhabited by mats to adjacent soil devoid of mat-forming fungi through mostly visual identification. In the seminal work, Cromack *et al.* (1979) examined *Hysterangium crassum* mat and non-mat areas in 40-65 year old Douglas-fir stands for differences in pH, oxalate content and mineral weathering. Oxalate was 20 times higher in *H. crassum* mats compared to uncolonized soil to a depth of 10 cm. They proposed oxalate abundance could accelerate primary mineral weathering from clays, particularly Fe and Al, improving host plant nutrition. Soil colonized by *H. crassum* also had significantly reduced pH (4.9) compared to uncolonized soil (6.1). Additionally, their quantification of mat occurrence found that mats can colonize >28% of the forest floor surface and >16% of the upper 10 cm of soil. Together with evidence from mat research in Canada (Fisher, 1972) and Finland (Hintikka & Haykki, 1967), a large effort to explore the biochemistry, diversity and dynamics of mats in the PNW began.

1.2.1. History of Pacific Northwest Ectomycorrhizal Fungal Mat Research

The 27 years spanning 1979-2006 yielded data on mats in the Oregon Coast Range and the Cascades, particularly the HJA that laid the foundation for recent research undertaken by the MO. Although microbial genetic methods became commonplace, the lapse in research during the late 1990's and early 2000's left many questions unanswered until 2007. Here, a summary of this early work is provided.

Following up on evidence from Cromack *et al.* (1979), Knutson *et al.* (1980) were interested in the co-occurrence of calcium oxalate-utilizing organisms with the mat-forming species *H. setchellii*, previously shown to produce large amounts of hyphal-associated oxalate crystals (Graustein *et al.*, 1977). They observed the highest quantities

of calcium-oxalate utilizing organisms from the mycorrhizosphere of *H. setchellii*, namely *Streptomyces* spp., which may utilize the low-energy salt as an energy source. This was the first study to address relationships between mats and associated microbiota.

Cromack *et al.* (1988) assessed soil animal populations of *H. setchellii* mats vs. non-mat areas in 50- to 75-year-old Douglas-fir forests and found increased abundance of soil invertebrates (mites, Collembola, nematodes) and protozoa (amoebae, ciliates) in mats. They hypothesized that these organisms may be opportunistically located to utilize nutrient rich fungal tissues. Additionally, these mat soils exhibited higher rates of respiration, phosphatase, protease and activity of enzymes capable of decomposing complex C-compounds.

Analysis of seasonal variations in the chemistry of soils associated with *H. setchellii* and adjacent non-mat soils was conducted by Griffiths *et al.* (1990) in 70-year-old Douglas-fir stands. Mats had higher respiration rates, microbial biomass carbon, acetylene reduction activity and mineralizable N, while having lower pH and denitrification rates. This may lead to the accumulation of N within mats. Additionally, peak mat respiration occurred during periods of maximum host photosynthetic activity (spring/fall) and the mats studied induced tighter N-cycling compared to non-mat areas.

To better gauge microbial biomass of mats, Ingham *et al*. (1991) compared direct versus fumigation-flush estimates for mat and non-mat soil. They found fumigation as an estimate of fungal hyphal biomass to be flawed as soils with distinctly visible hyphae from mats yielded low biomass numbers. However, the use of fumigation was found to be a useful measure of current soil fertility as it measures pools of available C or N. Direct measurements yielded 10- to 300-fold increases in fumigation biomass measures and *Hysterangium* mat rhizomorphic material was found to account for up to 50% of overall soil dry weight, whereas no rhizomorphic material was found in non-mat soils.

Entry *et al.* (1991b) explored litter decomposition and nutrient release in second-growth Douglas-fir forest *H. setchellii* mats and adjacent non-mat areas. Microbial biomass was four times greater in mat soils, and nutrient release from needles was higher in mat areas for N, P, K and Mg, suggesting that the microbial communities of mat

microenvironments more efficiently remove these nutrients from organic matter and may transfer them to host trees. In a second paper, Entry *et al.* (1991a) tracked ^{14}C-labeled lignin, cellulose and microbial biomass degradation in *H. setchellii* mat and adjacent non-mat soils. Microbial biomass and cellulose degradation rates were as much as six times higher in mat soils and lignin degradation was also greater. They suggested that the mat microenvironment increased microbial activity resulting in enhanced organic matter degradation of varying complexities. Entry *et al.* (1991b) further debate whether the nutrient release mechanism is direct saprotrophic action of *H. setchellii* or other saprotrophic organisms with which *H. setchellii* is in proximate association to capitalize on indirect nutrient release.

In these early studies, *H. setchellii* mats in soil litter was the primary focus. However, numerous mat types formed by other EmF and located in different areas of the soil profile had unique characteristics similar to *Hysterangium*. The first published efforts to differentiate between mat species in the PNW began in 1991. Griffiths *et al.*, (1991b) followed up on observations that *H. setchellii* mats were perennial features and explored other mat-forming species in the genus, as well as those formed by *Gautieria monticola* in the mineral soil, and microfloral associates of these mats. Differences between the two fungal mat-forming species were readily evident: (1) *G. monticola* mats were significantly drier than non-mat soils whereas there was no difference observed between non-mat and *H. setchellii* mats and (2) chloroform fumigation flush C was greater in *Hysterangium* samples than *G. monticola* and both were greater than non-mat soils. This study highlighted the uniquely hydrophobic nature of *G. monticola* mats, as compared to *H. setchellii*, and proposed different mat-forming EmF may have functionally varied roles; this may increase forest soil microbial community heterogeneity and thus overall diversity.

Combined with the above-mentioned study, Griffiths *et al.* (1991a) surveyed mid-successional Douglas-fir forests to quantify a previous observation that all establishing seedlings were intimately associated with mats of either *H. setchellii* or *G. monticola*. All Douglas-fir seedlings were associated with mats, whereas western hemlock seedlings

were either associated with mats or decaying wood, the latter being a life-history strategy for the species. The authors contend that mats may enable seedling establishment by increasing mycorrhization opportunity within mats, supplying carbohydrates to deeply shaded seedlings from established host tree photosynthate through the mycelial network and reduced root pathogen infection. This may be particularly important to the shade-intolerant Douglas-fir compared with a more shade-adapted western hemlock. Interestingly, the authors point out the probable diversity of EmF and soil microbial communities in non-mat areas, but contend that mat soil is characterized by a single mat-forming species and apparently excludes other EmF, decreasing diversity. Taken together, Griffiths *et al.* (1991a, b) suggest that mat communities, although having a reduced diversity themselves, may harbor unique microbial communities that enhance overall microbial diversity in PNW forests.

In a research summary, Griffiths and Caldwell (1992, references therein) highlighted elevated cations, dissolved organic carbon and oxalate associated with mats. They interpreted this as strong evidence organic acids enhance weathering in mats and fungal successional stage may also define nutrient use strategies, with late successional EmF possessing enhanced capabilities to access organic nutrients. Novel research by Griffiths and Caldwell (1992) examined enzyme activities of *Hysterangium* and *Gautieria* mats. Enzymes responsible for organic matter decomposition were higher in both mat types compared to non-mat areas. *Hysterangium* mats were higher than *Gautieria* for cellulase/proteinase whereas the opposite was true for peroxidase; phosphatase activity was higher than non-mat soils for both mat types but was geographically dependent between the two mat-forming genera. The heightened peroxidase activity may indicate capacity to access “recalcitrant” nutrients bound by complex organic molecules. Mats capability of hydrolyzing RNA was also found and provided functional support for increased phosphatase in mats. However, the authors contend that elevated mat enzyme activity still cannot be attributed to EmF directly and may be the result of associated saprotrophic organisms cohabitating mats. Unpublished

data reported in Griffiths and Caldwell (1992) note that siderophore-like compounds were elevated in mats and thus may enhance their competitive advantage.

Mats also efficiently retain nutrients and prevent leaching. This is particularly important in PNW old-growth forests where tight regulation of nutrient flows is necessary for continued forest productivity. Entry *et al.* (1992) examined microbial biomass and nutrient concentrations in *H. setchellii* mat and adjacent non-mat areas and found *H. setchellii* capable of concentrating nutrients in hyphal tissue. This may indicate productivity-limiting nutrients are released through organic matter decomposition and retained in mats; higher oxalate production in these mats may increase retention by binding free Al and Fe that would otherwise immobilize nutrients. These mats may act like a biochemically active filter membrane for the benefit of host plants.

Aguilera *et al.* (1993) found that as forest soils become enriched in organic N with age (>99.99% of total N), areas colonized by mats selectively remove soil organic N, leaving high C:N compounds behind; of the five age-classes examined, only old-growth showed statistically significant differences. These data led the researchers to deduce mineralized labile N in mat soils may be selectively removed by the EmF of mats and translocated to host trees more efficiently than in areas not colonized by mats.

Griffiths *et al.* (1994) examined the soil solution chemistry of *H. setchellii* and *G. monticola* mats as compared to non-mats in mid-successional Douglas-fir stands and found dissolved organic C, oxalate, phosphate, sulfate, H, Al, Fe, Cu, Mn and Zn to be higher in mat soil. Additionally, the correlation between dissolved organic carbon or oxalate and phosphate indicates these organic acids may be responsible for increased phosphate and trace nutrient weathering in mats. Of the two mats studied, *G. monticola* mats had much higher oxalate concentrations than did *H.* setchellii, suggesting enhanced mineral weathering capabilities; however, *H. setchellii* mats seemed better adapted to decompose organic matter.

Griffiths *et al.* (1995) surveyed the distribution of mats in old-growth Douglas-fir forests in relation to proximity of EmF, AmF and understory trees. They found mat incidence to be higher at the base of all trees, regardless of host mycorrhizal status, ant

that incidence was positively correlated with tree size. The authors hypothesized that mat enrichment may be the result of increased protection of young trees from root pathogens; stem-flow and canopy drip may locally elevate nutrient levels (particularly N) and/or litter deposition may be higher. In a follow-up paper, Griffiths *et al.* (1996) further examined mat spatial distribution and found them to be influenced by proximity of one mat to another, distance to the closest living tree, living tree density and forest successional age, the latter leading to increased yet varied mat size.

To summarize, research in the years spanning 1979-2006 demonstrated that mats: (1) are formed by multiple fungal genera that can occupy different areas of the soil profile; (2) may vary in their ecological and physiological roles; (3) have the capacity to accelerate mineral weathering and decompose organic matter to access tightly bound nutrients; (4) tend to have higher microbial biomass than non-mat soil; (5) can selectively remove organic N and other mineral elements from the soil, concentrating them in hyphal tissue; (6) produce large amounts of oxalate and support calcium-oxalate utilizing organisms; (7) support larger populations of soil animals; (8) may be perennial features in the PNW; (9) have lower pH and higher respirations rates; (10) efficiently retain nutrients and prevent loss; (11) support the establishment of Douglas-fir seedlings; (12) occur at a higher incidence at the base of trees and correlate with tree size and proximity to other mats; (13) have differing dynamics in old-growth and young forests; and (14) create specialized microhabitats that may locally decrease, but ultimately increase, microbial diversity in forest soil.

1.2.2. Microbial Communities Associated with Ectomycorrhizal Fungi and Ectomycorrhizal Fungal Mats

Ecosystem models are beginning to integrate the frequently described “black box” (Horton & Bruns, 2001) of soil microbial communities, particularly their roles as drivers and regulators of nutrient fluxes. A recent paper by Phillips RP *et al.* (2013) suggested the mycorrhizal status of temperate forest trees may be a fundamental predictor of carbon-nutrient cycles; forests dominated by EmF trees (e.g. PNW) function on an

organic nutrient economy caused by slow plant biomass turnover and efficient nitrogen utilization. EmF and associated microbial communities may be spatially structured in the soil profile to capitalize on microsite variation. It has repeatedly been shown that EmF (Dickie et al., 2002; Landeweert et al., 2003; Bueé et al., 2007), and other microbiota (Jumpponen et al., 2010; Eilers et al., 2012), exhibit vertical niche differentiation in the soil profile, a factor that contributes heavily to soil microbial diversity and establishes depth as a strong environmental gradient for microbial community structure and function. As organic matter decreases with depth, microbial communities likewise shift substrate decomposition capabilities. This idea is uniquely evident for mat-forming fungi in forests of the PNW, as *Hysterangium* and *Gauteria,* as well as *Piloderma* and *Ramaria* mats (discussed below), stratify in organic and mineral horizons, respectively.

A microbial ecology paradigm suggests that as a habitat changes the mode by which bacteria colonize that habitat will also change (Nazir *et al.,* 2010). Soil microhabitat heterogeneity is immense in type and scale, and varies based on pH, texture, organic matter, moisture, temperature and depth, to name a few. The mycosphere, and indeed the mycorrhizosphere, represent discrete microhabitats where bacteria cohabitate with fungi in associations distinct from bulk soil. Mats offer a unique opportunity to examine the structural differences in microbial community composition both within and outside of mats; mat hyphal proliferation almost guarantee fungal-bacterial associations occur with the mat-former. Nazir *et al.* (2010) contend the mycorrhizosphere represents a “nutritional hotspot” for soil bacteria in an otherwise C-limited environment due to their direct linkage to plant photosynthate. Interestingly, the mat microhabitat as a whole can be quite stable for associated microorganisms as they are persistent or perennial features in PNW forests (Dunham *et al.,* 2007; Trappe *et al*., 2012). In return for stable C-sources from tree to soil, as well as colonization sites, soil microbes may indeed enhance this tripartite partnership across kingdoms by solubizing phosphate, fixing nitrogen and acquiring other mineral nutrients (Nazir *et al.,* 2010). Indeed, as Kluber *et al.* (2010) point out, it would not be sensible to assume that EmF alone are responsible changes in mat soil, as associated organisms likely play important roles.

Early on, Mosse (1962) recognized bacterial selection by fungal-affected soils. Warmink *et al.* (2009) observed decreased mycosphere bacterial diversity compared to the bulk soil, suggesting mycosphere selection of a functional bacterial subset. On the other hand, associated bacteria may exert substantial influence on their fungal host (Johansson *et al*., 2004). In fact, numerous bacterial taxa are regarded as more conspicuously associated with fungi in various environments, including: (1) calcium-oxalate degrading *Streptomyces* with mats (Knutson *et al*., 1980); (2) the universal fungiphile *Rahnella aquaticus* (Warmink *et al*., 2009); (3) *Paenibacillus* spp. and certain *Pseudomonas* spp. with *Glomus intraradices* (Mansfeld-Giese *et al*., 2002); (4) members of the Burkholderiales including Oxalobacteriaceae, *Rubrivivax* and Comamonadaceae with AmF (Offre *et al., 2007* & 2008); (5) *Clostridium* spp. and *Azospirillum* spp., known nitrogen-fixers, with the EmF of Douglas-fir (Li & Hung, 1987); (6) numerous truffle brûlé associated taxa (Mello *et al.,* 2013); (7) distinct ectomycorrhizospheres harboring similar bacterial phyla and genera (Uroz *et al.,* 2012); and (8) different bacterial communities found in ectomycorrhizae and the surrounding soil (Vik *et al.,* 2013). The term "mycorrhiza-helper bacteria" describes active bacterial promotion of mycorrhizal development and function (Garbaye, 1994; Fey-Klett *et al.,* 2007; Barbieri *et al,* 2012), of which many aforementioned taxa belong. In a recent study, Uroz *et al.* (2013) performed enzyme assays on bacterial communities along a soil-mycorrhizosphere continuum and determined EmF preference for bacterial communities that functionally differ from the bulk soil. Mat-colonized soil provides a strikingly unique environment for fungal selection of bacterial taxa and, as such, has been recently explored.

1.2.3. Recent Ectomycorrhizal Fungal Mat Research in the Pacific Northwest

Nearly three decades of research addressed many questions, yet even more remained unanswered. Are the diversity of mat-forming EmF greater than previously determined and do these taxa differ with forest successional stage? Are there distinct microbial communities associated with mats and, if so, what taxa are responsible for those differences? What are the mechanisms responsible for the mineral nutrition of mats

and how do they differ between various mat types? Does the large proliferation of fungal hyphae play a role in the nutritional dynamics of mats? How do soil properties and other variables influence the diversity of mat types? How do mat communities influence forest soil respiration and affect forest soil biogeochemistry? What are the dynamics associated with the development and decline of mats and do these differ by horizon or mat-former?

Before the development of molecular tools to assess soil microbial diversity, Unestam (1991) described mats as homogeneous mycelia of interwoven hyphae and rhizomorphs all belonging to the same species, thus excluding other mycorrhizal fungi. Since then, it has become clear that mat mycorrhizal diversity, as well as the diversity of other fungi and bacteria, is not as restricted as previously thought. Trappe et al. (2012) clearly articulate this diversity in their assessment of mats at Crater Lakes National Park, Oregon, by noting the frequent occurrences of sporocarps from one taxon originating from the center of a mat of another taxon; the researchers further note that mats contain hyphae from numerous sources, although genetic methods are more reliable than the eye to capture phylogenetic diversity. Decades of research concerning the ways in which mats alter the soil environment support the idea that mats may possess unique microbial communities as compared to non-mat areas; recent research has endeavored to answer that question, as shown below.

In 2007, the first experiment to characterize mat-formers based on molecular typing of EmF root-tips and associated rhizomorphs was published (Dunham *et al.*, 2007), beginning the era of molecular-based diversity work on mats in the PNW. Previously, EmF species comprising mats were identified based on morphological characteristics or, when possible, fruiting bodies. Ultimately, Dunham *et al.* (2007) showed that mat-forming fungi had likely been misidentified in old-growth forests, as their data showed *Piloderma* spp. and *Ramaria* spp. to be the dominant mat-formers; *Piloderma* most frequently exhibited the characteristics thought to be *Hysterangium*-like while *Ramaria* assumed characters that typified *Gautieria*-like mats. In fact, *Piloderma* mats were found at 76.5% of old-growth sites, by far the most common genus. The diversity of mat-forming species was also high, particularly in mats formed in the mineral

soil; four unique *Piloderma* spp. formed mats in the organic horizon while 11 *Ramaria* spp. formed mineral horizon mats. New mat-forming genera were identified and their diversity increased with forest succession; old-growth harbored the highest diversity.

In old-growth forests of the Oregon Cascades the dominant rhizomorphic mat-forming fungi in the organic/mineral interface commonly belong to the genus *Piloderma* (Atheliaceae, Basidiomycota); the hydrophobic, powdery mats in the upper mineral horizon are now considered predominantly *Ramaria* (Gomphaceae, Basidiomycota) (Dunham *et al.,* 2007; Kluber *et al.,* 2010; Kluber *et al.,* 2011; Hesse, 2012). These two genera were responsible for about 80% of sampled mats in old-growth stands at the HJA (Dunham *et al.,* 2007) and represent two phylogenetically independent origins of the EmF habit (Hibbet & Matheney, 2009); other genera form mats less-frequently in these forests. The association of *Piloderma* and *Ramaria* with old-growth forests is not new; in fact, rhizomorphic structures of *Piloderma fallax* were found more frequently in old-growth than young or rotation-age Douglas-fir forests, while presence and quantity of coarse woody debris were highly correlated with frequency (Smith *et al.*, 1996; Smith *et al.*, 2000). Certain *Ramaria* species were entirely unique to old-growth (Smith *et al.*, 2002).

Kluber *et al.* (2010), comprehensively followed up on previous mat research and examined the extent to which horizon-specific mats altered forest soil biogeochemistry in old-growth and second-growth Doug-fir forests. Fungal associated enzymes, including phenoloxidase, β-glucosidase, chitinase, phosphatase, and protease, as well as a suite of soil chemical properties, were examined in both organic and mineral horizon mat and non-mat soils. The enzymatic profiles of organic horizon rhizomorphic mats were consistently greater than organic soil lacking mats, especially chitinase (1.7x), while the soil chemical properties of oxalate concentration and pH were higher and lower in mats, respectively. A different pattern was observed for enzymatic profiles of mineral horizon hydrophobic mats compared with non-mat soils. Mats expressed over two times higher chitinase, phosphatase and phenoloxidase activities; however, when normalized by microbial biomass carbon, the mineral enzyme profiles did not significantly differ,

whereas the organic profiles did. Soil chemical properties between mat and non-mat soils in the mineral horizon were also different; hydrophobic mats had 40 times higher oxalate content, higher ammonium concentration, higher soil organic matter and lower pH than non-mat soils. Most importantly, the data suggested regardless of mat-former identity, hydrophobic mineral mat soil exhibited similar enzymatic profiles, whereas the profiles of rhizomorphic mats in the organic horizon varied between mat-forming taxa. Ultimately, the authors hypothesized that the differing enzyme activities in mats compared to non-mat soil may be governed by distinct microbial communities.

In a follow-up study, Kluber *et al.* (2011) examined the fungal and bacterial communities of old-growth Douglas-fir forest *Piloderma* mats compared to non-mat soil using clone libraries and T-RFLP. Confirming previous results, chitinase activity was, on average, 1.4 times greater in *Piloderma* mats, with a high degree of seasonal variability. During the spring and fall, cool and wet seasons with peak photosynthesis and microbial activity, chitinase was higher in mats; no differences were observed during dry summer months when metabolic activity is low. For both soil fungal and bacterial communities, the authors found significant differences between *Piloderma* mat and non-mat soils while also noting substantial site-to-site variation. Interestingly, for soil fungi, removal of the mat former from the analysis did not change the results. Overall, *Piloderma* mat fungal communities were abundant in members of the Atheliales, while non-mat communities were high in Sebacinales, Russulales and Agaricales; *Cenococcum geophilum* was universally abundant, a common occurrence in PNW Douglas-fir forests (Trappe, 1964; Luoma & Eberhart, 2014). Myxotrichiaceae proved to be a strong indicator of non-mat soils. At the phylum level, percentages of Ascomycota and Basidiomycota were generally equal. *Piloderma* mat bacterial communities had higher abundance of γ-Proteobacteria, while Acidobacteria were more abundant in non-mat communities. Indicator species analysis indicated Sphingobacteria, Acidobacteria and an Actinobacterial taxon indicated non-mat soils while α-Proteobacteria, β-Proteobacteria and a different Actinobacterial taxon indicated *Piloderma* mats. Rarefaction curves revealed a trend of lower bacterial richness in *Piloderma* mats; overall fungal and

bacterial population sizes did not differ between *Piloderma* mat and non-mat soils, though seasonal fluctuations were strong and increased during metabolically active times (spring and fall). Kluber *et al.* (2011) concluded *Piloderma* mats host unique microbial communities compared to non-mat soils and although their populations and activities changed seasonally, composition remained the same. The question remained, however, as to the specific taxonomic identities of the fungi and bacteria responsible for differences in community composition, especially for *Ramaria* mats which were not examined by Kluber *et al.* (2011).

Hesse (2012) went further, by deep-sequencing fungal and bacterial communities of old-growth Douglas-fir *Piloderma* and *Ramaria* mats and adjacent non-mat soils. To date, this is the most intensive sampling and molecular sequencing effort on these soils. Hesse (2012) found distinct separation between soil fungal communities in *Piloderma* and *Ramaria* mat and respective non-mat soils, corroborating Kluber *et al*. (2011); mat groupings explained ~40% of community variation. Unlike Kluber *et al.* (2011), however, removal of the mat-forming taxa blended the differences between mat and non-mat communities while retaining the tight grouping of non-mat samples. Hesse (2012) noted that mat-former inclusion was more important with *Ramaria* mats, as *Piloderma* mats retained structure after *Piloderma* sequences were removed. Regardless of soil type, these fungal communities were hyperdiverse (highest pooled treatment richness was 799, 95% OTUs occurring more than four times) and spatially heterogeneous, indicating the need for high-throughput sequencing to accurately capture fungal communities and describe differences. The non-mat organic horizon soils exhibited highest fungal species richness, while *Ramaria* mat mineral horizon soils were least rich. As expected, *Piloderma* and *Ramaria* were found to be the most abundant taxa in their respective mats, while *Russula* frequently dominated non-mat soils; non-mat soils low in *Russula* were instead rich in *Inocybe*, *Hygrophorus*, *Cenococcum*, *Hydnum* and *Tricholoma*. Frequent, co-occurring taxa for all treatments included *Byssocorticium*, *Inocybe*, *Cenococcum*, *Hygrophorus*, *Tricholoma*, *Hygrocybe*, *Hydnum* and *Gautieria*, all of which are EmF.

Additionally, the active root-tip EmF community in mats was a good reflection of the relative abundance of those taxa in the bulk soil.

Hesse (2012) also showed that *Piloderma* and *Ramaria* mats influence the soil bacterial community in similar ways as the soil fungal community, yet painted a different picture. When analyzing only bacteria without regard for the fungal community, only *Piloderma* mat and respective non-mat communities were different; *Ramaria* mat bacterial communities did not represent a distinct group. Bacterial families strongly associated with *Piloderma* mat soil included Acidobacteria Gp1, Acetobacteraceae, Actinospicaceae, Sphingobacteriaceae and Microbacteriaceae, while those strongly associated with non-mat soil included Comamonadaceae, unknown Burkholderiales, Acidobacteria Gp17, Unknown Betaproteobacteria, Verrucomicrobia Subdivision 3, Acidobacteria Gp6, Unknown Spartobacteria and Acidobacteria Gp4. Soil pH and horizon were extremely strong correlates with bacterial community differences; acidophilic bacteria were more abundant in lower pH mat soils. When analyzing both bacterial families and fungal genera together, bacterial families strongly associated with *Piloderma* mats included Sphingobacteriaceae, Burkholderiaceae, Microbacteriaceae and Acetobacteriaceae, whereas none were associated with *Ramaria* mats; non-mat soils correlated with Acidobacteria Gp6 and an unclassified bacterium. Non-mat organic horizon soils harbored the highest number of unique bacterial OTUs; in contrast, both *Piloderma* and *Ramaria* mats had the lowest OTU richness. Ultimately, bacterial communities in *Piloderma* mat soils were qualitatively distinct from non-mat soils; *Ramaria* mats were quantitatively different.

Hesse (2012) conclude by noting mats in old-growth Douglas-fir forests of the PNW exhibit unique fungal and bacterial communities compared to their non-mat counterparts, with a stronger differentiation in the organic horizon for *Piloderma* mats. Bacterial communities, as opposed to fungal communities, were more strongly structured based on soil pH and horizon. Additionally, for the first time, and independent of mat status, this study showed the direct correlation of fungal and bacterial community richness in this, or any, forest system.

Moving away from community analyses, Phillips *et al.* (2012) quantified the *in situ* contribution of *Piloderma* mats on forest soil respiration; uniquely, as is inherent in laboratory incubations, roots were not severed from EmF host plants. Carbon flux from the organic horizon of these soils was substantial, about 73% of total soil respiration; mat-colonized soil represented about half of the total area. CO_2 efflux from *Piloderma* mats averaged 16% higher than non-mat soils across two growing seasons, a substantial difference ─ however, much lower than previous incubation studies (Griffiths *et al.*, 1990); the large discrepancies may be explained by disturbance caused by severing roots in lab incubations. Increased chitinase activity was also found in *Piloderma* mat soils and correlated with respiration rate. Interestingly, over two-years, a small number of previously identified *Piloderma* mats lost the visual characteristics that define this mat-type, while a few non-mat areas exhibited mat development.

Zeglin *et al.* (2013) explored the importance of chitin and *N*-acetyl glucosamine (NAG), the chitin monomer, turnover has on C and N cycling in old-growth Douglas-fir forest *Piloderma* mat and non-mat soils. *Piloderma* mat soils showed higher basal and induced biomass levels, respiration, N mineralization and chitinase rates in laboratory incubations where mycorrhizal roots were severed from their hosts. The authors noted the results may reflect inherent differences in fungal and bacterial community composition as previously determined (Kluber *et al.*, 2011; Hesse, 2012), and confirmed nutrient cycling rates differed between *Piloderma* mat and non-mat soil. Zeglin *et al.* (2013) note N limitation in these old-growth forest soils and fungal cell wall decomposition is primarily driven by N demand; however, non-mat soils did exhibit greater microbial C limitation than *Piloderma* mats with the addition of NAG and the authors hypothesized a “leaky” C recycling dynamic in mat soils as EmF access tree C. Importantly, potential chitinase activity did not increase with the addition of chitin, particularly in *Piloderma* mat soils; therefore, a saturation of natural chitinase production may exist.

In a follow-up incubation experiment, Zeglin and Myrold (2013) tracked the fate of chitin enriched fungal cell wall material in the same soils. They determined fungal cell

wall N was the primary substrate for microbial growth and N conservation in this old-growth system could be attributed to efficient microbial "recycling." Additionally, chitinaceous C significantly supported microbial metabolism. *Piloderma* mat soils showed higher assimilated chitinaceous C, cumulative respiration and potential chitinase activity; however, the differences between *Piloderma* mat and non-mat soil were small. The authors reiterated the idea that specific microbial taxa, which have been shown to differ between *Piloderma* mat and non-mat soils (Kluber *et al*., 2011; Hesse, 2012), may be responsible for the higher integration of fungal cell-wall C growth yield efficiency of *Piloderma* mats. They suggested further experimentation to determine taxa responsible for C/N retention and turnover within these two microbial communities.

Finally, Trappe *et al.* (2012) examined the diversity of mat-forming fungi at Crater Lake National Park, Oregon. This survey correlated fungal mats with soil properties, disturbance and ponderosa pine/white fir or mountain hemlock/noble fir forest ecotype. *Piloderma* mats, the most common mat-type and for which three distinct genotypes were identified, significantly and positively correlated with soil C:N ratio corroborating Aguilera *et al.* (1993). *Piloderma* mats were closely associated with coarse woody debris. *Ramaria* mats, both EmF and saprotrophic, were commonly found; however, analysis focused on the saprotrophic *Ramaria stricta* species complex. The authors suggested further evaluation of microbial communities associated with mat and adjacent non-mat areas to discern the functional roles of taxa responsible for differences.

1.2.4. Blanchard (2008) - Previous Research on the Current Project

In June, 2006, Blanchard (2008) began the experiment presented here. Refer to the methods section (Figure 1; Figure 2) or Blanchard (2008) for clarification of experimental design and hypotheses. A subset of seven old-growth sites (400+ years) at the HJA were selected from 17 evaluated by Dunham *et al.* (2007) for the presence of both *Piloderma* and *Ramaria* mats. At each site, two *Piloderma* (organic horizon), two *Ramaria* (mineral horizon) and respective adjacent non-mat soils were selected to accommodate four temporal sampling dates; soils were reciprocally transferred between

mat and non-mat areas in each horizon in an effort to track the development (birth) and decline (death) dynamics of each mat microbial community. T-RFLP profiles were used to track soil fungal and bacterial communities 10 months (April 2007), 16 months (October 2007) and 24 months (June 2008) after trial establishment. Blanchard (2008) hypothesized the birth of mats would gradually develop microbial communities that typify a natural mat community, while the death of mats would result in the rapid deterioration of the mat microbial community, ultimately becoming similar to non-mat soils.

Initial soil analyses (reproduced as Table 1) revealed both mat types exhibited increased P, K^+, Mg^{2+}, Na^+ and NH_4^- compared to their non-mat counterparts, while Ca, NO_3^- and total N did not differ between mat and non-mat soils. Soil pH was lower in both mats compared to their non-mat counterparts. After 24 months, birth treatments maintained pH levels resembling the non-mat soil from which they originated; however, death treatments showed a marginal pH increase.

Fungal community composition of organic horizon (*Piloderma*) treatments indicated a clear separation of *Piloderma* mat and non-mat soils at all sampling dates, confirming differences seen in other studies (Kluber *et al.*, 2011; Hesse, 2012); additionally, the removal of *Piloderma* T-RFLP peaks did not alter the results. The *Piloderma* death treatment quickly lost similarity to the *Piloderma* mat fungal community (by 6 months) and were indistinguishable from non-mat treatments after 24 months. Overall *Piloderma* mat community development was not documented in the *Piloderma* birth treatments, even after 24 months, as this treatment remained similar to non-mat soil fungal communities. The fungal community composition of mineral horizon (*Ramaria*) treatments behaved similarly to those in the organic horizon.

Bacterial community composition of organic horizon (*Piloderma*) treatments did not exhibit differences, except in the spring, when *Piloderma* mats differed from non-mat treatments. Significant seasonal separation in soil bacterial communities was observed; Blanchard (2008) posits that spring-season differences may have resulted from altering the dilution factor of bacterial DNA before sequencing. Mineral horizon (*Ramaria*)

treatment bacterial communities behaved similarly to the organic horizon; however, *Ramaria* mat and non-mat soils always had similar community composition.

Fungal and bacterial diversity was consistently lower in *Piloderma* mats than non-mat soils at all sampling times; *Piloderma* death treatments, though high in singletons, generally had the highest diversity and most closely resembled non-mat treatments. *Ramaria* mats exhibited reduced fungal and increased bacterial diversity compared to non-mat treatments; however, *Ramaria* death diversity was lower than both *Ramaria* mat and non-mat treatments. *Ramaria* birth diversity resembled that of *Ramaria* mat treatments; however, there was a decrease and increase in fungal and bacterial diversity over time, respectively.

Blanchard (2008) also quantified root-tip colonization by mat formers in each treatment. No colonization was found in birth treatments of either mat-type after 10 months. After 16 months, both mat types had root-tips colonized by the respective mat former, but these numbers were not significantly different from non-mat soil. After 24 months, the number of *Piloderma*-colonized root-tips in the birth treatment did not differ from *Piloderma* mat soils, while *Ramaria* birth remained lower than *Ramaria* mat treatments. Surprisingly, *Ramaria*-colonized root-tips began to appear in the death treatment after 24 months. Indicator T-RFLP peaks for *Piloderma* exhibited the same trend as root-tips in birth treatments. Unfortunately, *Ramaria* diversity prevented identification of T-RFLP peaks for this genus.

1.3. Project Objectives and Hypotheses

The rhizosphere has been subdivided to include the ubiquitous association of mycorrhizal fungi with plant root systems. The mycorrhizosphere and the hyphosphere describe the zones influenced by the root-mycorrhizal interface and the zone affected by extramatrical mycorrhizal hyphae, respectively (Linderman, 1988). Extramatrical mycelial networks of AmF are small compared to the extensive hyphal and rhizomorphic proliferations of some EmF, including *Piloderma* and *Ramaria* (Agerer, 2001). The ‘ectomycorrhizospheres’ formed by these fungi are substantial and have been classified

into the medium distance exploration mat-subtype (Agerer, 2001), where interwoven hyphae of the mat-forming fungus create distinct zones of influence. As previous research has both posited **(**Griffiths *et al.*, 1991a/b; Dunham *et al.*, 2008**)** and shown (Blanchard, 2008; Kluber *et al*, 2011; Hesse, 2012), mats of old-growth Douglas-fir forests formed by *Piloderma* (organic-horizon) and *Ramaria* (mineral-horizon) exert selective pressure on soil microbiota that support unique fungal and bacterial communities, in contrast to adjacent areas where mats are not present.

Little is known about how mat microbial communities develop (birth ─ colonize new soil not previously influenced by a mat) and decline (death ─ how microbial populations change as a once healthy mat senesces). The research presented here is the final sampling of the study established by Blanchard (2008), at 51-months (~ 4 years). Blanchard (2008) had not examined fungal and bacterial species directly, as T-RFLP profiles are limited, and did not focus on the EmF root-tip community aside from mat-formers.

For that reason, we sequenced the diversity of active EmF root-tips and used 454-pyrosequencing to obtain taxonomic profiles of soil fungi and bacteria to thoroughly examine mat development and decline dynamics. Birth treatments were of particular interest as their development to a mat state had not occurred after two years. We hypothesized: (1) mat and non-mat soils would harbor distinct microbial communities 4 years after identification of mat features; (2) birth treatments would have continued to develop mat characteristics such that their microbial communities were indistinguishable from those of natural mats; (3) death treatments would completely lose the mat community structure and closely resemble non-mat communities; (4) each microbial component would respond similarly; (5) reciprocal transfer disturbance would have a negligible effect on microbial community composition; and (6) *Piloderma* and *Ramaria* mats, in two separate soil horizons, would control microbial communities in unique ways. A hypothesis diagram is provided below for clarification of microbial community trajectories (Figure 1).

2. Manuscript Introduction:

The mycorrhizal association is diverse in both phylogeny and form; it is pervasive in land plants, occurring in over 80% of extant taxa (Wang & Qui, 2006), includes members in most major fungal and plant lineages (Cairney, 2000) and assumes numerous morphologies (Smith & Read, 2008). It is an ancient and ubiquitous symbiosis between plant and fungus that links above and below-ground biogeochemical processes and profoundly influences forest ecology. Ectomycorrhizas, the most frequent and widespread mycorrhizal type in temperate and boreal forests (Alexander, 2006), are formed when fungal hyphae ensheath the fine root-tips of host plants, forming a mantle, and establish an intercellular hyphal network between root cortical cells, known as the Hartig-net (Smith & Read, 2008). It is at this root-hyphal interface where the plant exchanges photosynthate in return for nutrients and water acquired by fungal associates in the soil matrix. Species of ectomycorrhizal fungi (EmF) have evolved functional differences to exploit unique ecological niches (Hobbie *et al.*, 2014; Koide *et al.*, 2014; Walker *et al.*, 2014). Along with ericoid mycorrhizal fungi (ErM), certain EmF have the capacity to decompose complex C substrates for use as a nutrient and/or C source (Bergero *et al.*, 2000; Koide et al., 2008; Talbot et al., 2008; Cullings & Courty, 2009; Baldrian & López-Mondéjar, 2014; Koide et al., 2014). EmF communities can even exhibit higher hydrolytic enzyme activity than saprotroph-dominated communities (Phillips et al., 2013), a function that may be attributable to mycorrhizosphere bacteria (Garbaye, 1994; Frey-Klett *et al.*, 2007; Barbieri *et al*, 2012) and fungi (Griffiths & Caldwell, 1992; Cairney & Meharg, 2002; Porras-Alfaro & Bayman, 2011). Differential functional traits may also be related to EmF exploration strategies, which range from contact (*Russula*) to long-distance (*Rhizopogon*) exploration types (Agerer, 2001/2006; Hobbie & Agerer, 2010).

In the Pacific Northwest (PNW), USA, evergreen coniferous forests are dominated by the ecologically and economically important EmF tree species Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*) west of the Cascade

Mountain crest (Smith *et al.*, 2009). Among the thousands of EmF colonizing these tree species (Smith & Read, 2008), a subset are capable of forming uniquely prominent, specialized profusions of hyphae on the forest floor known as ectomycorrhizal fungal mats (mats). EmF mats are globally widespread in forested regions, tropical to temperate (Hintikka & Naykki, 1967; Fisher, 1972; Högberg, 1982; Alexander & Högberg, 1986; Castellano, 1988; Dung, 2012), yet are particularly robust in the PNW where they have been the focus of over 30 years of research concentrated at the HJ Andrews Experimental Forest (HJA) (Cromack *et al.*, 1979; Cromack *et al.*, 1988; Griffiths *et al.*, 1991a/b; Griffiths *et al.*, 1994,1996; Dunham *et al.*, 2007; Kluber *et al.*, 2010/2011; Hesse, 2012). Descriptions and definitions for mats abound (Trappe *et al.*, 2012), but recent research in the region has coalesced around the definition proposed by Dunham *et al.* (2007): "dense profusions of rhizomorphs associated with obvious ectomycorrhizal root-tips that aggregate soil and alter its appearance and are uniform in structure and appearance for an area at least 0.5 m in diameter."

Approximately 25 EmF genera contain species capable of forming mats with varying morphologies and distributions in soil profile (Agerer, 2001; Dunham *et al.*, 2007; Trappe *et al.*, 2012). Both EmF (Dickie et al., 2002; Landeweert et al., 2003; Bueé et al., 2007; Dickie & Koide, 2014) and other microbiota (Jumpponen et al., 2010; Eilers et al., 2012) exhibit vertical niche differentiation in the soil profile, a factor that contributes heavily to soil microbial diversity and establishes depth as a strong environmental gradient for microbial community structure and function. The dominant mat-forming fungi in old-growth forests of the PNW, *Piloderma* (Atheliaceae, Basidiomycota) and *Ramaria* (Gomphaceae, Basidiomycota), stratify in organic and mineral soil horizons, respectively; *Piloderma* forms rhizomorphic mats and *Ramaria* forms powdery, hydrophobic mats (Dunham *et al.*, 2007; Kluber *et al.*, 2011/2010; Hesse, 2012).

Along with depth, the patchwork of mat and non-mat soil represents a strong microbial structuring gradient where fungal and bacterial community composition of *Piloderma* and *Ramaria* mats are distinctly different from adjacent non-mat soil in each

respective horizon (Kluber *et al.,* 2011; Hesse *et al.,* 2012). Early mat research in the PNW focused on the genera *Hysterangium* and *Gauteria*, which, at a superficial level, can be thought of as analogous to *Piloderma* and *Ramaria* in form and function. In fact, Dunham *et al.* (2007), after identifying EmF mat-forming fungi with molecular methods, concluded many *Piloderma* and *Ramaria* mats may have been misidentified based on visual appearance.

Past research has demonstrated that mats: (1) have the capacity to accelerate mineral weathering and decompose organic matter to access tightly bound nutrients by altering forest soil biogeochemistry through extracellular enzyme production (Cromack *et al.,* 1979; Cromack *et al.,* 1988; Entry *et al.,* 1991a; Griffiths & Caldwell, 1992; Kluber *et al.,* 2010); (2) tend to have higher microbial biomass and respiration rates and lower pH than non-mat soil (Cromack *et al.,* 1979; Cromack *et al.,* 1988; Griffiths *et al.,* 1990; Ingham *et al.,* 1991; Entry *et al.,* 1991b; Kluber *et al.,* 2010; Phillips *et al.,* 2012); (3) can selectively remove organic N and other mineral elements from the soil, concentrating them in hyphal tissue and preventing loss (Griffiths *et al.,* 1990; Entry *et al.,* 1991b; Entry *et al.,* 1992; Aguilera *et al.,* 1993); (4) produce large amounts of oxalate and support calcium-oxalate utilizing organisms (Knutson *et al.,* 1980; Griffiths *et al.,* 1994; Kluber *et al.,* 2010); (5) may be perennial features in the PNW (Griffiths *et al.,* 1991b; Griffiths *et al.,* 1994; Dunham *et al.,* 2007; Phillips *et al.,* 2012; Trappe *et al.,* 2012); (6) vary in their ecological and physiological roles based on mat-former (Griffiths *et al.,* 1991b; Kluber *et al.,* 2010;); (7) support the establishment of Douglas-fir seedlings (Griffiths *et al.,* 1991a); (8) occur at a higher incidence at the base of trees and correlate with tree size and proximity to other mats (Griffiths *et al.,* 1995); (9) can colonize as much as 57% of the forest floor (Phillips *et al.,* 2012); and (10) create specialized microhabitats that may locally decrease, but ultimately increase, microbial diversity in forest soil (Griffiths *et al.,* 1991 a,b).

The discrete boundaries, distinct biochemistries, local dominance and unique microbiota of soil colonized by mats (Kluber *et al.,* 2010/2011; Hesse, 2012), as well as the importance of *Piloderma* and *Ramaria* to old-growth coniferous forest ecosystem

processes, represents an exceptional system to study development (birth) and decline (death) dynamics, and response to disturbance, of these mats. Blanchard (2008) presented two years of data tracking *Piloderma* and *Ramaria* development and decline; however, even after 24-months, mat communities were unable to colonize non-mat soil. Here, we present the final sampling of a reciprocal soil transfer experiment at 51-months, where we sequenced the diversity of active EmF root-tips, soil fungi and soil bacteria to capture a complete picture of microbial community dynamics. We hypothesized: (1) mat and non-mat soils would harbor distinct microbial communities 4 years after identification of mat features; (2) birth treatments would have continued to develop mat characteristics such that their microbial communities were indistinguishable from those of natural mats; (3) death treatments would completely lose the mat community structure and closely resemble non-mat communities; (4) each microbial component would respond similarly; (5) reciprocal transfer disturbance would have a negligible effect on microbial community composition; and (6) *Piloderma* and *Ramaria* mats, in two separate soil horizons, would control microbial communities in unique ways.

3. Materials and Methods:

3.1. Study Site Description

This study was conducted at the H.J. Andrews Experimental Forest (HJA), a 6,400 ha watershed located in the central western Oregon Cascade Mountains northeast of Blue River in Lane County, Oregon (N44°13'59", W122°10'34"). The HJA is part of the National Science Foundation's Long-Term Ecological Research network and cooperatively administered by the USDA Forest Service's Pacific Northwest Research Station, Oregon State University and the Willamette National Forest. Situated in the Pacific Northwest-North Pacific Ocean Bioclimatic region, the maritime climate consists of three moist seasons (fall, winter and spring), and one dry season (summer) from June through August (Dyrness et al., 1974). Precipitation occurs mainly from November to March, ranging from 230 cm at low elevations to 355 cm at high elevations, and mean monthly temperatures range from 1°C in January to 18°C in July/August (8.7°C average). Lower-elevation bedrock is composed of Oligocene-lower Miocene volcanic rock while Miocene andesite lava flows typify higher elevations. Abrupt and rugged topography steeply carved by streams and historical glaciation led to weak development of Inceptisols, Alfisols and Spodosols (Dyrness, 2005).

Throughout the HJA, 183 sites were previously characterized for mat presence during an NSF-REU research project on soil characteristics (Griffiths, 2002; unpublished data). Dunham *et al.* (2007) examined 17 of Griffiths' old-growth (350+ years) sites to determine the species richness and community composition of mat-forming fungi. This study utilized a subset of seven old-growth Douglas-fir (*Pseudotsuga menziesii*) sites from Dunham *et al.* (2007) based on confirmed visual and molecular presence of both *Piloderma* and *Ramaria* mats. Douglas-fir and western hemlock (*Tsuga heterophylla*) were the only EmF hosts, limiting variations in host plants and stand age that effect soil microbial community structure (Bach *et al.*, 2009). Figure 3 shows study site distribution within the HJA watershed and Table 2 provides descriptive site attributes.

3.2. Sampling Design

This study was installed in June 2006 as part of the HJA Microbial Observatory, to monitor the development and decline of *Piloderma* and *Ramaria* EmF mat microbial communities over time. At each of the seven sites, two *Piloderma* and two *Ramaria* mats were identified in the organic and upper mineral soil horizons, respectively, along with adjacent non-mat soils. Characters used for *in situ* mat identification included dense profusions of rhizomorphs associated with obvious EmF root-tips (*Piloderma*) and powdery/ashy hydrophobic soils associated with obvious EmF root-tips (*Ramaria*). All mats were ~ 0.5 m in diameter and the dominant mat-forming genera were confirmed through molecular analysis of the fungal ITS region (Blanchard, 2008). Numerous pseudoreplications of each treatment were installed to accommodate four temporal samples at each site. The results of the first three samplings (6, 12 and 24 months) are reported by Blanchard (2008), who used T-RFLP profiles to compare fungal and bacterial communities. We report results of the final sampling at 51 months (September, 2010). Blanchard (2008) examined baseline soil chemistries for each site and treatment; those data are reproduced for reference and discussion in Table 1.

Mat and non-mat regions underwent a reciprocal soil core (10 cm diameter, 20 cm length) transfer experiment to examine microbial community dynamics associated with the development (birth) and decline (death) of mats; reciprocal transplant experiments are common in the literature (Weinbaum *et al.*, 1996; Hart, 2006; Bottomley *et al.*, 2006; Bradbury & Firestone, 2012; Zumsteg *et al.*, 2013). Mat soil of each type was enclosed in PVC pipe (open at the top and bottom) to kill the EmF mat community and was transferred to an adjacent non-mat area, hereby referred to as *death* cores. Non-mat soil was enclosed in a 2-mm mesh barrier to allow root/hyphal ingrowth and transferred into each mat type, hereby referred to as *birth* cores. Non-mat soil was similarly enclosed in 2-mm mesh and replaced to control for disturbance effects, hereby referred to as *non-mat disturbance control* cores. Additionally, natural soil cores from each mat type and non-mat soil were taken to represent native microbial communities, hereby referred to *background* cores. Due to stratification in the soil profile (spatial separation), only

Piloderma organic horizons and *Ramaria* mineral horizons were of interest. This design resulted in a total of 10 treatments at each site: (1) *Piloderma* background or Pil_Bck; (2) *Piloderma* death or Pil_Dth; (3) *Piloderma* birth or Pil_Bth; (4) non-mat disturbance control organic horizon or NMDst_O; (5) non-mat background organic horizon or NMBck_O; (6) *Ramaria* background or Ram_Bck; (7) *Ramaria* death or Ram_Dth; (8) *Ramaria* birth or Ram_Bth; (9) non-mat disturbance control mineral horizon or NMDst_M; and (10) non-mat background mineral horizon or NMBck_M. This design yielded 70 samples (7 sites x 10 treatments/site). A schematic of the sampling design and a treatment key are included for reference (Figure 2).

On September 20, 2010, destructive harvest of all sites and treatments was performed. All soil cores were separated by horizon (organic/mineral) based on color, texture, and visual interpretation of the presence of whole or partially decomposed organic material. Distinct surface litter layers were discarded on site. Soil samples were transferred in coolers from site to lab (< 8 h) and stored at 4°C. Within 48 h, soils were sieved (2 mm) to remove debris/roots; roots were set aside. Soil and hyphal material that passed through the sieve were assessed for root fragments and severed EmF root-tips for 3 min with the naked eye and 2 min with a stereo dissecting microscope at 10x magnification to standardize sampling effort. Discernible roots were combined with those captured by the sieve, placed in 95% ethanol and stored at 4°C for EmF morphotyping. Remaining soil from each sample was respectively homogenized. A subsample of each was used to determine gravimetric water content; dry weights were used to standardize soil dry mass for DNA extractions and qPCR amplifications. Soils were stored at -20°C for one year before molecular analyses.

This study ultimately analyzed the community dynamics of *Piloderma* and *Ramaria* mats as they developed (birth) and declined (death) after 51 months; this included the specific analysis of ectomycorrhizosphere root-tip EmF, as well as the interface of hyphosphere and bulk soil with fungal and bacterial pyrosequencing.

3.3. Ectomycorrhizal Root-Tip Analyses

3.3.1. Ectomycorrhizal Identification and Molecular Analyses

A stereo dissecting microscope was used to assess roots for EmF colonization. Initial root storage in 95% ethanol resulted in minimal desiccation and morphological disturbance; two measures were taken to ensure accurate morphotype differentiation: (1) roots were removed from ethanol and soaked in a 2x cetyltrimethylammonium bromide (CTAB) buffer solution at room temperature for one hour to restore turgidity and overall appearance and (2) a very conservative morphotyping approach was used, whereby morphological uncertainties were considered separate types for downstream analysis. Morphotyping characters included color, root branching, mantle texture and extramatrical hyphal ramification; these characters can overestimate EmF species richness (Burke *et al.*, 2005). One root-tip of each unique EmF morphotype was transferred to clean CTAB solution, cleared of all debris and contaminating fungi and underwent DNA extraction using an Extract-N-Amp™ Plant PCR Kit (Sigma Aldrich, St. Louis MO) following the manufacturer's instructions. Extracts were stored at -20°C until amplification.

The Internal Transcribed Spacer (ITS) region of the fungal rRNA gene was amplified using the fungal specific forward primer ITS1F (Gardes & Bruns, 1993), the general eukaryotic reverse primer ITS4 (White *et al.*, 1990) and GoTaq® DNA Polymerase (Promega, Madison, WI). PCR amplification was carried out in 35-µl reactions with the following reagents: template DNA (0.7 µl); dNTP's (2 mM, 2.8 µl); ITS1F primer (10 µM, 0.7 µl); ITS4 primer (10 µM, 0.7 µl); 5x GoTaq® buffer solution (7 µl); $MgCl_2$ (25 mM, 0.7 µl); dH_2O (16.6 µl); BSA (1mg/ml, 5.6 µl) and Taq (5 units/µl, 0.2 µl). After optimization using a DNA Engine® (Bio Rad, Hercules, CA) thermal-cycler, PCR conditions involved initial denaturation at 94°C for 1 min, 35 PCR cycles (denaturation, 95°C, 35 sec; annealing, 55°C, 55 sec; extension, 72°C, 60 sec), and a final extension at 72°C for 10 min. Positive and negative controls ensured target band amplification and purity of reagent cocktail. Amplicons were checked on a 1.5% agarose gel for a single, target fungal band. Samples that did not amplify had a maximum of three root-tips subjected to the above procedure before exclusion from further analyses.

Successfully amplified EmF root-tips underwent RFLP digests using two restriction enzymes, HinfI and DpnII, and followed the procedure of Gardes and Bruns (1993). The use of one enzyme may obscure differentiation between closely related taxa. Restriction digests were separated on 3% agarose gels, stained with ethidium bromide, digitally photographed and the resulting band patterns were visually compared across all root-tips. Due to conservative morphotyping, root-tips with similar RFLP patterns were grouped as the same phylotype; care was taken to maintain separation of RFLP types with questionable interpretation (e.g. blurred bands and uneven runs on a gel). An example RFLP gel is included (Figure 4). DNA from the cleanest and brightest of each unique RFLP type were cleaned with the Exo-Sap™ PCR product cleanup kit (Affymetrix, Santa Clara, CA) and submitted to the University of Washington's High Throughput Genomics Unit (Seattle, WA) for Sanger sequencing on an Applied Biosystems 3730xl DNA analyzer (Life Technologies, Grand Island, NY).

Fungal ITS sequences were manually compared, edited and fully processed using Geneious Pro v6.0.5 (Biomatters, Auckland New Zealand). Sequence alignment at 97% similarity was performed using MAAFT (Katoh *et al.*, 2005) to ensure similar RFLP patterns translated into molecular species. Those sequences that were ≥ 97% similar were grouped into the same molecular type and will hereby be referred to as EmF species. The GenBank (Benson *et al.*, 2012) MegaBlast search feature for highly similar sequences was used to assign taxonomies to EmF species; final taxonomic identities were compared to the curated UNITE database (Abarenkov *et al.*, 2010). Taxonomies were assigned based on overall BLAST consensus if the majority of the fungal ITS1 and ITS2 regions aligned with a GenBank sequence as well as the following criteria: (1) 97-100% minimum identity for species-level; (2) 95-96% identity for genus-level; (3) 90-94% identity for family-level; and (4) sequences below 90% similarity were named to the closest consensus level unless confirmed by UNITE. Although the use of taxonomic limits is arbitrary and may not truly define molecular species as biological species, this is a common procedure in EmF literature (Nunez *et al.*, 2009; Kipfer *et al.*, 2010; Karpati *et*

al., 2011). EmF root-tips that failed to produce quality sequences after multiple attempts were referred to as unknown or "Unk," followed by a sequential number.

3.3.2. Ectomycorrhizal Root-Tip Community Analyses and Statistical Methods

A data matrix was created in Microsoft Excel 2010 of the presence/absence (1/0) of EmF species in each of the 70 samples for import into PC-ORD v6.07 (McCune & Mefford, 2011). Non-metric multidimensional scaling (NMS) ordinations (Kruskal, 1964; Mather, 1976) were used to visualize EmF community differences and to account for non-linear, non-normal data, where treatments were ordinated in EmF species space. Initially, a large number of treatments contained no live EmF roots (e.g. organic horizon death cores and thin organic horizons); this was coupled with extreme data heterogeneity (many singleton species) to make a proportional city-block distance measure (e.g. Sørensen) unsuitable for calculating a distance matrix. Therefore, a Euclidean metric was used to capture the distance matrix between treatments and species. After the omission of treatments with no EmF species (not ecologically relevant), Sørensen distance could be used; however, Euclidean distance was selected for three reasons: (1) the removal of soil cores with no live root-tips left an unequal sample design which is incompatible with the Sørensen distance measure; (2) the non-proportionality of Euclidean distance is less of an issue with binary data (presence/absence) as a similar result is reached with proportional Sørensen distance; and (3) although Euclidean distance is based on squared distance, weighting abundant species more heavily, this is not an issue with a binary dataset as the square of 1/0 are 1/0. Additionally, site 41was determined to be an outlier for the following reasons: (1) EmF background mats were not definitively identified in the original study at these sites (Blanchard, 2008); (2) no discernable mats or typical rhizomorphic/hydrophobic mat-features were identified during EmF root-tip analyses; and (3) the mat-forming genera *Piloderma* and *Ramaria* were not identified on root-tips in the background mats. It was removed from future analyses, reducing sample size to 60. EmF root-tip ordinations were executed with all treatments from both horizons in the data matrix. Autopilot mode was used, which

assessed 250 runs of real/random data, starting from random configurations, shuffling the main matrix each time for a total of 500 iterations with stability criterion of 0.00001 (McCune & Mefford, 2011). A Monte-Carlo test compared real and random data with a $p < 0.05$ considered significant. A second ordination grouped by treatment used similar methods, but species within a treatment were averaged to yield non-binary data. This latter matrix was converted to presence/absence and used to create a two-way hierarchical cluster dendrogram for root-tip EmF species and treatments to illustrate clustering relationships; Euclidean distance and Ward's linkage were used.

Multi-response permutation procedures (MRPP) were used to assess statistical differences between treatments, sites and other groupings of interest (Biondini, 1988), and were executed separately for each soil horizon. Comparisons were considered significant if both occurred: (1) the probability of a smaller or equal delta to that observed was $p \leq 0.05$; and (2) the chance-corrected within group agreement was $A \geq 0.05$. The A-value describes the effect size independent of the sample size while the p-value is dependent on sample size. Heterogeneous ecological data tend to have A-values < 0.1 (McCune & Grace, 2002); therefore A-values close to 0.1 were considered substantial. Because of the extreme heterogeneity of the data and treatment manipulation, and a desire to control type II errors, a $p < 0.05$ was considered significant. Euclidian distance was used for MRPP to keep comparisons consistent with NMS.

3.4. Soil Fungal and Bacterial Community Analyses

3.4.1. DNA Extraction, Amplicon Library Preparation and Pyrosequencing

Total microbial DNA was extracted from sieved soil samples using the MoBio PowerSoil® DNA isolation kit (MoBio Laboratories, Carlsbad, CA) following the manufacturer's protocol. Each of 59 soil samples were homogenized and extractions performed in triplicate on 0.33 g soil dry-weight, for a total of 1 g per sample or 177 individual extractions. Ram_Dth from site 137 was not included in further analyses because of a processing error. To control for amplification of contaminant fungi, a blank sample underwent the extraction protocol and future amplification steps. A NanoDrop®

ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) was used to quantify gDNA; each extract was diluted to 20 ng/µl for downstream analysis based on optimization of template concentrations.

For soil fungi, the ITS region of fungal rRNA gene was amplified in a two-step procedure using the fungal specific forward primer ITS1F (Gardes & Bruns, 1993), the general eukaryotic reverse primer ITS4 (White *et al.*, 1990) and GoTaq® Hot Start DNA Polymerase (Promega, Madison, WI). The first amplification was carried out in 25-µl reactions with the following reagents: template DNA (20 ng/µl, 5 µl); dNTP's (2 mM, 2.5 µl); ITS1F primer (10 µM, 2.5 µl); ITS4 primer (10 µM, 2.5 µl); 5x GoTaq® buffer solution (5 µl); $MgCl_2$ (25 mM, 2.5 µl); dH_2O (5.0 µl); Taq (5 units/µl, 0.2 µl). After optimization using a DNA Engine® (Bio Rad, Hercules, CA) thermal-cycler, PCR conditions involved an initial denaturation step at 94°C for 4 min, 30 PCR cycles (denaturation, 94°C, 60 sec; annealing, 54°C, 45 sec; extension, 72°C, 120 sec), and a final extension at 72°C for 8 min. Positive and negative controls were included to ensure target band amplification and purity of reagent cocktail. Amplicons were checked on a 1.5% agarose gel; a subset were quantified on a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) to ensure even amplification.

A second amplification was performed to attach the Roche 454 adaptors. The forward adaptors contain unique 10-bp multiplex identification (MID) tags to differentiate samples after unidirectional sequencing (Figure 5). A two-step PCR decreases the probability of a 3'-end bias, where one in four DNA tags would have an exact match to regions in the template DNA (Ari Jumpponen, personal communication). The second amplification was carried out in 25-µl reactions with the following reagents: template DNA (10 µl); dNTP's (2 mM, 2.5 µl); ITS1F primer with the 454 adaptor sequence A and a unique 10-bp MID (10 µM, 1.5 µl); ITS4 primer with the 454 adaptor sequence B (10 µM, 1.5 µl); 5x GoTaq® buffer solution (5 µl); $MgCl_2$ (25 mM, 2.5 µl); dH_2O (1.6 µl); Taq (5 units/µl, 0.4 µl). PCR conditions involved an initial denaturation step at 94°C for 4 min, 5 PCR cycles (denaturation, 94°C, 60 sec; annealing, 54°C, 60 sec; extension, 72°C, 120 sec) and a final extension at 72°C for 10 min.

For soil bacteria the highly conserved region of the 16S small subunit rRNA gene, analogous to the 18S region in fungi, was amplified using the primer pair 27F and 338R based on *Escherichia coli* numbering (Liu *et al.*, 2007; Fierer *et al.*, 2008) and performed in a similar two-step procedure to fungi. The region of rDNA targeted by this primer pair has been considered superior for the accurate taxonomic identification of bacteria from environmental samples without sequence misclassifications observed using other regions (Hamady *et al.*, 2007; Lauber *et al.*, 2009). Amplification methodology was similar to fungi except where noted. The first amplification involved a different primer set and the substitution of AmpliTaq Gold® 360 DNA Polymerase (Life Technologies, Grand Island, NY) for GoTaq®. After extensive PCR cycle optimization and contaminant troubleshooting, PCR conditions involved an initial denaturation step at 95°C for 10 min, 30 PCR cycles (denaturation, 95°C, 20 sec; annealing, 52°C, 20 sec; extension, 67°C, 60 sec) and a final extension at 67°C for 7 min. PCR conditions for the second amplification, to attach Roche 454 adaptors, involved an initial denaturation step at 95°C for 10 min, 5 PCR cycles (denaturation, 95°C, 30 sec; annealing, 52°C, 30 sec; extension, 67°C, 60 sec), and a final extension at 67°C for 10 min.

For both fungi and bacteria, barcoded PCR product was compared, side-by-side, to product from the first PCR amplification on 1.5% Agarose gel to ensure the addition of 70-bp tags to the amplified rDNA. Equal amounts of each triplicate were pooled (59 samples) and cleaned using a 0.7:1 ratio of Agencourt AMPure XP® beads (Beckman Coulter, Brea, CA) to template. Samples were quantified using a Qubit® 2.0 Fluorometer (Life Technologies, Grand Island, NY) and pooled by treatment at equimolar concentrations. The 10 pooled treatments were cleaned a second time using AMPure XP® beads at 0.7:1 ratio, quantified using a Qubit® 2.0 Fluorometer, diluted to 5 ng/ul and submitted to the Center for Genome Research and Biocomputing Core Laboratories (CGRB) at Oregon State University (Corvallis, OR) for sequencing. Briefly, quality control included qPCR DNA quantification using the Applied Biosystems 7500/7500 Fast Real-Time PCR System (Life Technologies, Grand Island, NY). qPCR product was analyzed using the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) to

ensure target region quality and the absence of short fragments not visible on an agarose gel. Samples were pooled in equimolar concentrations based on qPCR results. Sequencing was performed at the Oregon State University CGRB on the Roche GS Jr. Platform (Roche, South San Francisco, CA).

3.4.2. qPCR of Soil Fungi and Bacteria

Fungal and bacterial rDNA copy numbers were used as a proxy for biomass in each soil sample. DNA extractions for fungi and bacteria followed the procedures for 454 above. The three pseudoreplicate extracts/sample from 454 analysis were quantified on a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and diluted to 25 ng/µl. qPCR procedures generally followed Boyle *et al.* (2008), adapted from Fierer *et al.* (2005). Briefly, DNA was amplified in 25-µl reactions with an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara CA) at Oregon State University CGRB using the following reagents: (1) 2x Brilliant SYBR® Green qPCR Master Mix (Agilent Technologies, Santa Clara, CA) (12.5 µl); template DNA (2 µl); the forward primers ITS1-F (fungi) and Eub338 (bacteria) (10 µM, 2.5 µl); the reverse primers 5.8S (fungi) and Eub518 (bacteria) (10 µM, 2.5 µl); 1/50 diluted ROX reference dye (Affymetrix, Santa Clara, CA) (0.5 µl); dH_2O (7.5 µl). The following PCR conditions were used: (1) 50°C, 2 min; (2) 95°C, 10 min; (3) 40 cycles at 95°C, 30 sec, 53°C 30 sec and 72°C 30 sec; and a final disassociation step of 95°C, 15 sec and 60°C, 20 sec. SYBR® Green was quantified during the 72 °C elongation step. Standard curves were generated from plasmid purified *Haematanectria haematocoeca* (fungi) and *Pseudomonas aeruginosa* (bacteria); plasmid concentrations ranged from 5.0×10^{-1} to 5.0×10^{-7} ng DNA. Analysis of standard curves from each run to ensure r^2 values > 0.95 indicated efficiency of 95% to 102% and single peak dissociation curves.

3.4.3. Data Processing - Soil Fungi

After sequencing, initial read quality filtering was performed using the Roche GS FLX software v2.3 shotgun pipeline (Roche, 2010): Briefly, low quality bases were

trimmed from the 3' end until an error rate of ~1% was observed, resulting in 143,682 variable length amplicons and an average read length of 564 bp. Further data analyses were performed using a software combination that included the Quantitative Insights into Microbial Ecology (QIIME) bioinformatics toolkit (Caporaso *et al.*, 2010; v1.5.0), the MEtaGenome ANalyzer (MEGAN) (Huson *et al.*, 2011), PC-ORD v6.11 (McCune & Mefford, 2011) and Geneious Pro v6.0.5 (Geneious, 2012).

Raw sequences were subjected to stringent quality control using QIIME. Briefly, split_libraries.py was used to demultiplex sequences based on unique 10-bp MIDs and had Roche adaptors and primers trimmed. Sequences shorter than 250 bp and longer than 1000 bp, with more than six ambiguous bases, one primer mismatch and/or homopolymeric runs exceeding six were discarded. Sequences with an average quality score within a 50-bp sliding window below 25 were truncated at the beginning of each respective window, resulting in 68,318 sequences. These sequences underwent three separate processing pathways which were later compared for consistency downstream: (1) trimmed to 250 bp to cluster only the highest quality sections of each amplicon due to read call deterioration common in the Roche 454 machine; (2) run through the fungal ITS extractor (Nilsson *et al.*, 2010), a Perl script developed to isolate the fungal ITS1 and ITS2 regions, to cluster sequences based only on the variable ITS1 region and eliminate clustering based on the more conserved small subunit and 5.8S regions of fungal rDNA; and (3) left unaltered from the results of split_libraries.py in an effort to retain the most sequence information (e.g. reads long enough to capture partial ITS2).

The USEARCH pipeline (Edgar, 2010; Edgar *et al.*, 2011) was used within pick_otus.py to denoise sequences, check for chimeras (reference and denovo) and cluster amplicons based on 97% sequence similarity. This resulted in 67,880 sequences and 2,235 operational taxonomic units (OTUs), which will hereby be considered molecular species. A representative sequence from each OTU was chosen using the most abundant criterion within pick_rep_set.py. These sequences were assigned taxonomic affiliation using BLAST classification software (Altschul *et al.*, 1990) and the UNITE/QIIME 12_11 ITS release reference database within assign_taxonomy.py. An

OTU table was created with taxonomic assignments using make_otu_table.py and exported to Microsoft Excel 2010 for manual comparison of the three processing pathways. Unassigned OTUs were manually subjected to UNITE/BLAST searches to parse possible reasons for missing taxonomies. Where possible, manual identification to higher taxonomic levels were made based on result quality. All singleton OTUs were discarded, resulting in 1,246 fungal species from 65,230 sequences. A consensus taxonomy data matrix was created, separated into organic and mineral soil horizons (*Piloderma* and *Ramaria*, respectively), collapsed to the genus level and used for downstream analyses, unless otherwise noted. Any taxon above the genus level was kept separate and denoted to the highest taxonomy with a sequential number (e.g. Gomphaceae 1). This resulted in 653 fungal OTUs. OTU table analyses were completed using QIIME scripts and exported to previously mentioned programs.

3.4.4. Fungal Ecology Assignment

Putative ecologies were assigned to fungal taxa based on current mycological knowledge (Hoff *et al.*, 2004; Agerer, 2006; Cannon & Kirk, 2007; Hyde & Soytong, 2008; Rinaldi *et al.*, 2008; Smith & Read, 2008).

3.4.5. Data Processing - Soil Bacteria

Initial read quality filtering mirrored fungal methods, resulting in 109,747 variable length amplicons and an average read length of 341 bp. Raw sequence quality control also followed fungal methods, except that the minimum acceptable sequence length was reduced to 200 bp. This resulted in a total of 57,184 sequences. These sequences were trimmed to 250 bp to cluster only the highest quality sections of each amplicon due to read call deterioration common with Roche 454-pyrosequencing. Denoising and chimera checking followed fungi and 18,553 bacterial singletons were removed from future analyses. This resulted in 38,631 sequences and 4,324 OTUs, hereby considered molecular species. A representative sequence from each OTU was chosen with the most abundant criterion within the pick_rep_set.py script. These

sequences were assigned to a taxonomic affiliation using BLAST classification software (Altschul *et al.*, 1990) and the Greengenes 16S rRNA gene database (DeSantis *et. al*., 2006) within assign_taxonomy.py. An OTU table was created with taxonomic assignments using make_otu_table.py; this OTU table was collapsed at the family level to yield 217 bacterial families and all subsequent downstream analyses were completed in the same way as fungi at the bacterial family level, unless otherwise noted.

3.5. Statistical Analyses of 454 Data - Soil Fungi and Bacteria

All statistical analyses, where applicable, were performed using a significance criterion of $p \leq 0.05$ unless otherwise noted.

3.5.1. Rarefaction and Diversity Analyses

Rarefaction curves were created for the soil fungal dataset using the QIIME pipeline (Caporaso *et al.,* 2010) with the following scripts in order: (1) multiple_rarefactions.py; (2) alpha_diversity.py; (3) alpha_rarefaction.py scripts; and (4) make_rarefaction_plots.py. The first script randomly subsamples or rarefies an OTU table; the second script calculates alpha diversity, or within-sample diversity from rarefied OTU tables; the third script joins multiple files produced by step 2; and the fourth script constructs data visualizations included here. Rarefactions were performed using a step size of 10 with 10 iterations at each step. Rarefaction curves were visualized using an .html viewer. Average sample richness and overall treatment richness (singletons included) are reported on the respective graphs for each treatment.

Overall diversity statistics for fungal genera, bacterial families and 97% OTUs (singletons removed) were calculated in PC-ORD v6.07 (McCune & Mefford, 2011). Treatment averages for richness, evenness, Shannon’s diversity, Simpson’s diversity, percent empty cells, skewness and kurtosis are presented.

3.5.2. Community Ordination Analyses

Ordination permits the compression of high-dimensional data into low-dimensional space by isolating the strongest structure among variables, representing it in a few dimensions (McCune & Grace, 2002). As opposed to other commonly misused ordination methods in community ecology, such as principal components (Pearson, 1901) or canonical correspondence (ter Braak, 1986), non-metric multidimensional scaling (NMS) (Kruskal & Wish, 1978) is more effective for ecological community data (McCune & Grace, 2002). Especially in analyses involving the structuring of hyperdiverse microbial communities, NMS is particularly useful as it depends only on biologically relevant data representation (Clarke, 1993). NMS is a non-parametric data visualization technique, adept at dealing with non-normal, non-linear data with the additional advantage of avoiding the "zero truncation problem," where only measures of "0" are possible beyond the extremes of species tolerance and information on environmental unfavorability is absent (McCune & Grace, 2002).

In community ecology, a primary data matrix (species matrix) signifies sample units by species. Cells represent a numerical measurement of species presence, absence or abundance. NMS iteratively searches for the best species distribution based on a distance matrix and compresses dimensionality by minimizing "stress" (a measurement of departure from monotonicity) and distance in ordination space. Once variation explained by successive ordination axes becomes statistically insignificant the final ordination dimensionality is determined (typically ≤ 3). A Monte Carlo randomization test statistically evaluates whether the axes determined by NMS are stronger than expected by chance (McCune & Grace, 2002).

NMS ordinations were universally used for soil fungal and bacterial 454 analyses and were performed within PC-ORD v6.07 (McCune & Mefford, 2011). These were used to assess relationships in soil microbial community composition. Separate ordinations were performed for each soil horizon (organic/mineral) and each microbial group (soil EmF, soil fungi, and soil bacteria), for a total of six reported ordinations. Soil EmF were extracted from the overall fungal dataset based on putative taxonomic and

ecological assignments, excluding unidentified groups. All data were relativized by sample-unit (treatment) total to account for uneven sampling effort. The Sørensen city-block dissimilarity (distance) measure was used to calculate distance matrices as is generally applied in molecular sampling studies with abundance data. All NMS ordinations were performed in autopilot mode with the "slow and thorough" setting. Each ordination was run in triplicate and compared against the others for similarity using a Mantel test under "compare scores" to evaluate the correlation between inter-point ordination distances. The final ordination represented the lowest stress and instability of the triplicate set. For all ordinations, a two or three-dimensional solution was achieved and the reported two-dimensional solutions represent the axes explaining the majority of the variation in ordination space. Due to heavy site-site variability, site centroids were translated to the origin to better visualize treatment differences. Final ordination axis scores are reported as pre-translation scores as this manipulation inhibits proper calculation, though the original values remain relevant (McCune, personal communication). Visual inspection of ordination plots and overlay of joint plot vectors to simultaneously plot "species" and treatment scores were used to identify patterns, where the angle and length of the lines indicates the direction and strength, respectively, of the relationship. Joint plot vectors were scaled to 100% and are displayed for varying correlation strengths ($0.2 > r^2 < 0.4$; see individual ordinations).

3.5.3. Multi-response Permutation Procedures

Multi-response permutation procedure (MRPP) is a nonparametric statistical method used to test the null hypothesis of no difference between pre-existing groups inherent to the sample design (Biondini, 1988). It is commonly used as a second-step to NMS ordinations because data do not need to meet distributional assumptions, such as multivariate normality and variance homogeneity, rare in ecological community data (McCune & Grace, 2002). Additionally, it provides a statistical measure to group visually evident NMS separation without spatial limitations. MRPP test statistics include the A-value, a measure of effect size independent of sample size or chance-corrected

within-group homogeneity, and the p-value which is dependent on sample size. An A-value of 1 indicates that all group members are identical; an A-value of 0 indicates that differences between group members are as expected by chance; a negative A-value indicates there was less agreement within groups than expected by chance. Heterogeneous ecological data tend to have A-values < 0.1 and p-values should always be considered within this context (McCune & Grace, 2002).

Here MRPP were used to assess statistical differences between treatments, sites, and other *a priori* groupings of interest. NMS ordinations were assessed using MRPP with the Sørensen distance to be compatible with NMS. Comparisons were considered significant if both the probability of a smaller or equal delta to that observed was $p \leq 0.05$ and the chance-corrected within group agreement was $A \geq 0.05$.

3.5.4. Indicator Species Analyses

Indicator species analysis (ISA) (Dufrene & Legendre, 1997) is a common tool in community ecology used to detect and describe the importance of particular taxa that indicate environmental variables or *a priori* groupings (McCune & Grace, 2002). Here, it was used to investigate whether certain fungal genera or bacterial families tended to associate with treatments and was performed within PC-ORD v6.07 (McCune & Mefford, 2011). The combination of MRPP and ISA are commonly used in community ecology (McCune & Grace, 2002) and are complementary; the latter by isolating statistically relevant taxa responsible for group differences identified by MRPP. Taxon indicator values are the product of a taxon's relative abundance and relative frequency; therefore rare taxa will inherently have low indicator values, expressed as a percent of perfect indication. Blocked ISA was used on organic horizon soils because of the balanced design, thus reducing site-to-site variation; mineral horizon soils could only be analyzed by regular ISA due to sampling error, which omitted one Ram_Dth sample from site 137. A Monte Carlo randomization test using a random start and 100 permutations was also performed to evaluate the statistical significance of each indicator value; p-values < 0.15 were reported and their relevance discussed.

The function of an ISA is to detect and describe the significance of taxa in predicting environmental conditions; here, this involves the presence and absence of mat-forming fungi. The study design may fundamentally obscure ISA results when considering all treatments together; inherent in the hypotheses are a trajectory by which certain treatments may become more similar or different to others (Figure 1). Therefore, certain pairwise treatment comparisons are likely to yield results that support, contradict and/or build upon results from an overall ISA. Additionally, the arbitrary combination of treatments hypothesized to be similar (e.g. Pil_Bck/Pil_Bth and NMBck_O/Pil_Dth) may be misleading and was not performed. Though mineral soils exhibited noteworthy treatment effects, organic soils with and without *Piloderma* mats warranted further investigation, particularly for the fungal component. Pairwise ISAs were performed on all organic-horizon soil fungal communities (10 total) as well as background organic horizon bacterial communities to identify deeper trends in community composition; ISA parameters were kept consistent with those mentioned above and p-values < 0.10 were reported.

3.5.5. Taxonomic Visualization of Soil Fungal and Bacterial Communities

OTU tables with taxonomic values and identities from QIIME (Caporaso *et al.*, 2010) were exported to MEGAN (Huson *et al.*, 2011). MEGAN was used to visualize taxonomic distributions of soil fungi and bacteria based sample or treatment. In all cases, unequal sampling effort was accounted for by normalizing based on sample unit total.

3.5.6. qPCR of Soil Fungi and Bacteria

Fungal and bacterial rDNA copy numbers were analyzed in MiniTab® (MiniTab v.16.1.1) and compared across treatments using two-way ANOVA and the general linear model for the organic and mineral horizons, respectively. Due to non-normal distribution of rDNA copy numbers, data were first log-transformed to meet normality and equal variance assumptions of ANOVA. Post-hoc means comparisons were performed using Tukey's HSD test where appropriate. Fungal:bacterial ratios were analyzed similarly.

Because of the interest in mat and non-mat background communities as distinct entities, post-hoc comparisons using one-tailed t-tests were performed to explore differences between natural communities and test the hypotheses that EmF mat soils have higher fungal biomass and lower bacterial biomass than non-mat soils. Manipulated treatments were assumed to fall between the natural background soils, thus generating less significant ANOVA p-values. Additionally, a post-hoc comparison was performed on Pil_Bck and Pil_Dth fungal biomass due to the inherent isolation of Pil_Dth soils from fungal ingrowth, especially EmF.

3.5.7. Additional Comparisons of Soil Fungal and Bacterial Communities

As the mat-forming fungus in the organic horizon, abundance of *Piloderma* rRNA gene copy numbers for each treatment were compared using ANOVA in MiniTab® (MiniTab v.16.1.1), after relativization by soil core total to account for unequal sampling effort. Due to non-normal rRNA gene copy number distribution, data were Johnson transformed (similar to Pearson transformation) to meet normality and equal variance criteria (equation = 0.784171+0.302465*Ln((X+0.0000173293)/(0.264908-X))); this transformation optimizes the function from three flexible distribution families and the selected data distribution is used to meet normality (Chou *et al.*, 1998). A two-way ANOVA and a post-hoc means comparison using Tukey's HSD to examine treatment differences were performed.

To test whether *Piloderma* mat communities were competitively excluding *Russula*, the relative abundance of *Piloderma* and *Russula* in three treatments (Pil_Bck, Pil_Bth and NMBck_O) were compared using a Friedman test in MiniTab® (MiniTab v.16.1.1), a non-parametric version of a two-way ANOVA. Post-hoc means comparisons for significantly different results were analyzed using a Kruskal Wallace test.

4. Results:

4.1. Ectomycorrhizal Root-Tips

4.1.1. RFLP/Sanger Sequencing and Diversity Statistics

A total of 780 EmF root-tips from 70 soil cores yielded 131 unique RFLP types which were later condensed into 111 unique species in 32 genera. The merging of RFLP types was based on 97% sequence similarity. PCR amplification success was 96%, while RFLP identification was 88% as some root-tips contained multiple fungi prohibiting unique RFLP/sequence identification; of the 111 unique EmF species, 20 remained unknown (18%) due to multiple unsuccessful sequencing attempts. One species, *Glomus* sp., is arbuscular mycorrhizal while another genus, *Mycena*, is known to be saprotrophic or form orchid mycorrhizae (Ogura-Tsujita *et al.,* 2009) and represented 5 species; these, not being EmF, were removed from further analyses. Thus, a final number of 105 species in 30 genera were analyzed. According to the mean jackknife estimate (first-order = 154; second order = 180), overall EmF diversity observed in this study was ~70% of expected. A species-area curve indicated sampling effort was not sufficient to capture full EmF diversity (Figure 6). In the organic horizon, EmF species richness followed the sequence Pil_Bck, Pil_Bth, NMDst_O, NMBck_O and Pil_Dth, while mineral horizon species richness followed the sequence Ram_Bck, NMBck_M, Ram_Bth, NMDst_M and Ram_Dth (Table 3); in both horizons, background mat communities had the greatest EmF species richness.

4.1.2. NMS Ordinations

Soil cores were used as replicates within each treatment (sample units) and yielded a significant 3-dimensional ordination (final stress = 11.1, final instability < 0.000; $p = 0.02$), with axes 1-3 representing 45.3%, 20.4% and 20.3% of the variation, respectively (total = 86%) (Figure 7); the Pil_Dth treatment was excluded for previously mentioned reasons. A Mantel Test was used to evaluate the redundancy between three sample unit distributions in ordination space (avg. 96.8% redundancy). Axis-3 of the

NMS ordination was not included because it was the only axis correlated with the most ubiquitous EmF species that does not form a mat, *Cenococcum geophilum* ($r = -0.417$). Although treatment distances in species space were not large, clustering indicated similarity between: (1) Pil_Bck/Pil_Bth and NMBck_O/NMDst_O in the organic horizon (Figure 7, Panel A); (2) Ram_Bck/Ram_Bth and NMDst_M/Ram_Dth/NMBck_M for the mineral horizon (Figure 7, Panel B). Grouping in ordination space was wider in the mineral than the organic horizons; treatment centroids and convex hulls are provided for visual reference (Figure 7). Data were not structured such that a statistically meaningful ISA could be performed.

A subsequent NMS analysis, where all soil-core treatment replicates were grouped by the sum of EmF species, supported results from above with individual soil cores as replicates (Appendix 2). Data were not structured such that a Monte Carlo test yielded a p-value < 0.05; axes 1-2 consistently had p-values ~ 0.1 while axes 3-5 had $p < .05$, but stress was inherently low and could not be reduced, a common occurrence with sample sizes this small (i.e. n=9). Mantel comparisons of three NMS ordinations forced into 2-dimensional solutions yielded similarity values no lower than 99.99% (final stress = 5.84, final instability < 0.000), with axes 1-2 representing 29.9% and 61.1% of the variation, respectively (total = 91%). It was not possible to perform MRPP on these data because there was no within treatment replication. A two-way cluster analysis of treatments with singletons removed (Figure 8) confirmed relationships from NMS analyses. Overall, most *Piloderma* spp. had strong negative correlations with the second axis ($r < -0.6$), supporting Pil_Bck placement at the bottom of axis-2. *Ramaria celerivirescens*, and Gomphaceae spp. had a strong positive correlation with axis-1 ($r = 0.73$, $r = 0.71$), supporting the placement of Ram_Bck at the extreme right of axis-1. EmF species strongly negatively correlated with axis 2 included *Piloderma* sp. 2/4/6, *Ramaria formosa*, *Sebacina* sp. 1, *Helvella lacunosa*, and *Russula turci* ($r \leq -0.9$), indicating species highly associated with *Piloderma* mat communities. EmF species strongly positively correlated with axis-1 included *Byssocorticium atrovirens*, *Piloderma* sp. 4/10/11, *Rhizopogon vesiculosus*, *Tomentella* sp. 3, *Tricholoma saponaceum*,

Albatrellaceae sp. 1, *Cortinarius* sp. 4, *Russula amethystina, Inocybe* sp. 3, *Sistotrema muscicola,* Ceratobasidiaceae sp. 1 and unknowns 3/7/19 ($r \geq 0.71$), indicating species highly associated with *Ramaria* mat communities. Finally, species strongly negatively correlated with axis-1 included *Wilcoxina rehmii, Tomentellopsis echinospora, Russula* sp. 8 and Thelephoraceae sp. 1 ($r \leq -0.72$), to name a few non-mat associates.

4.1.3. MRPP Analyses

MRPP results can be found in Table 4 (column 1). In the organic horizon Pil_Bck significantly differed from NMBck_O (MRPP; A = 0.08, p = 0.04) and NMDst_O (MRPP; A = 0.07, p = 0.03), which supported placement in the NMS ordination (Figure 7). Results confirm the general developmental trend of Pil_Bth EmF root-tip communities toward Pil_Bck; Pil_Bth was found to be more similar to Pil_Bck (MRPP; A = -0.02, p = 0.72) than to NMBck_O (MRPP; A = 0.04, p = 0.23). Pil_Dth treatments were not included in these analyses because the experimental design limited root growth into the organic horizon.

Mineral horizon results were similar to the organic horizon. Ram_Bck differed significantly from both NMBck_M (MRPP; A = 0.09, p = 0.03) and NMDst_M (MRPP; A = 0.08, p = 0.01); although within-treatment variation was noticeably larger than the organic horizon in the two axes represented in the NMS ordination (Figure 7), MRPP results were strong. Unlike the organic horizon, Ram_Bth EmF root-tip communities were significantly different from NMBck_M (MRPP; A = 0.04, p = 0.05) and quite similar to Ram_Bck (MRPP; A = -0.04, p = 0.81). Proximity to the root-zone permitted root ingrowth into Ram_Dth cores; this root-tip community was not different from any other treatment, but most closely resembled NMDst_M (MRPP; A = -0.04, p = 0.88). A graphical summary of EmF root-tip results is included for reference (Figure 9).

4.2. Sequencing and Diversity of Soil Fungi

A total of 67,880 sequences passed filtering and quality control thresholds, with samples ranging from 296-2150 sequences. Sample submission for 454-pyrosequencing,

given the wide sequence variation between individual samples, yielded fairly similar treatment totals (range 6301-7558). A total of 2,235 OTUs were identified while the OTU count per sample ranged from 69-277; 781 were singletons (35.0% OTUs, 1.2% sequences), 385 were doubletons (17.2% OTUs, 1.1% sequences), 176 were tripletons (7.9% OTUs, 0.8% sequences), 105 were quadrupeltons (4.7% OTUs, 0.6% sequences) and the remaining 788 OTUs occurred > 4 times (35.3% OTUs, 96.3% sequences). Non-singleton OTUs were phylotyped to the lowest acceptable classification; 207 at the Kingdom level (14.2% OTUs, 2.8% sequences), 128 to Phylum (8.8% OTUs, 2.5% sequences), 85 to Class (5.9% OTUs, 3.5% sequences), 128 to Order (8.8% OTUs, 3.8% sequences), 82 to Family (5.6% OTUs, 4.3% sequences), and the remaining 824 to Genus or Species level (56.6% OTUs, 83.2% sequences). Identification of the most abundant taxa was thorough, with 323/333 (97%) OTUs that occurred more than 25 times identified to the class-level or higher. Groupings were analyzed at the genus level in PC-ORD leaving higher taxonomic identification at the respective level indicated (e.g. sp. 1, sp. 2 etc.), and included 653 "genera." A summary of average fungal OTU richness, evenness and diversity statistics for each treatment can be found for "genera" (Table 5) and 97% OTU's (Table 6); henceforth "genera" will be referred to as genera.

In the organic horizon, richness and Shannon's diversity of fungal genera, as well as 97% OTUs, were highest for the NMBck_O and the Pil_Dth treatments; Pil_Bck and NMDst_O were intermediate, while Pil_Bth was lowest (Tables 5, 6). There was no statistical difference in richness between treatments when comparing fungal genera ($F = 1.48$; $p = 0.239$) or 97% OTUs ($F = 0.82$; $p = 0.523$). Shannon's diversity followed similar trends (Genera; $F = 1.57$, $p = 0.213$ - 97% OTUs; $F = 0.75$, $p = 0.567$). In the mineral horizon NMBck_M, Ram_Bck and Ram_Dth had similar richness and diversity in both groupings; whereas, Ram_Bth and NMDst_M were lower (Tables 5, 6). Similarly to the organic horizon, there was no statistical difference in richness between treatments when comparing fungal genera ($F = 1.3$; $p = 0.298$) or 97% OTUs ($F = 1.68$; $p = 0.186$). However, Shannon's diversity of fungal genera was slightly different ($F = 2.93$; $p = .042$), while Shannon's diversity of 97% OTUs was marginally different ($F =$

2.41; p = 0.077). Post-hoc means comparisons did not yield significant differences. For both fungal richness and Shannon's diversity, the organic horizon showed high site-to-site variation, particularly for richness; for the mineral horizon, richness was more variable than Shannon's diversity by site (data not shown).

Rarefaction curves for 97% fungal OTUs depict individual samples within each treatment (Organic = Figure 10; Mineral = Figure 11). Average sample richness can also be found in Table 6, while Table 7 lists the shared 97% OTUs between treatments. In the organic horizon, overall treatment richness proportionally tracked average sample richness with Pil_Dth and Pil_Bth being the most and least diverse, respectively. Pil_Bck shared the lowest number of 97% OTUs with NMDst_O and Pil_Bth, while Pil_Bth shared an approximately equal number of OTUs with all treatments. NMBck_O shared fewer OTUs with NMDst_O than it did with Pil_Bck, but shared the fewest with Pil_Bth and the most with Pil_Dth. Overall, the smallest number of shared OTUs occurred between the two hypothesized transitional treatments, Pil_Bth and Pil_Dth.

4.3. Soil Fungal Community Composition

4.3.1. Organic Horizon (*Piloderma*)

In the organic horizon, an NMS ordination of treatments in soil fungal genus-space and subsequent MRPP analyses (refer to Figure 12 and Table 4 column 2b throughout) clearly indicate overall fungal community treatment differences (MRPP; A = 0.10, p = 0.01). The community composition of Pil_Bth became more like Pil_Bck (MRPP; p = 0.65) and, to a lesser extent, Pil_Dth became more like NMBck_O (MRPP; p = 0.11). Pil_Bck fungal community composition was distinct from Pil_Dth (MRPP; p < 0.01), NMBck_O (MRPP; p = 0.04) and NMDst_O (MRPP; p = 0.02), while Pil_Bth composition was different from Pil_Dth (MRPP; p = 0.04), and NMBck_O (MRPP; p = 0.05), yet exhibited no difference from the NMDst_O (MRPP; p = 0.45). The act of disturbing non-mat soil, enclosing it in 2-mm mesh, and putting it back in place appeared to unexpectedly shift the fungal community from its hypothesized position as analogous

to NMBck_O (Figure 12); compared to the other treatments, NMDst_O most closely resembled Pil_Dth (MRPP; p = 0.60) and Pil_Bth (MRPP; p = 0.45).

Overlaying joint-plot vectors on the two principle NMS axes (Figure 12) highlighted fungal genera responsible for treatment separation. *Piloderma*, *Oidiodendron* and Dermateaceae *7* were negatively correlated with axis-1 and were most abundant in Pil_Bck and Pil_Bth treatments. *Rhizopogon*, *Cenococcum*, and Zygomycota *7* were positively correlated with axis-2 and were more prominent in Pil_Bth and, to a lesser extent, NMDst_O and Pil_Bck. *Thysanophora*, Helotiales *8* and *Ascomycota 21/57* were negatively correlated with axis-2, while *Thysanophora* was also positively correlated with axis-1 and were indicative of NMBck_O and Pil_Dth treatments. Treatment separation along the two NMS axes represented 60% of total variation (Figure 12). A graphical summary of soil fungal results is included for reference (Figure 13).

An ISA (refer to Table 8 throughout) of soil fungi was performed to highlight treatment differences between fungal genera that may not have been apparent on the two primary axes represented by NMS (Figure 12). A total of 14 fungal genera were identified as strong indicators of treatment type ($p < 0.05$); an additional 20 fungal genera had p-values < 0.15 and were reported as their probable importance may have been underrepresented with small sample size. Similarities between NMS and ISA include *Piloderma* as a strong indicator for Pil_Bck/Pil_Bth (IV = 49/33; $p < 0.01$), *Pseudotomentella* strongly indicated NMBck_O (IV = 50; p = 0.02) and *Thysanophora* as a reasonable indicator for NMBck_O/Pil_Dth (IV = 33/30; p = 0.13); *Cenococcum* was a reasonable indicator of Pil_Bck (IV = 38; p = 0.15) although ubiquitous. Unique ISA indicators included Agaricomycetes 15 and *Neofabraea* for Pil_Bck treatments (IV = 48, 43; p = 0.01, 0.02), while Capnodiales 1 and Dermateaceae 2 were good indicators (IV = 41, 40; p = 0.08, 0.09); Pil_Bth did not contain any unique indicator genera though it shared the second highest IVs with Pil_Bck for *Piloderma*, *Neofabraea* and *Cenococcum.* *Mortierella* (IV = 44; $p < 0.01$) was a strong indicator of Pil_Dth while Mitosporic Helotiales 9, Ceratobasidiaceae 2 and Helotiaceae 13 were good indicators (IV = 39, 42, 33; p = 0.05, 0.06, 0.08). Considering non-mat treatments, *Sphaerobolus*, *Leohumicola*,

Auricularia and Pezizomycetes 12 (IV = 56, 46, 43, 42; p = 0.01, 0.04, 0.04, 0.04) were strong indicators of NMBck_O, while *Biscogniauxia*, Dermateaceae 5 and *Hyalodendriella* (IV = 38, 33, 30; p = 0.14, 0.14, 0.14) were reasonable indicators. Pezizomycetes 3 and *Pseudeurotium* (IV = 36, 40; p = 0.04, 0.05) were strong indicators of NMDst_O, while Agaricomycetes 3 (IV = 30; p = 0.11) was a reasonable indicator. *Wallemia* had a notably low IV in Pil_Bck treatments (IV = 7) while Pil_Bth seemed to be transitionally losing *Wallemia* compared to non-mat treatments and Pil_Dth.

Focusing on only EmF genera from the soil fungal dataset yielded similar results to the whole fungal community (MRPP; A = 0.12, $p < 0.01$); however, notable differences were found (refer to Figure 14 and Table 4 column 2a throughout). The similarity between Pil_Bck and Pil_Bth EmF communities was slightly stronger (MRPP; p = 0.69) while Pil_Bck was strongly distinct from NMDst_O (MRPP; $p < 0.01$). EmF community similarity was much greater between Pil_Dth vs. NMBck_O (MRPP; p = 0.31) and NMBck_O vs. NMDst_O (MRPP; p = 0.53) than for all soil fungi. Much of the similarity between the Pil_Bth and NMDst_O soil communities was removed when only considering EmF (MRPP; p = 0.07), indicating disturbance effects. A graphical summary of soil EmF results is included for reference (Figure 15).

Joint-plot vectors for the EmF community (Figure 14) indicated *Piloderma*, which was negatively correlated with both axes 1 and 2, as the strongest genus determining Pil_Bck and Pil_Bth treatment similarities. *Wilcoxina*, with a strong negative correlation with axis-2, was notably less abundant in NMBck_O and NMDst_O treatments. *Sebacina* and *Amphinema* were positively correlated with axis-1 and were less important in Pil_Bck and Pil_Bth communities. *Pseudotomentella*, *Cadophora* and *Russula* were positively correlated with axis-1 and were significant members of NMBck_O and, to a lesser extent, NMDst_O communities.

A hierarchical cluster dendrogram for soil fungi illustrates treatment relationships with individual soil cores (Figure 16); NMDst_O was excluded from this analysis for cluster clarity. Two major groups were immediately evident (a = non-mat; b = *Piloderma* mat) and reflect results from NMS (Figure 12) and MRPP (Table 4 column 2b). One

Pil_Bck and one Pil_Bth community clustered in group ‘a,’ while two NMBck_O and one Pil_Dth community clustered in group ‘b.’ Otherwise there was very strong clustering of hypothesized community shifts and, in general, respective mat and non-mat communities from the same site clustered together, supporting high site-to-site variation.

Fungal relative abundance in the organic horizon as broken down by treatment (Figure 17) and individual soil core (Figure 18), permits visualization of NMS, MRPP and ISA trends; however, only soil fungi classified to the genus level were included in Figures 17 and 18. Indicator genera are quite apparent in these figures; most notably are *Piloderma*, *Russula, Mortierella*, *Pseudotomentella*, *Leohumicola*, *Auricularia* and *Biscogniauxia*. At the phylum level (Figure 19) the Basidiomycota are more abundant in Pil_Bck than NMBck_O and the Pil_Bth community more closely resembles Pil_Bck. Transferring a *Piloderma-mat* to non-mat soil (Pil_Dth) increases Ascomycota and Fungi *incerti cedis* while decreasing Basidiomycota.

4.3.2. Mineral Horizon (*Ramaria*)

An NMS ordination of all soil fungal genera (refer to Figure 20 throughout) showed a separation of Ram_Bck from all other treatments. However, this was not represented in subsequent MRPP analyses (refer to Table 4 column 2b throughout), which indicated no pairwise treatment comparison was different from any other. Joint-plot overlays were able to identify fungal genera that resulted in reasonable separation of Ram_Bck communities. *Tricholoma*, *Hymenoscyphus*, *Syzygospora*, *Troposporella*, Atheliaceae 9, four Hyaloscyphaceae spp., Capnodiales 8, Dothideomycetes 3 and Helotiaceae 3 were negatively correlated with axis-2, while *Mortierella* and to a lesser extent, *Russula* and *Wallemia*, were positively correlated. The Ram_Bck centroid was negatively associated with axis-2, though ordination distances within the treatment were variable; one Ram_Bck sample even clustered with non-mat and Ram_Bth communities.

An ISA (refer to Table 9 throughout) examined fungal community differences between treatments that may not have been represented on the two primary NMS axes (Figure 20). Seventeen fungal genera strongly indicated treatment type ($p < 0.05$); an

additional 16 fungal genera had p-values < 0.15 and were reported as their probable importance may have been underrepresented with a small sample size. For Ram_Bck *Thysanophora*, *Mycena*, Agaricomycetes 6, Helotiales 3, *Libertella* and Dothideomycetes sp. 3 (IV = 70, 60, 36, 41, 44, 40; p = < 0.01, < 0.01, 0.04, 0.04, 0.04, 0.04) were all strong indicators, Dothideomycetes sp. 4, *Tricholoma* and *Cladosporium* (IV = 58, 41, 35; p = 0.06, 0.08, 0.08) were good indicators and *Syzygospora* (IV = 28; p = 0.13) was a reasonable indicator. *Ramaria* was absent from both NMS vectors and the ISA. Ram_Dth also had a large number of indicator species: Sordariomycetes sp. 9, *Pyrenochaeta*, Glomeromycota sp. 12, *Rhizophagus* and *Kuzuhaea* (IV = 51, 40, 40, 40, 39; p = 0.01, 0.02, 0.03, 0.03, 0.05) were strong indicators, Basidiomycota sp. 7 and Helotiales sp. 10 (IV = 37, 31; p = 0.07, 0.08) were good indicators and Helotiaceae sp. 9 and Herpotrichiellaceae sp. 8 (IV = 37, 33; p = 0.11, 0.12) were reasonable indicators. Ram_Bth, NMDst_M and NMBck_M had fewer indicator genera. Basidiomycota sp. 3 and *Neofabraea* (IV = 49, 45; p = 0.01, 0.03) were strong indicators of Ram_Bth and Mitosporic Ascomytota sp. 4 (IV = 41; p = 0.06) was a good indicator. Only Tricholomaceae sp. 2 and Hyaloscyphaceae sp. 7 (IV = 29, 29; p = 0.13, 0.14) were found to be reasonable indicators of NMDst_M. *Stibella* was a strong indicator of NMBck_M (IV = 60; p < 0.01) and *Inocybe* was a reasonable indicator (IV = 49; p = 0.08).

Focusing only on EmF genera from the soil fungal dataset yielded somewhat similar results to the entire soil fungal community (refer to Figure 21 and Table 4 column 2a throughout). The NMS ordination (Figure 21) visually shows a greater separation in treatment centroids; however within-treatment scatter remained high. Ram_Bck was the only treatment with reasonable grouping in ordination space, being negatively correlated with axis-2. Specific joint-plot vectors of note include *Tricholoma*, *Piloderma* and Atheliaceae 4 associating with Ram_Bck, while *Leohumicola*, *Leucogaster*, *Gautieria* and *Lyophyllum* do not. Additionally, *Russula* is strongly negatively associated with axis-1 and seems to indicate non-mat communities.

Fungal relative abundance in the mineral horizon as broken down by treatment (Figure 22) and individual soil core (Figure 23) permits visualization of the trends from NMS, MRPP and ISA; however, only soil fungi classified to the genus level were included in Figures 22 and 23 whereas higher taxonomies were excluded. Many indicator genera are represented in these figures; most notably are *Tricholoma*, *Mycena*, *Lecythophora*, *Inocybe* and *Wilcoxina*, while *Ramaria* was absent. Fungal phyla were evenly distributed among treatments in the mineral horizon and soil manipulation did not shift community composition at this taxonomic level (Figure 24).

4.4. Sequencing and Diversity of Soil Bacteria

A total of 18,766 OTUs were identified from 57,184 sequences that passed filtering and quality control thresholds; 14,442 were singletons (80.0% OTUs, 25.3% sequences) and were removed (4,324 non-singleton OTUs); 1,799 were doubletons (10.0% OTUs, 6.3% sequences), 679 were tripletons (3.6% OTUs, 3.6% sequences), 381 were quadrupeltons (2.0% OTUs, 2.7% sequences) and the remaining 1,465 OTUs occurred > 4 times (7.8% OTUs, 62.2% sequences). The remaining sequences ranged from 204-1487 per sample with a OTU range of 148-716. The number of sequences per treatment ranged from 2404-5137. OTUs were phylotyped to the lowest acceptable classification; groupings were ultimately analyzed at the family level in PC-ORD, leaving higher taxonomic identification at each respective level, which resulted in 217 families. The organic horizon contained 3,340 non-singleton 97% OTUs whereas the mineral horizon contained 3,467. A summary of average bacterial OTU richness, evenness and diversity statistics can be found for “families” (Table 10) and 97% OTU’s (Table 6); henceforth “families” will be referred to as families.

In the organic horizon, bacterial family richness was lowest for Pil_Bck and Pil_Bth, while 97% OTUs showed only Pil_Bth as having a markedly lower richness; similar results were found for Shannon’s diversity (Tables 10 and 6). Bacterial family richness was statistically different between all treatments ($F = 3.16$; $p = 0.031$), however a post-hoc means comparison did not yield further separation; 97% OTU richness did not

yield treatment differences (F = 1.6; p = 0.206). Similar results were found for Shannon's diversity (Family F = 3.12, p = 0.033; 97% OTUs F = 1.64, p = 0.194), however Pil_Bth was found to have statistically lower bacterial family Shannon's diversity than Pil_Dth following post-hoc means comparisons; other treatments were intermediate between the two. In the mineral horizon, Ram_Bck generally showed decreased richness and Shannon's diversity compared to non-mat soils for all analyses (Tables 10 and 6). Significant treatment differences in bacterial family richness (F = 3.66; p = 0.018), 97% OTU richness (F = 3.12; p = 0.034) and Shannon's diversity of 97% OTUs (F = 5.27; p = 0.003) were observed, while no treatment difference in Shannon's diversity of bacterial families was observed (F = 1.4; p = 0.265). Post-hoc means comparisons showed that Ram_Bck consistently had statistically lower metrics than Ram_Dth and NMBck_M for all but 97% OTU Shannon's diversity. Additionally, the Ram_Bth 97% OTUs had a higher Shannon's diversity than Ram_Bck. Unlike soil fungi, bacterial richness and Shannon's diversity were not substantially affected by site-site variation (data not shown).

4.5. Soil Bacterial Community Composition

4.5.1. Organic Horizon (*Piloderma*)

An NMS ordination of treatments in soil bacterial family-space and subsequent MRPP analyses (refer to Figure 25 and Table 4 column 3 throughout) suggest overall bacterial community treatment differences (MRPP; A = 0.06, p = 0.09); however, strong fungal community trends did not translate to bacteria. Taken as a whole, centroid positions on the bacterial NMS ordination (Figure 25) along the first two axes of a three dimensional result, show similar trends to the fungal ordination (Figure 12); Pil_Bth groups closer to Pil_Bck while Pil_Dth groups closer to NMBck_O. NMDst_O, again, was the most scattered treatment in ordination space. Overall there were three bacterial community treatment pairs that strongly differed; Pil_Bck vs. Pil_Dth (MRPP; p = 0.04), Pil_Bth vs. Pil_Dth (MRPP; p < 0.01) and NMDst_O vs. Pil_Dth (MRPP; p = 0.03). The only other reasonable bacterial community treatment difference occurred between

Pil_Dth and NMBck_O (MRPP; $p = 0.10$). Therefore, the bacterial communities in Pil_Dth treatments were unique from all other treatments, although marginally for NMBck_O. One unexpected comparison was between Pil_Bck and NMBck_O, which appeared to be visually distinct in the two dimensions represented in the NMS ordination; however these two communities were not unique when analyzed with MRPP (MRPP; $p = 0.55$). This result may compromise interpretation of the trajectories of Pil_Bth and Pil_Dth treatment communities; however, a few notable trends appear relevant. Pil_Bck and Pil_Bth treatments shared bacterial communities that yielded the most analogous pairwise comparison (MRPP; $p = 0.76$), whereas Pil_Bth was more dissimilar to NMBck_O than even Pil_Bck (MRPP; $p = 0.27$). No significant changes were observed when comparing higher or lower bacterial taxonomic groupings, even 97% OTUs; above the family level (e.g. order) treatment differences were further obscured (data not shown).

Joint-plot vectors for the bacterial community highlight families that correlate with treatment centroids (Figure 25). Pil_Bck treatments are associated with Acidobacteriaceae and Coxiellaceae, while being notably absent in Hyphomicrobiaceae and Chloracidobacteria; the latter two are associated with Pil_Dth. Acidobacteria 6 (iii-15) is highly correlated with Pil_Dth and to a lesser extent NMBck_O, while Chitinophagaceae and Xanthomonadaceae are highly correlated with NMBck_O and Pil_Dth. The Pil_Bth communities have a strong correlation with Bradyrhizobiaceae, Mycobacteriaceae, Ellin329 and Actinobacteria 1, while some Pil_Bck and NMDst_O communities contain these taxa. A graphical summary of soil bacterial results is included for reference (Figure 26).

A blocked ISA (refer to Table 11 throughout) of soil bacteria was performed to examine community differences between treatments not apparent on the two primary NMS axes (Figure 25). A total of 14 bacterial families were identified as strong indicators of treatment type ($p < 0.05$); an additional 18 bacterial families had p-values < 0.15 and were reported as their probable importance may have been underrepresented with a small sample size. Pil_Bck possessed one strong indicator, Coxiellaceae (IV = 35; $p < 0.01$), and one reasonable indicator, Micrococcaceae (IV = 32; $p = 0.13$). Pil_Bth

shared Actinobacteria-1 with Pil_Bck as a reasonable indicator (IV = 25, 28; p = 0.11), yet contained no unique indicator families. TM7-1 and Actinobacteria-7 were strong indicators of NMBck_O (IV = 38, 39; p = 0.03, 0.04), while Kineosporiaceae, SC3, Ellin6067 and Pseudonocardiaceae were good indicators (IV = 34, 40, 34, 37; p = 0.06, 0.07, 0.07, 0.08) and EW055 and Rhodobacteriaceae were reasonable indicators (IV = 33, 32; p = 0.13, 0.14). NMDst_O contained the strong indicators CCU21, Sporichthyaceae and Solirubrobacteraceae (IV = 43, 50, 45; p = 0.03, 0.04, 0.04), a good indicator, *C111* (IV = 40; p = 0.08) and two reasonable indicators, Gemm-1 and ZB2 (IV = 36, 34; p = 0.10, 0.14). Finally, Pil_Dth contained the highest number of strong indicators with FFCH4570, SVA0725, Nitrospiraceae and Koribacteraceae (IV = 50, 57, 46, 30; p = < 0.01, < 0.01, 0.04, 0.04) and shared two reasonable indicators, Xanthomonadaceae with NMBck_O (IV = 36, 28; p = 0.11) and Oxalobacteriaceae with Pil_Bth (IV = 36, 19; p = 0.13). Chitinophagaceae, Mycobacteriaceae and Ellin329 were rather cosmopolitan in importance among treatment groups and likely separated strongly on NMS axis-2 due to within-treatment separation or their relevance to the two primary axes of the ordination; Solibacteraceae and PRR-10 were also cosmopolitan.

Bacterial relative abundance in the organic horizon as broken down by treatment (Figure 27) permits visualization of NMS, MRPP and ISA trends; however, only soil bacteria classified to the family level were included. Bacterial distributions appear relatively uniform at the family level with slight variations in relative abundance.

4.5.2. Mineral Horizon (*Ramaria*)

An NMS ordination of soil bacterial families (refer to Figure 28 throughout) did not show visual separation of treatments. Individual sample scatter was high, a result reflected in the overall MRPP analysis (refer to Table 4 column 3 throughout: MRPP; A = -0.01, p = 0.55). The only reasonable statistical difference between treatments was Ram_Bck vs. Ram_Bth (MRPP; p = 0.05), an unexpected result that corresponds to that of the soil EmF community. Otherwise, the only trend in treatment differences can be seen with Ram_Bck, where the lowest pairwise p-values occurred between

Ram_Bck/NMBck_M (MRPP; p = 0.29) and Ram_Bck/NMDst_M (MRPP; p = 0.15); Ram_Bck and Ram_Dth were quite similar (MRPP; p = 0.69). In the NMS analysis, centroids for NMBck_M and Ram_Bth were almost overlapping, while Ram_Dth and NMDst_M were equidistant in opposite directions along axis-1. Treatment scatter appeared less pronounced in non-mat treatments. One extreme outlier, 4Ram_Bck, highly skewed the ordination in the negative direction on axis-1, thus compressing real distances among other samples; we found no reason to exclude this sample.

Interpretation of joint-plot vectors for bacterial community treatments is questionable; however, general observations are relevant. The outlier, 4Ram_Bck, strongly correlated with a large number of families that were not as prominent in other samples and therefore absent from the ISA (Table 12); these will not be mentioned. Mycobacteriaceae and Ellin329 were positively correlated with axis-2 and had a higher relative abundance in certain Ram_Bck, NMDst_M and Ram_Dth samples. On the other hand Kineosporiaceae, Chloracidobacteria and Acidobacteria iii1-15 were negatively correlated with axis-2 and positively correlated with axis-1 indicating affinity for non-mat and some Ram_Bth samples.

An ISA (refer to Table 12 throughout) of soil bacteria was performed to examine community differences between treatments not apparent on the two primary NMS axes (Figure 28). A total of 12 bacterial families were identified as strong indicators of treatment type ($p < 0.05$); an additional 11 bacterial families had p-values < 0.15 and were reported as their probable importance may have been underrepresented with a small sample size. Ram_Bck had no unique bacterial indicators. Ram_Dth harbored the highest number of strong indicators including SJA-28, MLE1-12, BD7-11, Solirubrobacteraceae, Hyphomonadaceae, Holophagaceae and KD8-87 (IV = 57, 51, 40, 40, 40, 35, 31; p = < 0.01, < 0.01, 0.03, 0.03, 0.03, 0.05, 0.05). Gallionellales strongly indicated Ram_Bth (IV = 48; p = 0.01), while Entotheonellaceae and WPS-2 were good indicators (IV = 33, 35; p = 0.05, 0.05). TK17 was a strong indicator of NMDst_M (IV = 67; $p < 0.01$) and Xanthobacteriaceae was a good indicator (IV = 37; p = 0.06). NMBck_M had one strong indicator, Phyllobacteriaceae (IV = 38; p = 0.03), one good

indicator, RB40 (IV = 37; p = 0.07), and one reasonable indicator, Kineosporiaceae (IV = 29; p = 0.13). More cosmopolitan families included Thermogemmatisporaceae, Nitrospirales, Hyphomicrobiaceae, Beijerinckiaceae and Ellin6529.

Bacterial relative abundance in the mineral horizon as broken down by treatment (Figure 29) permits visualization of NMS, MRPP and ISA trends; however, only soil bacteria classified to the family level were included. Bacterial distributions appear relatively uniform at the family level with slight variations in relative abundance.

4.6. qPCR Results

Fungal ITS copy numbers (Figure 31A) showed significant treatment differences in the organic horizon (ANOVA; F = 3.34, p = 0.026), but no difference in the mineral horizon (ANOVA; F = 1.3, p = 0.301). A post-hoc means comparison including all treatments in the organic horizon did not reflect differences when correcting for multiple comparisons. However, a targeted post-hoc means comparison performed only on background communities (Pil_Bck vs. NMBck_O; Ram_Bck vs. NMBck_M) using a one-tailed t-test indicated Pil_Bck community has significantly greater fungal rDNA copy numbers than the NMBck_O community (1.7x; p = 0.048). Fungal rDNA copy numbers in Ram_Bck and NMBck_M soils did not differ using the same analysis (p = 0.47), supporting ANOVA results. Pil_Bck soils also had significantly higher fungal biomass than Pil_Dth (2.7x; p = 0.004).

Bacterial 16S rRNA gene copy numbers (Figure 31B) showed no significant difference among treatments in the organic (ANOVA; F = 1.32, p = 0.290) or mineral (ANOVA; F = 0.40, p = 0.804) horizons, respectively. Post-hoc means comparisons using a one-tailed t-test of background treatments (Pil_Bck vs. NMBck_O; Ram_Bck vs. NMBck_M) did not differ for the organic (p = 0.11) or mineral (p = 0.15) horizons, though there was a trend for higher bacterial rRNA gene copy numbers in Pil_Bck compared to NMBck_O; the opposite was true for Ram_Bck compared to NMBck_M.

Fungal:Bacterial ratios (Figure 31C) showed significant treatment differences in the O-horizon (ANOVA; F = 3.21, p = 0.03), but no difference in the mineral horizon

(ANOVA; F = 0.74, p = 0.572). A post-hoc means comparison including all treatments in the organic horizon did not reflect a difference when correcting for multiple comparisons. Targeted post-hoc means comparisons on background communities (Pil_Bck vs. NMBck_O; Ram_Bck vs. NMBck_M) using a one-tailed t-test indicated no difference in the fungal:bacterial ratios in either soil horizon (organic p = 0.22; mineral p = 0.21), although both appeared to trend higher in background mat soils. Pil_Dth soils may have a lower fungal:bacterial ratio than Pil_Bck soils (p = 0.085).

4.7. Other Community Results

4.7.1. Influence of *Piloderma* in the Organic Horizon

Due to inherently high *Piloderma* relative abundance in mat-soils of the organic horizon, ITS copy numbers belonging to *Piloderma* were removed, relativized by sample unit and compared across treatments (Figure 31). Significant treatment differences existed (ANOVA; F = 9.13, p < 0.001) and post-hoc means comparisons indicated Pil_Bck and Pil_Bth were statistically similar while being different from NMBck_O. As expected, *Piloderma* ITS copy numbers increased for Pil_Bth and decreased for Pil_Dth compared to original background treatments. *Piloderma* copy number was naturally low in NMBck_O and NMDst_O communities (relative abundance, 2.7%, 2.3%); however, non-mat samples at site 147 possessed a relative abundance of *Piloderma* typical of Pil_Bck (NMBck_O = 14.4%; NMDst_O = 11.8%). Additionally, Pil_Bck from site 82 had atypically low relative abundance of *Piloderma* (2.8%). Over the course of 51 months, these communities may have naturally shifted from the original identification.

An additional MRPP analysis was run for the soil fungal community in the organic horizon after *Piloderma* OTU removal to assess the impact of this genus on community structure (compare to Table 4 column 2b). Removing *Piloderma* muted original treatment differences (MRPP; A = 0.06, p = 0.09). Pairwise comparisons showed increased similarities between Pil_Bck/NMBck_O (MRPP; A = 0.04, p = 0.19) and Pil_Bth/Pil_Dth (MRPP; A = 0.04, p = 0.19), while increasing slightly between Pil_Bck/Pil_Dth (MRPP; A = 0.05, p = 0.13) and Pil_Bth/NMBck_O (MRPP; A = 0.07,

p = 0.11). Treatment differences became more pronounced for NMBck_O/NMDst_O (MRPP; A = 0.08, p = 0.07) and NMBck_O/Pil_Dth (MRPP; A = 0.06, p = 0.06). The remaining comparisons generally remained unchanged (data not shown).

Similarly, an MRPP analysis was run for the soil fungal EmF community in the organic horizon after the removal of the *Piloderma* OTU (compare values to Table 4 column 2b). Again, this diminished overall differences (MRPP; A = 0.04, p = 0.17); however, the Pil_Bck EmF community remained unique compared to NMBck_O (MRPP; A = 0.08, p = 0.04) unlike the entire soil fungal community. There was also no change in soil EmF communities between Pil_Bth/Pil_Bck and NMBck_O/NMDst_O, which remained similar. Pil_Bth and NMBck_O also had substantially increased EmF community similarity (MRPP; A = 0.02, p = 0.32). Pil_Dth EmF became more dissimilar to NMBck_O (MRPP; A = 0.04, p = 0.14) and NMDst_O (MRPP; A = 0.04, p = 0.17), while becoming more similar to Pil_Bck (MRPP; A = 0.02, p = 0.22) and Pil_Bth (MRPP; A = -0.02, p = 0.56). The remaining comparisons were unchanged.

4.7.2. Competitive Exclusion of *Russula* in *Piloderma* Mats

The presence of *Piloderma* mats has been hypothesized to competitively exclude the EmF genus *Russula* from mat communities (Hesse, 2012). We found the relative abundance of *Piloderma* and *Russula* ITS copy numbers to be similar in NMBck_O (p = 0.748), an indication that *Piloderma* was increasing and *Russula* was decreasing in Pil_Bth (p = 0.149) and that *Piloderma* relative abundance was significantly greater than *Russula* in Pil_Bck (p = 0.004). The relative abundance of *Russula* in Pil_Bck was 0.8%; of the 20 *Russula* OTUs identified from the soil, and the 10 species identified on EmF root-tips, only those with identities closest to *Russula turci* were present in Pil_Bck, indicating that a small number of species within the genus may be capable of colonizing *Piloderma* mats. It appears *Piloderma* mats are competitively excluding *Russula* in these forests (Figure 32).

4.7.3. Pairwise Indicator Species Analyses of Organic Horizon Soil Fungal Communities

A pairwise ISA performed on all organic-horizon soil fungal treatments (10 total) identified deeper trends in community composition; the original ISA included all treatments and yielded 34 fungal genera that were strong, good and reasonable treatment indicators (Table 8; $p < 0.05, < 0.10, < 0.15$, respectively). Pairwise comparisons of the same treatments yielded 41 genera that were strong and good indicators ($0.05 < p < 0.10$), increasing both the number and significance of indicator genera from the original 34. Of the original 34, 23 were present in pairwise comparisons, 11 were unique to the overall ISA and 18 were unique to pairwise comparisons.

The most important of these 10 pairwise comparisons was Pil_Bck and NMBck_O fungal communities; as mentioned, differences in these communities were inherent to the project design. Twelve genera significantly differed between the two treatments. Genera strongly indicating Pil_Bck communities included *Piloderma, Leptodontidium, Neofabraea, Oidiodendron* and Agaricomycetes sp. 15 (IV = 84, 82, 77, 77, 67; p = 0.02, 0.03, 0.02, 0.08, 0.06), while strong indicators of NMBck_O included *Russula*, *Pseudotomentella, Leohumicola, Cadophora*, Dermateaceae sp. 5, *Sphaerobolus* and *Thysanophora* (IV = 84, 81, 73, 73, 67, 63, 62; p = 0.02, 0.01, 0.06, 0.08, 0.06, 0.06, 0.09). Of the pairwise genera identified, only *Oidiodendron* and *Russula* were absent as significant indicators when all five treatments were included because both genera were shared with Pil_Bth. Of the 10 genera represented in both ISAs, *Neofabraea*, *Leptodontidium*, *Cadophora* and *Cenococcum* were meaningfully represented in other treatments. *Piloderma* was shared between Pil_Bck and Pil_Bth, *Sphaerobolus* was unique to NMDst_O while Agaricomycetes sp. 15 was unique to Pil_Bck and Dermateaceae sp. 5 was shared between NMBck_O and Pil_Dth. Of particular note was the overwhelming association of *Russula* and *Pseudotomentella* with NMBck_O soils, as well as *Leptodontidium* and *Neofabraea* with Pil_Bck.

Pairwise ISAs identified differences that may still exist between the hypothesized community trajectories after 51 months (e.g. Pil_Bth→Pil_Bck, Pil_Dth→NMBck_O). Some of these taxa may contribute to a transitional community structure should they be

absent from background communities. Six genera differed between each of the treatment pairs. For Pil_Bck and Pil_Bth, Dermateaceae sp. 2, Agaricomycetes sp. 15 and *Gymnopus* proved to be strong indicators of Pil_Bck (IV = 67, 64, 63; p = 0.06, 0.07, 0.06), while *Geomyces*, Leotiomycetes sp. 7 and *Umbelopsis* (IV = 67, 67, 64; p = 0.06, 0.09, 0.06) were strong indicators of Pil_Bth, though *Umbelopsis* was of notable importance in Pil_Bck as well (IV = 36). Of this indicator group *Gymnopus*, Leotiomycetes sp. 7 and *Umbelopsis* were not present in the overall ISA. For Pil_Dth and NMBck_O, *Mortierella*, Hyaloscyphaceae sp. 3 and *Oidiodendron* were strong indicators of Pil_Dth (IV = 79, 75, 70; p = < 0.01, 0.05, 0.08); *Leohumicola*, *Pseudotomentella* and *Russula* were strong indicators of NMBck_O (IV = 83, 80, 80; p = 0.02, 0.03, 0.04). Of this pairwise indicator group only *Russula* and *Oidiodendron* were not represented in the overall treatment ISA.

Pairwise ISAs also identified differences in hypothesized trajectories away from the soil fungal community of origin. In the first comparison, Pil_Bck/Pil_Dth, the four strong indicators of Pil_Bck were *Piloderma*, *Cenococcum*, *Neofabraea* and *Cortinarius* (IV = 91, 83, 78, 65; p = < 0.01, 0.02, 0.02, 0.08), while the six strong indicators of Pil_Dth were *Mortierella*, *Sagenomella*, *Phialocephala*, *Wallemia*, *Amorphotheca* and *Thysanophora* (IV = 85, 83, 78, 71, 66, 59; p = 0.01, 0.02, 0.02, 0.09, 0.09, 0.09). Of these *Cortinarius*, *Sagenomella* and *Phialocephala* were absent from the overall ISA. Of the six genera indicating Pil_Dth only one, *Thysanophora*, indicated NMBck_O when compared to Pil_Bck. In the second comparison, NMBck_O→Pil_Bth, Pil_Bth was characterized by *Oidiodendron*, *Piloderma*, *Umbelopsis*, Leotiomycetes 7 and *Leptodontidium* (IV = 80, 77, 74, 73, 72; p = 0.03, 0.07, < 0.01, 0.07, 0.09) and NMBck_O was characterized by *Cadophora*, *Thysanophora*, *Sphaerobolus* and *Gymnopus* (IV = 72, 67, 67, 64; p = 0.08, 0.06, 0.06, 0.05), but not *Russula*. Of the five genera indicating Pil_Bth two, *Umbelopsis* and Leotiomycetes sp. 7, did not indicate Pil_Bck when compared to NMBck_O.

Finally, to capture fungal genera that may have been affected by disturbance in non-mat communities a comparison of NMBck_O and NMDst_O was performed.

Genera strongly indicating NMBck_O include *Mycena* and *Pseudotomentella* (IV = 75, 67; p = 0.04, 0.09), while strong indicators of NMDst_O were *Umbelopsis*, *Lecythophora*, Chaetothyriales sp. 4, *Penicillium* and Dothideomycetes sp. 4 (IV = 76, 75, 75, 66, 60; p = < 0.01, 0.04, 0.04, 0.05, 0.09). Of this group, only *Lecythophora* and *Pseudotomentella* were included as indicators when considering all five treatments; the former was notably absent from Pil_Bck and the latter was the best NMBck_O indicator. Additionally, *Umbelopsis* was of some importance in NMBck_O (IV = 24).

4.7.4. Pairwise Indicator Species Analyses of Organic Horizon Soil Bacterial Community Background Treatments

Although Pil_Bck and NMBck_O were similar when considering the entire bacterial community, a pairwise ISA was performed on bacterial families for O-horizon background treatments, as previous studies have shown *Piloderma* mat and non-mat bacterial communities to be different (Kluber *et al.*, 2011; Hesse, 2012). A total of five families were identified as strong indicators of treatment type ($p < 0.05$); an additional six were good indicators ($0.05 < p < 0.10$). The indicator taxa represented here may have been obscured by in the overall ISA due to transitional treatments (e.g. Pil_Bth and Pil_Dth), as evidenced for soil fungi. Bacterial families indicating Pil_Bck communities included an unknown phylum Actinobacteria, Acetobacteriaceae, Coxiellaceae and Methylocystaceae (IV = 73, 71, 63, 63; p = 0.03, 0.01, 0.04, 0.09), while strong indicators of NMBck_O included unknown class TM7-1, unknown order Ellin6067, Beijerinckiaceae, unknown class SC3, unknown order Sva0725, unknown class Actinobacteria and unknown order IS-44 (IV = 84, 77, 68, 68, 67, 67, 63; p = 0.02, < 0.01, 0.07, 0.07, 0.06, 0.06, 0.06).

When looking at bacterial genera in the same way a larger number of indicators were evident, although taxonomic certainty at the genus level was rarely possible. Indicators of Pil_Bck were genera in the Acetobacteraceae, Methylocystaceae, an unknown Actinobacteria, Coxiellaceae and Sinobacteraceae (IV = 74, 71, 71, 63, 60; p = 0.02, 0.05, 0.02, 0.04, 0.02), while marginal indicators included an unknown

Alphaproteobacteria, Bradyrhizobiaceae and Syntrophobacteraceae (IV = 58, 55, 50; p = 0.10, 0.13, 0.12). Indicators of NMBck_O were genera in class TM7-1, Beijerinckiaceae, unknown order Ellin6067, *Sphingomonas*, unknown order IS-44; Comamonadaceae, unknown class Actinobacteria, unknown class Chloracidobacteria and Chitinophagaceae (IV = 83, 77, 76, 75, 74, 73, 67, 63, 60; p = 0.06, 0.08, 0.02, 0.08, 0.05, 0.02, 0.05, 0.08, 0.04), while marginal indicators included Flammeovirgaceae and an unknown order Sva0725 (IV = 74, 67; p = 0.13, 0.11). This indicates specific genera within the family level groupings differ between Pil_Bck and NMBck_O.

As a final ISA, 97% OTUs were compared between the background communities to assess differences present at the putative species level. Far fewer indicator taxa were present at the species level than either genus or family. Pil_Bck contained six indicators including one species within *Mycobacterium* (IV = 69), *Steroidobacter* (IV = 64), an unknown Acetobacteraceae (IV = 46), *Bradyrhizobium* (IV = 67), *Rhizobium* (IV = 64) and *Acidisphaera* (IV = 67); NMBck_O contained only one indicator, a species in the phylum Bacteroidetes (IV = 51). All p-values equaled 0.08. *Piloderma* mats may be selecting for specific bacterial taxa that are less abundant and, therefore, do not differentiate treatments based on overall bacterial community composition.

4.7.5. Influence of *Ramaria* in the Mineral Horizon

The genus *Ramaria* was surprisingly absent from soil fungal 454 sequences. There were only 79 ITS rDNA copy numbers that were identified as *Ramaria* in the mineral horizon, distributed among 7/29 soil cores (25%); *Ramaria* was present in three Ram_Bck cores, three Ram_Bth cores and one Ram_Dth core. The only simultaneous presence of *Ramaria* in Ram_Bck and Ram_Bth soil cores occurred at site-147; the only Ram_Dth core that contained *Ramaria* also occurred at this site. In fact, *Ramaria* ITS copy numbers were found in higher overall abundance in the organic horizon (204 copies in four soil cores). *Ramaria* did not surface as an indicator of Ram_Bck soils, or any other treatment for that matter. These findings bring into question the long-term stability of *Ramaria* mats.

4.8. Summary of Results Based on Original Treatment Hypotheses

Four diagrams at the end of the results section graphically depict each microbial communities response to experimental manipulation (Figures 9, 15, 13 and 26), which can be compared to the original hypotheses (Figure 1). These will be explained briefly in the text below and summarize results from NMS, MRPP and ISA.

EmF on root-tips in the organic horizon were the only microbial group where Pil_Dth treatments were omitted for aforementioned reasons. Pil_Bck remained distinct from non-mat communities while the transition of Pil_Bth to Pil_Bck was incomplete; Pil_Bth remained similar to both NMBck_O and NMDst_O, though to a lesser extent than Pil_Bck. The birth of *Piloderma* mat active EmF root-tip communities was occurring. There was also a high degree of similarity between NMBck_O and NMDst_O, indicating that disturbance and enclosure in 2 mm mesh did not affect root-tip EmF community structure after 51 months (Figure 9, left panel). EmF root-tip fungi in the mineral horizon underwent similar community shifts to the organic horizon. Ram_Bck remained distinct from non-mat communities while the transition of Ram_Bth to Ram_Bck was more definitive than for the organic horizon. Ram_Bth only shared similarities with NMDst_M yet had a high degree of similarity to Ram_Bck. The transition of Ram_Dth to a non-mat community and Ram_Bth to a *Ramaria* mat community led to similarities between the two, apparently still in transition. Ram_Dth also retained similarities to Ram_Bck. NMBck_M and NMDst_M remained quite similar, which again indicates a lack of disturbance affect for the active EmF root-tip community (Figure 9, right panel).

Soil EmF in the organic horizon behaved fairly similarly to EmF root-tips. Pil_Bck remained distinct from both non-mat treatments. The Pil_Dth community fully transitioned from Pil_Bck to become more like the non-mat communities, which remained very similar themselves. Pil_Bth nearly transitioned to Pil_Bck, however had a marginal similarity to NMDst_O. A slight similarity also remained between Pil_Bth and Pil_Dth. Thus, the soil EmF community appears to have mostly confirmed our hypotheses after 51 months (Figure 15, left panel). Soil EmF in the mineral horizon did

not respond neatly to the original hypotheses. Ram_Bck was not distinct from non-mat communities; it was found to be similar to both NMBck_M and NMDst_M. In fact, the only community differences were found between Ram_Bck/Ram_Bth and Ram_Bck/Ram_Dth, though the latter was marginal (Figure 15, right panel).

Soil fungi in the organic horizon behaved in the same way as soil EmF with two notable exceptions; Pil_Dth did not retain any similarities with Pil_Bck or show transitional community similarities with Pil_Bth. Interestingly, NMBck_O and NMDst_O showed the highest dissimilarity among soil fungi than other microbial groups and may indicate soil saprotrophic response to disturbance. Overall, the soil fungal community responses in the organic horizon were closest to the original hypotheses (Figure 13, left panel). Soil fungal communities in the mineral horizon showed no differences between any of the treatments (Figure 13, right panel).

Soil bacterial communities did not respond similarly to fungal communities. In the organic horizon, Pil_Bck was found to have similar bacterial communities to both non-mat treatments. Pil_Dth, interestingly, was found to be significantly different from all treatments except NMBck_O, from which it was marginally different. Otherwise, no other communities were unique (Figure 26, left panel). In the mineral horizon the only treatment difference was Ram_Bck and Ram_Bth. Otherwise, all bacterial communities were similar (Figure 26, right panel).

5. Discussion:

5.1. The Perennial Status of Ectomycorrhizal Fungal Mats

It has been suggested that mats are perennial features (Griffiths *et al.*, 1994; Phillips *et al*, 2012; Trappe *et al.*, 2012), and may migrate to (colonize) novel resource-pools through time (Trappe *et al*, 2012; Hintikka & Näykki, 1967; personal observation). Phillips *et al.* (2012) also observed the deterioration of a small number of *Piloderma* mats, as well as mat establishment in areas not previously colonized over two growing seasons calling into question mat longevity and lending support for mat migration. The continued growth and senescence of fine-roots within mat-soil can allow long-term colonization and support for mat-forming fungi. Mean fine-root lifespan has been estimated at 108 days in an Alaskan black spruce forest (Ruess *et al.*, 2003) and 400 days in a Swedish Norway spruce forest (Majdi *et al.*, 2001); tight nutrient recycling in mats may capitalize on this turnover (Griffiths *et al.*, 1994). Ectomycorrhizal fungal mat stability over time was a key assumption of this study, as the development (birth) and decline (death) of mats was tracked over 51 months and initial mat presence or absence was expected to persist (background treatments).

Initial data from this experiment (Blanchard, 2008) clearly indicated each mat-type possessed different fungal communities than adjacent non-mat soil in respective horizons. Our data demonstrate that *Piloderma*-mat and non-mat organic communities remained distinct after 51 months for every soil fungal community analyzed; additionally, *Piloderma* relative ITS copy numbers were highest in Pil_Bck cores, coupled with visual observations of dense white hyphae and rhizomorphs indicating mat presence. Mats formed by other species have been observed as long-lived perennial features, perhaps decades for *Hydnellum ferruginieum* (Hintikka & Häykki, 1967). We believe this is the first genetic-based empirical evidence that *Piloderma* mats are long-lived perennial features capable of supporting unique fungal communities, as compared to adjacent non-mat soil, for over four years.

Long-term *Piloderma* mat persistence may find support in the ability of *Piloderma* spp. to act as functional saprotrophs (detailed later). It is generally accepted that certain EmF have the capacity to decompose complex C substrates for use as a nutrient and/or C source (Koide *et al.*, 2008; Talbot *et al.*, 2008; Cullings & Courty, 2009; Koide *et al.*, 2014); EmF communities can even exhibit higher hydrolytic enzyme activity than saprotroph-dominated communities (Phillips LA *et al.*, 2013). *Piloderma* spp. are particularly capable of functional saprotrophy (Tedersoo *et al.*, 2003; Cullings *et al.*, 2010; Phillips LA *et al.*, 2013), likely permitting the mycelial mat structure to persist during variations in host plant C supply. However, enzyme production does not guarantee released C uptake, and is probably the result of mining tightly bound mineral nutrients. Further support may lie in stable isotope signatures of mat-forming genera such as *Hydnellum* spp., as those for *Piloderma* have not been studied, perhaps because of resupinate sporocarp inaccessibility (Tedersoo *et al.*, 2010). EmF with higher $\delta^{15}N$ values can use organic N-sources more thoroughly than EmF exhibiting lower $\delta^{15}N$ values (Lilleskov *et al.*, 2002); *Hydnellum* spp. have considerably higher average $\delta^{15}N$ values than *Russula* spp. ($\delta^{15}N = 8$ vs. 3.7 in Taylor *et al.*, 2003; $\delta^{15}N = 7.5$ vs. 2.1 in Trudell *et al.*, 2004**).** Hobbie and Agerer (2010) were able to relate high biomass, exploratory strategies and hydrophobic hyphae to higher $\delta^{15}N$ values, traits describing *Piloderma*. $\delta^{13}C$ values for *Hydnellum* spp. have also been shown to equal those of some saprotrophs (Taylor *et al.*, 2003). An important next step to understanding *Piloderma* persistence is gathering stable C and N isotopic signatures from *Piloderma* spp.

Dissimilar results were found with *Ramaria*-mat and non-mat mineral communities. Only the composition of active EmF roots differed, whereas soil EmF and soil fungi exhibited a high degree of similarity. Blanchard (2008) initially typed all mineral horizon fungal mats in this experiment to six *Ramaria* species. Here, after 51 months, only three *Ramaria* species were identified on EmF root-tips. *Ramaria formosa* was identified in Pil_Bck and Pil_Bth treatments from site 41, which led to the exclusion of this site from further analyses on the basis of mat misidentification and likelihood of having mixed mats at the site. The other two species, *Ramaria celerivirescens* and

Ramaria sp. 3, were found only at site 147 in Ram_Bck, Ram_Bth and Ram_Dth samples. Pyrosequencing of soils revealed only a quarter of *Ramaria* ITS copy numbers were found in the mineral horizon; *Ramaria* EmF have previously been reported in association with mixed-mat organic soil (Dunham *et al.*, 2007; Hesse, 2012), but we cannot rule out cross-contamination during soil horizon differentiation in the laboratory. Kluber *et al.* (2010) noticed variations in mat growth habits, where dominance by one mat-former was not absolute and root-tips were colonized by multiple mat-forming fungi; without proper consideration, these variations may complicate sampling and limit data analysis. Since initial EmF typing did not indicate mixed mats (Blanchard, 2008), a more likely scenario is the development of mixed mats during the experiment.

Hesse (2012) identified *Ramaria* as the overwhelmingly dominant fungal taxa in *Ramaria* mats, an assumed trait of Ram_Bck treatments here; however, *Ramaria* was the 39th most abundant fungal genus overall, representing only 0.41% of fungal sequences and was more strongly represented in the organic horizon. In fact, only half of Ram_Bck mats contained *Ramaria*: sites 82, 120 and 147, represented < 1% of sequence relative abundance at each site. These data point to the possibility that *Ramaria* mats are more ephemeral than *Piloderma* mats and the visual identification of their hydrophobic, powdery structure may, at times, be a legacy effect (Myrold, personal communication) of historical mat presence, a remnant of the physical alteration of the soil environment.

Another possibility is the presence of other mat-formers (Trappe *et al.*, 2012) or mat-former succession. For instance, *Tricholoma* was isolated from EmF root-tips and was almost exclusively found in Ram_Bck soils (87% of all sequences), particularly at sites 120 and 125, where it occupied 18% and 4% of sequence relative abundance, respectively; however, this genus is known to form mats resembling the dense, rhizomorphic structure of *Piloderma* mats in organic soil (Trappe *et al.*, 2012). Site 137 contained *Sistotrema* on EmF root-tips and in soil of Ram_Bck and Ram_Bth treatments, representing 3% relative abundance. *Sistotrema* forms mats visually similar to *Ramaria* (Trappe *et al.,* 2012) and has been identified as a mat-former at the HJA (Dunham *et al.*, 2008). *Gautieria* was not present in Ram_Bck soils, ruling out the possibility of this

visually and spatially similar mat-former, which dominates early-successional forests in the region (Griffiths *et al.,* 1994).

There appears to be differential persistence of *Piloderma* and *Ramaria* mats in the current study. To the authors' knowledge, this is the first time a dichotomy in pattern of persistence based on molecular evidence has been documented. *Piloderma* mats are perennial features, but the same cannot be said about *Ramaria* mats. As differences in soil fungal and bacterial communities have previously been identified for each *Piloderma* and *Ramaria* mat and non-mat soil in the respective horizon (Hesse, 2012), it was expected that mat behavior would be consistent. Due to the rejection of the underlying hypothesis, that Ram_Bck and NMBck_M soils would harbor unique fungal and bacterial communities after 51 months, further discussion on mineral horizon *Ramaria* mat soils is not warranted. It is not relevant to compare Ram_Bth and Ram_Dth community change when the background communities were not unique themselves and the mat-former of interest was almost entirely absent after 51 months. Results from mineral horizon soils were included in this thesis to allow public data access, but further discussion will focus on the microbial communities of *Piloderma* mat and non-mat soil in the organic horizon, and the effect of Pil_Bth and Pil_Dth treatments on those communities.

5.2. Reciprocal Soil Transfer Experiments and Root Disruption - Effects of Soil Enclosure in PVC and Mesh on Fungal and Bacterial Biomass (qPCR)

The act of transferring soil between distinct environments to assess microbial community responses is widely practiced in microbial ecology (Bottomley *et al.,* 2006; Kageyama *et al.,* 2013; Zumsteg *et al.,* 2013); studies with root colonized soil showed fungi had a more significant transfer response than bacteria within the same vegetation type (Kageyama *et al.,* 2013). Roots, and indeed their direct C inputs through decay, leaching and translocation to mycorrhizal fungi, are known to exert substantial control on soil microbiota worldwide and in old-growth Douglas-fir forests of the PNW. This is true for both organic (Siira-Pietikäinen *et al.,* 2001) and mineral (Brant *et al.,* 2006) soil horizons.

5.2.1. *Piloderma* Mat Death Treatments (Pil_Dth)

Non-mat soils have exhibited lower fungal biomass than O-horizon rhizomorphic mats, whether the mat former was *Hysterangium* (Ingham *et al.*, 1991; Entry *et al.*, 1991b; Entry *et al.*, 1992) or *Piloderma* (Zeglin *et al.*, 2013). However, Kluber *et al.* (2011) found no difference between *Piloderma* mat and non-mat fungal biomass as represented by ITS copy number. Our data agree with Zeglin *et al.* (2013) for *Piloderma*, where Pil_Bck contained significantly more fungal ITS copy numbers than NMBck_O. Blanchard (2008) documented an almost immediate transition of Pil_Dth fungal communities away from Pil_Bck, finding Pil_Dth indistinguishable from NMBck_O after only six months. This was attributed to the loss of *Piloderma* and would suggest Pil_Dth would exhibit a reduction of fungal biomass from Pil_Bck such that it is indistinguishable from NMBck_O. Our data support this hypothesis; however, enclosure of Pil_Dth soils in PVC pipe could confound these results

In a reciprocal soil transfer experiment where soil cores were enclosed in PVC pipe, Bottomley *et al.* (2006) observed significantly lower fungal and higher bacterial biomass levels in closed vs. open cores, regardless of treatment type, after two years; the researchers suggested this may be attributable to loss of mycorrhizal fungi and enhanced C availability, nitrification potential, inorganic N and water content of closed cores. Root access to soil enclosed in PVC could occur only from the bottom, the mineral horizon, as the PVC pipe extended above the soil surface. To enter the organic horizon, roots would have had to penetrate up through the soil core, bringing with them microbiota ill-adapted to compete in the organic horizon. Problems with this approach were apparent in EmF analyses, where all treatments were omitted from root-tip analysis due to lack of root colonization. Issues are also apparent with proportions of fungal ecological groupings. Pil_Bck harbored 35% each of EmF and saprotrophic fungi (0.98:1 ratio), while NMBck_O contained 25% EmF and 45% saprotroph (0.57:1 ratio). Pil_Dth experienced a substantial EmF reduction (18%) and an increase in saprotrophic fungi (51%) (0.36:1 ratio). This may have reduced Pil_Dth biomass beyond NMBck_O and had an impact on fungal community composition as well, as suggested by Bottomley *et al.* (2006).

After 51 months, our study found no bacterial biomass differences in the O-horizon. This is opposed to Kluber *et al.* (2011), who documented significantly greater bacterial populations in non-mat vs. rhizomorphic mat soils in the fall, a similar sampling time to ours. Bacterial biomass followed the trend Pil_Bck > Pil_Dth > NMBck_O, indicating a transitional state where Pil_Dth was losing the higher bacterial biomass from its soil of origin (Pil_Bck) and becoming more like NMBck_O. However, the Pil_Dth soil bacterial community was significantly different from Pil_Bck, Pil_Bth and NMDst_O and marginally different from NMBck_O. In this fungal-dominated system it appears that although bacterial biomass was unaffected by enclosure in PVC pipe, the exclusion of roots and associated EmF may have led to novel bacterial community development in Pil_Dth. Bacterial communities in these systems may be more closely associated with roots and the mycorrhizosphere than expected.

5.2.2. Mineral Horizon (*Ramaria*) Treatments

Mineral horizon treatments did not respond similarly to the organic horizon and we found no treatment differences in fungal or bacterial biomass. Here, our results oppose those of Kluber *et al.* (2010), who found higher overall microbial biomass in hydrophobic mat than non-mat mineral soil. As stated previously, this may be attributed to the fact that Ram_Bck communities did not persist during the experiment, indicated by microbial community composition, which makes further comparison difficult.

Pil_Dth cores had nearly no colonized EmF root-tips, while substantial numbers were found in Ram_Dth. The short distance required to penetrate Ram_Dth samples allowed ample EmF root colonization, evidenced by substantial EmF root-tips, a smaller shift in EmF/saprotrophic fungal abundance and minimal shifts in fungal and bacterial biomass. These data support a high degree of root/fungal structuring of bacterial communities in the mineral horizon, which were nearly identical between Ram_Dth and NMBck_M, and less so between Ram_Dth and Ram_Bck. Enclosing soil in PVC should be approached with caution and appropriately reflect the question at hand; in this case,

root exclusion may have obscured the structuring effect of EmF and roots on soil bacteria (Pil_Dth), while allowing similar communities to develop in root presence of (Ram_Dth).

5.2.3. Organic Non-Mat Disturbance Control Treatments (NMDst_O)

Included in this experiment was a non-mat disturbance control treatment (NMDst_O), intended to ensure disruption of soil caused by coring and mesh enclosure would not drastically alter the NMBck_O microbial community. We are unaware of any microbial community study that has included a disturbance control treatment of this kind. Therefore, no study has ever quantified or qualified the microbial response to root severing and enclosure in mesh screens after regrowth has occurred.

In our study, the community composition of EmF root-tips, soil EmF and soil bacteria were unaffected by disturbance after 51 months. This is supported by NMS, MRPP, EmF root-tip, bacterial family richness and bacterial biomass data. The overall soil fungal community, however, was more vulnerable to disturbance. MRPP results indicated soil fungal communities were on the lower end of similarity between the NMBck_O and NMDst_O, while the soil fungal NMS showed more substantial scatter for NMDst_O than any other treatment; in fact, half of the samples clustered more with Pil_Bck and Pil_Bth than NMBck_O and Pil_Dth. Richness was also reduced in NMDst_O and overall fungal biomass exhibited the largest variation of any treatment. NMDst_O fungal taxonomic distributions more closely resembled Pil_Dth than they did NMBck_O, the result of increased saprotrophic taxa capitalizing on organic matter inputs from root severing. This will be discussed in greater detail below.

5.3. Fungal Communities of Organic Horizon (*Piloderma*) Soils

5.3.1. Do Piloderma Mats Competitively Exclude *Russula*?

As defined in this and previous mat studies, non-mat areas are simply soil devoid of visible mats. Therefore, non-mat fungal community structure may be inherently variable, as no visually dominant fungus identifies unique areas. Surprisingly, one fungal genus has been consistently detected in non-mat communities. *Russula* emerged as a

strong non-mat indicator and a compliment to *Piloderma* in mats formed by that genus in this study and those of Hesse (2012) and Kluber *et al.* (2011).

Our data support the hypothesis by Hesse (2012) that *Piloderma* mats competitively exclude *Russula*. *Russula* did not indicate a particular treatment in the overall ISA, likely due to the presence of transition treatments; however, *Russula* strongly indicated NMBck_O when compared to Pil_Bck, but not Pil_Bth, using pairwise ISAs. *Russula* occupied only 0.08% of the relative sequence abundance in Pil_Bck, and comprised 4.6% of NMBck_O communities. After 51 months, non-mat soil with high *Russula* and low *Piloderma* populations transferred to *Piloderma* mats (Pil_Bth - carrying *Russula* mycorrhizas, hyphae and spores) showed a competitive exclusionary trend, where *Piloderma* increased and *Russula* decreased; *Russula* occupied 3.9% relative abundance in Pil_Bth. Competitive exclusion of *Russula* by *Piloderma* may result from physical/biochemical alteration of soil by mature *Piloderma* mats (Kluber *et al.,* 2010). A senescent spore bank may have contributed to *Russula* sequence abundance in Pil_Bth treatments as *Russula* relies heavily on spore production (Redecker *et al.,* 2001), fruiting abundantly (O'Dell *et al.*, 1992; Smith *et al.,* 2002) and having limited extramatrical hyphal production (Agerer, 2001). Samples taken after 51 months would be expected to further exclude *Russula* from the system. As *Russula* is one of the most dominant EmF genera in many forest ecosystems (Horton & Bruns, 2001; Peter *et al.,* 2001; Avis *et al.*, 2003) and the sixth most abundant genus in the current study, this exclusion may provide a competitive advantage for *Piloderma* mat patchiness. This idea is not new as Agerer *et al.* (2002) evidenced competitive exclusion at small scales (cm), where pairs of EmF species never co-associated; this is supported by Pickles *et al.* (2012) and our data indicate competitive exclusion may occur at larger scales in this system.

5.3.2. Characterization of Organic Horizon Background Fungal Communities (Pil_Bck and NMBck_O)

Previous studies have found significant differences in soil fungal communities between conspicuously distinct *Piloderma* mats and adjacent non-mat areas (Blanchard,

2008; Kluber *et al*., 2011; Hesse, 2012). Taxonomic groupings highly correlated with *Piloderma* mats included the Atheliales genera *Piloderma* (Hesse, 2012) and *Leptosporomyces* (Kluber *et al*., 2011). Non-mat organic horizon taxonomic groupings have included Sebacinales, Myxotrichiaceae and Agaricales (Kluber *et al.,* 2011), as well as *Russula* (Kluber *et al.,* 2011; Hesse, 2012). This study confirms earlier results (aside from *Leptosporomyces* and the Myxotrichiaceae) and shows individual fungal community components ─ root-tip EmF, soil EmF and soil fungi ─ are indeed different. The current study identified *Piloderma, Neofabraea, Leptodontidium*, *Oidiodendron* and Agaricomycetes sp. 15 as strong indicators of Pil_Bck; *Russula*, *Pseudotomentella*, *Leohumicola, Cadophora*, Dermateaceae sp. 5, *Sphaerobolus* and *Thysanophora* strongly indicated NMBck_O when a pairwise treatment comparison was performed. Therefore, these taxa constitute the unique assemblages of each background organic horizon fungal community. Had *Piloderma* not been the most abundant genus in Pil_Bck, mat presence may not have persisted for 51 months. The unique fungal assemblage of Pil_Bck communities may form an ecological partnership to establish *Piloderma* mats and allow them to persist as perennial features, while the putative ecological functions of the observed taxonomic complexes of background treatments, Pil_Bck and NMBck_O, may explain their spatial separation in these forests.

5.3.2.1. The Major Taxa (*Piloderma* = Pil_Bck; *Russula* = NMBck_O)

Piloderma, responsible for the characterization of organic-horizon mats in this study, is a corticioid EmF genus with global ecological importance (Erland & Taylor, 1999). It is commonly found on coarse woody debris (Smith *et al.,* 2000; Tedersoo *et al.,* 2003; Trappe *et al*., 2012), other complex C substrates (Larsen *et al.,* 1997) or in the upper layers of forest soil profiles (Landeweert, 2003). *Piloderma* spp. are generally nitrophobic and restricted to low N sites (Lilleskov *et al.,* 2002; Nygren *et al.,* 2008) such as old-growth conifer forests of the PNW. Compared to other abundant EmF taxa in this study, *Piloderma* exhibits strong functionally saprotrophic capabilities, which may explain its prominent position in these O-horizon soil microbial communities.

Piloderma spp. have been shown to: (1) contain lignolytic genes including multiple laccases whose transcription was confirmed with RT-PCR (Chen *et al.,* 2003), manganese peroxidase and numerous lignin peroxidases. Of the 48 taxa examined, *Piloderma* was the only EmF genus to have two laccase and any manganese peroxidase genes (Bending & Read, 1997; Chen *et al.,* 2001); (2) have the highest acid phosphatase and leucine aminopeptidase activities of the Norway spruce associated fungi studied (Velmala *et al.,* 2014), while producing high phosphatase, laccase and leucine aminopeptidase activities in lodgepole pine stands of British Columbia (Jones *et al.,* 2012); (3) contain nitrate reductase genes for active NO_3^- assimilation and transport over long distances through hydrophobic hyphae (Nygren *et al.,* 2008); (4) hydrolyze fatty acid esters via esterase production and grow on these substances as a sole C substrate (Caldwell *et al.,* 1991); (5) correlate with cellobiohydrolase, glucosidase and xylosidase activity in sub-boreal spruce forests of British Columbia (Phillips LA *et al.,* 2013); (6) liquefy gelatin via gelatinase production and degrade casamino acids (Hutchison, 1990); (7) increase chitinase activity in the surrounding soil (Kluber *et al.,* 2010); (8) produce proteolytic enzymes capable of mobilizing N from organic compounds (Dahlberg *et al.,* 1997; Lilleskov *et al.,* 2011); (9) grow in pure culture detached from host plants (Kropp, 1982), on mixtures of glucose with ammonium, nitrate or BSA protein (Finlay *et al.,* 1992) or with protein as the sole N source (Bending & Read, 1996); and (10) exhibit low $^{14}CO_2$ pulse label incorporation from Scots pine seedlings into roots and hyphae while showing obvious mycorrhization and soil exploration (Heinonsalo *et al.,* 2004).

These data indicate that *Piloderma* spp. have strong potential to decompose lignin, cellulose, hemicellulose, soil humic polymers, suberin, cutin, chitin, proteins, amino acids, and reduce NO_3^-; *Piloderma* may be particularly adept at accessing organic forms of N and P and may be able to survive for some period disconnected from plant host C. Data from Heinonsalo *et al.* (2004) suggest that because *Suillus* and non-mycorrhizal roots were a strong ^{14}C sink and *Piloderma* was not, this fungus may have accessed C necessary for growth from soil organic matter. The latter point is not generally supported in the literature and remains under considerable debate.

In addition to organic substrate decomposition, *Piloderma* spp. are adept mineral nutrient miners; colonized root-tips (Rosling *et al.,* 2003) and hyphae (Landeweert *et al.,* 2003) frequently extend into mineral soil from the mat feature (Dunham *et al.,* 2007; Phillips *et al.,* 2012). This was evident in our study by active *Piloderma* root-tips and *Piloderma* ITS gene presence in many mineral horizon treatments. Previous research has shown *Piloderma* capable of: (1) creating micropores in feldspar to extract P, K, Ca and Mg (Van Breemen *et al.,* 2000); (2) extracting K from biotite and Mg from chlorite (Glowa *et al.,* 2003); (3) stimulating higher NH_4^+, K^+, Mg^{2+}, Fe^{3+}, Mn^{2+}, Al^{3+} and SO_4^{2-} uptake in the ectomycorrhizosphere of Norway spruce seedlings, possibly transforming chlorite and mica into 2:1 clays (Arocena *et al.,* 2004); (4) directly connecting calcium feldspars to root-tips (Jongmans *et al.,* 1997); (5) solubizing quartz, potassium feldspar, apatite, tricalcium phosphate and marble using C transported to mineral surfaces from host trees (Rosling & Finlay, 2005); (6) enhancing the cation exchange capacity of ectomycorrhizosphere soils, notably Ca, Mg, and K (Arocena *et al.,* 1999); (7) solubizing wood ash to capture Ca, K and Mg, but not necessarily P (Mahmood *et al.,* 2001, 2002; Hagerberg *et al.,* 2005), perhaps with associated microbial assistance (Mahmood *et al.,* 2003); and (8) actively mediating oxalate release and creation under varying P conditions, exuding more oxalate under P-limited conditions, storing calcium oxalate crystals on hyphae, and resorbing Ca under high-P conditions (Tuason & Arocena, 2009).

As these studies suggest, *Piloderma* can be considered “rock eating” (Jongmans *et al.,* 1997), stimulating higher inorganic mineral nutrient uptake for host plants (Arocena *et al.,* 2004), while simultaneously decomposing organic matter in other areas. This ability among rhizomorphic, mat-forming fungi has been shown for *Hydnellum* in Jack pine forests of Canada (Fisher, 1972) and may be related to differential gene expression throughout the fungal network (Wiemken & Boller, 2002). Mineral nutrient acquisition by *Piloderma* is likely related to production and secretion of oxalate (Cromack *et al.,* 1979; Griffiths *et al.,* 1994; Kluber *et al.,* 2010), a low molecular weight organic acid capable of increasing solubility of Al, Fe and Ca and making complexed P, S and trace nutrients more available (Griffiths *et al.,* 1994; Dutton & Evans, 1996).

Kluber *et al.* (2010) found 2.7 times more oxalate in rhizomorphic mats (mostly *Piloderma*) than non-mat soils; aside from pH it was the only soil chemistry metric that differed. It is possible that, although Kluber *et al.* (2010) found drastically higher oxalate in hydrophobic mats (40x, mostly *Ramaria*) compared to non-mat soils, *Piloderma* is concentrating organic acid excretions on hyphal surfaces as complexed calcium oxalate crystals (Tuason & Arocena, 2009). Kluber *et al.* (2010) sieved soils at 4 mm in the organic and 2 mm in the mineral horizon. The rhizomorphic nature of *Piloderma* mats form strong hyphae that do not easily break and pass through sieve gaps, whereas the powdery nature of *Ramaria* mats allows hyphal fragments to readily pass through 2 mm gaps (personal observation). This may be the case, as Cromack *et al.* (1979) found 20x more oxalate in *Hysterangium crassum* mats, morphologically similar to *Piloderma*; however, these researchers removed soil from mat material and performed chemical analyses on hyphae. *Piloderma* oxalate composition is similar to that of *Hysterangium crassum* (Arocena *et al.*, 2001). Griffiths *et al.* (1994) found results similar to Kluber *et al.* (2010) for *Hysterangium setchellii* using similar extraction methods. Therefore, as evidenced by the results of Tuason and Arocena (2009) and Arocena *et al.* (2004), sample proximity to root-tips (or hyphae) may confound oxalate results in *Piloderma*, as compared to *Ramaria* colonized soils, and *Piloderma* values may be closer to Cromack *et al.* (1979). The supposition of Kluber *et al.* (2010) ― that *Piloderma* mats exhibited marginally elevated oxalate compared to non-mats because they do not directly contact weatherable minerals ― may need further consideration, as oxalate is also involved in early-stage lignin decomposition (Dutton & Evans, 1996), of which *Piloderma* exhibits potential. *Piloderma* mats may also support unnoticed exploration into the mineral soil to simultaneously utilize oxalate as an organic and inorganic nutrient acquisition agent, with transport between soil horizons. This idea is in need of future research.

As previously mentioned, *Russula* has proved to be a strong indicator of non-mat soils in old-growth PNW conifer forests (Kluber *et al.*, 2011; Hesse, 2012) and was the strongest indicator of NMBck_O treatments in the current study. The genus *Russula* is comprised of ecologically variable species that range from short to medium distance

exploration types (Agerer, 2001; Avis *et al.,* 2003; Wallander *et al.,* 2013), making overarching ecological characterizations difficult. However, some important functional attributes can be considered in the context of contrasting soils dominated by *Russula* with those of *Piloderma*. Differences exist for both organic and inorganic nutrient acquisition, which may explain the spatial separation and characterization of O-horizon non-mat communities by *Russula*.

The saprotrophic capabilities of *Russula* differ from those of *Piloderma* in a number of ways. Unlike *Piloderma* spp., *Russula* spp. have been historically difficult to grow in pure culture (Kropp, 1982; Nygren *et al.,* 2007). Concerning complex phenolic breakdown, *Russula* spp. were found to contain only one laccase (Chen *et al.,* 2003), one lignin peroxidase and no manganese peroxidase (Chen *et al.,* 2001) coding genes; laccases were also purified from *Russula delica* (Matsubara & Iwasaki, 1972) and class II peroxidase encoding genes were found in two *Russula* sp. (Bödeker *et al.,* 2009). Two *Russula* isolates from an old-growth hardwood forest expressed minimal β-glucosidase and chitinase activities, yet activities of phenol oxidase and acid phosphatase that were higher than many saprotrophic taxa (Burke *et al.,* 2014); no *Piloderma* species were included for comparison. The production of laccase, peroxidase and phenol oxidase by *Russula* spp. is supported by Gramss *et al.* (1998); however, when including members of the Atheliaceae, Russulaceae had lower acid phosphatase/leucine aminopeptidase activities, with equivalent cellobiohydrolase activity (Tedersoo *et al.,* 2012).

Russula spp. were unable to degrade pectin, lipids, amylose, gelatin, casamino acids or urea (Hutchison, 1990); although they grew slowly, *Russula* was found to produce some extracellular proteases (Nygren *et al.,* 2007) and utilize protein (Lilleskov *et al.,* 2011). This may explain the results of Hobbie *et al.* (2014), which indicated *Russula* only incorporated current year photosynthate or recent, litter-derived C, perhaps from more easily accessible proteinaceous organic matter. Higher chitin concentration of fungal tissue has been found to increase decomposition rates (Fernandez & Koide, 2012); *Piloderma* hyphae tend to have lower chitin concentrations than *Russula* hyphae (Wallander *et al.,* 1997). This may be an N recycling strategy of *Piloderma* in these

systems, where lower levels of chitin in mat tissue can prevent scavenging by other fungi in hyphal mats. Interestingly, Baldrian *et al.* (2012) discovered inactivity of the Russulales during organic matter decomposition, whereas members of the Atheliales remained active; this highlights differing saprotrophic capacity of these two fungi.

Russula spp. have smooth hydrophilic mantles (Kernaghan *et al.*, 1997) and lack the capacity for long distance nutrient transport; rather, they efficiently acquire diffused nutrients. Peay *et al.* (2011) suggest in areas of higher root density *Russula* may be advantageous due to their short exploration strategies in the soil matrix (Agerer, 2001). Low hyphal CEC of *Russula* spp. may be a response to their growth habit, enabling better uptake of monovalent rather than divalent cations (McKnight *et al.*, 1990). External *Russula* mantle surfaces are adorned with thin-walled, swollen cystidia that contain chemical deterrents; upon injury, these cells produce antibiotic sesquiterpenoids (Taylor & Alexander, 2005). Production of these compounds is more prominent in nitrophilic, fetid *Russula* species (Avis, 2012), which have been found in old-growth mixed conifer forests (Izzo *et al.*, 2005). Cystidia may also prevent fungivory and arthropod feeding, or aid in nutrient acquisition, particularly P, through oxalate production (Massicotte *et al.*, 2005; Avis, 2012); however, no significant oxalate production by *Russula* spp. has been found, except perhaps Rineau & Garbaye (2010).

Russula, as opposed to *Piloderma,* contained no nitrate reductase genes and may be better suited for NH_4^+ utilization (Nygren *et al.*, 2008); however, *Russula* was highly correlated with NO_3^- when associated with Scotts pine (Rudawska *et al.*, 2011). Nygren *et al.* (2008) defended this unexpected result by noting NH_4^+ uptake occurs via passive membrane diffusion on the smooth, contact-type hydrophilic *Russula* hyphae and proposed a similar (undetermined) mechanism for NO_3^-. On the other hand, nitrate reductase potential is necessary for *Piloderma*, whose hydrophobic exploratory hyphae assimilate NO_3^- to avoid toxicity for transport to the host. Host C savings by *Russula* over short distances may be evident by enhanced sporocarp production potential, whereas host C cost by *Piloderma* may be similarly rewarded due to greater exploitation of the soil matrix and better capability of *Piloderma* to saprotrophically access C.

From the discussion above, and as Hobbie *et al.* (2012) suggest, hydrophilic *Russula* hyphae are adapted for soluble nutrient uptake, whereas hydrophobic exploratory hyphae (e.g. *Piloderma*) can use insoluble, complex organic nutrients. This may be the crux of *Piloderma* mat and non-mat spatial separation in old-growth HJA forests. For heterogeneous forest soils (e.g. this study), Velmala *et al.* (2014) found functional complementarity in the EmF community and suggested host trees gain a great advantage by associating with EmF that simultaneously access organic and inorganic nutrient pools, particularly N (Nygren *et al.*, 2008). The importance of functional complementarity here may involve multiple levels, both within and between *Piloderma* mat and non-mat areas.

Lilleskov *et al.* (2002) further suggest that, along an Alaskan N deposition gradient, the EmF community shifts from N uptake specialists in low-N conditions (*Piloderma*) to specialists for P uptake in high-N conditions (Russulaceae). At the stand level, Twieg *et al.* (2009) found no relationship between EmF community structure and soil nutrients and suggested little spatial niche partitioning among species; however, these authors expect niche partitioning at finer spatial scales. Results from Avis *et al.* (2003) indicate certain *Russula* spp. may respond positively to higher N supply when there is no soil acidification, cation loss and/or P limitation. *Piloderma* mats ─ by reducing pH (Hesse, 2012; Kluber *et al.*, 2010), having strong cation uptake capacity (Arocena *et al.*, 1999) and expressing efficient P acquisition enzymes (Jones *et al.*, 2012; Velmala *et al.*, 2014) ─ may control niche partitioning of the mat stand-level patchwork and result in broader functional complementarity within the system, as *Piloderma* and *Russula* exhibit similarities and differences in enzymatic capacity yet have broadly different ecological strategies (Burke *et al.*, 2012). Here we have only speculated on functional reasons for the mat patchwork, based on the two major EmF taxa, to provide promising avenues for future research; definitive evidence is beyond the reach of this study.

5.3.2.2. Co-Associates (Indicator Taxa) of Pil_Bck

The dark septate endophytic genus *Leptodontidium* is indicative of Pil_Bck treatments and accounted for 1.6% of O-horizon sequence abundance. *Leptodontidium* is

a polyphyletic genus in the Helotiales with varying ecological strategies; free living saprotrophy, as well as mycorrhiza-like associations with both orchids and members of the Pinaceae (Fernando & Currah, 1995) have been observed, while the majority of fungus-plant interactions have been considered neutral or beneficial to host plants (Hou & Guo, 2008). In fact, association with ectomycorrhizas is common among *Leptodontidium* species (Tedersoo *et al.,* 2009). A species of *Leptodontidium* has even been isolated from whiteveined wintergreen (*Pyrola picta*), which simultaneously hosts *Piloderma* (Zimmer *et al.,* 2007); *P. picta* is a common understory species at the HJA (Cappellazzi *et al.,* 2007; http://plants.usda.gov/core/profile?symbol=PYPI2). *Leptodontidium* commonly associates with coarse-textured, nutrient-poor soils rich in organic residues (Fernando & Currah, 1996), similar to *Piloderma*. Taxa in this genus are capable of decomposing cellulose, chitin and lignin, the latter through polyphenol oxidase production (Graf-Wimark, 2010; Fernando & Currah, 1996, 1995). Differing substrate preference has been observed among species in this genus, with the majority favoring chitin (Graf-Wimark, 2010); however, *Leptodontidium* were also abundant in fresh and decayed conifer needles (Aneja *et al.,* 2006) among other substrates. Although this genus was found in all Pil_Bck and NMBck_O soil cores, 83% of sequences occurred in Pil_Bck, indicating strong preference for the *Piloderma* mat environment.

The Pil_Bck indicator genus *Oidiodendron* accounted for 3.6% of overall O-horizon sequence abundance. *Oidiodendron* spp., even a single genet, have been found to associate with both ErM and EmF roots (Bergero *et al.,* 2000; Lacourt *et al.,* 2001). Bergero *et al.* (2000) suggest the enhanced ability of ErM fungi to mobilize nitrogen from complex organic substrates may confer nutritional significance for the EmF symbiosis, either as root-associated saprotrophs or mycorrhizal fungi. *Oidiodendron griseum* has been isolated as an ErM fungus from salal (*Gaultheria shallon*), one of the most common understory plants at the HJA (Xiao & Berch, 1996), and Lacourt *et al.* (2001) suggest *Oidiodendron* may facilitate complex belowground interactions between multiple plants of different mycorrhizal types. Fungi in this genus have also been isolated from soil organic matter, decaying wood and bark (Hambleton *et al.,* 1998),

similar substrate preferences to *Piloderma*. Species in the genus are highly capable of: (1) decomposing cellulose via aggressive cellobiohydrolase and β-glucosidase production (Koukol & Baldrian, 2012); (2) readily utilizing chitin as the sole substrate and nitrogen source (Leake & Read, 1990); (3) mobilizing protein bound to tannic-acids through protease production (Bending & Read, 1996); and (4) degrading lignin via peroxidase (Bending & Read, 1997). Bending and Read (1996) further suggest the large quantity of tannin in wood may ensure abundant complexation with organic N, a well-adapted scenario for *Oidiodendron*; in fact, the addition of organic fertilizers to soil remarkably increased *Oidiodendron* frequency (Lee *et al.*, 2012). Read and Perez-Moreno (2003) propose that ErM in forest soils may "unmask" nutrients inaccessible to EmF through enhanced decomposition pathways and support both ErM and EmF plants. However, Kluber *et al.* (2011) identified *Oidiodendron* as more closely associated with non-mat soils; this discrepancy should be evaluated in future studies.

A third, less-abundant (0.3% of O-horizon sequences) Pil_Bck indicator was *Neofabraea*, a genus in the poorly understood Ascomycete family, Dermateaceae. Species believed to be bark endophytes (Abeln *et al.*, 2000; Wang *et al.*, 2006) have recently been found in high abundance in forest soils (Buée *et al.*, 2009) and as ericaceous fungal endophytes (Wurzburger *et al.*, 2011). *Neofabraea* spp. frequently infect pome fruit (Kider *et al.*, 2011) and may be adapted for simple C compound utilization. Members of the genus have also been found in the soil beneath *Boletus edulis* sporocarps (Peintner *et al.*, 2007) and have clustered with ErM/EmF in phylogenetic analyses (Anderson *et al.*, 2003). They have been isolated from decomposing pine litter (Zheng *et al.*, 2010) and as ErM of *Rhododendron* involved in the decomposition of organic matter (Lin *et al.*, 2011). In a study of EmF community composition in boreal mixed-wood forests, one *Piloderma* and one *Neofabraea* type were intimately associated with each other and only found under conifers (DeBellis *et al.*, 2006).

Other taxa indicated Pil_Bck treatments; however, limited taxonomic identification restricted functional extrapolation. Agaricomycetes sp. 15 was most closely associated with an uncultured fungus from a spruce forest that may play a role in

phosphorus acquisition (GenBank ID #EF521224.1). Capnodiales sp. 1 and Dermateaceae sp. 2 are putative saprotrophs based on order and family groupings; these OTUs were of negligible abundance.

5.3.2.3. Co-Associates (Indicator Taxa) of NMBck_O

Pseudotomentella, a resupinate, rhizomorphic and predominantly EmF genus in the Thelephoraceae (Agerer, 2006), was the second strongest indicator of NMBck_O treatments; it represented approximately 0.7% of sequence abundance with 70% of O-horizon sequences in NMBck_O soils. Interestingly, *Pseudotomentella* presence on active EmF root-tips did not reflect its soil distribution. Research is lacking on specific ecological functions of this genus. As the closely related genus *Tomentella* typically fruits on dead wood (Vasiliauskas *et al.*, 2007), *Pseudotomentella* may play a role in decomposition; however, Baldrian *et al.* (2012) found substantial reduction in Thelephorales that were active during decomposition, similar to the Russulales. In one study on EmF selection of bacterial and ascomycete microflora, Izumi and Finlay (2011) found distinct bacterial communities colonizing *Piloderma* and *Pseudotomentella* root-tips, which indicates differing functional capacity of the species and may explain their spatial separation in these forests. Additionally, Kranabetter *et al.* (2009) found an antagonistic relationship between *Piloderma fallax* and *Tomentella* spp. in a British Columbian boreal forest.

Cadophora, representing 1% of O-horizon sequence abundance, is a fungal endophyte in the Ascomycota. *C. finlandia*, of which the majority of OTUs found in this study belong, can form ErM and EmF (Gorfer *et al.*, 2007). Previously this species fungal complex was referred to as Mycelium radices atrovirens or dark septate endophytes, whose varied ecological roles include soil and wood saprobes, as well as mutualists (Vrålstad *et al.*, 2002). *C. finlandia* has been reported to promote host growth and survival on acidic or Fe contaminated soils (Vrålstad *et al.*, 2002, references therein). *Cadophora* and *Pseudotomentella* both exhibit melanized cell walls which can act to prevent physical, chemical and biological stress in the soil environment (Erland &

Taylor, 1999; Vrålstad *et al.,* 2002). In a late successional Norway spruce forest with experimental N addition, *Cadophora* increased and Atheliales decreased in abundance on bilberry roots with N fertilization (Ishida & Nordin, 2010), though N-additions had no effect on the overall fungal community.

Leohumicola was first described by Hambleton *et al.* (2005) as globally dispersed endophytes of EmF and ErM roots (Hambleton *et al.,* 2005; Tedersoo *et al.,* 2009). It represented 0.4% of O-horizon sequence abundance in this study, while the most abundant OTUs were *L. minima* and *L. verrucosa*. These species have been isolated from volcanic ash soils in Chile (*L. minima*) and soils under various ericaceous shrubs or members of the Pinaceae (*L. verrucosa*), are able to grow on potato dexatrose or oatmeal agar and form ErM in vitro (Hambleton *et al.,* 2005). Recent discoveries indicate *L. minima* as an orchid root endophyte only isolated from plants in the autumn, while other species form ErM (Kohout *et al.,* 2013 and references therein). Other than presence, little else is known about the ecology of this genus, which makes its greater abundance in non-mat vs. *Piloderma* mat fungal communities interesting.

Other less-abundant indicator fungal genera of NMBck_O soils included: (1) *Thysanophora*, an early stage conifer litter saprotroph (Kasal *et al.,* 1995; Zifcakova *et al.*, 2011); (2) *Sphaerobolus*, a cosmopolitan genus with coprophilous and lignicolous ecology (Geml *et al.*, 2005); (3) *Auricularia*, a wood-inhabiting white rot genus (Floudas *et al.,* 2012); (4) *Biscogniauxia*, a pathogenic genus; and (5) *Hyalodendriella*, a lignicolous and/or endophytic genus (Crous *et al.,* 2007). These five genera accounted for only 0.5% of all fungal sequences in the O-horizon and likely play minor roles in non-mat fungal communities.

5.3.2.4. Conclusions on Pil_Bck and NMBck_O Fungal Communities

From the indicator species assemblages of Pil_Bck and NMBck_O, broad community characterizations are possible. Of course, these are not the only fungi with significant functional roles; however, their chance-corrected presence is important. In organic horizon mat systems, *Piloderma* may recruit fungi better adapted to decompose

complex organic substrates, particularly protein-polyphenol complexes, like *Leptodontidium*, *Oidiodendron* and *Neofabraea*, to either circumvent evolutionary limitations in direct N or P acquisition (Wu *et al.,* 2011) or, as a more likely scenario, increase acquisition efficiency of an inherently capable enzymatic profile to garner maximum host support. In return, these fungal associates may benefit from leached photosynthate or recycling nutrients from *Piloderma* mat hyphae. In non-mat organic soils, the fungal functional complex is less certain. *Russula* and *Pseudotomentella* may be unable to compete for host roots in the *Piloderma* mat environment due to the physical alteration of the soil matrix (Kluber *et al.,* 2010). Specific ErM and endophytic differences are less clear and warrant further study.

5.3.3. Characterization of Organic Horizon Birth and Death (Pil_Bth and Pil_Dth) Fungal Communities

The primary objectives of this study were to determine whether Pil_Bth and Pil_Dth treatments further transitioned toward respective background fungal communities between 24 and 51 months, as well as identify responsible taxa by sequencing EmF root-tips and soil fungi. Blanchard (2008) noticed death of the *Piloderma* mat community quickly (6 months), and NMS analysis indicated Pil_Dth and NMBck_O communities were indistinguishable after 24 months. However, Pil_Bth fungal communities more closely resembled NMBck_O after 24 months, even though *Piloderma* T-RFLP peaks indicated significant colonization by *Piloderma*.

5.3.3.1. Co-associates (Indicator Taxa) of Pil_Bth

Our data indicate, after 51 months, *Piloderma* mat fungal communities (Pil_Bck) were able to fully colonize transplanted non-mat soil (Pil_Bth) and out-compete the native non-mat community. The overall fungal communities between Pil_Bck and Pil_Bth were indistinguishable, with development occurring between 24 and 51 months. The Pil_Bck indicator taxa, *Piloderma, Neofabraea* and *Oidiodendron* colonized Pil_Bth from Pil_Bck, whereas *Russula* remained prominent in Pil_Bth from NMBck_O. The

presence of *Russula* in Pil_Bth may represent a residual spore bank, as discussed earlier; only one third of Pil_Bth cores contained *Russula* colonized EmF root-tips. Of the 10 *Russula* EmF species found on root-tips overall, only one species was found in each core.

The ubiquitous Zygomycete genus *Umbelopsis* was slightly more abundant in Pil_Bck than NMBck_O, but significantly increased in Pil_Bth. Hesse (2012) found this genus correlated with *Ramaria* mats, however we found no indication of this; in fact, *Umbelopsis* represented 2.6% of overall O-horizon sequence abundance with 56% of all sequences found in this horizon. *Umbelopsis* was dominant among Douglas-fir woody root endophytes in the Washington Cascades (Hoff *et al.,* 2004) and is a common woody root associate (Toju *et al.,* 2013). *Umbelopsis* is known to decompose soil organic matter and is suggested to possess ruderal characteristics such as stress tolerance, rapid dispersal and efficient nutrient uptake (Hoff *et al.,* 2004). In an extracellular enzyme profiling study of O-horizon forest soils, *Umbelopsis* spp. were capable of cellulose and chitin degradation but not lignin; however, these abilities were some of the lowest measured for the non basidiomycetous microfungi tested (Baldrian *et al.,* 2011). These characteristics, as well as its indication of the disturbance control treatment, NMDst_O, indicate the ability of *Umbelopsis* to capitalize on disturbance and proliferate even after 51 months.

5.3.3.2. Co-Associates (Indicator Taxa) of Pil_Dth

Our data largely confirm Blanchard (2008) for Pil_Dth. After 51 months Pil_Dth fungi were only slightly similar to NMBck_O yet entirely dissimilar to Pil_Bck and Pil_Bth. In fact, Pil_Dth was more similar to NMDst_O than NMBck_O, a result of disturbance fungal taxa. A large number of non-EmF indicator taxa were unique to this treatment. Given the lack of live root-tips, EmF were likely captured as spores or exploratory hyphae. The only indicator genus shared with Pil_Bck was *Oidiodendron*, while *Thysanophora* was the only indicator genus shared with NMBck_O.

Mortierella is a genus of widespread soil-inhabiting zygomycetous saprobes (Wagner *et al.,* 2013). Many species are chitinolytic (DeBoer *et al.,* 1999), lignolytic (Phillips LA *et al.,* 2013) and/or endophytic (Grigoriev *et al.,* 2011), and this genus is

closely related to *Umbelopsis* (Hoff *et al.*, 2004). It is one of the 10 most frequently encountered genera in soil metagenomics (Nagy *et al.*, 2011) and the 11th here. Although ubiquitous in soil cores, its high abundance in Pil_Dth made *Mortierella* the best overall indicator of this treatment. Abundance trends indicated it was more heavily excluded from *Piloderma* mat than non-mat soils. This may be an indication that after 51 months Pil_Dth samples are still high in dead organic material, remnants of root severing and enclosure in PVC pipe. The ubiquitous nature of *Mortierella*, its fast growth habit and substrate abundance may have guaranteed higher abundance in Pil_Dth cores. Interestingly, the lack of living root mass in these treatments suggests the ability of *Mortierella* spp. to proliferate without live root tissue.

Wallemia was ubiquitous and the most abundant genus in the study (12.3% of all sequences), with higher indication of Pil_Dth. *Wallemia* spp. are xerotolerant molds present in air, soil, wood and hypersaline environments (Zalar *et al.*, 2005). It has also been found as a root endophyte of wild rice (Yuan *et al.*, 2010). The high abundance of *Wallemia* in coniferous forest soils has not been documented before and may be the result of contamination, although no other data indicate this. Another possibility is the misidentification of these OTUs by QIIME. Hesse (2012), studying the same soil microbial communities, report a ubiquitous, unidentified Basidiomycete as the fourth most abundant genus, representing 10% of sequences. *Wallemia* may be widely distributed and functionally important in Oregon coniferous forests.

Other Pil_Dth indicator taxa were: (1) *Porotheleum*, a genus of wood decay fungi (Rubino & McCarthy); (2) *Amorphotheca*, a common conifer soil saprotroph (Fürst *et al.*, 1998); and (3) seven putative soil saprotrophs with incomplete taxonomic depth to describe further. The presence of these taxa suggests heavy decay of severed roots during transplant and the lack of colonization by new roots from enclosure in PVC pipe.

5.3.4. Other Important Fungal Taxa in Organic Horizon Soils

Taxa indicating specific treatments are certainly not the only important players in the fungal community. One of the first high-throughput sequencing studies of forest soil fungi identified the genera *Cenococcum, Russula, Mortierella, Cryptococcus,*

Ceratobasidium, *Lactarius*, *Scleroderma*, *Neofabraea*, and *Inocybe* as the most prominent in a French temperate forest (Buée *et al.*, 2009); the first four genera each represented over 1% of fungi in these forest soils and the most abundant genus in the group, *Russula*, was 3.66%.

Previous research has identified *Leptosporomyces*, a saprotrophic fungal genus within the Atheliales, as closely associated with *Piloderma* mats (Kluber *et al.*, 2011); although being the ninth most abundant fungal genus here (3.4% of O-horizon sequences), it did not indicate any treatment. However, *Leptosporomyces* sequence abundance increased from NMBck_O→Pil_Bth and decreased from Pil_Bth→Pil_Bck, indicating this genus may play a larger role during *Piloderma* mat development.

Rosling *et al.* (2011) recently classified the ubiquitous soil ascomycetous genus *Archaeorhizomycetes*. It has been characterized from surface sterilized coniferous EmF root-tips and is locally diverse, with up to 20 OTUs at a given research site (Rosling *et al.*, 2011). It is believed that a mismatch in the ITS4 binding site in *Archaeorhizomycetes* can significantly reduce their abundance in ITS, as opposed to LSU studies (Rosling *et al.*, 2013). Nevertheless, *Archaeorhizomycetes*, comprising 40 OTUs, was the third most abundant in this study (4.9% of sequences), evenly distributed between organic and mineral soil. The ecological role of these fungi can vary from endophytic to saprotrophic on wood, and some may be uniquely associated with particular EmF species, even mycoparasitic (Rosling *et al.*, 2013). Our data indicate higher relative abundance of this genus in NMBck_O than Pil_Bck, and appear unchanged in Pil_Bth. Future mat studies should pay particular attention to this genus as we elucidate its functional ecology.

Typical of EmF studies worldwide, *Cenococcum* was ubiquitous. Although *Cenococcum* is not always the most abundant EmF genus, it tends to have even spatial distribution and occurs in the majority of soil cores (Horton & Bruns, 2001). It was the second (Kluber *et al.*, 2011) and seventh (Hesse, 2012) most abundant genus from the same region in clone libraries and environmental sequences, respectively, and colonized up to 96% of total mycorrhizal root-tips in a silver fir stand (Vogt *et al.*, 1981). Our study confirms this distribution pattern, where it was present on active root-tips in over

half of our cores regardless of horizon (1.8% total sequences) and 83% of O-horizon soil cores, excluding Pil_Dth. This highly melanized genus can resist decomposition and tolerate water stress (Koide *et al.*, 2014). *Cenococcum* had higher relative abundance in NMBck_O vs. Pil_Bck; however, this may be chance-related.

Finally, *Wilcoxina* is particularly effective at chitin degradation (Velmala *et al.*, 2014), which may explain its higher relative abundance in Pil_Dth treatments. One might expect *Piloderma* mats to harbor a higher proportion of chitin-degrading fungi, resulting from the hyphal mat structure; however, these samples were taken during the fall, a time of intense hyphal growth and minimal senescence. In the Pil_Dth samples, mat hyphae from Pil_Bck may still be decomposing.

5.3.5. The Role of Saprotrophic Fungi

EmF and ErM fungi are known to possess a wide array of saprotrophic capabilities that vary by species (Baldrian & López-Mondéjar, 2014); EmF may even supply host plants with soil C during times of high demand and low photosynthesis (Courty *et al.*, 2007). Saprotrophic fungi intimately interact with mycorrhizal fungi in the soil matrix and profoundly affect mycorrhizosphere processes, either cooperatively or competitively (Cairney & Meharg, 2002). EmF and saprotrophic fungi have been found to dominate spatially separate vertical niches in the soil, where saprotrophs dominate upper litter layers while EmF occupy fragmented litter and below (Lindahl *et al.*, 2007); this has recently been confirmed as a global phenomenon (McGuire *et al.*, 2013). Substrate preference (e.g. EmF access host C to decompose marginal organic matter), antagonism or avoidance of competition may drive vertical differentiation (McGuire *et al.*, 2013). Explanations of saprotrophic fungi in non-litter soil, therefore, range from synergism to competitive tolerance. It has been suggested that EmF associated microflora may be responsible for the majority of the saprotrophic abilities exhibited by EmF, in return benefiting from the C flow from host plant to EmF mycelium (Cairney & Meharg, 2002). This may be particularly important in the *Piloderma* mat system.

Strict saprotrophic fungal associations with O-horizon treatments were rare. The only exception was Pil_Dth, where EmF and viable root loss increased saprotrophic

dominance. Our data indicate Pil_Bck soils harbored the highest and lowest relative abundance of EmF and saprotrophic sequences, respectively. This was accompanied by higher ErM relative abundance, which may exceed EmF in saprotrophic capacity (Read & Perez-Moreno, 2003). It is possible that the EmF cohort in *Piloderma* mats is larger than non-mat soils, the result of decreased substrate nutritional status. On the other hand, NMS and MRPP analyses indicated stronger separation of Pil_Bck from NMBck_O when the EmF community was isolated. This may indicate the EmF community is more particular of mat vs. non-mat microhabitat than the saprotrophic community and *Piloderma* mat EmF are stronger saprotrophic competitors than non-mat EmF.

Surprisingly, putative fungal saprotrophs in our study did not decrease in percent relative abundance between organic and mineral soil (both avg ~ 46%), perhaps due to the fact that soil samples were limited to 20 cm and mineral soils were homogenized between upper and lower portions. Even so, Blanchard (2008) found percent C in mineral soil (avg 6%) to be significantly lower than percent C in organic soil (avg 27%); 6% mineral soil C may be sufficient to maintain high saprotrophic fungal relative abundance. The EmF:saprotrophic fungal ratio increased from organic to mineral horizons, indicating greater dominance by EmF in mineral soil.

5.3.6. Uncommon Fungal Associations

Our data do not indicate higher abundance or co-association of lichenaceous fungi with *Piloderma* mats. Hintikka and Näykki (1967) observed co-association of *Cladonia* with *Hydnellum ferrugineum* mats, particularly near the center of mats where senescent or dead mycelia were found; however, the soil DNA analyzed here may not represent the visual observations of Hintikka and Näykki (1967). These authors also documented small *Hydnellum ferrugineum* mats to have “vigorous and continuous” moss cover on the mycelium. Cappellazzi *et al.* (2007) found active EmF root-tip abundance significantly decreased one year after forest floor moss was removed. The proliferation of *Piloderma* root-tips within mats may increase overall root-tip abundance when compared to non-mat soil; however, mat presence was not considered by Cappellazzi *et al.* (2007). The

correlation of mats with moss cover has not been studied. Taken together, it is possible forest floor moss cover is more established over *Piloderma* mat soil in old-growth coniferous forests of the PNW, with interesting implications on forest nutritional dynamics (see discussion in Cappellazzi *et al.*, 2007).

AmF host plants were less abundant than EmF hosts in the forests studied; however, AmF rarity is interesting. We found our pyrosequencing database to contain only 0.3% AmF sequences overall in these soils. Hesse (2012) found similarly low AmF abundance in the same region, ruling out the possibility of primer bias by using multiple primer sets with similar results. Compared to AmF, EmF are known to dominate coniferous forest ecosystems worldwide (Smith & Read, 2008), but the negligible occurrence of AmF given host presence is intriguing.

5.4. Bacterial Communities of Organic Horizon (*Piloderma*) Soils

Compared to fungi, our data show no difference between overall Pil_Bck and NMBck_O soil bacterial community composition; this was true for bacterial families as well as higher and lower taxonomic groupings (data not shown). Ectomycorrhizal fungi have previously been hypothesized to exert selective influence over bacterial microflora (Mosse, 1962; Warmink *et al.*, 2009); associations between host plant, EmF and bacteria have been aptly referred to as a tripartite partnership (Nazir *et al.*, 2010). In one study of common subarctic EmF on *Betula pubescens*, Izumi and Finlay (2011) showed bacterial communities associated with *Piloderma* and *Pseudotomentella* root-tips significantly differed from each other and a grouping of three other common EmF; these results suggested strong fungal control, explained perhaps by differential C allocation from host plant to EmF to bacteria. In this study, *Piloderma* and *Pseudotomentella* were strong indicators of Pil_Bck and NMBck_O, respectively. This would suggest bacterial differences between the two background organic communities, which have previously been documented (Kluber *et al.*, 2011; Hesse, 2012). Blanchard (2008), however, found only seasonal rather than treatment differences in background bacterial communities.

Our data support those of Blanchard (2008) and call into question the differences observed by Hesse (2012) and Kluber *et al.* (2011).

Burke *et al.* (2008) found no indication EmF taxa colonizing Douglas-fir root-tips had any effect on associated bacterial communities; the majority of these EmF were in the Russulaceae, many in the genus *Russula*, suggesting EmF support similar bacterial communities regardless of taxonomy. Including broader EmF taxonomic diversity may have led to the different results of Izumi and Finlay (2011). Similarly to Burke *et al.* (2008), Izumi *et al.* (2007) found analogous bacterial communities on EmF tips of *Tomentellopsis submollis* and *Suillus variegates* using culture dependent and independent analyses. EmF selective pressure imposed on bacteria should decrease as the scale moves from root-tip to mycelium to bulk soil. Samples from this study included extramatrical mycelium, as hyphal material rubbed through the sieve during soil processing. It is possible bacterial associations with root-tips or the ectomycorrhizosphere were diluted after removing roots and blending exploratory hyphae with bulk soil. Uroz *et al.* (2012) support this idea; these authors discovered ectomycorrhizospheres (e.g. EmF root-tips, emanating hyphae and attached soil) of two EmF species were similar in terms of phyla and genera, but were significantly enriched in Alpha-, Beta- and Gammaproteobacteria compared to the bulk soil. Vik *et al.* (2013) also found differing bacterial taxa associated with EmF and the surrounding soil. Interestingly, Hesse (2012) and Kluber *et al.* (2011) used similar soil processing methodology as we did here.

Metabolically active bacteria are often missed by the DNA techniques used here (Izumi *et al.*, 2007); however, both previous studies examining *Piloderma* mat and non-mat soils used rDNA; Kluber *et al.* (2011) used clone libraries and T-RFLP profiles and Hesse (2012) used 454-pyrosequencing. The only community characterization difference was bacterial primer set choice. Variations in soil processing methodology as well as primer choice may, in part, explain the differences in bacterial community results of this study with previous studies (Kluber *et al.*, 2011; Hesse, 2012).

5.4.1. Nuanced Differences between Pil_Bck and NMBck_O

At the phylotype level (97% OTUs), Uroz *et al.* (2012) found clear differences between the ectomycorrhizosphere bacterial communities associated with *Xerocomus pruinatus* and *Scleroderma citrinum*, which were not apparent at higher taxonomic levels; we explored this possibility by performing pairwise ISAs on Pil_Bck and NMBck_O bacterial communities at the family, genus and 97% OTU level. We found a larger cohort of bacterial indicators at the genus than family level, but a much smaller cohort at the 97% OTU level, perhaps the result of high site-site variation; these data indicate bacterial functional traits are strongly selected for at the genus level as few differences in higher level bacterial taxonomic relative abundance were found (data not shown).

Different selection pressures between the O-horizon background communities dominate within the Proteobacteria, which had the highest overall relative abundance (~50%). The α-Proteobacteria indicator complex suggests *Piloderma* mats select for two families (Acetobacteraceae and Methylocystaceae), the genus *Bradyrhizobium* (Bradyrhizobiaceae), and two unknown species within *Rhizobium* (Rhizobiaceae) and *Acidisphaera* (Acetobacteraceae). Non-mat O-horizon soils broadly select the Beijerinckiaceae, a single genus within that family, and the genus *Sphingomonas* (Sphingomonadaceae). The β-Proteobacteria indicator complex suggests non-selectivity in *Piloderma* mats, while non-mat soils are highly selective. NMBck_O selected three families (Comamonadaceae and two unknowns), one unknown genus within the Comamonadaceae, and two unknown genera within the orders Ellin6067 and IS-44. δ-proteobacteria were evenly selected for by *Piloderma* mats, a genus within the Syntrophobacteraceae, and non-mat areas, the family Myxococcaceae. Conversely, the γ-Proteobacteria indicator complex suggests highly specific discrimination by *Piloderma* mats, where they select for one family, Coxiellaceae, one unknown genus within Coxiellaceae and a particular species in the genus *Steroidobacter* (Sinobacteraceae).

Background communities were found to select for members of four other phyla to a much lower degree than Proteobacteria. Considering the phylum Actinobacteria (~15%), *Piloderma* mats selected for one family within the Actinomycetales

(Mycobacteriaceae) and a species in the genus *Mycobacterium*; non-mat soils were slightly less specific, associating closely with a single, unknown family within the class Actinobacteria and a specific genus within that family. No Acidobacteria were unique to *Piloderma* mats, the second most abundant phylum (~22%), whereas non-mat soils selected for an unknown family within the class Sva0725 and an unknown genus within that family, while also selecting for an unknown genus within Chloracidobacteria. Non-mats also selected a single, unknown species within the phylum Bacteroidetes; this was the fourth most abundant phylum (~11%) with the highest relative abundance represented by NMBck_O. Finally, the candidate division TM7 was only selected for by non-mat soils, which associated with two unknown families, in the classes TM7-1 and SC3 and more specifically, one unknown genus within the unknown family of TM7-1. Unfortunately, in contrast to the majority of our fungal data at the genus level, functional characterizations of bacteria at the family level are tenuous. It would be even harder to extrapolate differential functions in *Piloderma* mat and non-mat soils, as we have only commented on possible fungal functional roles based on unique species complexes.

Identification of bacterial taxa as *Piloderma* mat or non-mat associates generally support, and add to, data from Hesse (2012) and Kluber *et al.* (2011) for old-growth Douglas-fir forests in the PNW. This is the first mat study where overall bacterial communities were not found to significantly differ between background O-horizon soils, even though fungal communities were distinct. These data support Warmink *et al.* (2009), who identified universal and species-specific bacterial fungiphiles in the mycosphere; at a broader spatial scale, while the majority of bacterial taxa were either shared or randomly distributed between *Piloderma* mat and non-mat soils (universal), unique microhabitats capable of selecting bacterial subsets (species or microhabitat specific) invariably influence overall function. An important next step would be to compare bacterial communities at decreasing spatial scales to discern place and process of indicator bacteria within and outside *Piloderma* mats. A worthy comparison would be bulk soil with hyphal material (represented here), extramatrical hyphae and individual *Piloderma* root-tips. If the mycorrhizosphere represents a “nutritional hotspot” (Nazir *et*

al., 2010) for soil bacteria, differentiating the effect of two clearly different fungal communities on specific bacterial assemblages is crucial to understanding the functional complexity of these ectomycorrhizospheres (Frey-Klett *et al.*, 2007).

5.5. Common Mycorrhizal Networks, Ectomycorrhizal Fungal Mats and Forest Connectivity ─ Perspectives and Future Research Needs

Underground plant linkages between shared cohorts of mycorrhizal fungi, known as common mycorrhizal networks (CMNs), are well established (Newman, 1988; Molina *et al.*, 1992; Smith & Read, 2008; Toju *et al.*, 2013) and complex intergenerational, multi-species, fungal-plant assemblages can form (Beiler *et al.*, 2010). These assemblages are particularly relevant within the diverse old-growth, nutrient-limited, EmF conifer forests in this study, where networks may form conduits for interplant resource transfer crucial to ecosystem function. Past EmF network research has identified: (1) net C-transfer between heterospecific (Simard *et al.*, 1997) and conspecific (Teste *et al.*, 2010) trees; (2) water reallocation to support drought-stressed trees (Bingham & Simard, 2012); (3) nitrogen transfer from older trees to seedlings (Teste *et al.*, 2009); (4) support and recruitment of seedlings by mother trees of the same species (Nara, 2006); (5) mediation of overstory-understory competition between trees, where coexistence is promoted between some species at the expense of others (Booth, 2004); (6) the ecologically important network between conifers and ericaceous shrubs (Villarreal-Ruiz *et al.*, 2004; Grelet *et al.*, 2009); and (7) the plausible universality among EmF species to link multiple plants (Nara, 2006). It would be reasonable to conclude that individual plants connected to CMNs would gain access to larger nutrient pools (Newman, 1988); however, this also presents an ecological and evolutionary paradox, where connections to a CMN may help competitors (Selosse *et al.*, 2006). This latter notion may have to be reevaluated as species survival is not separate from the community to which it belongs; rather, it is intimately intertwined. Thus, it would also be reasonable to conclude that species succession, leading to the stability of old-growth forests, is the direct result of cooperation and sharing between the functional ecological layers of the

forest network, an idea that is not absent from scientific thought (Leimar & Hammerstein, 2010).

Differential allocation of resource pools among plants and fungi engaged in CMNs has support in both EmF and AmF systems. Kiers *et al.* (2011) showed both plants and fungi involved in an AmF network were able to detect, discriminate and reward the best partner with carbon and nutrients. Host plants were also able to preferentially allocate photosynthate to the more beneficial of two AmF partners (Bever *et al.*, 2009). Similarly, in an EmF system, C transfer to the fungus and N transfer to the plant differed between birch and spruce connected by a mycorrhizal network; birch trees transferred the most C to EmF and, in return, received the most N (Ek *et al.*, 1996). These phenomena may find support in *Piloderma* mats of this study. As mats colonize resource pools with unique microbial communities (Pil_Bth) that concentrate microbial influence and efficiently acquire nutrients, the plant reward may be represented in ample C allocation to *Piloderma* which supports massive hyphal proliferation ─ the mat structure. As *Piloderma* mats are born into non-mat areas (colonization), the mat community may have a competitive edge through these reciprocal rewards.

The propensity for multiple-host fungi in Douglas-fir systems (Horton & Bruns, 1998; Kennedy *et al.*, 2003; Smith and Read, 2008) broadens the ecological significance of EmF networks to the forest-scale and may have led to the evolution of multiple resource-specific acquisition complexes, each capable of targeted capture and transfer to host plants. Within the context of the forest network, these seemingly separate niche complexes may be more interconnected than they appear. The patchwork of mat occurrence in old-growth forests of the PNW, as well as the stratification of *Piloderma* and *Ramaria* mats in organic and mineral soil, lends support to this idea. *Piloderma* mat and non-mat areas host unique EmF taxonomic assemblages with differential foraging abilities (e.g. *Piloderma* and *Russula*). As non-mat soils with distinctly different communities were transferred within *Piloderma* mats (Pil_Bth), mat species directly competed with non-mat microbial communities and ultimately colonized these soils such that Pil_Bck and Pil_Bth exhibited few differences after 51 months.

Clearly, direct plant linkages via EmF networks are not the limit of resource pool mobility in these systems. As He *et al.* (2006) describe, N transfer in their EmF/AmF system occurred beyond the strict boundaries of common mycorrhizal linkages; thus indirect pathways must be involved, highlighting the functional hierarchy between microbes in the soil system. Direct associations between EmF and saprotrophic fungal mycelia (Cairney & Meharg, 2002), as well as bacterial taxa (Deveau *et al.,* 2012), add countless levels to the network hypothesis. This study found that, indeed, particular saprotrophic fungi and bacteria strongly indicated microhabitat preference for *Piloderma* mats and non-mat areas. As an example, reciprocal rewards may exist between *Piloderma* mycelia and those of associated microbes to permit decomposition of complex, N-limited substrates; this may include leakage of energy-rich C compounds from tree photosynthate via *Piloderma* hyphae in return for cohabitation and benefit from enhanced enzymatic capabilities.

It is reasonable to envision connection between Douglas-fir and western hemlock in these systems; in late successional forests these species associate with the same EmF symbionts, and putative *Pseudotsuga* specific genera (e.g. *Rhizopogon*) have been shown to associate with western hemlock in pure culture synthesis experiments (Horton *et al.*, 2005, references therein). Kennedy *et al.* (2003) suggest a strong potential for common EmF networks to form between Douglas-fir and the tanoak understory, a phenomenon that has found stronger support recently (Simard *et al.*, 2012). At a deeper level of network potential, a connection may exist between plants of varying mycorrhizal types to form "superorganisms" (van der Heijden & Horton, 2009), and evidence for this has recently been found (Toju *et al.,* 2013). In the system studied here, connections between ErM understory shrubs and EmF overstory trees are of particular importance, as genetic and functional distinctions between ErM and EmF are becoming less certain (Grelet *et al.,* 2010). The accumulation of evidence suggests co-colonization of ErM and EmF plant roots by the same fungus (Grelet *et al.*, 2010; Villarreal-Ruiz *et al.*, 2004; Bergero *et al.,* 2000), as well as the proper functioning of ErM in carbon and nitrogen mobilization and translocation after being isolated from an EmF root (Grelet *et al.*, 2009).

In fact, a *Piloderma* sp. has been isolated from ericaceous plants in a subarctic heathland (Kjøller *et al.*, 2010). These data provide evidence of close association of specific ErM with *Piloderma* mats (e.g. *Oidiodendron*) and others with non-mat soil (e.g. *Leohumicola*). Our data also suggest higher proportion of ErM fungi in *Piloderma* mats (5.1%) than in non-mat areas (2.1%). Considering orchid mycorrhizas, non-mat areas host one orchid mycorrhizal complex (e.g. *Russula*, *Sebacina* and *Pseudotomentella*), while *Piloderma* mats may support different orchid mycorrhizas (e.g. *Leptodontidium*). As *Piloderma* mats undergo colonization (Pil_Bth) of non-mat soil, it appears *Russula* remains in the community even after 51 months, albeit to a much lesser extent, further supporting the idea of *Piloderma* mats as unique microbial networking centers.

Beiler *et al.* (2010) described a complex and robust networking system between *Rhizopogon* and multiple age classes of Douglas-fir trees, suggesting resource shuttling to expanding mycelial fronts; there was an almost universal interconnection between trees in the sample area, reinforced with repeated network loops. Over the course of 51 months, we documented the colonization of non-mat soil by *Piloderma* mats (Pil_Bth), including a proliferation of *Piloderma* hyphae. The foraging capacity of these mats necessitates resource allocation to developmental zones, which can be strong C sinks. Beiler *et al.* (2011) also reported identifying roots from multiple tree genotypes in a single *Rhizopogon* tubercle, an unexpected result that requires further investigation. It would seem that some networking EmF, such as *Rhizopogon*, may allow multiple trees to intermingle within a single tubercle, supporting the possibility of *Rhizopogon* acting as resource and/or signal networking points for the greater mycorrhizal network. *Piloderma* species form rhizomorphs capable of long-distance exploration, transport and dominance on small spatial scales (e.g. mats). It is unclear how many *Piloderma* genets interact within an individual mat; however, like *Rhizopogon*, these genets are perennial and can maintain network connection. This is in stark contrast to *Russula* which forms small genets (Redecker *et al.*, 2001) and has limited hyphal exploration in the soil matrix (Agerer, 2001). It is possible that mats exhibit similar network functionality to *Rhizopogon*, especially those formed by *Piloderma*. In this study, there was no

relationship between *Rhizopogon* and any of the organic horizon treatments; rather, distribution of *Rhizopogon* was more strongly associated with site.

Previous research provides strong support for the role of mats in forest CMNs. The incidence of mats was higher at the base of three understory tree species, regardless of mycorrhizal status (Griffiths *et al.,* 1995), while mat distribution was found to be influenced by proximity to other mats and the nearest living tree, as well as the tree density of a stand (Griffiths *et al.,* 1996). Griffiths *et al.* (1991) also found all establishing Douglas-fir seedlings intimately associated with mats, and western hemlock seedlings were either associated with a mat or originating from buried or decayed wood, substrates which *Piloderma* spp. are adept at colonizing (Smith *et al.,* 2000). In this spatial context, the patchwork of *Piloderma* mats in old-growth Douglas-fir forests; their ability to colonize over 27% of the forest floor (Cromack *et al.,* 1979) with over 50% of soil dry weight comprised of hyphae/rhizomorphs (Ingham *et al.,* 1991); their exploratory habit; and their association with developing saplings of dominant late-successional tree species exemplifies a distribution uniquely capable of forming crucial underground network connections. The mat structure, especially the stable, perennial mats formed by *Piloderma*, provides a visually striking *in situ* laboratory to study EmF networking and may help illuminate the socialism-capitalism continuum of EmF network facilitation (van der Heijden & Horton, 2009). The potential for the distinctive fungal communities of *Piloderma* mat and non-mat areas to function as discrete, yet networked, entities is an exciting and necessary area for future research.

6. Conclusion:

This thesis represents a unique methodological approach to explore development and decline dynamics of *Piloderma* and *Ramaria* mat microbial communities in old-growth Douglas-fir forests of the Pacific Northwest, USA. This was accomplished by sequencing the major soil microbial components including EmF root-tips, soil fungi and soil bacteria. These methods may be adapted to study other microbial phenomena.

Foremost, we documented differential persistence of *Piloderma* (organic) and *Ramaria* (mineral) mats. Unique *Piloderma* mat fungal communities persisted for 51-months, dominated by *Piloderma* both visually and molecularly. *Ramaria* mat fungal communities originally distinct from non-mat mineral soils, while visually apparent, were almost entirely devoid of the molecular presence of *Ramaria* and were no different from non-mat soils after 51-months. We suggest this dichotomy may extend to mats formed by other fungi and relate to the functional saprotrophic capabilities of the mat-forming fungus and associated microbial community. Therefore, the majority of discussion and analysis was restricted to *Piloderma* mat and non-mat soils in the organic horizon, as differences between these microbial communities were inherent to the study design.

Piloderma mats were found to colonize transplanted non-mat soil and develop a fungal community indistinguishable from mature mats within four years (birth). This process involved altering the soil environment and outcompeting the non-mat fungal community. To compliment *Piloderma*, the best indicator of *Piloderma* mats, we identified *Russula* as a major indicator of non-mat soils and present strong evidence *Piloderma* mats competitively exclude *Russula* from the system. The presence of *Russula* in the soil of birth treatments may be the result of a senescent spore bank, as few colonized root-tips were found.

Piloderma mat death occurred quickly (Blanchard, 2008); after 51-months *Piloderma* mat soil transplanted into non-mat soil was more similar to non-mat treatments. However, enclosure in PVC pipe, thereby excluding roots and mycorrhizal connections, had a strong impact on the soil fungal community. Saprotrophic fungal taxa

proliferated at the expense of EmF. Soil fungal communities in these forests are heavily influenced by the ectomycorrhizosphere.

Inclusion of a disturbance control treatment highlights the substantial impact of root-severing even after 51-months. The fungal community composition of these treatments also shifted strongly toward saprotrophic taxa. This was not the case for similarly-disturbed non-mat soil transplanted into *Piloderma* mats, suggesting a strong competitive ability of either the prolific growth form of *Piloderma* or associated EmF.

Soil bacterial communities in the organic horizon responded quite differently to fungi. The *Piloderma* death treatment, where mat soil was enclosed in PVC pipe, had a differing bacterial community to all other treatments; again, this was likely the result of root and mycorrhizal fungal exclusion from the system. Otherwise, no differences were found between soil bacterial communities of any other treatment as a whole. At a higher level of specificity, *Piloderma* mats and non-mat soils were found to exert differing selective pressures on a subset of bacterial families, genera and species. The bacterial indicator complexes of *Piloderma* mat and non-mat soils warrant further study, perhaps on a more detailed spatial scale than the bulk soil analysis here.

The high heterogeneity of microbial communities makes comparisons on this scale extremely difficult. In this thesis, we have tried to provide functional discussion for the observed taxonomic trends we hope will guide future mat research. Because of the high site-to-site differences in soil microbiota in this study — even when considering a single watershed, forest age-class and plant species composition — it may be wise to design future projects at smaller spatial scales. Defining sites as individual mat and adjacent non-mat soil within an acre (arbitrary bounds) may have reduced microbial community variation and strengthened the observed mat development and decline dynamics.

Bibliography

Abarenkov K, Nilsson RH, Larsson KH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Ursing BM, Vrålstad T, Liimatainen K, Peintner U, Kõljalg U. 2010. The UNITE database for molecular identification of fungi - recent updates and future perspectives. *New Phytologist* **186(2):**281-285.

Abeln ECA, de Pagter MA, Verkley GJM. 2000. Phylogeny of *Pezicula*, *Dermea* and *Neofabraea* inferred from partial sequences of the nuclear ribosomal RNA gene cluster. *Mycologia* **92(4):**685-693.

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.

Agerer R, Grote R, Raidl S. 2002. The new method 'micromapping', a means to study species-specific associations and exclusions of ectomycorrhizae. *Mycological Progress* **1(2):**155-166.

Agerer R. 2006. Fungal relationships and structural identity of their ectomycorrhizae. *Mycological Progress* **5:**67-107.

Aguilera LM, Griffiths RP, Caldwell BA. 1993. Nitrogen in ectomycorrhizal mat and non-mat soils of different-age Douglas-fir forests. *Soil Biology and Biochemistry* **25(8):**1015-1019.

Alexander IJ, Högberg P. 1986. Ectomycorrhizas of tropical angiospermous trees. *New Phytologist* **102:**541-549.

Alexander IJ. 2006. Ectomycorrhizas – out of Africa? *New Phytologist* **172:**589-591.

Allen MF, Swenson W, Querejeta JI, Egerton-Warburton LM, Treseder KK. 2003. Ecology of mycorrhizae: a conceptual framework for complex interactions among plants and fungi. *Annual Review of Phytopathology* **41:**271-303.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* **215(3)**:403-410.

Aneja MK, Sharma S, Fleischmann F, Stich S, Heller W, Bahnweg G, Munch JC, Schloter M. 2006. Microbial colonization of beech and spruce litter - Influence of decomposition site and plant litter species on the diversity of microbial community. *Microbial Ecology* **52:**127−135.

Anderson IC, Campbell CD, Prosser JI. 2003. Diversity of fungi in organic soils under a moorland - Scots pine (*Pinus sylvestris* L.) gradient. *Environmental Microbiology* **5(11):**1121-1132.

Anderson IC, Cairney JG. 2007. Ectomycorrhizal fungi: exploring the mycelial frontier. *FEMS Microbiology Reviews* **31:**388-406.

Arocena JM, Glowa KR, Massicotte HB, Lavkulich L. 1999. Chemical and mineral composition of ectomycorrhizosphere soils of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) in the Ae horizon of a Luvisol. *Canadian Journal of Soil Science* **79:**25-35.

Arocena JM, Glowa KR, Massicotte HB. 2001. Calcium-rich hypha encrustations on *Piloderma*. *Mycorrhiza* **10:**209-215.

Arocena JM, Göttlein A, Raidl S. 2004. Spatial changes of soil solution and mineral composition in the rhizosphere of Norway-spruce seedlings colonized by *Piloderma croceum*. *Journal of Plant Nutrition and Soil Science* **167:**479-486.

Avis PG, McLaughlin DJ, Dentinger BC, Reich P B. 2003. Long-term increase in nitrogen supply alters above-and below-ground ectomycorrhizal communities and increases the dominance of Russula spp. in a temperate oak savanna. *New Phytologist* **160(1):**239–253.

Avis PG. 2012. Ectomycorrhizal iconoclasts: the ITS rDNA diversity and nitrophilic tendencies of fetid Russula. *Mycologia* **104(5):**998-1007.

Bach LH, Grytnes J, Halvorsen R, Ohlson M. 2009. Tree influence on soil microbial community structure. *Soil Biology and Biochemistry* **42:**1934-1943.

Bahram M, Koljalg U, Courty PE, Diedhiou AG, Kjoller R, Polme S, Ryberg M, Veldre V, Tedersoo L. 2013. The distance decay of similarity in communities of ectomycorrhizal fungi in different ecosystems and scales. *Journal of Ecology* **101:**1335-1344.

Baldrian P, Voriskova J, Dobiasova P, Merhautova V, Lisa L, Valaskova V. 2011. Production of extracellular enzymes and degradation of biopolymers by saprotrophic microfungi from the upper layers of forest soil. *Plant and Soil* **338:**111-125.

Baldrian P, Kolarik M, Stursova M, Kopecky J, Valaskova V, Vetrovsky T, Zifcakova L, Snajdr J, Ridl J, Vlcek C, Voriskova J. 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME Journal* **6(2):**248-258.

Baldrian P, López-Mondéjar R. 2014. Microbial genomics, transcriptomics and proteomics: new discoveries in decomposition research using complementary methods. *Applied Microbiology and Biotechnology*. **Published Online.**

Barbieri E, Ceccaroli P, Palma F, Agostini D, Stocchi V. 2012. Ectomycorrhizal helper bacteria: the third partner in the symbiosis. In: *Edible Ectomycorrhizal Mushrooms* (Zambonelli A, Bonito GM eds.). *Soil Biology* **34:**125-141.

Beiler KJ, Durall DM, Simard SW, Maxwell SA, Kretzer AM. 2010. Architecture of the wood-wide web: *Rhizopogon* spp. genets link multiple Douglas-fir cohorts. *New Phytologist* **185:**543-553.

Beiler JK. 2011. The complex socio-spatial architecture of *Rhizopogon* spp. mycorrhizal networks in xeric and mesic old-growth interior Douglas-fir forest plots. PhD Dissertation, The University of British Colombia **pp**172.

Bending GD, Read DJ. 1996. Nitrogen mobilization from protein-polyphenol complex by ericoid and ectomycorrhizal fungi. *Soil Biology and Biochemistry* **28:**1603-1612.

Bending GD, Read DJ. 1997. Lignin and soluble phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. *Mycological Research* **101(11):**1348-1354.

Benson DA, Karsch-Mizrachi I, Clark K, Lipman DJ, Ostell J, Sayers EW. 2012. GenBank. *Nucleic Acids Research* **40**:D48-D53.

Bergero R, Perotto S, Girlanda M, Vidano G, Luppi AM. 2000. Ericoid mycorrhizal fungi are common root associates of a Mediterranean ectomycorrhizal plant (Quercus ilex). *Molecular Ecology* **9(10):**1639-1649.

Bever JD, Richardson SC, Lawrence BM, Holmes J, Watson M. 2009. Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecology Letters* **12:**13-21.

Bingham MA, Simard S. 2012. Ectomycorrhizal networks of *Pseudotsuga menziesii* var. *glauca* trees facilitate establishment of conspecific seedlings under drought. *Ecosystems* **15:**188-199.

Bödeker IT, Nygren CM, Taylor AF, Lindahl BD. 2009. ClassII peroxidase-encoding genes are present in a phylogenetically wide range of ectomycorrhizal fungi. *ISME Journal* **3(12):**1387-1395.

Booth MG. 2004. Mycorrhizal networks mediate overstorey-understorey competition in a temperate forests. *Ecology Letters* **7:**538-546.

Boyle SA, Yarwood RR, Bottomley PJ, Myrold DD. 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.**

Bidartondo MI, Read DJ, Trappe JM, Merckx V, Ligrone R, Duckett JG. 2011. The dawn of symbiosis between plants and fungi. *Biology Letters* **7:**574-577.

Blanchard JH. 2008. Episodic dynamics of microbial communities associated with the birth and death of ectomycorrhizal mats in old-growth Douglas-fir stands. M.S. Thesis, Oregon State University.

Brant JB, Myrold DD. 2006. Root controls on soil microbial community structure in forest soils. *Oecologia* **148:**650-659.

Bottomley PJ, Yarwood RR, Kageyama SA, Waterstripe KE, Williams MA, Cromack Jr. K, Myrold DD. 2006. Responses of soil bacterial and fungal communities to reciprocal transfers of soil between adjacent coniferous forest and meadow vegetation in the Cascade Mountains of Oregon. *Plant and Soil* **289:**35-45.

Bradbury, D. C. & Firestone, M. K. 2012. Responses of redwood soil microbial community structure and N transformations to climate change. In R. B. Standiford, T. J. Weller, D. D. Piirto & J. D. Stuart tech. cords. Proceedings of the coast redwood forests in a changing California: a symposium for scientists and managers. General Technical Report PSW-GTR-238. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station.

Brundrett M. 2004. Diversity and classification of mycorrhizal associations. *Biology Reviews*. **49:**473-495.

Buée M, Courty PE, Garbaye DMJ. 2007. Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community. *Soil Biology and Biochemistry* **39:**1947-1955.

Buée M, Reich M, Murat C, Morin E, Nilsson RH, Uroz S, Martin F. 2009. 454 pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytologist* **184:**449-456.

Burke DJ, Martin KJ, Rygiewicz PT, Topa MA. 2005. Ectomycorrhizal fungi identification in single and pooled root samples: terminal restriction fragment length polymorphism (TRFLP) and morphotyping compared. *Soil Biology and Biochemistry* **37:**1683-1694.

Burke DJ, Dunham SM, Kretzer AM. 2008. Molecular analysis of bacterial communities associated with the roots of Douglas fir (*Pseudotsuga menziesii*) colonized by different ectomycorrhizal fungi. *FEMS Microbial Ecology* **65:**299-309.

Burke DJ, Smemo KA, López-Gutiérez JC, Hewins CR. 2012. Soil enzyme activity in an old-growth northern hardwood forest: Interactions between soil environment, ectomycorrhizal fungi and plant distribution. *Pedobiologia* **55(6):**357-364.

Burke DJ, Smemo KA, Hewins CR. 2014. Ectomycorrhizal fungi isolated from old-growth northern hardwood forest display variability in extracellular enzyme activity in the presence of plant litter. *Soil Biology and Biochemistry* **68:**219-222.

Cairney JWG. 2000. Evolution of mycorrhiza systems. *Naturwissenschaften*. **87:**467-475.

Cairney JWG, Meharg AA. 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(8):**803-809.

Caldwell BA, Castellano MA, Griffiths RP. 1991. Fatty acid esterase production by ectomycorrhizal fungi. *Mycologia* **83(2):**233-236.

Cannon PF, Kirk PM. 2007. Fungal Families of the World**.** Wallingford, Oxfordshire, UK. CAB International. **456pp.**

Cappellazzi, JE; Kimmerer, RW; Horton, TR. 2007. The Influence of Forest Floor Moss Cover on Mycorrhizal Abundance in Douglas-fir Forests of the Central-Western Oregon Cascades. The *State University of New York College of Environmental Science and Forestry Honors Thesis*. **147pp.**

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7(5):**335-336.

Castellano MA. 1988. The taxonomy of the genus *Hysterangium* (Basidiomycotina, Hysterangiaceae) with notes on its ecology. *Ph.D. thesis. Oregon State University, Corvallis, OR, USA.* **238pp.**

Chen DM, Taylor AFS, Burke RM, Cairney JWG. 2001. Identification of genes for lignin peroxidases and manganese peroxidases in ectomycorrhizal fungi. *New Phytologist* **152:**151-158.

Chen DM, Bastias BA, Taylor AFS, Cairney WG. 2003. Identification of laccase-like genes in ectomycorrhizal basidiomycetes and transcriptional regulation by nitrogen in *Piloderma byssinum*. *New Phytologist* **157:**547-554.

Chou Y, Polansky AM, Mason RL. 1998. Transforming non-normal data to normality in statistical process control. *Journal of Quality Technology* **30:**133-141.

Clarke KR. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*. **18:**117-143.

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

Courty PE, Breda N, 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 PE, Buée M, Diedhiou AG, Frey-Klett P, Le Tacon F, Rineau F, Turpault MP, Uroz S, Garbaye J. 2010. The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. *Soil Biology and Biochemistry* **42(5):**679-698.

Cromack K, Sollins P, Graustein WC *et al.* 1979. Calcium oxalate accumulation and soil weathering in mats of the ectomycorrhizal fungus *Hysterangium crissum* (Tul. And Tul.) Fischer. *Soil Biology and Biochemistry* **11:**463-468.

Cromack K, Fitcher BL, Moldenke AM, Entry JA, Ingham, ER. 1988. Interactions between soil animals and ectomycorrhizal fungal mats. *Agriculture, Ecosystems and Environment* **24:**161-168.

Crous PW, Braun U, Schubert K, Groenewald JZ. 2007. Delimiting *Cladosporium* from morphologically similar genera. *Studies in Mycology* **58:**33-56.

Cullings K, Courty PE. 2009. Saprotrophic capabilities as functional traits to study functional diversity and resilience of ectomycorrhizal community. *Oecologia* **161:**661-664.

Cullings K, Ishkahanova G, Ishkahanov G, Henson J. 2010. Induction of saprophytic behavior in the ectomycorrhizal fungus *Suillus granulatus* by litter addition in a *Pinus contorta* (Lodgepole pine) stand in Yellowstone. *Soil Biology and Biochemistry* **42:**1176-1178.

Dahlberg A, Jonsson L, Nylund JE. 1997. Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old-growth Norway spruce forest in south Sweden. *Canadian Journal of Botany* **75(8):**1323-1335.

DeBellis T, Kernaghan G, Bradley R, Widden P. 2006. Relationships between stand composition and ectomycorrhizal community structure in boreal mixed-wood forests. *Microbial Ecology* **52(1):**114-126.

DeBoer W, Gerards S, Klein Gunnewiek PJA, Modderman R. 1999. Response of the chitinolytic microbial community to chitin amendments of dune soils. *Biology and Fertility of Soils.* **29(2):**170-177.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. 2006. Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. *Applied Environmental Microbiology* **72:**5069-5072.

Deveau A, Plett JM, League V, Frey-Klett P, Martin F. 2012. Communication between plant, ectomycorrhizal fungi and helper bacteria. *Biocommunication of Fungi* **pp.**229-247.

Dickie IA, Koide RT. 2014. Deep thoughts on ectomycorrhizal fungal communities. *New Phytologist* **201:**1083-1085.

Dickie IA, Xu B, Koide RT. 2002. Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. *New Phytologist* **156:**527-535.

Dung NQ. 2012. *Hysterangium* mats and associated bacteria under *Eucalyptus gomphocephala* in south-western Australia. *Faculty of Science and Engineering Master's Thesis* Murdoch University. **119pp.**

Dunham SM, Larsson K, Spatafora JW. 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.

Dutton MV, Evans CS. 1996. Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. *Canadian Journal of Microbiology* **42:**881-895.

Dyrness CT, Franklin JF, Moir WH. 1974. A preliminary classification of forest communities in the central portion of the western cascades in Oregon. *United States International Biological Program, Coniferous Forest Biome, Bulletin No. 4. University of Washington.* **123pp.**

Dyrness CT. 2005. Soil descriptions and data for soil profiles in the Andrews Experimental Forest, selected reference stands, Research Natural Areas, and National Parks: Long-Term Ecological Research. [Database]. Corvallis, OR: Forest Science Data Bank: SP001. Available from: http://www.fsl.orst.edu/lter/data/abstract.cfm?dbcode=SP001S.

Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26(19):**2460-2461.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **btr381**.

Ek H, Andersson S, Söderström. 1996. Carbon and nitrogen flow in silver birch and Norway spruce connected by a common mycorrhizal mycelium. *Mycorrhiza* **6:**465-467.

Eilers KG, Debenport S, Anderson S, Fierer N. 2012. Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. *Soil Biology and Biochemistry* **50:**58-65.

Entry JA, Donnely PK, Cromack Jr.K. 1991a. Influence of ectomycorrhizal mat soils on lignin and cellulose degradation. *Biology and Fertility of Soils* **11:**75-78.

Entry JA, Rose CL, Cromack Jr.K. 1991b. Litter decomposition and nutrient release in ectomycorrhizal mat soils of a Douglas-fir ecosystem. *Soil Biology and Biochemistry* **23(3)**:285-290.

Entry JA, Rose CL, Cromack Jr.K. 1992. Microbial biomass and nutrient concentrations in hyphal mats of the ectomycorrhizal fungus *Hysterangium setchellii* in a coniferous forest soil. *Soil Biology and Biochemistry* **24(5):**447-453.

Erland S, Taylor AFS. 1999. Resupinate ectomycorrhizal fungal genera. *Ectomycorrhizal Fungi: Key Genera in Profile.* 347-363.

Fernandez CW, Koide RT. 2012. The role of chitin in the decomposition of ectomycorrhizal fungal litter. *Ecology* **93(1):**24-28.

Fernando AA, Currah RS. 1995. *Leptodontidium orchidicola* (mycelium radices atrovirens complex): aspects of its conidiogenesis and ecology. *Mycotaxon* **104:**287-294.

Fernando AA, Currah RS. 1996. A comparative study of the effects of the root endophytes *Leptodontidium orchidicola* and *Phialocephala fortinii* (fungi imperfecti) on the growth of some subalpine plants in culture. *Canadian Journal of Botany* **74:**1071-1078.

Fierer N, Jackson JA, Vilgalys R, Jackson RB. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology* **71(7):**4117-4120.

Fierer N, Hamady M, Lauber CL, Knight R. 2008. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. *PNAS.* **105(46):**17994-17999.

Finlay RD, Frostegård A, Sonnerfeldt AM. 1992. Utilization of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in symbiosis with *Pinus contorta* Dougl. Ex Loud. *New Phytologist* **120:**105-115.

Fisher RF. 1972. Spodosol development and nutrient distribution under Hydnaceae fungal mats. *Soil Science Society of America Proceedings* **36:**492-495.

Floudas D. 2012. The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science* **336:**1715-1719.

Frank AB. 1885. Über die auf Würzelsymbiose beruhende Ehrnährung gewisser Bäum durch unterirdische Pilze. *Berichte der Deutschen Botanischen Gesellschaft* **3**:128–145.

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

Fürst HM, Kraepelin G, Görisch H. 1998. Detection of *Amorphotheca resinae* in German soil by an improved selective isolation method. *Mycological Research* **102(3):**323-326.

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

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for Basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2(2)**:113-118.

Geml J, Davis DD, Geiser DM. 2005. Systematics of the genus *Sphaerobolus* based on molecular and morphological data, with the description of *Sphaerobolus ingoldii* sp. nov. *Mycologia* **97(3):**680-694.

Geneious Pro Version 6.0.5 created by Biomatters. 2012. Available from http://www.geneious.com/

Glowa KR, Arocena JM, Massicotte HB. 2003. Extraction of potassium and/or magnesium from selected soil minerals by *Piloderma*. *Geomicrobiology Journal* **20:**99-111.

Gorfer M, Klaubauf S, Bandian D, Strauss J. 2007. *Cadophora finlandia* and *Phialocephala fortinii*: *Agrobacterium*-mediated transformation and functional GFP expression. *Mycological Research* **111:**850-855.

Graf-Wimark DRH. 2010. Substrate utilization, ammonium mineralization and phylogenetic relationships of the fungi *Leptodontidium* sp. found in boreal forest soil. *M.S. Thesis: Swedish University of Agricultural Sciences*. 55pp.

Gramss F, Günther TH, Fritsche W. 1998. Spot tests for oxidative enzymes in ectomycorrhizal, wood-, and litter decay fungi. *Mycological Research* **102(1):**67-72.

Graustein WC, Cromack Jr.K., Sollins P. 1977. Calcium oxalate: Occurrence in soils and effect on nutrient and geochemical cycles. *Science* **198:**1252-1254.

Grelet GA, Johnson D, Paterson E, Anderson IC, Alexander IJ. 2009. Reciprocal carbon and nitrogen transfer between an ericaceous dwarf shrub and fungi isolated from *Piceirhiza bicolorata* ectomycorrhizas. *New Phytologist* **182:**359-366.

Grelet GA, Johnson D, Varlstad T, Alexander IJ, Anderson IC. 2010. New insights into the mycorrhizal *Rhizoscyphus ericae* aggregate: spatial structure and co-colonization of ectomycorrhizal and ericoid roots. *New Phytologist* **188:**210-222.

Griffiths RP, Caldwell BA, Cromack K, Morita RY. 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 RP, Castellano MA, Caldwell BA. 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 RP, Ingham ER, Caldwell BA, Castellano MA, Cromack Jr.K. 1991b. Microbial characteristics of ectomycorrhizal mat communities in Oregon and California. *Biology and Fertility of Soils* **11:**196-202.

Griffiths RP, Caldwell BA. 1992. Mycorrhizal mat communities in forest soils. *In: Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) Mycorrhizas in ecosystems.* **Pp.98-105** Cambridge University Press, Cambridge.

Griffiths RP, Baham JE, Caldwell BA. 1994. Soil solution chemistry of ectomycorrhizal mats in forest soil. *Soil Biology and Biochemistry* **26(3):**331-337.

Griffiths RP, Chadwick AC, Robatzek M, Schauer K, Schaffroth KA. 1995. Association of ectomycorrhizal mats with Pacific yew and other understory trees in coniferous forests. *Plant and Soil* **173:**343-347.

Griffiths RP, Bradshaw GA, Marks B, Lienkaemper GW. 1996. Spatial distribution of ectomycorrhizal mats in coniferous forests of the Pacific Northwest, USA. *Plant Soil* **180:**147-158.

Griffiths RP. 2002. Chemical and microbiological properties of soils in the Andrews Experimental Forest (1994 REU Study). *Long-Term Ecological Research. Forest Science Data Bank, Corvallis, OR. [Database]*. Available: http://andrewsforest.oregonstate.edu/data/abstract.cfm?dbcode=SP006.

Grigoriev V, Nordberg H, Shabalov I, Aerts A, Cantor M, Goodstein D, Kuo A, Minovitsky S, Nikitin R, Ohm RA, Otillar R, Poliakov A, Ratnere I, Riley R, Smirnova T, Rokhsar D, Dubchak I. 2011. The genome portal of the department of energy joint genome institute. *Nucleic Acids Research* gkr947v1-gkr947.

Hagerberg D, Pallon J, Wallander H. 2005. The elemental content in the mycelium of the ectomycorrhizal fungus *Piloderma* sp. during the colonization of hardened wood ash. *Mycorrhiza* **15:**387-392.

Hamady M, Walker JJ, Harris JK, Gold NJ, Knight R. 2007. Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. *Nature Methods*. **5(3):**235-237.

Hambleton S, Egger KN, Currah RS. 1998. The genus *Oidiodendron*: species delimitation and phylogenetic relationships based on nuclear ribosomal DNA analysis. *Mycologia* **90(5):**854-869.

Hambleton S, Nickerson NL, Seifert KA. 2005. *Leohumicola*, a new genus of heat-resistant hyphomycetes. *Studies in Mycology* **53:**29-52.

Hart CS. 2006. Potential impacts of climate change on nitrogen transformations and greenhouse gas fluxes in forests: a soil transfer study. *Global Change Biology* **12:**1032-1036.

He X, Bledsoe CS, Zasoski RJ, Southworth D, Horwath WR. 2006. Rapid nitrogen transfer from ectomycorrhizal pines to adjacent ectomycorrhizal and arbuscular mycorrhizal plants in a California oak woodland. *New Phytologist* **170:**143-151.

Heinonsalo J, Hurme KR, Sen R. 2004. Recent ^{14}C-labelled assimilate allocation to Scots pine seedling root and mycorrhizosphere compartments developed on reconstructed podzol humus, E- and B- mineral horizons. *Plant and Soil* **259(1-2):**111-121.

Hesse CN. 2012. Characterization of fungal and bacterial communities associated with mat-forming ectomycorrhizal fungi from old-growth stands in the H.J. Andrews Experimental Forest. *Oregon State University Dissertation*. **185pp.**

Hibbett DS, Matheney PB. 2009. The relative ages of ectomycorrhizal mushrooms and their plant hosts estimated using Bayesian relaxed molecular clock analyses. *BMC Biology.* **7(13)**.

Hintikka V, Naykki O. 1967. Notes on the effects of the fungus *Hydnellum ferrugineum* (Fr.) Karst. On forest soil and vegetation. *Communications Instituti Forestalis Fenniae* **62:**1-22.

Hobbie EA, Agerer R. 2010. Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. *Plant and Soil* **327:**71-83.

Hobbie EA, Ouimette AP, Schuur EAG, Kierstead D, Trappe JM, Bendiksen K, Ohenoja E. 2013. Radiocarbon evidence for the mining of organic nitrogen from soil by mycorrhizal fungi. *Biogeochemistry* **114(1-3):**381-389.

Hobbie EA, van Diepen LT, Lilleskov EA, Ouimette AP, Finzi AC, Hofmockel KS. 2014. Fungal functioning in a pine forest: evidence from a (15) N-labeled global change experiment. *New Phytologist* **201(4):**1431-1439.

Hoff JA, Klopfenstein NB, McDonald GI, TonnJR, Kim MS, Zambino PJ, Hessburg PF, Rogers JD, Peever TL, Carris LM. 2004. Fungal endophytes in woody roots of Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*). *Forest Pathology* **34:**1-17.

Högberg P. 1982. Mycorrhizal associations in some woodland and forest trees and shrubs in Tanzania. *New Phytologist* **92:**407-415.

Horton TR, Bruns TD. 1998. Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas fir (*Pseudotsuga menziesii*) and bishop pine (*Pinus muricata*). *New Phytologist* **139:**331-339.

Horton TR, Bruns TD. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology* **10(8)**:1855-1871.

Horton TR, Molina R, Hook K. 2005. Douglas-fir ectomycorrhizae in 40 and 400 year-old stands: mycobiont availability to late successional western hemlock. *Mycorrhiza* **15:**393-403.

Hou X, Guo S. 2008. Interaction between a dark septate endophytic isolate from *Dendrobium* sp. and roots of *D. nobile* seedlings. *Journal of Integrative Plant Biology* 8pp.

Huson DH, Mitra S, Ruscheweyh HJ, Weber N, Schuster SC. 2011. Integrative analysis of environmental sequences using MEGAN 4**.** *Genome Research* **21:**1552-1560.

Hutchinson LJ. 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(7):**1522-1530.

Ingham ER, Griffiths RP, Cromack K, Entry JA. 1991. Comparison of direct vs fumigation incubation microbial biomass estimates from ectomycorrhizal mat and non-mat soils. *Soil Biology and Biochemistry* **23(5):**465-471.

Ishida TA, Nordin A. 2010. No evidence that nitrogen enrichment affect fungal communities of *Vaccinium* roots in two contrasting boreal forest types. *Soil Biology and Biochemistry* **42:**234-243.

Izumi H, Moore ERB, Killham K, Alexander IJ, Anderson IC. 2007. Characterization of endobacterial communities in ectomycorrhizas by DNA- and RNA-based molecular methods. *Soil Biology and Biochemistry* **39:**891-899.

Izumi H, Finlay RD. 2011. Ectomycorrhizal roots select distinctive bacterial and ascomycete communities in Swedish subarctic forests. *Environmental Microbiology* **13(3):**819-830.

Izzo A, Agbowo J, Bruns TD. 2005. Detection of plot-level changes in ectomycorrhizal communities across years in an old-growth mixed-conifer forest. *New Phytologist* **166(2):**619-629.

Johansson JF, Paul LR, Finlay RD. 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbial Ecology* **48:**1-13.

Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism parasitism continuum. *New Phytologist* **135:**575-585.

Jones MD, Smith SE. 2004. Exploring the functional definitions of mycorrhizas: Are mycorrhizas always mutualisms? *Canadian Journal of Botany*. **82(8):**1089-1109.

Jones MD, Phillips LA, Treu R, Ward V, Berch SM. 2012. Functional responses of ectomycorrhizal fungal communities to long-term fertilization of lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. latifolia Engelm.) stands in central British Columbia. *Applied Soil Ecology* **60:**29-40.

Jongmans AG, van Breemen N, Lundström U, van Hees PAW, Finlay RD, Srinivasan M, Unestam T, Giesler R, Melkerud PA, Olsson M. 1997. Rock-eating fungi. *Nature* **389:**682-683.

Jumpponen A, Jones KL, Blair J. 2010. Vertical distribution of fungal communities in tallgrass prairie soil. *Mycologia* **102(5):**1027-1041.

Kageyama SA, Posavats NR, Jones SS, Waterstripe KE, Bottomley PJ, Cromack Jr K, Myrold DD. 2013. Effects of disturbance scale on soil microbial communities in the Western Cascades of Oregon. *Plant and Soil* **372(1-2):**459-471.

Karpati AS, Handel SN, Dighton J, Horton TR. 2011. Quercus rubra-associated ectomycorrhizal fungal communities of disturbed urban sites and mature forests. *Mycorrhiza* **21:**537-547.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAAFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30(14):**3059-3006.

Kennedy PG, Izzo AD, Bruns TD. 2003. There is high potential for the formation of common mycorrhizal networks between understory and canopy trees in a mixed evergreen forest. *Journal of Ecology* **91:**1071-1080.

Kernaghan G, Currah RS, Bayer RJ. 1997. Russulaceous ectomycorrhizae of *Abies lasiocarpa* and *Picea engelmannii*. *Canadian Journal of Botany* **75:**1843-1850.

Kider JT, Raja S, Badler NI. 2011. Fruit senescence and decay simulation. *Eurographics* **30(2):**10pp.

Kiers TE, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bücking H. 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* **333:**880-882.

Kipfer T, Egli S, Ghazoul J, Moser B, Wohlgemuth T. 2010. Susceptibility of ectomycorrhizal fungi to soil heating. *Fungal Biology* **114:**467-472.

Kjøller R, Olsrud M, Michelsen A. 2010. Co-existing ericaceous plant species in a subarctic mire community share fungal root endophytes. *Fungal Ecology* **3:**205-214.

Kluber LA, Tinnesand KM, Caldwell BA, Dunham SM, Yarwood RR, Bottomley PJ, Myrold DD. 2010. Ectomycorrhizal mats alter forest soil biogeochemistry. *Soil Biology and Biochemistry* **42:**1607-1613.

Kluber LA, Smith JE, Myrold DD. 2011. Distinctive fungal and bacterial communities are associated with mats formed by ectomycorrhizal fungi. *Soil Biology and Biochemistry* **43:**1042-1050.

Knutson DM, Hutchins AS, Cromack K. 1980. The association of calcium oxalate-utilizing *Streptomyces* with conifer ectomycorrhizae. *Antonie van Leeuwenhoek* **46:**611-619.

Kohout P, Tesitelova T, Roy M, Vohnik M, Jersakova J. 2013. A diverse fungal community associated with *Pseudorchis albida* (Orchidaceae) roots. *Fungal Ecology* **6:**50-64.

Koide RT, Sharda JN, Herr JR, Malcolm GM. 2008. Ectomycorrhizal fungi and the biotrophy-saprotrophy continuum. *New Phytologist* **178:**230-233.

Koide RT, Fernandez C, Malcolm G. 2014. Determining place and process: functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. *New Phytologist* **201:**433-439.

Kottke I, Oberwinkler F. 1986. Mycorrhiza of forest trees - structure and function. *Trees* **1(1):**1-24.

Kranabetter JM, Durall DM, MacKenzie WH. 2009. Diversity and species distribution of ectomycorrhizal fungi along productivity gradients of a southern boreal forest. *Mycorrhiza* **19(2):**99-111.

Kropp BR. 1982. Fungi from decayed wood as ectomycorrhizal symbionts of western hemlock. *Canadian Journal of Forest Research*. **12:**36-39.

Kropp BR, Trappe JM. 1982. Ectomycorrhizal fungi of *Tsuga heterophylla*. *Mycologia* 74:479-488

Kruskal JB, Wish M. 1978. Multidimensional scaling. Sage publications. Beverly Hills, CA. 93pp.

Koukol O, Baldrian P. 2012. Intergeneric variability in enzyme production of microfungi from pine litter. *Soil Biology and Biochemistry* **49:**1-3.

Lacourt I, Girlanda M, Perotto S, Pero MD, Zuccon D, Luppi AM. 2001. Nuclear ribosomal sequence analysis of *Oidiodendron*: towards a redefinition of ecologically relevant species. *New Phytologist* **149:**565-576.

Landeweert R, Leeflang P, Kuyper TW, Hoffland E, Rosling A, Wernars K, Smit E. 2003. Molecular identification of ectomycorrhizal mycelium in soil horizons. *Applied and Environmental Microbiology* **69(1):**327-333.

Larsen MJ, Smith JE, McKay D. 1997. On *Piloderma bicolor* and the closely related *P. byssinum, P. croceum,* and *P. fallax*. *Mycotaxon* **63:**1-8.

Lauber CL, Hamady M, Knight R, Fierer N. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied Environmental Microbiology*. **75(15):**5111.

Leake JR, Read DJ. 1990. Chitin as a nitrogen source for mycorrhizal fungi. *Mycological Research* **94(7)**:993-995.

Lee E, Eo J, Lee C, Eom A. 2012. Effect of soil ameliorators on ectomycorrhizal fungal communities that colonize seedlings of *Pinus densiflora* in abandoned coal mine spoils. *Microbiology* **40(3):**166-172.

Leimar O, Hammerstein P. 2010. Cooperation for direct fitness benefits. *Philosophical Transactions of the Royal Society of Britain* **365:**2619-2626.

Li CY, Hung LL. 1987. Nitrogen-fixing (acetylene-reducing) bacteria associated with ectomycorrhizae of Douglas-fir. *Plant and Soil* **98:**425-428.

Lilleskov EA, Hobbie EA, Fahey TJ. 2002. Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. *New Phytologist* **154(1):**219-231.

Lilleskov EA, Hobbie EA, Horton TR. 2011. Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. *Fungal Ecology* **4(2):**174-183.

Lindahl B, Ihrmark K, Boberg J, Trumbore SE, Hogberg P, Stenlid J, Finlay RD. 2007. Spatial separation of litter decomposition and mycorrhizal nutrient uptake in a boreal forest. *New Phytologist* **173:**611-620.

Lin L-C, Lee M-J, Chen J-L. 2011. Decomposition of organic matter by the ericoid mycorrhizal endophytes of Formosan rhododendron (*Rhododendron formosanum* Hemsl.). *Mycorrhiza* **21:**331-339.

Liu, Z. Z., C. Lozupone, M. Hamady, F. D. Bushman, and R. Knight. 2007. Short pyrosequencing reads suffice for accurate microbial community analysis. *Nucleic Acids Research.* **35:**e120.

Luoma DL, Eberhart JL. 2014. Relationships between Swill needle cast and ectomycorrhizal fungus diversity. *Mycologia* In Press.

Majdi H, Damm E, Nylund JE. 2001. Longevity of mycorrhizal roots depends on branching order and nutrient availability. *New Phytologist.* **150:**195-202.

Mansfeld-Giese K, Larsen J, Bodker L. 2002. Bacterial populations associated with mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices*. *FEMS Microbiology and Ecology* **41:**133-140.

Mahmood S, Finlay RD, Erland S, Wallander H. 2001. Solubilisation and colonization of wood ash by ectomycorrhizal fungi isolated from a wood ash fertilized spruce forest. *FEMS Microbiology and Ecology* **35:**151-161.

Mahmood S, Finlay RD, Wallander H, Erland S. 2002. Ectomycorrhizal colonization of roots and ash granules in a spruce forest treated with granulated wood ash. *Forest Ecology and Management* **160:**65-74.

Mahmood S, Finlay RD, Fransson AM, Wallander H. 2003. Effects of hardened wood ash on microbial activity, plant growth and nutrient uptake by ectomycorrhizal spruce seedlings. *FEMS Microbiology and Ecology* **43:**121-131.

Marks GC, Kozlowski TT. 1973. Ectomycorrhizae: their ecology and physiology. *Academic Press,* London, UK. **444pp.**

Marschner P, Jentschke G, Godbold DL. 19-98. Cation exchange capacity and lead sorption in ectomycorrhizal fungi. *Plant and Soil* **205:**93-98.

Massicotte HB, Melville LH, Peterson RL. 2005. Structural features of mycorrhizal associations in two members of the Monotropoideae, Monotropa uniflora and Pterospora andromedea. *Mycorrhiza* **15(2):**101-110.

Matsubara T, Iwasaki H. 1972. Occurrence of laccase and tyrosinase in fungi of Agaricales and comparative study of laccase from Russula delica and Russula pseudodelica. *The Botanical Magazine Tokyo* **85:**71-83.

McCune B, Mefford M J. 2011. PC-ORD. Multivariate Analysis of Ecological Data. Version 6.06. MjM Software, Gleneden Beach, Oregon, U.S.A.

McGuire KL, Allison SD, Fierer N, Treseder KK. 2013. Ectomycorrhizal-dominated boreal and tropical forests have distinct fungal communities, but analogous spatial patterns across soil horizons. *PLOS One* **8(7):**e68278.

McKnight KB, McKnight KH, Harper KT. 1990. Cation exchange capacities and mineral element concentrations of macrofungal stipe tissue. *Mycologia* **82(1):**91-98.

Mello A, Ding G, Piceno YM, Napoli C, Tom LM, DeSantis TZ, Andersen GL, Smalla K, Bonfante P. 2013. Truffle brûlés have an impact on the diversity of soil bacterial communities. *PLOS One* **8(4):**e61945.

"Microbial Observatory at the HJ Andrews LTER." Oregon State University. http://cropandsoil.oregonstate.edu/content/microbial-observatory-hj-andrews-lter. 5/25/13.

Minitab 16.1.1 Statistical Software. 2010. [Computer software]. State College, PA: Minitab, Inc. (www.minitab.com)

Molina R, Massicotte H, Trappe JM. 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen MF, ed. Mycorrhizal Functioning. New York, USA: Chapman and Hall pp. 357-423.

Mosse B. 1962. Establishment of vesicular-arbuscular mycorrhiza under aseptic conditions. *Microbiology* **27:**509-520.

Nagy LG, Petkovits T, Kovacs GM, Voigt K, Vagvolgyi C, Papp T. 2011. Where is the unseen fungal diversity hidden? A study of *Mortierella* reveals a large contribution of reference collections to the identification of fungal environmental sequences. *New Phytologist* **191(3):**789-794.

Nara K. 2006. Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytologist* **169:**169-178.

Nazir R, Warmink JA, Boersma H, van Elsas JD. 2010. Mechanisms that promote bacterial fitness in fungal-affected soil microhabitats. *FEMS Microbiology Ecology*. **71:**169-185.

Newman EI. 1988. Mycorrhizal links between plants: their functioning and ecological significance. *Advances in Ecological Research* **18:**243-270.

Nilsson RH, Veldre V, Hartmann M, Unterseher M, Amend A, Bergsten J, Kristiansson E, Ryberg M, Jumpponen A, Abarenkov K. 2010. An open source software package for automated extraction of ITS1 and ITS2 from fungal ITS sequences for use in high-throughput community assays and molecular ecology. *Fungal Ecology* **3:**284-287.

Nuñez MA, Horton TR, Simberloff D. 2009. Lack of belowground mutualisms hinders Pinaceae invasions. *Ecology* **90(9):**2352-2359.

Nygren CM, Edgvist J, Dlfstrand M, Heller G, Taylor AF. 2007. Detection of extracellular protease activity in different species and genera of ectomycorrhizal fungi. *Mycorrhiza* **17(3):**241-248.

Nygren CM, Eberhardt U, Karlsson M, Parrent JL, Lindahl BD, Taylor AFS. 2008. Growth on nitrate and occurrence of nitrate reductase-encoding genes in a phylogenetically diverse range of ectomycorrhizal fungi. *New Phytologist* **180(4):**875-889.

O'Dell TE, Luoma DL, Molina RJ. 1992. Ectomycorrhizal fungal communities in young, managed, and old-growth Douglas-fir stands. *Northwest Environmental Journal* **8(1):**166-168.

Offre P, Pivato B, Siblot S, Gamalero E, Corberand T, Lemanceau P, Mougel C. 2007. Identification of bacterial groups preferentially associated with mycorrhizal roots of *Medicago truncatula*. *Applied Environmental Microbiology* **73:**913-921.

Offre P, Pivato B, Mazurier S, Siblot S, Berta G, Lemanceau P, Mougel C. 2008. Microdiversity of Burkholderiales associated with mycorrhizal and nonmycorrhizal roots of *Medicago truncatula*. *FEMS Microbiology and Ecology* **65:**180-192.

Pearson K. 1901. On Lines and Planes of Closest Fit to Systems of Points in Space. *Philosophical Magazine.* **2(11):559–572.**

Peay KG, Kennedy PG, Bruns TD. 2011. Rethinking ectomycorrhizal succession: are root density and hyphal exploration types drivers of spatial and temporal zonation? *Fungal Ecology* **4(3):**233-240.

Peintner U, Iotti M, Klotz P, Bonuson E, Zambonelli A. 2007. Soil fungal communities in a *Castanea sativa* (chestnut) forest producing large quantities of *Boletus edulis sensu lato* (porcini): where is the mycelium of porcini? *Environmental Microbiology* **9(4):**880-889.

Peter M, Ayear F, Egli S. 2001. Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. **149:**311-325.

Peterson RL, Massicotte HB. 2004. Exploring structural definitions of mycorrhizas, with emphasis on nutrient-exchange interfaces. *Canadian Journal of Botany* **82(8):**1074-1088.

Phillips CL, Kluber LA, Martin JP, Caldwell BA, Bond BJ. 2012. Contributions of ectomycorrhizal fungal mats to forest soil respiration. *Biogeosciences* **9:**1635-1666.

Phillips LA, Ward V, Jones MD. 2013. Ectomycorrhizal fungi contribute to soil organic matter cycling in sub-boreal forests. *ISME Journal* **Published Online**:1-15.

Phillips RP, Brzostek E, Midgley MG. 2013. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon-nutrient couplings in temperate forests. *New Phytologist* **199(1):**41-45.

Pickles BJ, Genney DR, Anderson IC, Alexander IJ. 2012. Spatial analysis of ectomycorrhizal fungi reveals that root-tip communities are structured by competitive interactions. *Molecular Ecology* **20:**5110-5123.

Pirozynski, KA; Malloch, DW. 1975. The origin of land plants: a matter of mycotrophism. *Biosystems* **6(3):**153-164.

Porras-Alfaro A, Bayman P. 2011. Hidden fungi, emergent properties: Endophytes and microbiomes. *The annual Review of Phytopathology* **49:**291-315.

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

Redecker D, Szaro TM, Bowman RJ, Bruns TD. 2001. Small genets of *Lactarius xanthogalactus*, *Russula cremoricolor* and *Amanita francheti* in late-stage ectomycorrhizal successions. *Molecular Ecology* **10:**1025-1034.

Rosling A, Landeweert R, Lindahl BD, Larsson KH, Kuyper TW, Taylor AFS, Finlay RD. 2003. Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. *New Phytologist* **159(3):**775-783.

Rosling A, Finlay R D. 2005. Responses of ectomycorrhizal fungi to mineral substrates. *Geochimica et Cosmochimica Acta* **69(10):**A232 - Supplement 1, Goldschmidt Conference Abstracts.

Rosling A, Cox F, Cruz-Martinez K, Ihrmark K, Grelet G, Lindahl BD, Menkis A, James TY. 2011. Archaeorhizomycetes: Unearthing an ancient class of ubiquitous soil fungi. *Science* **333:**876-879.

Rosling A, Timling I, Taylor L. 2013. Archaeorhizomycetes: Patterns of distribution and abundance in soil. In. Genomics of Soil- and Plant-Associated Fungi. Soil Biology **36:** Chapter 14, 333-349.

Rubino DL, McCarthy C. 2003. Composition and ecology of macrofungal and myxomycete communities on oak woody debris in a mixed-oak forest of Ohio. *Canadian Journal of Forest Research* **33:**2151-2163.

Rudawska M, Leski T, Stasinska M. 2011. Species and functional diversity of ectomycorrhizal fungal communities on Scots pine (*Pinus sylvestris* L.) trees on three different sites. *Annals of Forest Science* **68(1):**5-15.

Ruess RW, Hendrick RL, Burton AJ, Pregitzer KS, Sveinbjornsson B, Allen MF, Maurer GE. 2003. Coupling fine root dynamics with ecosystem carbon cycling in black spruce forests of interior Alaska. *Ecological Monographs* **73(4):**643-662.

Schüßler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. *Mycological Research* **105(12):**1413-1421.

Selosse MA, Richard F, He X, Simard SW. 2006. Mycorrhizal networks: *des liaisons dangereuses? Trends in Ecology and Evolution* **21(11):**621-628.

Siira-Pietikäinen A, Haimi J, Kanninen A, Pietikäinen J, Fritze H. 2001. Responses of decomposer community to root-isolation and addition of slash. *Soil Biology and Biochemistry* **33:**1993-2004.

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R. 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* **388:**579-582.

Simard SW, Beiler KJ, Bingham MA, Deslippe JR, Philip LJ, Teste FP. 2012. Mycorrhizal networks: mechanisms, ecology and modeling. *Fungal Biology Reviews* **26:**39-60.

Smith JE, Molina R, McKay D, Luoma D, Castellano M. 1996. Ectomycorrhizal fungus diversity in Douglas-fir forests of the Oregon Cascades. In: Azcon-Aguilar, C.; Barea, J. M., eds. Mycorrhizas in integrated systems from genes to plant development: Proceedings of the fourth European Symposium on mycorrhizas; 1994 July 11-14; Granada. Brussels: European Commission: 141-143.

Smith JE, Molina R, Huso MMP, Larsen MJ. 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 JE, Molina R, Huso MMP, Luoma DL, McKay D, Castellano MA, Lebel T, 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, U.S.A. *Canadian Journal of Botany* **80:**186-204.

Smith SE, Read DJ. 2008. Mycorrhizal symbiosis (3rd ed). *Academic Press*, London, UK. **787pp.**

Smith, WB; Miles, PD; Perry, CH; Pugh, SA. 2009. Forest Resources of the United States, 2007. Gen. Tech. Rep. WO-78. Washington, DC: U.S. Department of Agriculture, Forest Service, Washington Office. 336 p.

Talbot JM, Allison SD, Treseder KK. 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Functional Ecology* **22(6):**955-963.

Taylor AFS, Fransson PM, Högberg P, Högberg MN, Plamboeck AH. 2003. Species level patterns in ^{13}C and ^{15}N abundance of ectomycorrhizal and saprotrophic fungal sporocarps. *New Phytologist* **159(3):**757-774.

Taylor AFS, Alexander I. 2005. The ectomycorrhizal symbiosis: life in the real world. *Mycologist* **19(3):**102-112.

ter Braak CJF. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology.* **67:**1167-1179.

Tedersoo L, Kõljalg U, Hallenberg N, Larsson KH. 2003. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. *New Phytologist* **159(1):**153-165.

Tedersoo L, Pärtel K, Jairus T, Gates G, Põldmaa K, Tamm H. 2009. Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the *Helotiales*. *Environmental Microbiology* **11(12):**3166-3178.

Tedersoo L, May TW, Smith ME. 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza* **20:**217-263.

Tedersoo L, Naadel T, Bahram M, Pritsch K, Buegger F, Leal M, Kõljalg U, Põldmaa K. 2012. Enzymatic activities and stable isotope patterns of ectomycorrhizal fungi in relation to phylogeny and exploration types in an afrotropical rain forest. *New Phytologist* **195:**832-843.

Teste FP, Simard SW, Durall DM, Guy RD, Jones MD, Schoonmaker AL. 2009. Access to mycorrhizal networks and roots of trees: importance for seedling survival and resource transfer. **90(10):**2808-2822.

Teste FP, Simard SW, Durall DM, Guy RD, Berch SM. 2010. Net carbon transfer between *Pseudotsuga menziesii* var. *glauca* seedlings in the field is influenced by soil disturbance. *Journal of Ecology* **98:**429-439.

Toju H, Yamamoto S, Sato H, Tanabe AS, Gilbert GS, Kadowaki K. 2013. Community composition of root-associated fungi in a *Quercus*-dominated temperate forest: "codominance" of mycorrhizal and root-endophytic fungi. *Ecology and Evolution* **13pp.**

Trappe MJ, Cromack K Jr, Caldwell BA, Griffiths RP, Trappe JM. 2012. Diversity of mat-forming fungi in relation to soil properties, disturbance, and forest ecotype at crater lake national park, Oregon, USA. *Diversity* **4:**196-223.

Trappe JM. 1964. Mycorrhizal hosts and distribution of *Cenococcum graniforme*. *Lloydia* **27:**100-106.

Trappe JM. 1996. What is a mycorrhiza? Proceedings of the 4th European Symposium on mycorrhizae, Grenada, Spain, EC Report EUR 16728:3-9.

Trudell SA, Rygiewicz PT, Edmonds RL. 2004. Patterns of nitrogen and carbon stable isotope ratios in macrofungi, plants and soils in two old-growth conifer forests. *New Phytologist* **164(2):**317-335.

Tsujita-Ogura Y, Gebauer G, Hashimoto T, Umata H, Yukawa T. 2009. Evidence for novel and specialized mycorrhizal parasitism: the orchid *Gastrodia confusa* gains carbon from saprotrophic *Mycena*. *Proceedings of the Royal Society Biology*. **276:**761-767.

Tuason MMS, Arocena JM. 2009. Calcium oxalate biomineralization by *Piloderma fallax* in response to various levels of calcium and phosphorus. *Applied and Environmental Microbiology* **75(22):**7079-7085.

Twieg BD, Durall DM, Simard SW, Jones MD. 2009. Influence of soil nutrients on ectomycorrhizal communities in a chronosequence of mixed temperate forests. *Mycorrhiza* **19(5):**305-316.

Unestam T. 1991. Water repellency, mat formation, and leaf-stimulated growth of some ectomycorrhizal fungi. *Mycorrhiza* **1:**13-20.

Uroz S, Oger P, Morin E, Frey-Klett P. 2012. Distinct ectomycorrhizospheres share similar bacterial communities as revealed by pyrosequencing-based analysis of 16S rRNA genes. *Applied Environmental Microbiology* **78(8):**3020-3024.

Uroz S, Courty PE, Pierrat JC, Peter M, Buée M, Turpault MP, Garbaye J, Frey-Klett P. 2013. Functional profiling and distribution of forest soil bacterial communities along the soil mycorrhizosphere continuum. *Microbial Ecology* **66:**404-415.

van Breemen N, Finlay R, Lundström U, Jongmans AG, Giesler R, Olsson M. 2000. Mycorrhizal weathering: A true case for mineral plant nutrition? *Biogeochemistry* **49:**53-67.

van der Heijden MGA, Horton TR. 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology* **97:**1139-1150.

Vasiliauskas R, Menkis A, Finlay RD, Stenlid J. 2007. Wood-decay fungi in fine living roots of conifer seedlings. *New Phytologist* **174:**441-446.

Velmala SM, Rajala T, Heinonsalo J, Taylor AFS, Pennanen T. 2014. Profiling functions of ectomycorrhizal diversity and root structuring in seedlings of Norway spruce (*Picea abies*) with fast- and slow-growing phenotypes. *New Phytologist* **201:**610-622.

Vik U, Logares R, Blaalid R, Halvorsen R, Carlsen T, Bakke I, Kolstø A, Økstad OA, Kauserad H. 2013. Different bacterial communities in ectomycorrhizae and surrounding soil. Nature *Scientific Reports* **3:**3471.

Villareal-Ruiz L, Anderson IC, Alexander IJ. 2004. Interaction between an isolate from the *Hymenoscyphus* ericae aggregate and roots of *Pinus* and *Vaccinium*. *New Phytologist* **164(1):**183-192.

Vogt KA, Edmonds RL, Grier CC. 1981. Dynamics of ectomycorrhizae in *Abies amabilis* stands: The role of *Cenococcum graniforme*. *Holarctic Ecology* **4:**167-173.

Vrålstad T, Schumacher T, Taylor AFS. 2002. Mycorrhizal synthesis between fungal strains of the *Hymenoscyphus ericae* aggregate and potential ectomycorrhizal and ericoid hosts. *New Phytologist* **153:**143-152.

Wagner L, Stielow B, Hoffmann K, Petkovits T, Papp T, Vagvolgyi GS, de Hoog G, Voigt K. 2013. A comprehensive molecular phylogeny of the Mortierellales (Mortierellomycotina) based on nuclear ribosomal DNA. *Persoonia* **30:**77-93.

Walker JKM, Cohen H, Higgins LM, Kennedy PG. 2014. Testing the link between community structure and function for ectomycorrhizal fungi involved in a global tripartite symbiosis. *New Phytologist* **202(1):**287-296.

Wallander H, Massicotte HB, Nylund JE. 1997. Seasonal variation in protein, ergosterol and chitin in five morphotypes of *Pinus sylvestris* L. ectomycorrhizae in a mature Swedish forest. *Soil Biology and Biochemistry.* **29(1):**45-53.

Wallander H, Ekblad A, Godbold DL, Johnson D, Bahr A, Baldrian A, Björk RG, Kieliszewska-Rokicka B, Kjøller R, Kraigher H, Plassard C, Rudawska M. 2013. Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forest soils - A review. *Soil Biology and Biochemistry* **57:**1034-1047.

Wang B, Qiu YL. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* **16:**299-363.

Wang Z, Binder M, Schoch CL, Johnston PR, Spatafora JW, Hibbett DS. 2006. Evolution of helotialean fungi (Leotiomycetes, Pezizomycotina): A nuclear rDNA phylogeny. *Molecular Phylogenetics and Evolution* **41:**295-312.

Warmink JA, Nazir R, van Elsas JD. 2009. Universal and species-specific bacterial 'fungiphiles' in the mycospheres of different basidiomycetous fungi. *Environmental Microbiology* **11(2):**300-312.

Weinbaum BS, Allen MF, Allen EB. 1996. Survival of Arbuscular Mycorrhizal Fungi Following Reciprocal Transplanting Across the Great Basin, USA. *Ecological Applications* **6(4):**1365-1372.

Wiemken V, Boller T. 2002. Ectomycorrhiza: gene expression, metabolism and the wood-wide web. *Current Opinion in Plant Biology* **5:**1-7.

Wright DP, Johansson T, Le Quéré A, Söderström B, Tunlid A. 2005. Spatial patterns of gene expression in the extramatrical mycelium and mycorrhizal root-tips formed by the ectomycorrhizal fungus *Paxillus involutus* in association with birch (*Betula pendula*) seedlings in soil microcosms. *New Phytologist* **167:**579-576.

Wu J, Liu Z, Wang X, Sun Y, Zhou L, Lin Y, Fu S. 2011. Effects of understory removal and tree girdling on soil microbial community composition and litter decomposition in two Eucalyptus plantations in South China. *Functional Ecology* **25(4):**921-931.

Wu T. 2011. Can ectomycorrhizal fungi circumvent the nitrogen mineralization for plant nutrition in temperate forest ecosystems? *Soil Biology and Biochemistry* **43(6):**1109-1117.

Wurzburger N, Higgins BP, Hendrick RL. 2011. Ericoid mycorrhizal root fungi and their multicopper oxidases from a temperate forest shrub. *Ecology and Evolution* **2(1):**65-79.

Xiao G, Berch SM. 1996. Diversity and abundance of ericoid mycorrhizal fungi of *Gaultheria shallon* on forest clearcuts. *Canadian Journal of Botany* **74:**337-346.

Yuan ZL, Zhang CL, Lin FC, Kubicek CP. 2010. Identity, diversity, and molecular phylogeny of the endophytic mycobiota in the roots of rare wild rice (*Orzya granulate*) from a nature reserve in Yunnan, China. *Applied and Environmental Microbiology* **76(5):**1642-1652.

Zalar P, de Hoog GS, Schroers HJ, Frank MJ, Gunde-Cimerman N. 2005. Taxonomy and phylogeny of the xerophilic genus *Wallemia* (Wallemiomycetes and Wallemiales, cl. Et ord. nov.). *Antonie van Leeuwenhoek* **87:**311-328.

Zifcakova L, Dobiasova P, Kolarova Z, Koukol O, Baldrian P. 2011. Enzyme activities of funi associated with *Picea abies* needles. *Fungal Ecology* **4:**427-436.

Zimmer K, Hymson NA, Gebauer G, Allen EB, Allen MF, Read DJ. 2007. Wide geographic and ecological distribution of nitrogen and carbon gains from fungi in pyroloids and monotropoids (Ericaceae) and in orchids. *New Phytologist* **175:**166-175.

Zeglin LH, Kluber LA, Myrold D. 2013. The importance of amino sugar turnover to C and N cycling in organic horizons of old-growth Douglas-fir forest soils colonized by ectomycorrhizal mats. *Biogeochemistry.* **112(1-3):**679-693.

Zeglin LH, Myrold D. 2013. Fate of decomposed fungal cell wall material in organic horizons of old-growth Douglas-fir forest soils. *Soil Biology and Biochemistry.* **77(2):**489-500.

Zheng JQ, Han SJ, Wang Y, Zang CG, Li MH. 2010. Composition and function of microbial communities during the early decomposition stages of foliar litter exposed to elevated CO_2 concentrations. *European Journal of Soil Science* **61:**914-925.

Zobel DB, McKee A, Hawk GM. 1976. Relationships of environment to composition, structure, and diversity of forest communities of the central western cascades of Oregon. *Ecological Monographs* **46:135-156.**

Zumsteg A, Bååth E, Stierli B, Zeyer J, Frey B. 2013. Bacterial and fungal community responses to reciprocal soil transfer along a temperature and soil moisture gradient in a glacier forefield. *Soil Biology and Biochemistry*. **61:**121-132.

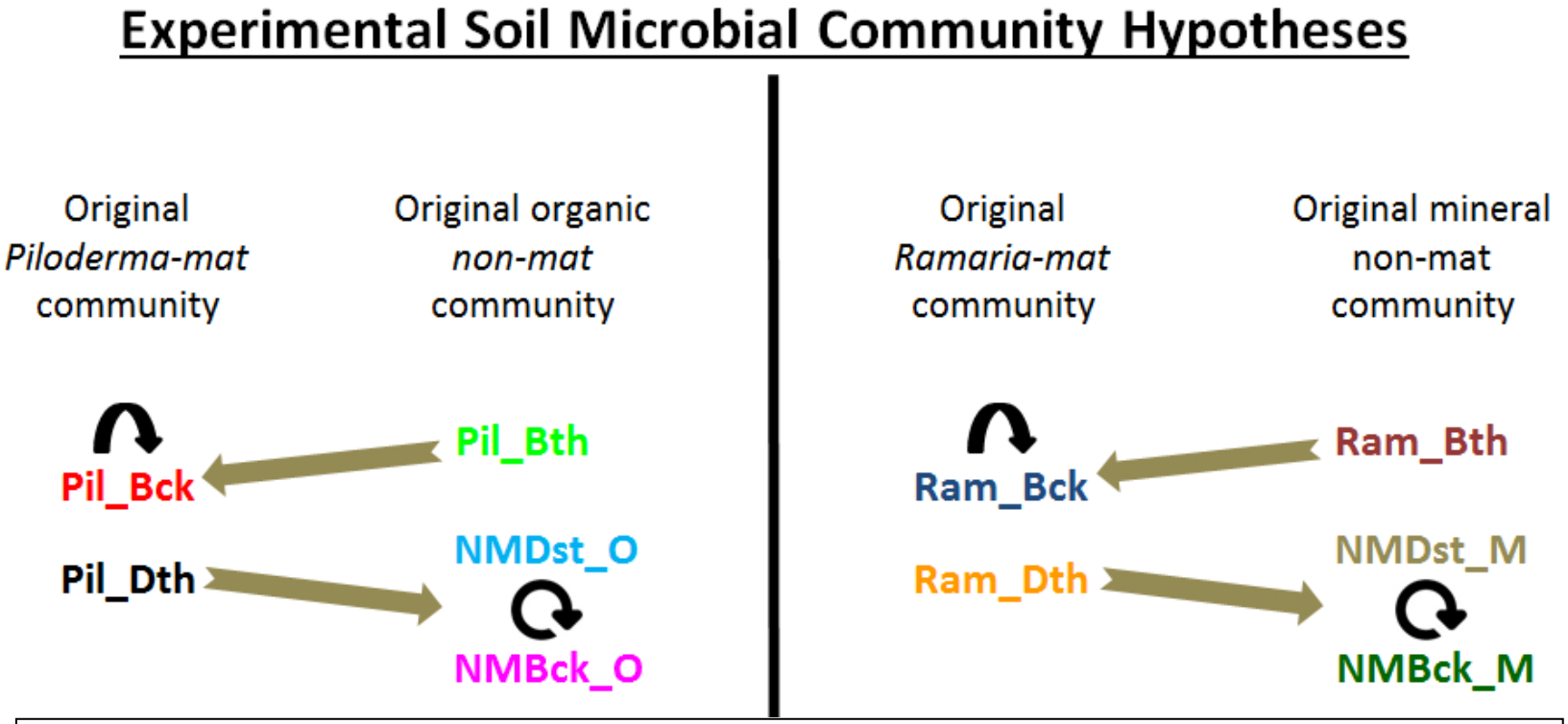


Figure 1: Schematic diagram of hypothesized soil microbial community trajectories for the reciprocal soil transplant experiment. Each column represents soils that were originally hypothesized to possess similar communities. Arrows signify the trajectory by which communities were expected to remain unique or change; half black arrows indicate uniqueness, circular black arrows indicate similarity of communities on either side of the arrow and brown arrows indicate change. Generally, background mat communities are predicted to be unique from background non-mat communities. Death soils are expected to become more like the non-mat communities and birth soils are expected to become more like the mat communities. Figures 9, 15, 13 and 26 depict actual results for root-tip EmF, soil EmF, soil fungal and soil bacterial analyses, respectively. Compare with NMS and MRPP analyses as well. Treatments include *Piloderma* Background (**Pil_Bck**), *Piloderma* Death (**Pil_Dth**), *Piloderma* Birth (**Pil_Bth**), Non-Mat Organic Disturbance Control (**NMDst_O**), Non-Mat Organic Background (**NMBck_O**), *Ramaria* Background (**Ram_Bck**), *Ramaria* Death (**Ram_Dth**), *Ramaria* Birth (**Ram_Bth**), Non-Mat Mineral Disturbance Control (**NMDst_M**) and Non-Mat Background Mineral Background (**NMBck_M**).

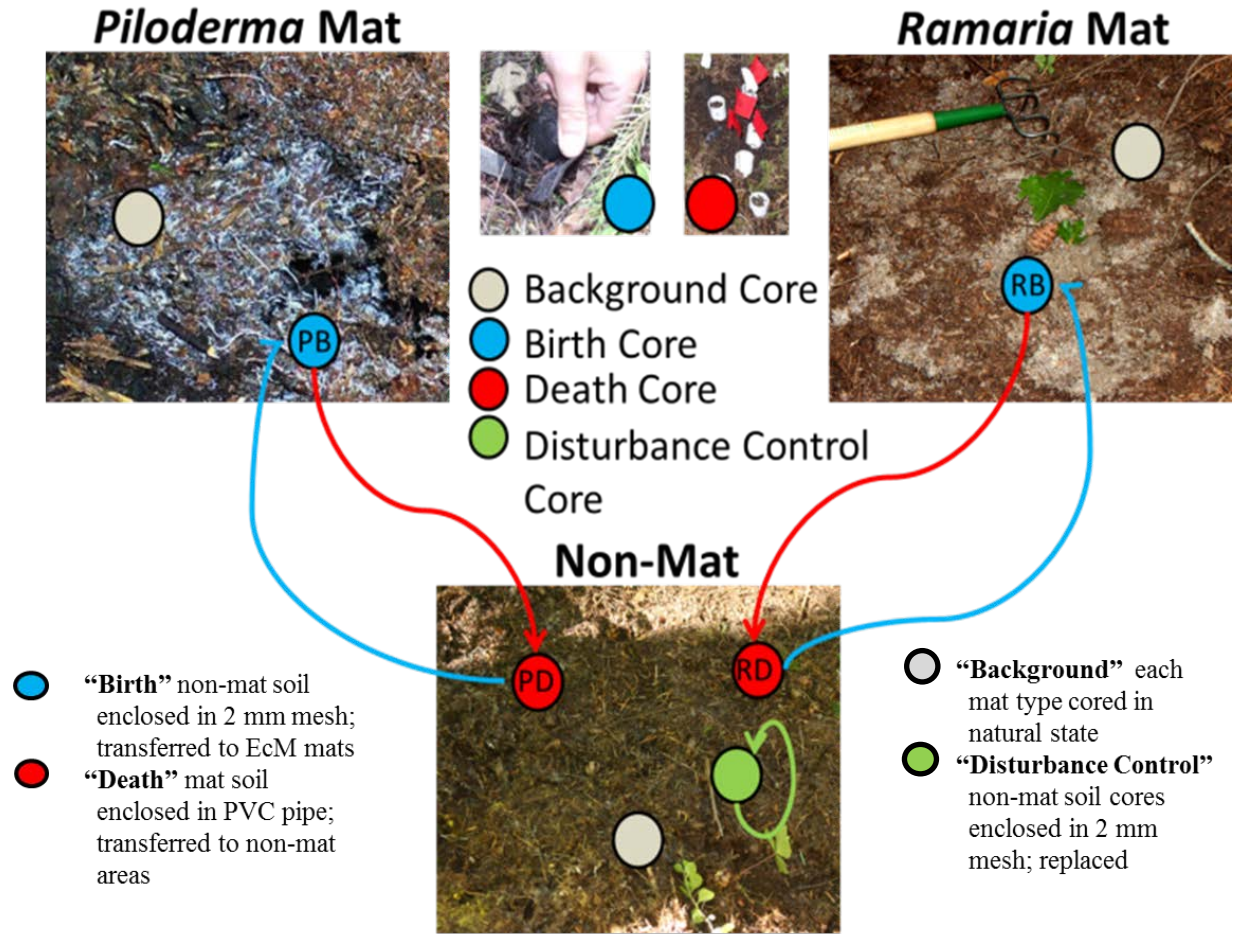


Figure 2: Schematic representation of study design with treatments and mat vs. non-mat soils identified. The mesh bags used for birth and PVC pipe used for death treatments are shown. Treatments include *Piloderma* Background (**Pil_Bck**), *Piloderma* Death (**Pil_Dth**), *Piloderma* Birth (**Pil_Bth**), Non-Mat Organic Disturbance Control (**NMDst_O**), Non-Mat Organic Background (**NMBck_O**), *Ramaria* Background (**Ram_Bck**), *Ramaria* Death (**Ram_Dth**), *Ramaria* Birth (**Ram_Bth**), Non-Mat Mineral Disturbance Control (**NMDst_M**) and Non-Mat Background Mineral Background (**NMBck_M**).

Treatment (average n=7)	Bray-P ppm (SE)	K ppm (SE)	Mg ppm (SE)	Na ppm (SE)	Ca ppm (SE)	NO_3^--N ppm (SE)	NH4-N ppm (SE)	C % (SE)	N % (BSE)
Background Piloderma Mat (Pil_Bck)	81 (14)	610 (36)	296 (25)	33 (2.6)	4184 (420)	0.5 (0.1)	44 (6)	27 (2)	0.7 (0.1)
Background Organic Non-mat (NMBck_O)	65 (6)	530 (54)	270 (28)	32 (2.5)	4170 (691)	0.8 (0.2)	38 (3)	27 (3)	0.7 (0.1)
Background Ramaria Mat (Ram_Bck)	19 (4)	338 (51)	223 (89)	30 (2.1)	1615 (547)	0.4 (0.1)	9 (1)	*7 (1)	0.2 (0.0)
Background Mineral Non-mat (NMBck_M)	16 (5)	289 (58)	198 (48)	26 (2.0)	1869 (397)	0.3 (0.0)	8 (1)	*5 (1)	0.2 (0.0)

Table 1: Baseline soil chemical analyses averaged by treatment at time of installation. Reproduced from Blanchard (2008). *Significant at $p \leq 0.05$.

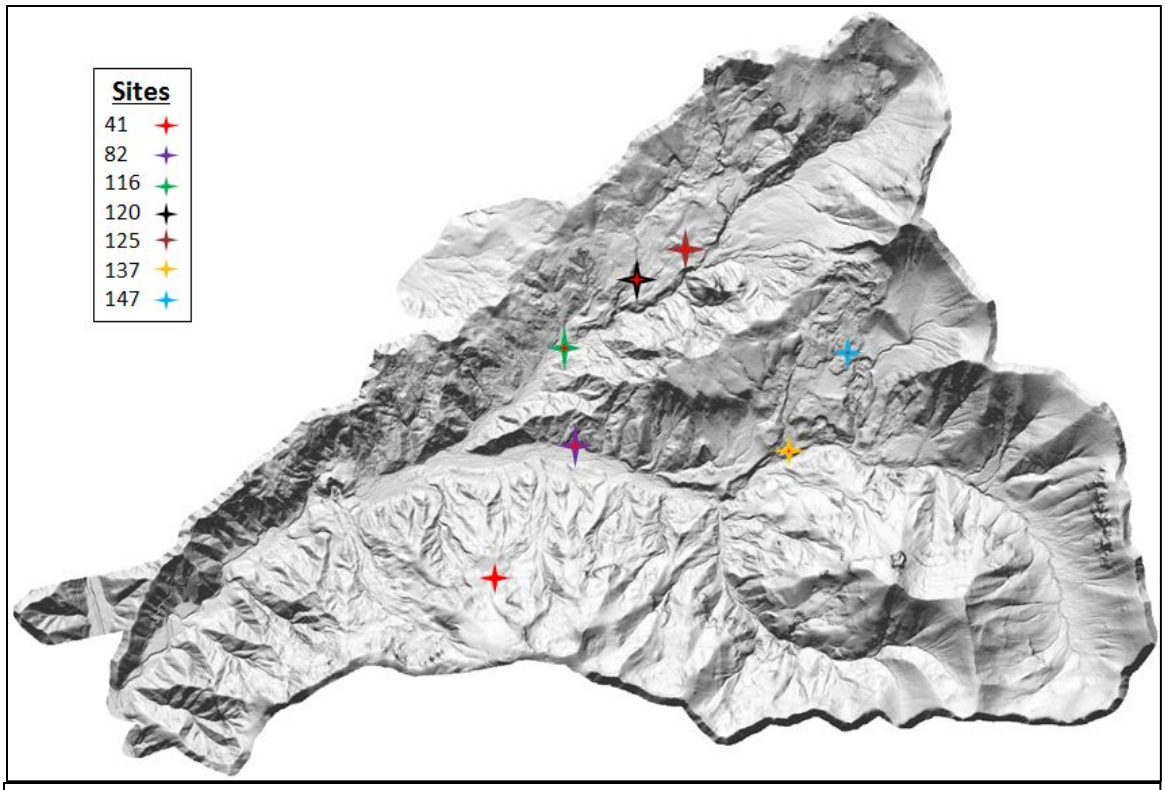


Figure 3: LIDAR relief map of the HJ Andrews LTER showing site locations.

	Current Study					July 11th 1994 Soil Survey (Background Data)								
Site #	Latitude	Longitude	Elevation (m)	Aspect	Stand Age	pH	Bulk Density (g/ml)	Soil Organic Matter (%)	Forest Floor Moss Cover	Soil Temperature °C (2 day avg; July 11-12, 1994)	Denitrification Potential (ug/g*hour C)	Net Mineralizable Nitrogen (ug/g)	Sodalime Respiration (g/m^2*day)	7 Week Accumulated CO_2 (g/m^2)
41	44 13.095	122 12.134	976	NW	Old Growth	5.2	0.44	25.66	N	10.3	3.38	130.22	2.11	349.4
82	44 14.165	122 11.433	682	SW	Old Growth	5	0.69	21.32	N	15	1.06	73.92	2.29	317.9
116	44 14.965	122 11.437	722	SE	Old Growth	4.5	0.91	23.08	N	18.61	0.92	42.04	2.03	268
120	44 15.506	122 10.734	795	S	Old Growth	4.7	0.6	22.77	N	16.94	0.64	75.19	0.97	321.8
125	44 15.848	122 10.465	908	S	Old Growth	5.3	0.57	21.61	N	15.83	1.82	79.58	1.15	288.9
137	44 14.196	122 9.675	816	S	Old Growth	5	0.44	20.67	N	17.78	1.41	17.79	0.62	119.9
147	44 15.163	122 9.215	1192	SE	Old Growth	4.9	0.4	23.48	N	15	1.08	86.72	0.44	363.6

Table 2: Descriptive traits for sampling sites at the HJ Andrews LTER. The left side contains location and age attributes of each site while the right side contains soil chemical and microbiological data assessed in July of 1994 by Robert P. Griffiths and Bruce A. Caldwell (http://andrewsforest.oregonstate.edu/data/abstract.cfm?dbcode=SP006&topnav=97); these data are almost 20 years old and are intended to provide attributes that may differentiate sites.

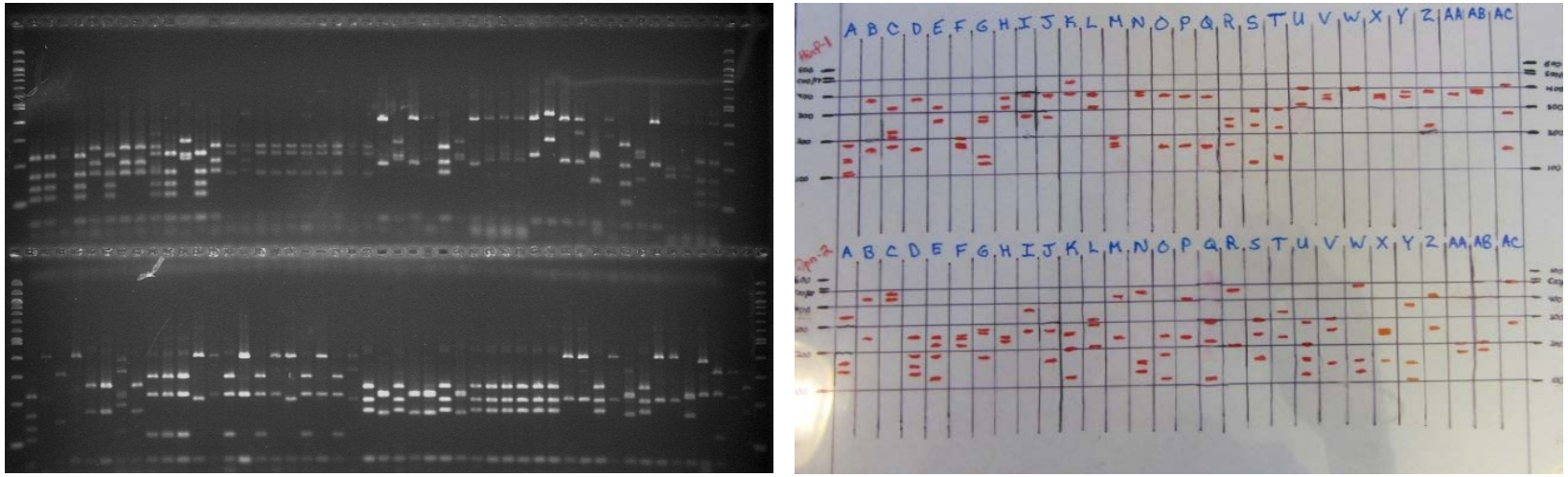


Figure 4: EmF root-tip restriction digests. (a) 96 lane 3% agarose gel using the restriction enzyme DpnII. (b) Method of visual comparison for both restriction enzymes. Visually ambiguous RFLP patterns (eg. RFLP types AA and AB) were separated, sequenced individually and combined only if sequences were 97% similar.

454 Forward Adaptor-MID-Primer Sequence (5' to 3')

CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAGTGCGTCTTGGTCATTTAGAGGAAGTAA
454 Adaptor Sequence A **MID** **ITS1F Primer**

454 Reverse Adaptor-Primer Sequence (5' to 3')

CCTATCCCCTGTGTGCCTTGGCAGTCTCAGTCCTCCGCTTATTGATATGC
454 Adaptor Sequence B **No MID** **ITS4 Primer**

Figure 5: Roche 454 adaptors and multiplex tags. Fungal ITS primers used for reference.

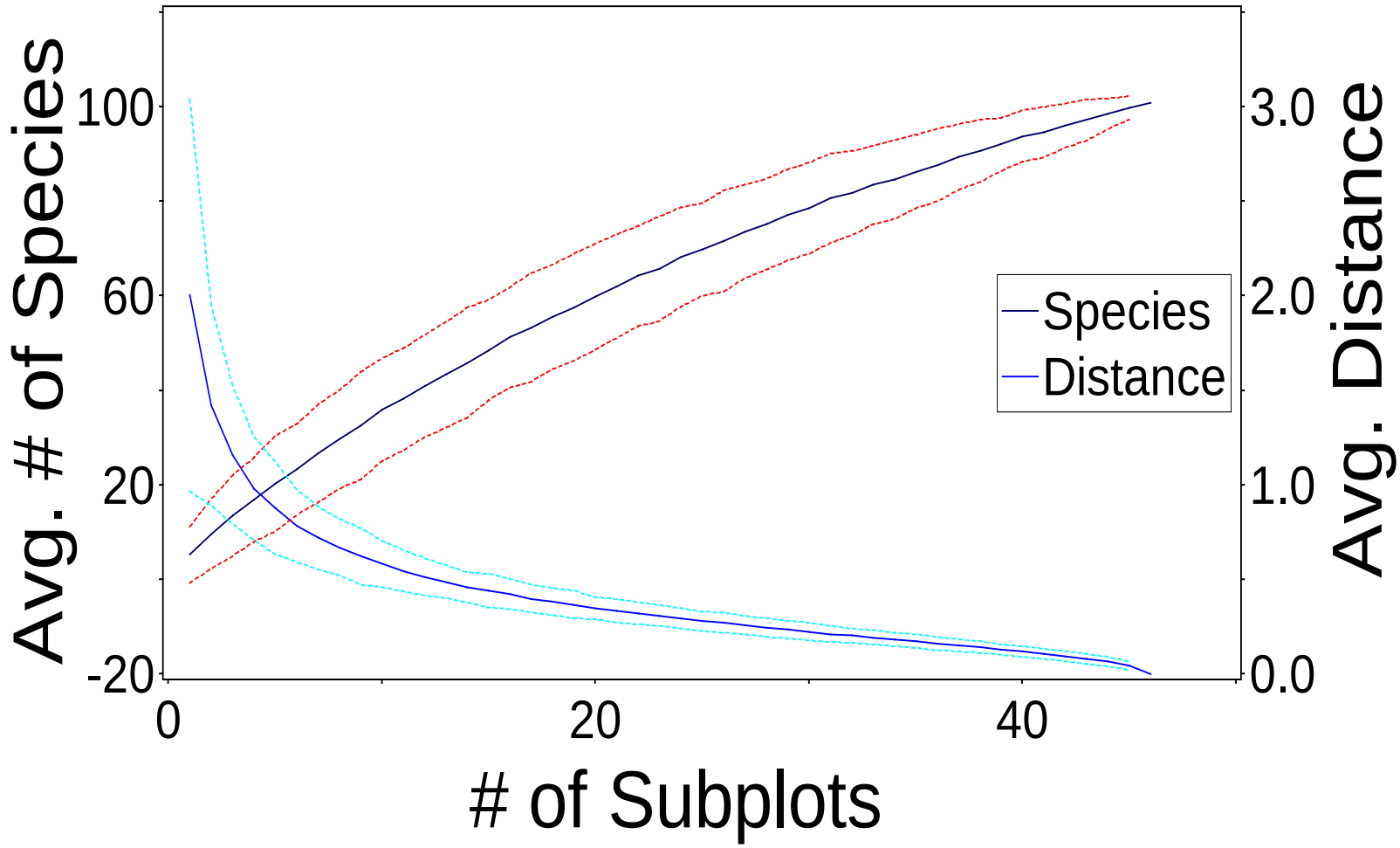


Figure 6: Species area curve of all EmF root-tip species and soil cores. Top line represents the species accumulation curve. Bottom line represents the average distance between the subsample and the whole. Dotted lines represent confidence bands +/- 2 S.D. Expected species richness was not observed (~70% captured).

Horizon	Treatment Name	Treatment Type	Sample Size (n)	Species Richness
Organic	Piloderma Background Core	▲Pil_Bck	7	27
	Piloderma Death Core	▲Pil_Dth	7	5
	Piloderma Birth Core	▲Pil_Bth	7	20
	Non-mat Disturbance Control Core	▲NMDst_O	7	16
	Non-Mat Background Core	▲NMBck_O	7	10
Mineral	Ramaria Background Core	▲Ram_Bck	7	33
	Ramaria Death Core	▲Ram_Dth	6	17
	Ramaria Birth Core	▲Ram_Bth	7	23
	Non-mat Disturbance Control Core	▲NMDst_M	7	23
	Non-Mat Background Core	▲NMBck_M	7	29

Table 3: Treatment identifiers used throughout text with full descriptors and horizons. Sample size indicates the final number used in statistical analyses. Observed species richness the result of unique root-tip sequences regardless of the number of root-tips found in each sample (e.g. Pil_Dth had very few live root-tips from any sample.)

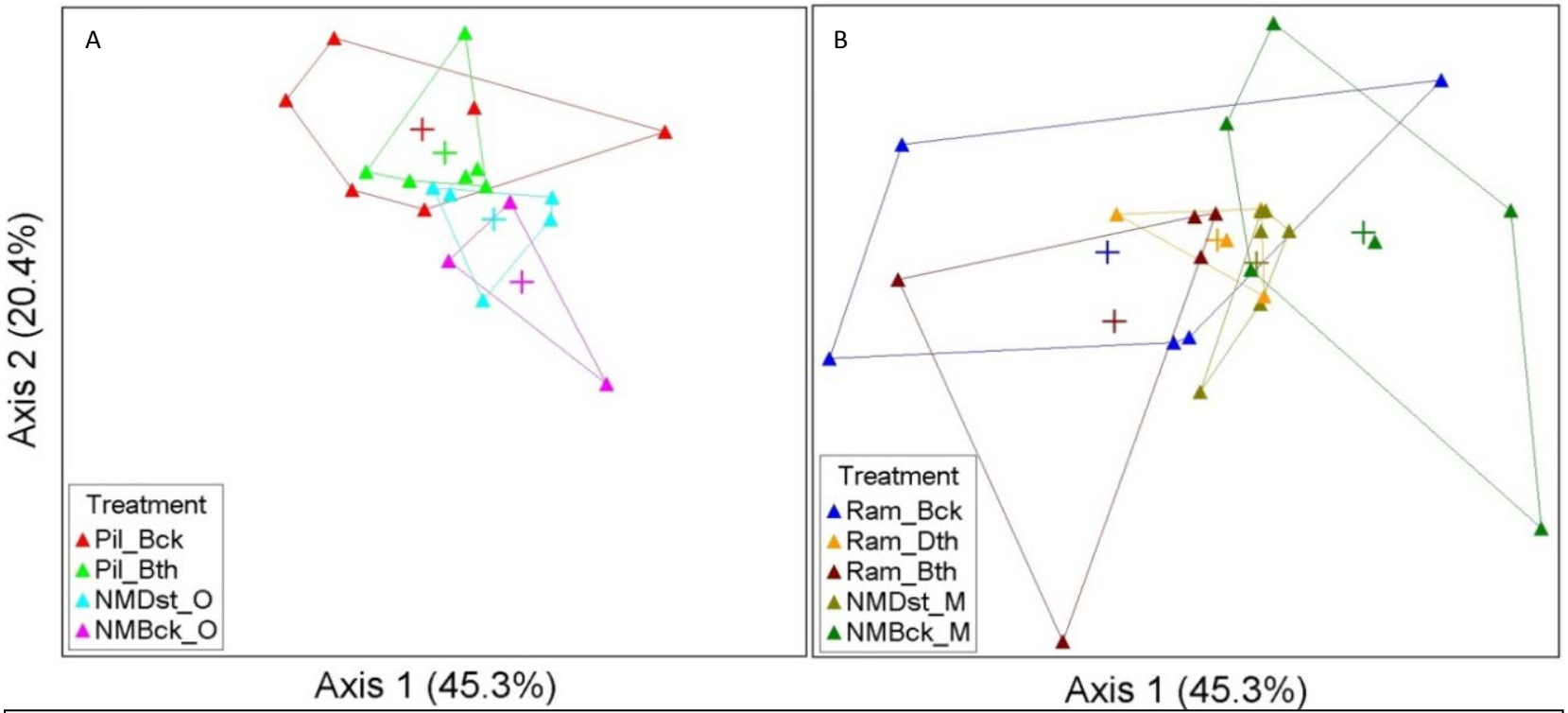


Figure 7: NMS ordination of reciprocal soil core treatments in EmF root-tip species space. Panel A represents the organic horizon (*Piloderma*) and panel B represents the mineral horizon (*Ramaria*). Both panels are in the same ordination space, separated for ease of viewing and can be visualized as if overlaid. Treatment averages (centroids) are expressed by tick marks of the same color while convex hulls indicate within treatment variability. The figure represents the first two axes of a three-dimensional solution (total 86% of variation explained); percent variance explained by each axis is represented in parentheses, while axis-3 explained 20.3% (stress = 11.1, $p = 0.02$; instability < 0.00001).

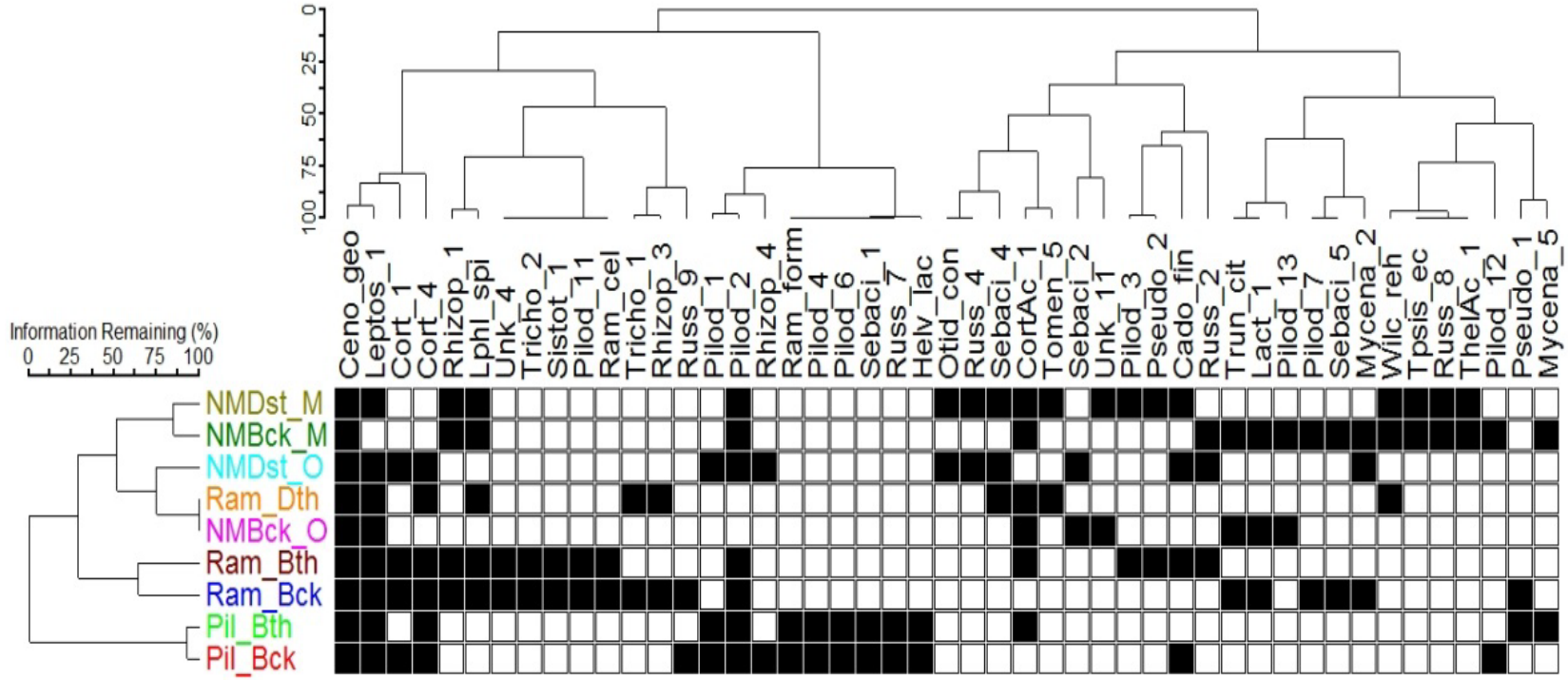


Figure 8: Two-way cluster analysis of grouped treatments and EmF species present and active on root-tips. Full species names can be found in Appendix 2. The matrix is presence-absence with dark boxes indicating presence and white boxes absence. Clusters reflect similarities in NMS ordinations.

Treatment Comparisons			(1) EmF Root Tips Org (A = 0.04 p = 0.1443) Min (A = 0.04 p = 0.0627)		(2a) Soil EmF Org (A = 0.12 p = 0.0071) Min (A = 0.02 p = 0.3081)		(2b) Soil Fungi Org (A = 0.10 p = 0.0143) Min (A = -0.05 p = 0.8376)		(3) Soil Bacteria Org (A = 0.06 p = 0.0953) Min (A = -0.01 p = 0.5488)	
			A-value	p-value	A-value	p-value	A-value	p-value	A-value	p-value
NMBck_O	vs.	Pil_Bck	**0.08***	**0.04***	**0.17****	**0.00****	**0.1****	**0.04***	-0.01	0.55
NMBck_O	vs.	Pil_Bth	**0.04**	0.23	**0.12*****	**0.04***	**0.1****	**0.05***	0.03	0.27
NMBck_O	vs.	Pil_Dth	n/a	n/a	0.02	0.31	**0.05**	**0.11**	**0.05**	**0.10**
NMBck_O	vs.	NMDst_O	-0.06	0.84	-0.01	0.53	**0.06**	**0.11**	0.02	0.30
Pil_Bck	vs.	Pil_Bth	-0.02	0.72	-0.03	0.69	-0.03	0.65	-0.04	0.76
Pil_Bck	vs.	Pil_Dth	n/a	n/a	**0.11****	**0.02***	**0.15****	**0.00****	**0.11****	**0.04***
Pil_Bck	vs.	NMDst_O	**0.07***	**0.03***	**0.22****	**0.00****	**0.13****	**0.02***	-0.04	0.73
Pil_Bth	vs.	Pil_Dth	n/a	n/a	**0.06**	**0.11**	**0.09***	**0.04***	**0.15****	**0.00****
Pil_Bth	vs.	NMDst_O	0.01	0.34	**0.10***	**0.07**	0.00	0.45	-0.03	0.68
Pil_Dth	vs.	NMDst_O	n/a	n/a	0.03	0.26	-0.02	0.60	**0.12****	**0.03***
NMBck_M	vs.	Ram_Bck	**0.09***	**0.03***	-0.01	0.59	-0.03	0.71	0.02	0.29
NMBck_M	vs.	Ram_Bth	**0.04**	**0.05***	-0.02	0.64	**0.04**	0.21	-0.02	0.55
NMBck_M	vs.	Ram_Dth	0.01	0.40	**0.05**	0.19	-0.01	0.54	-0.10	0.99
NMBck_M	vs.	NMDst_M	-0.01	0.72	-0.03	0.65	-0.08	0.92	-0.04	0.75
Ram_Bck	vs.	Ram_Bth	-0.04	0.81	**0.09***	**0.05***	0.03	0.25	**0.07***	**0.05***
Ram_Bck	vs.	Ram_Dth	0.01	0.37	**0.12****	**0.06**	0.03	0.25	-0.02	0.69
Ram_Bck	vs.	NMDst_M	**0.08***	**0.01***	-0.02	0.53	-0.05	0.78	**0.04**	**0.15**
Ram_Bth	vs.	Ram_Dth	0.02	0.25	0.02	0.32	-0.06	0.91	-0.04	0.77
Ram_Bth	vs.	NMDst_M	0.02	0.24	-0.01	0.44	-0.06	0.84	-0.02	0.58
Ram_Dth	vs.	NMDst_M	-0.04	0.88	-0.04	0.65	-0.08	0.95	-0.04	0.81

Table 4: Results of MRPP analyses for all experiments: (1) Root-tip EmF community; (2a) Soil fungal EmF community; (2b) Soil fungal community; and (3) 454-Pyrosequencing of soil bacteria. Pil_Dth cores were not analyzed in (1) because of sample design (see methods) and are labeled "n/a." Overall A and p-values are above each respective pane while values for individual comparisons are in the table. Comparisons are considered significant if both occur: (1) A-value ≥ 0.05; and (2) p-value ≤ 0.05. Significant treatments are in bold with the following markings: ($0.05 \leq A < 0.15$*); ($A \geq .15$**); ($0.05 \geq p > 0.01$*); ($p < .01$**); treatments in bold without asterisks are considered marginally different. Not all comparisons are of interest but were reported for reference and discussion of the overall soil community picture in response to treatments.

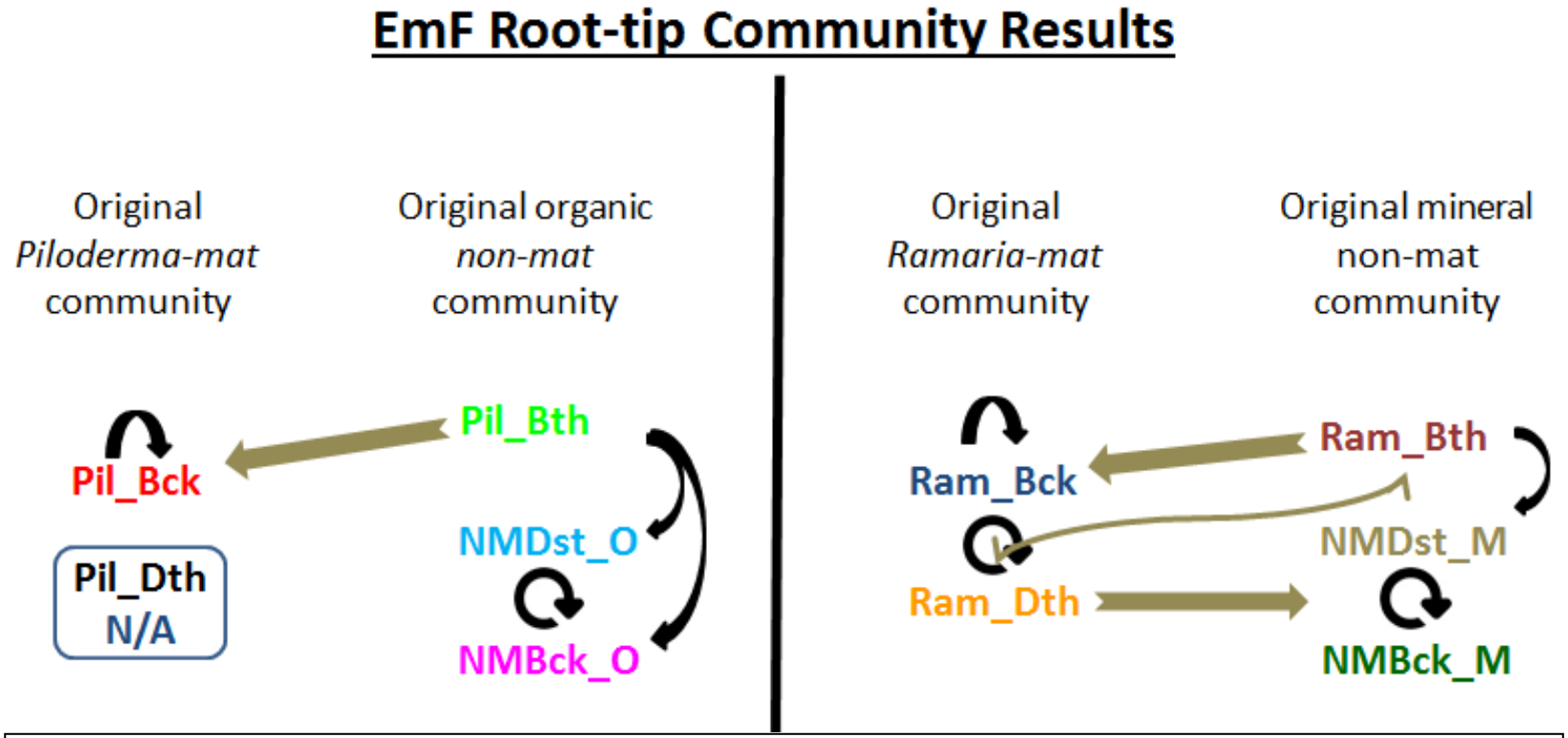


Figure 9: Schematic diagram of EmF root-tip community results for the reciprocal soil transplant experiment. Each column represents soils that were originally hypothesized to possess similar communities. Arrows signify the trajectory by which communities remained unique or changed; black arrows indicate uniqueness, brown arrows indicate change. Blue box around Pil_Dth treatment signifies omission from analysis due to lack of root ingrowth. One-way black arrows indicate birth treatments that retained some similarities to original non-mat community composition. Compare to Figure 1, MRPP and NMS results. Refer to results text for written explanation.

	Treatment	Richness	Evenness	Shannon's Diversity	Simpson's Diversity	% Empty Cells	Skewness	Kurtosis
Organic	Pil_Bck	82.80	0.724	3.161	0.9154	83.466	10.840	138.302
	Pil_Dth	95.70	0.737	3.346	0.9243	80.905	12.257	182.669
	Pil_Bth	70.70	0.730	3.100	0.9244	85.895	9.381	102.952
	NMDst_O	82.70	0.722	3.151	0.9139	83.500	11.265	151.302
	NMBck_O	96.00	0.759	3.455	0.935	80.838	9.607	120.383
Mineral	Ram_Bck	84.70	0.718	3.177	0.9168	81.831	11.436	156.821
	Ram_Dth	83.20	0.714	3.144	0.9185	82.146	10.535	135.539
	Ram_Bth	73.00	0.705	3.000	0.9083	84.355	11.020	142.249
	NMDst_M	70.70	0.703	2.983	0.9008	84.835	11.842	166.744
	NMBck_M	85.50	0.730	3.236	0.9249	81.688	10.163	123.044

Table 5: Soil fungal community (genera and above) statistics grouped by horizon. The phylogenetic groupings here were the same as those used in NMS and MRPP comparisons of fungal 454 data. Values are averages (n=6) from each treatment except Ram_Dth (n=5). The organic horizon included 501 genera and the mineral horizon included 466 Genera (total for both horizons = 653).

		Treatment	Richness	Evenness	Shannon's Diversity	Simpson's Diversity	% Empty Cells	Skewness	Kurtosis
Soil Fungi	Organic	Pil_Bck	164.5	0.778	3.92	0.953	92.6	20.1	507.82
		Pil_Dth	191.7	0.787	4.11	0.959	91.4	23.5	709.20
		Pil_Bth	143.3	0.777	3.85	0.958	93.6	17.3	376.23
		NMDst_O	163.7	0.774	3.89	0.951	92.7	22.1	636.23
		NMBck_O	178.2	0.790	4.08	0.961	92.0	17.8	407.92
	Mineral	Ram_Bck	162.0	0.753	3.83	0.946	92.8	23.5	673.92
		Ram_Dth	174.6	0.750	3.86	0.948	92.2	22.3	622.61
		Ram_Bth	141.3	0.748	3.69	0.945	93.7	21.5	559.06
		NMDst_M	139.2	0.736	3.62	0.931	93.8	26.5	851.89
		NMBck_M	169.2	0.763	3.90	0.952	92.4	21.9	597.78
Soil Bacteria	Organic	Pil_Bck	365.0	0.935	5.47	0.991	89.1	16.3	413.75
		Pil_Dth	334.2	0.952	5.50	0.993	90.0	15.1	389.23
		Pil_Bth	287.3	0.934	5.25	0.989	91.4	20.4	645.59
		NMDst_O	432.2	0.934	5.63	0.992	87.1	17.8	487.87
		NMBck_O	356.3	0.940	5.51	0.992	89.3	16.2	425.30
	Mineral	Ram_Bck	244.0	0.944	5.15	0.990	93.0	15.6	389.79
		Ram_Dth	490.0	0.929	5.74	0.992	85.9	18.6	515.05
		Ram_Bth	417.0	0.941	5.63	0.993	88.0	15.9	411.37
		NMDst_M	393.3	0.946	5.58	0.993	88.7	15.2	370.26
		NMBck_M	468.3	0.938	5.74	0.994	86.5	14.3	311.82

Table 6: Soil fungal (green; 2,235) and soil bacterial (red; 4,324) 97% OTU community diversity statistics separated by horizon, bacterial singletons removed. Values are averages (n=6) from each treatment except Ram_Dth (n=5). The organic horizon included 1,249 fungal OTUs and 3,340 bacterial OTUs. The mineral horizon included 1,439 fungal OTUs and 3,467 bacterial OTUs.

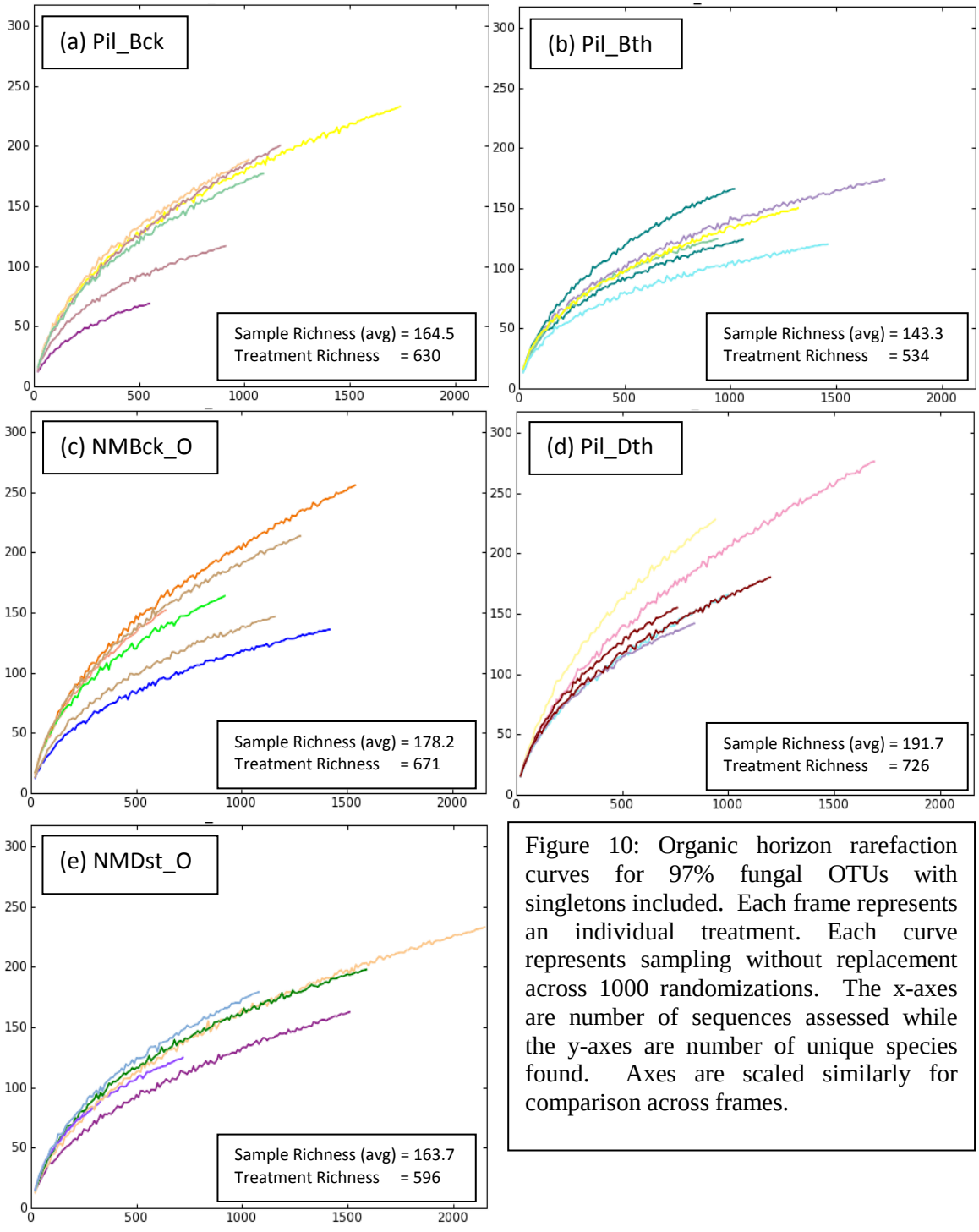


Figure 10: Organic horizon rarefaction curves for 97% fungal OTUs with singletons included. Each frame represents an individual treatment. Each curve represents sampling without replacement across 1000 randomizations. The x-axes are number of sequences assessed while the y-axes are number of unique species found. Axes are scaled similarly for comparison across frames.

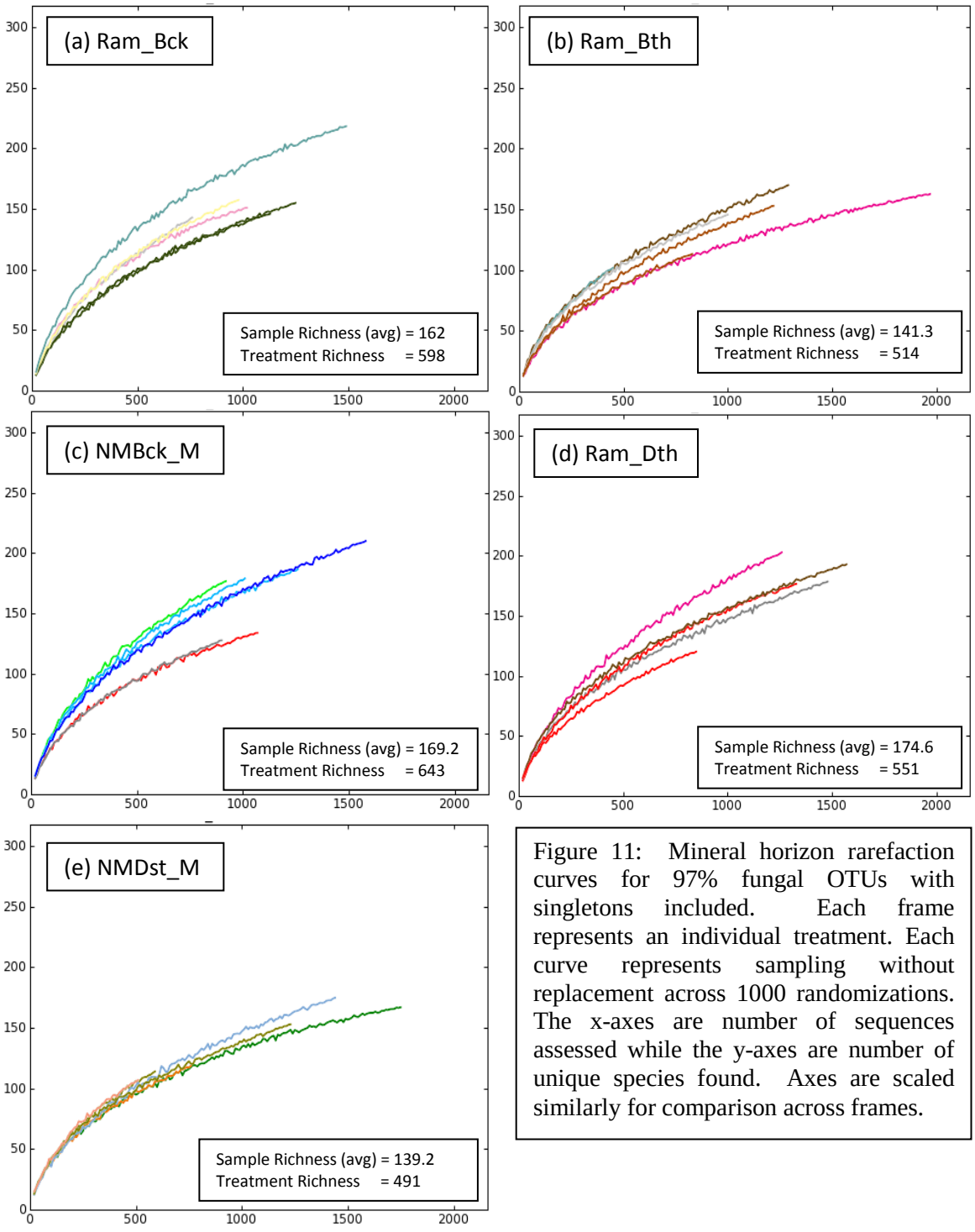


Figure 11: Mineral horizon rarefaction curves for 97% fungal OTUs with singletons included. Each frame represents an individual treatment. Each curve represents sampling without replacement across 1000 randomizations. The x-axes are number of sequences assessed while the y-axes are number of unique species found. Axes are scaled similarly for comparison across frames.

Organic Horizon Treatments					
Treatments	Pil_Bck	Pil_Dth	Pil_Bth	NMDst_O	NMBck_O
Pil_Bck	502				
	1303				
Pil_Dth	315	523			
	607	1305			
Pil_Bth	265	239	437		
	552	518	1123		
NMDst_O	258	275	257	463	
	711	686	641	1656	
NMBck_O	309	314	256	290	541
	687	637	589	781	1535

Mineral Horizon Treatments					
Treatments	Ram_Bck	Ram_Dth	Ram_Bth	NMDst_M	NMBck_M
Ram_Bck	490				
	1047				
Ram_Dth	237	397			
	561	1584			
Ram_Bth	251	223	396		
	540	807	1552		
NMDst_M	229	224	214	389	
	544	784	771	1483	
NMBck_M	287	245	230	252	509
	609	833	847	834	1732

Table 7: Shared fungal (brown) and bacterial (green) 97% OTUs between treatments in each soil horizon.

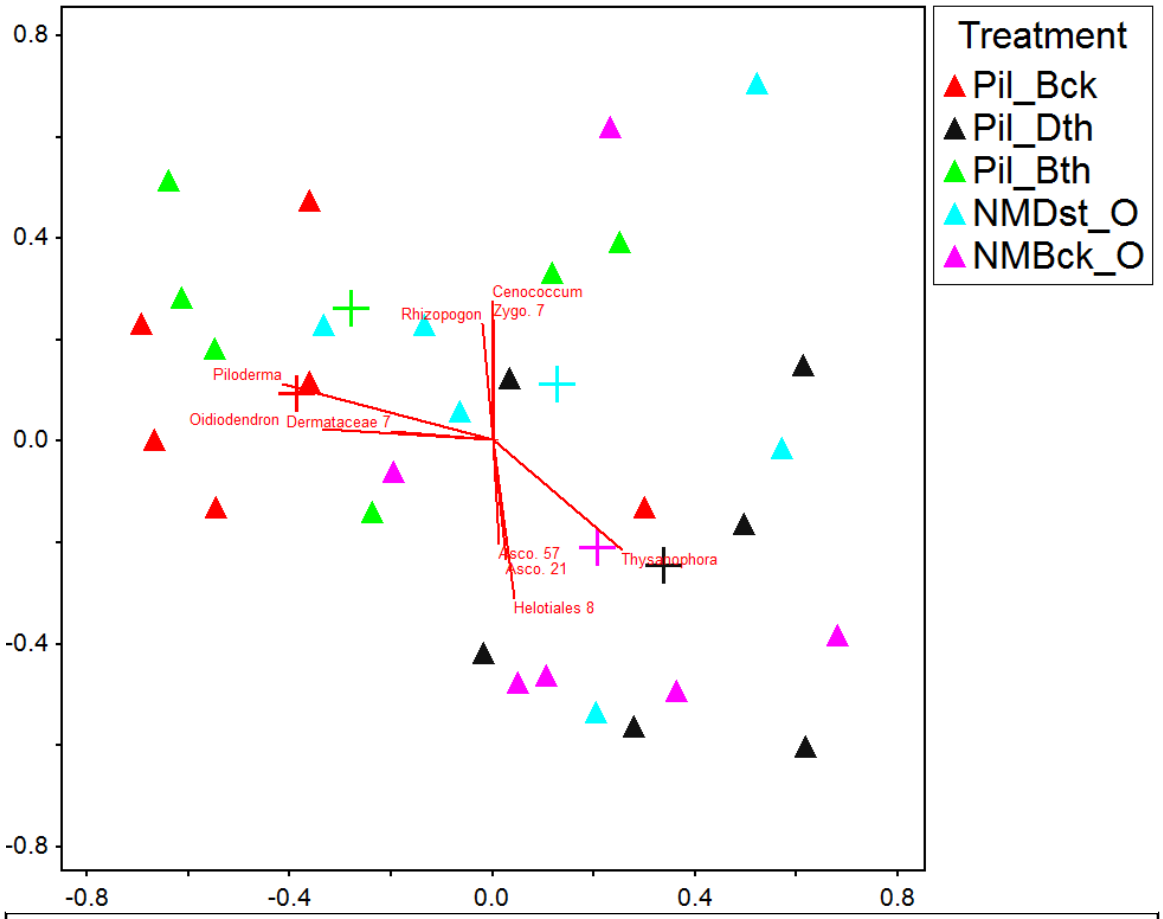


Figure 12: NMS ordination of organic horizon soil cores (triangles) in soil fungal genus-space (501 genera). The figure represents the first two axes of a three-dimensional solution (total 75.6% of variation explained); axis-1 (x-axis) explained 37.3% and axis-2 (y-axis) explained 22.7% (stress = 14.50, p = 0.004; instability < 0.00001). Treatment centroids are expressed by tick marks of the same color. Spatial (site) variation was corrected for by translating site centroids to the origin; therefore axis scores are not reported on the graph. Joint plot vectors are scaled to 100% and displayed for $r^2 > 0.25$.

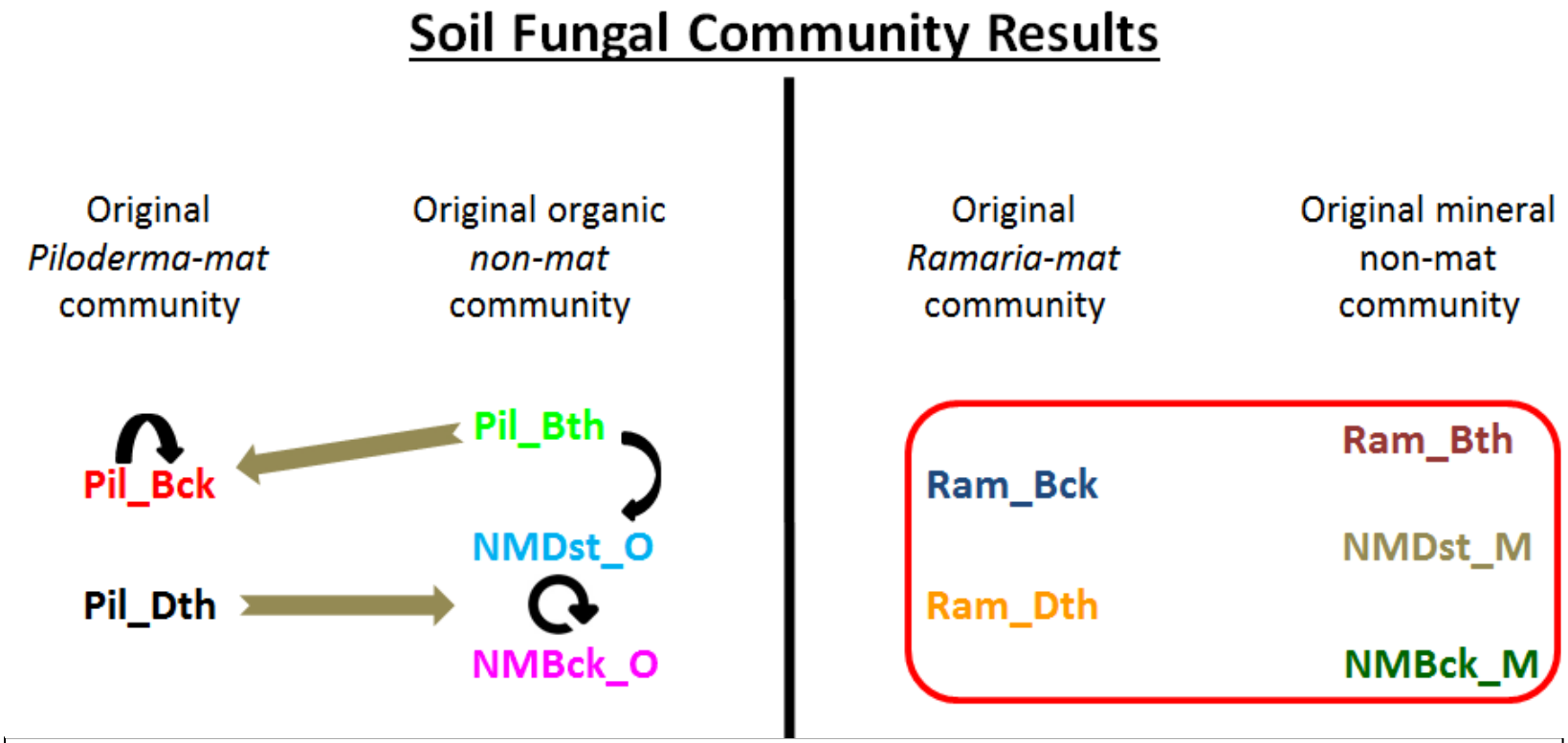


Figure 13: Schematic diagram of soil fungal community results for the reciprocal soil transplant experiment. Each column represents soils that were originally hypothesized to possess similar communities. Arrows signify the trajectory by which communities remained unique or changed; black arrows indicate uniqueness, brown arrows indicate change. Red box around *Ramaria* indicates overall similarities between all treatments. Compare to Figure 1, MRPP and NMS results. Refer to results text for written explanation.

Blocked Indicator Species Analysis for Soil Fungi in the Organic Horizon - Vertical Order Based on Significance Test										
					Treatment	Pil_Bck	Pil_Dth	Pil_Bth	NMDst_O	NMBck_O
					n	6	6	6	6	6
OTU #	Taxon Name	Avg IV	p *	Maximum IV	MaxGrp					
476	Mortierella	20	0.0016	44	Pil_Dth	8	44	12	22	14
529	Piloderma	20	0.0022	49	Pil_Bck	49	6	33	5	5
412	Lecythophora	20	0.0062	41	NMDst_O	7	20	16	41	15
582	Sphaerobolus	12	0.0074	56	NMBck_O	2	0	0	1	56
20	Agaricomycetes sp. 15	12	0.0136	48	Pil_Bck	48	12	0	0	0
544	Pseudotomentella	14	0.0186	50	NMBck_O	0	5	9	7	50
490	Neofabraea	18	0.022	43	Pil_Bck	43	11	19	8	11
638	Wallemia	20	0.0266	33	NMDst_O	9	24	15	33	19
516	Pezizomycetes sp. 3	13	0.0386	36	NMDst_O	9	7	7	36	7
413	Leohumicola	15	0.0396	46	NMBck_O	10	1	10	10	46
61	Ascomycota sp. 28	10	0.0402	33	Pil_Dth	14	33	0	0	1
111	Auricularia	9	0.0408	43	NMBck_O	1	0	0	1	43
507	Pezizomycetes sp. 12	9	0.042	42	NMBck_O	0	3	0	0	42
541	Pseudeurotium	10	0.0486	40	NMDst_O	1	0	3	40	7
471	Mitosporic Helotiales sp. 9	11	0.0546	39	Pil_Dth	7	39	3	0	4
181	Ceratobasidiaceae sp. 2	11	0.0582	42	Pil_Dth	4	42	0	1	9
524	Phialocephala	18	0.0728	38	Pil_Dth	14	38	12	13	14
281	Helotiaceae sp. 13	9	0.0778	33	Pil_Dth	6	33	6	0	0
165	Capnodiales sp. 1	9	0.078	41	Pil_Bck	41	0	0	0	3
434	Leptodontidium	20	0.0804	35	Pil_Bck	35	18	20	17	10
538	Polyporales sp. 2	11	0.0806	30	Pil_Dth	0	30	5	16	1
579	Sordariomycetes sp. 1	15	0.0862	39	Pil_Dth	6	39	3	21	5
225	Dermateaceae sp. 2	9	0.0926	40	Pil_Bck	40	3	0	2	2
539	Porotheleum	13	0.105	38	Pil_Dth	14	38	3	1	10
24	Agaricomycetes sp. 3	7	0.112	30	NMDst_O	4	1	1	30	0
158	Cadophora	18	0.117	39	NMBck_O	10	14	9	17	39
36	Amorphotheca	12	0.124	36	Pil_Dth	8	36	13	0	4
366	Hyaloscyphaceae sp. 3	17	0.128	41	Pil_Dth	19	41	18	5	3
593	Thysanophora	13	0.1332	33	NMBck_O	0	30	0	0	33
152	Biscogniauxia	8	0.1362	38	NMBck_O	4	0	0	0	38
228	Dermateaceae sp. 5	11	0.1372	33	NMBck_O	0	15	3	4	33
54	Ascomycota sp. 21	8	0.138	38	NMBck_O	0	0	0	4	38
361	Hyalodendriella	8	0.1406	30	NMBck_O	3	8	0	1	30
179	Cenococcum	17	0.1488	38	Pil_Bck	38	1	20	7	18

Table 8: Blocked indicator species analysis for soil fungi in the organic horizon based on putative taxonomic affinities. All treatments were analyzed together. "IV" stands for importance value based on relative abundance and relative frequency. A Monte Carlo randomization test based on 5,000 permutations was performed to identify statistically important genera; reported as "p *"; a high IV with a corresponding p-value < 0.05 is considered strong indication, $0.05 < p < 0.1$ is good indication and $0.1 < p < 0.l5$ is a reasonable indicator. High indicator values are colored green in each treatments respective column; the treatment with the highest IV is denoted in column "MaxGrp."

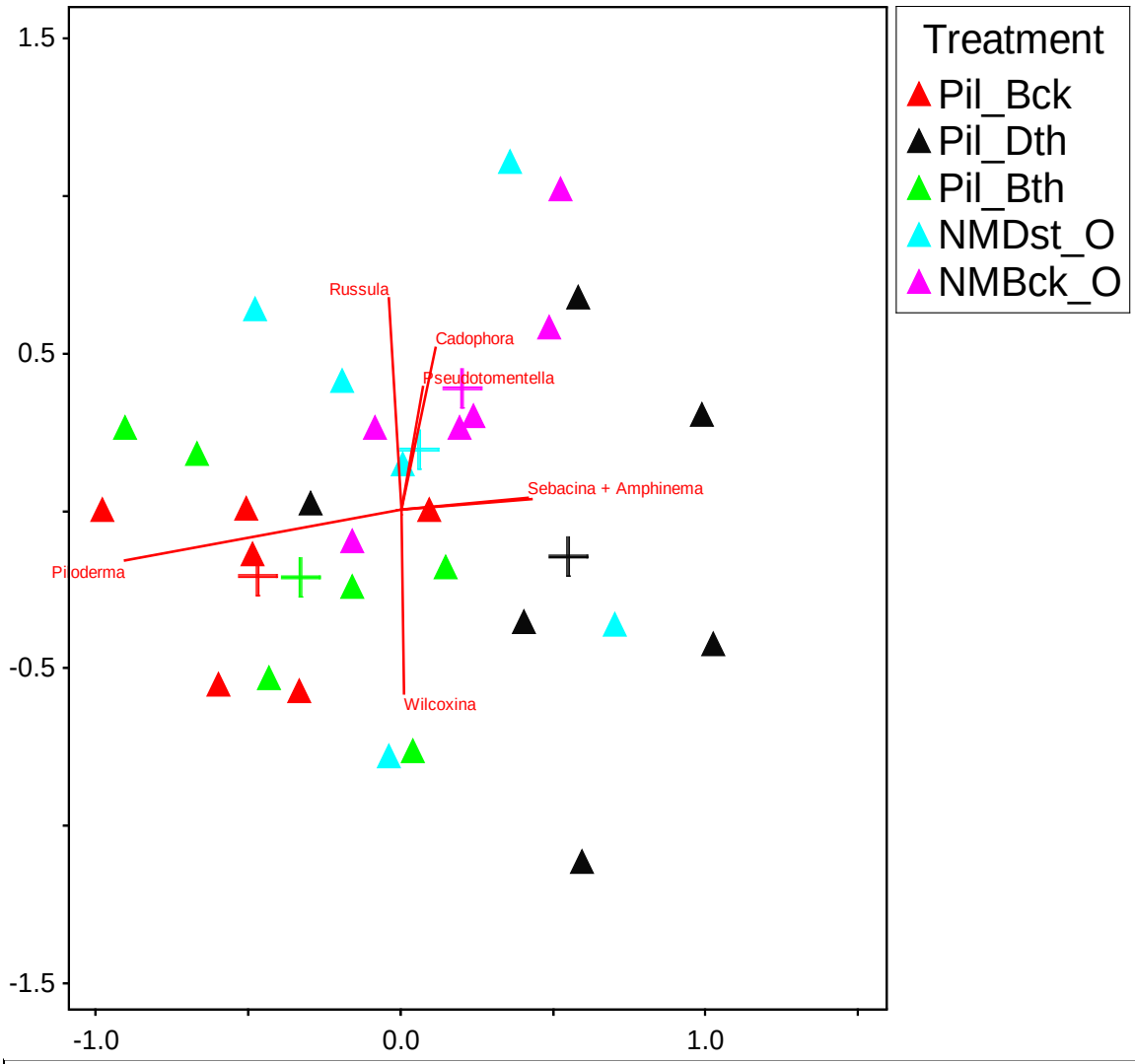


Figure 14: NMS ordination of organic horizon soil cores (triangles) in soil EmF genus-space (49 genera). Those genera whose ecologies were determined to be ectomycorrhizal were isolated and ordinated here. The figure represents the first two axes of a three-dimensional solution (total 79.3% of variation explained); axis-1 (x-axis) explained 33.1% and axis-2 (y-axis) explained 22.3% (stress = 14.34, p = 0.003; instability < 0.00001). Treatment centroids are expressed by tick marks of the same color. Spatial (site) variation was corrected for by translating site centroids to the origin; therefore axis scores are not reported on the graph. Joint plot vectors are scaled to 100% and displayed for $r^2 > 0.25$. Because the study design limited root growth in Pil_Dth cores, the higher scatter for this treatment is expected.

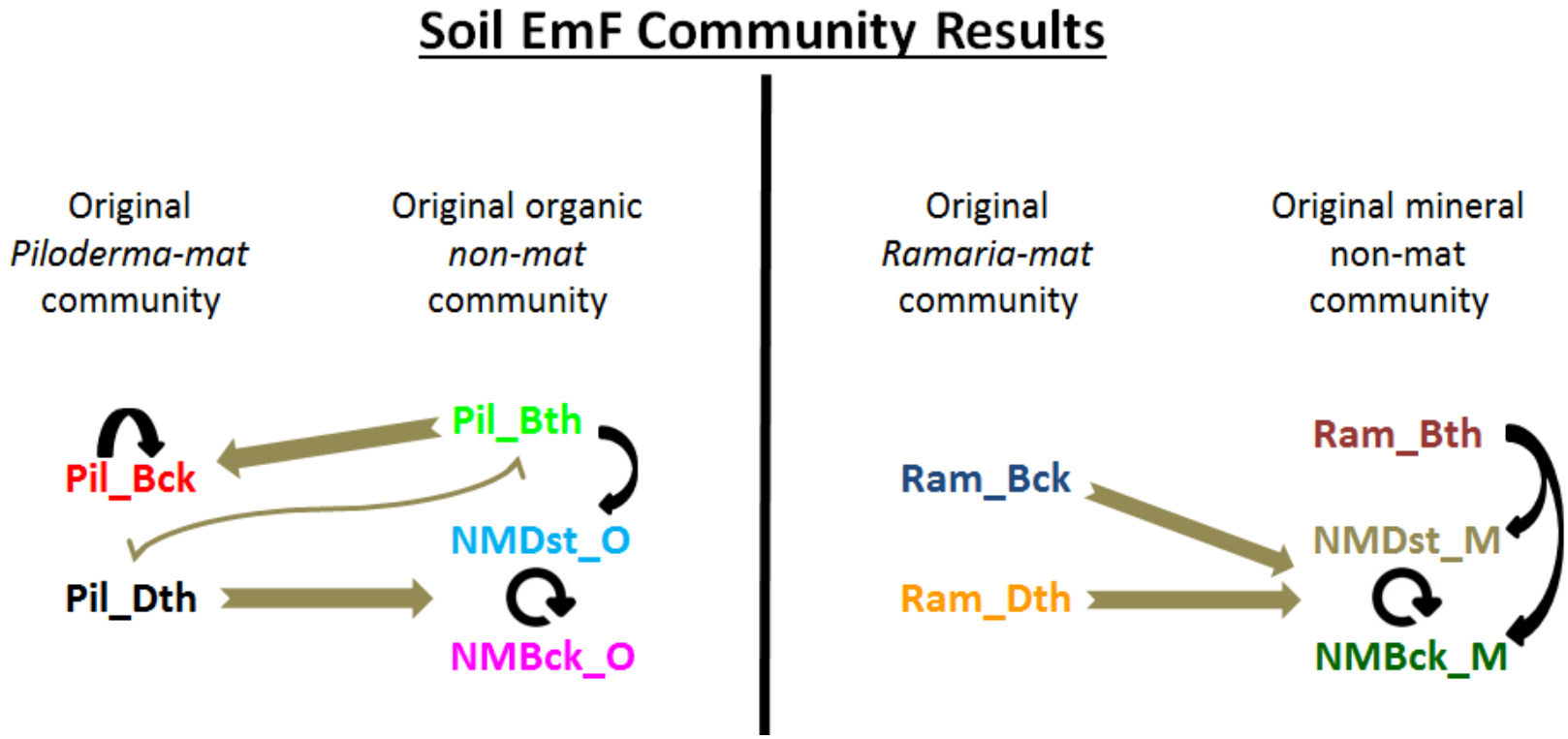


Figure 15: Schematic diagram of soil EmF community results for the reciprocal soil transplant experiment. Each column represents soils that were originally hypothesized to possess similar communities. Arrows signify the trajectory by which communities remained unique or changed; black arrows indicate uniqueness, brown arrows indicate change. Compare to Figure 1, MRPP and NMS results. Refer to results text for written explanation.

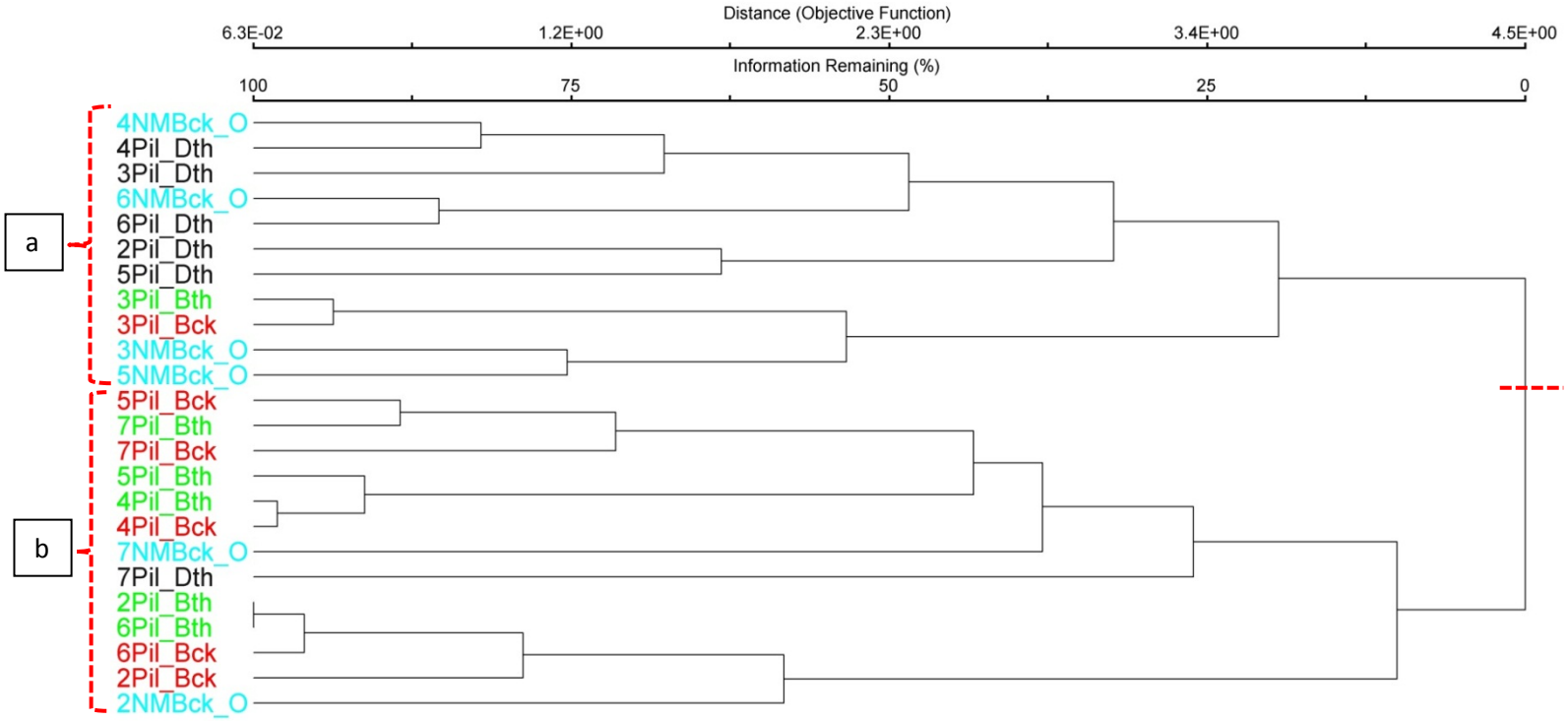


Figure 16: Hierarchical one-way cluster dendrogram of treatments based on the relative abundance of soil fungal genera. A Sørensen distance measure and a flexible beta (-0.25) group linkage method were used. Total percent chaining was 5.35. Treatments with shorter branches are more similar to one another; for example 4Pil_Bth and 4Pil_Bck are quite similar while being quite different from 7NMBck_O. Numbers preceeding treatment names indicate the site of origin: Site 82 = 2; Site 116 = 3; Site 120 = 4; Site 125 = 5; Site 136 = 6; Site 147 = 7. Dotted red lines indicate major treatment groupings in the dendrogram into non-mat (a) and *Piloderma* mat (b) communities.

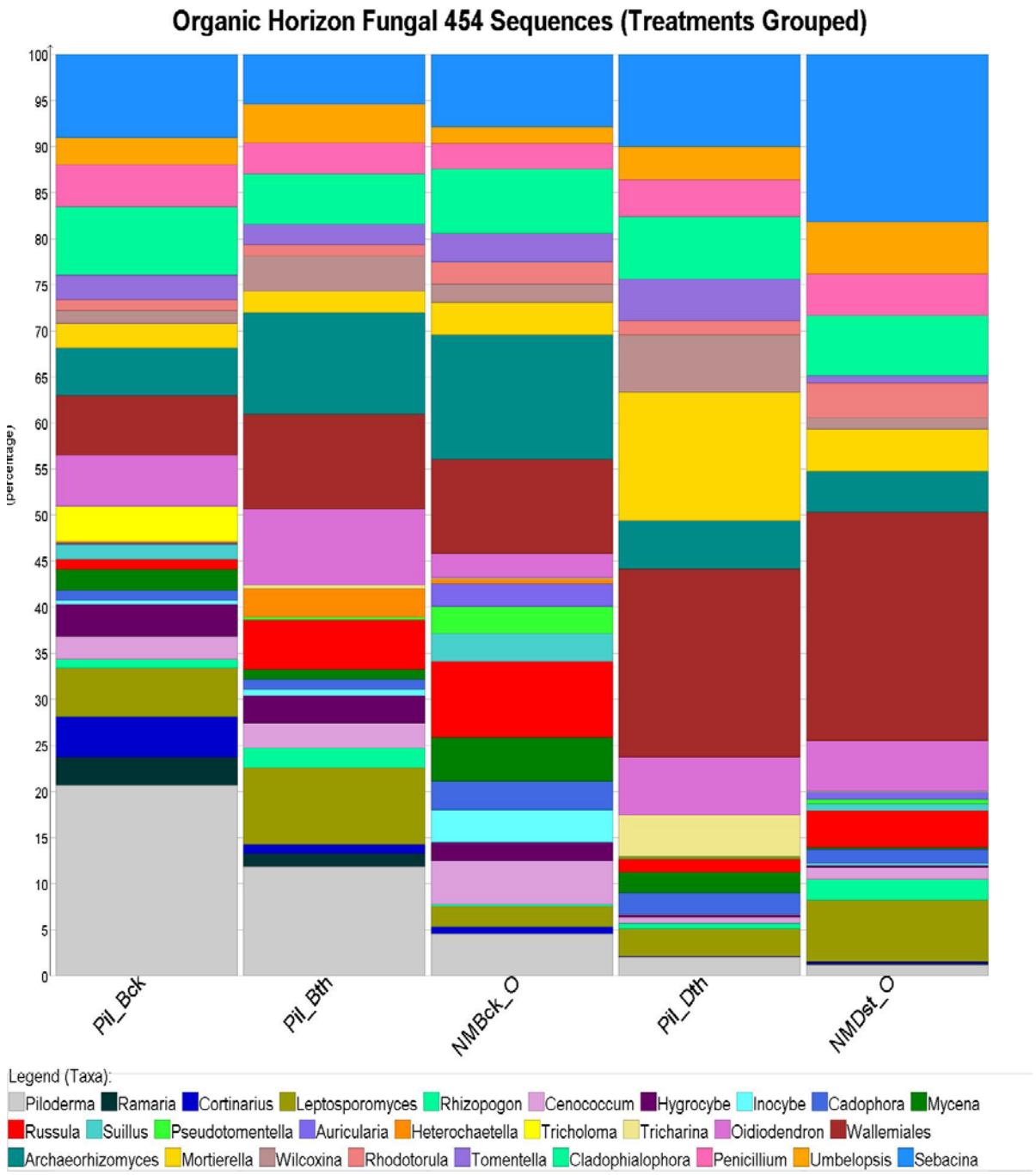


Figure 17: Organic horizon soil fungal genera grouped by treatment. Each was relativized to the treatments total to account for uneven sampling depth. The 28 taxa above represent at least 80% of all encountered taxa in each treatment. The remaining genera occupy less than 20% of fungal abundance in each treatment.

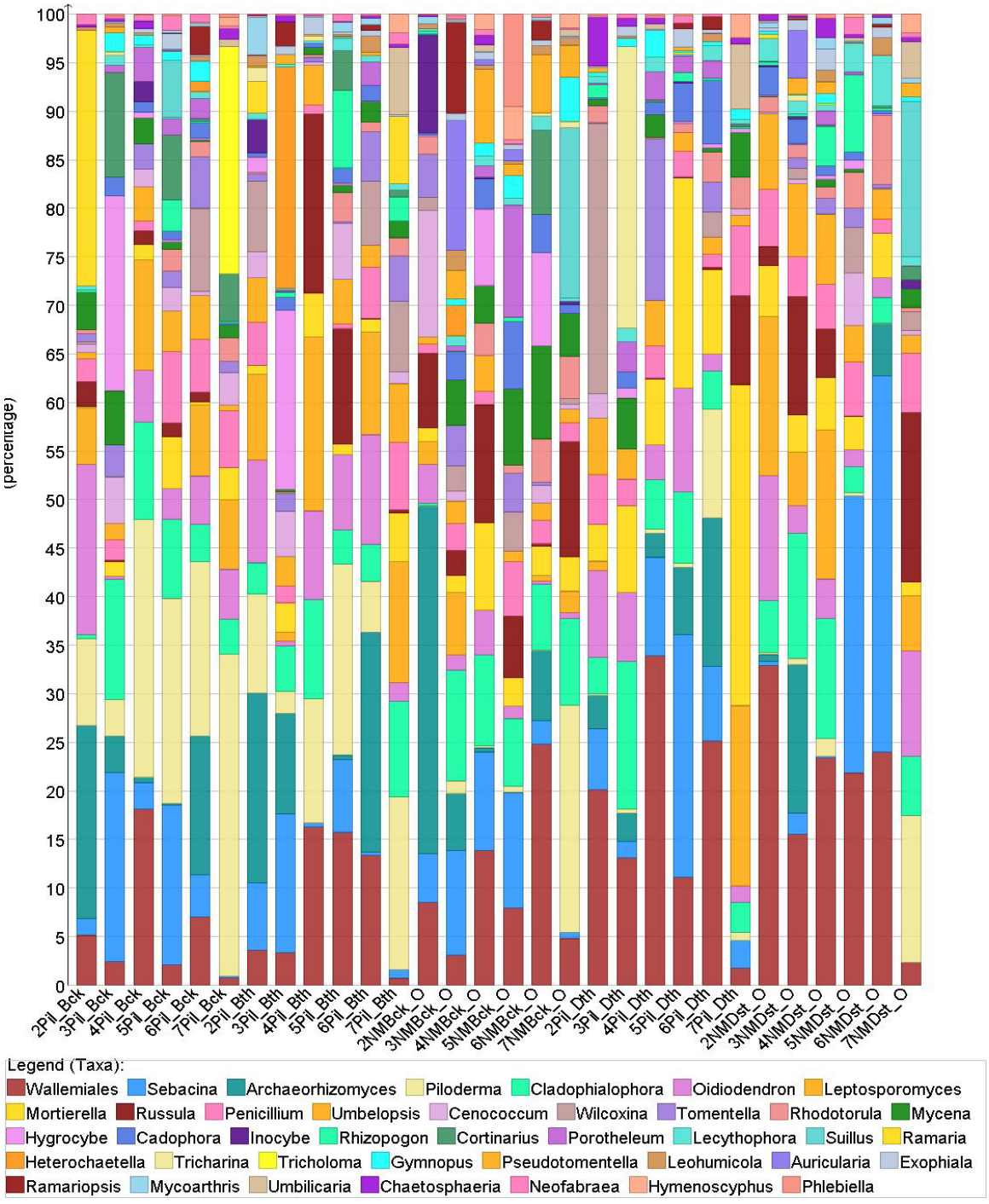


Figure 18: Organic horizon soil fungal genera separated by individual sample. Each sample was relativized to its total to account for unequal sampling depth. Genera represented here are the 40 most abundant in the organic horizon and account for over 80% of the sequence abundance for each respective sample.

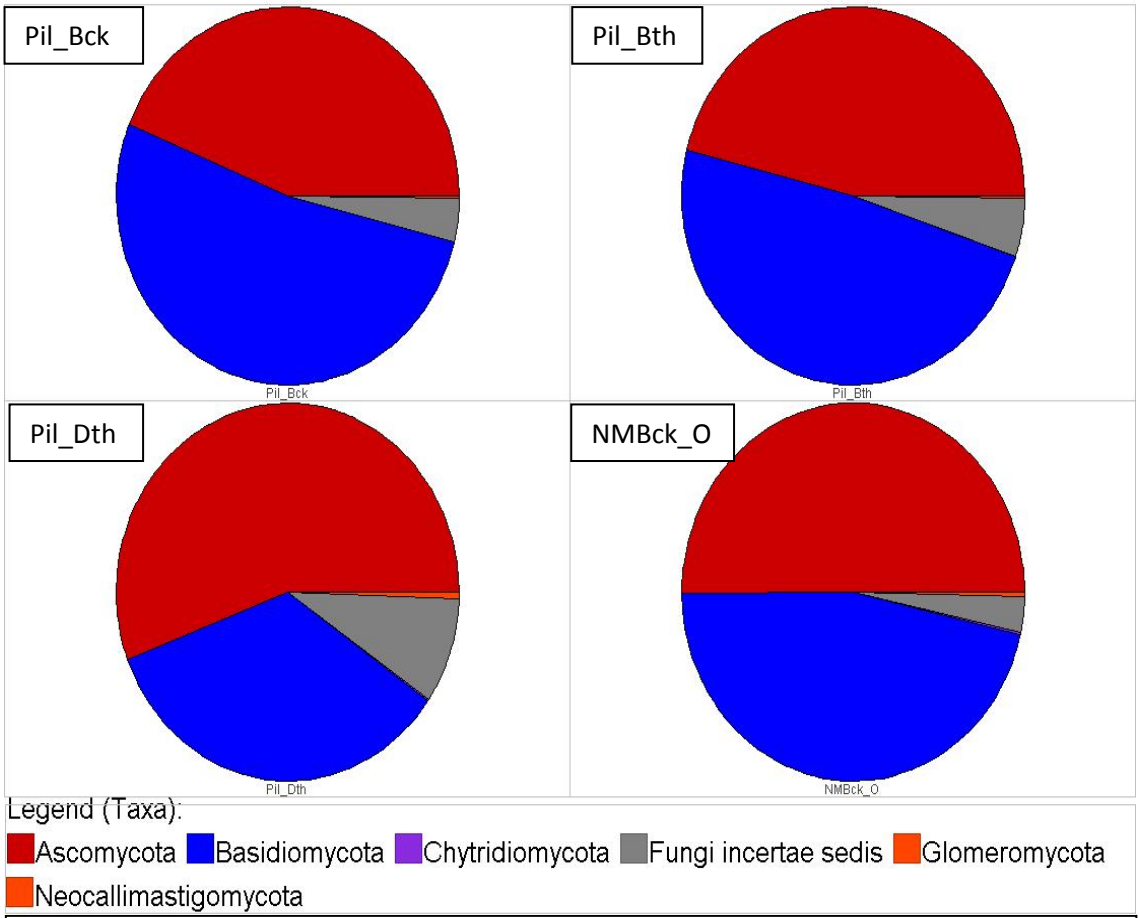


Figure 19: Soil fungal phyla in the organic horizon grouped by treatment. Treatment NMDst_O is omitted.

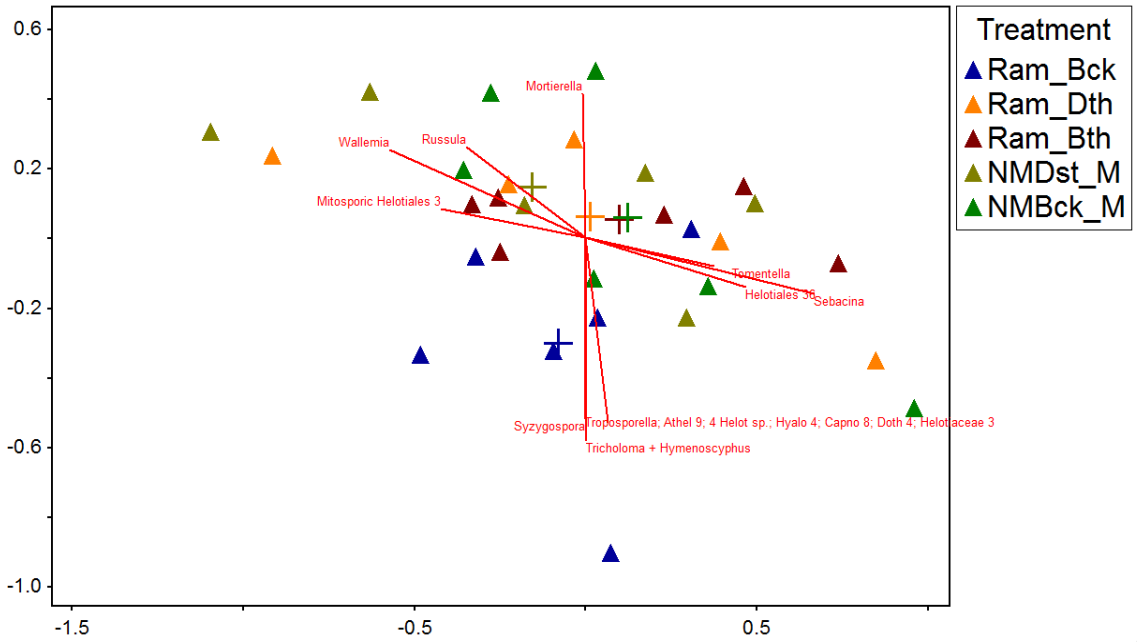


Figure 20: NMS ordination of mineral horizon soil cores (triangles) in fungal genus-space (466 genera). The figure represents the first two axes of a three-dimensional solution (total 79.7 % of variation explained); axis-1 (x-axis) explained 38.4% and axis-2 (y-axis) explained 26.1% (stress = 14.21, p = 0.004; instability < 0.00001). Treatment centroids are expressed by tick marks of the same color. Spatial (site) variation was corrected for by translating site centroids to the origin; therefore axis scores are not reported on the graph. Joint plot vectors are scaled to 100% and displayed for $r^2 > 0.3$.

Indicator Species Analysis for Soil Fungi in the Mineral Horizon - Vertical Order Based on Significance Test										
					Treatment	Ram_Bck	Ram_Dth	Ram_Bth	NMDst_M	NMBck_M
					n	6	5	6	6	6
OTU #	Taxon Name	Avg IV	p *	Maximum IV	MaxGrp					
593	Thysanophora	15	0.0016	70	Ram_Bck	70	0	1	0	2
587	Stibella	12	0.0034	60	NMBck_M	0	0	2	0	60
139	Basidiomycota sp. 3	11	0.0108	49	Ram_Bth	0	0	49	4	0
478	Mycena	14	0.0114	60	Ram_Bck	60	1	1	3	8
579	Sordariomycetes sp. 9	14	0.0156	51	Ram_Dth	1	51	17	0	2
158	Cadophora	19	0.0166	42	Ram_Bck	42	11	11	13	18
324	Helotiales sp. 38	14	0.0224	45	Ram_Bck	45	0	0	0	23
412	Lecythophora	20	0.023	39	NMDst_M	16	13	15	39	16
545	Pyrenochaeta	8	0.0232	40	Ram_Dth	0	40	0	0	0
259	Glomeromycota sp. 12	8	0.0254	40	Ram_Dth	0	40	0	0	0
552	Rhizophagus	8	0.0254	40	Ram_Dth	0	40	0	0	0
490	Neofabraea	12	0.0266	45	Ram_Bth	4	1	45	4	7
27	Agaricomycetes sp. 6	8	0.0386	36	Ram_Bck	36	0	2	3	0
439	Libertella	9	0.0404	41	Ram_Bck	41	0	0	0	6
1	Absconditella	9	0.0406	44	Ram_Bck	44	0	0	0	2
239	Dothideomycetes sp. 3	9	0.0426	40	Ram_Bck	40	2	1	4	0
394	Kuzuhaea	12	0.0496	39	Ram_Dth	5	39	9	5	1
638	Wallemia	20	0.0572	25	NMDst_M	20	21	18	25	16
557	Rhodotorula	20	0.0606	44	NMDst_M	7	19	13	44	16
240	Dothideomycetes sp. 4	12	0.0636	58	Ram_Bck	58	0	2	1	1
456	Mitosporic Ascomycota sp. 4	10	0.0642	41	Ram_Bth	2	3	41	1	1
119	Basidiomycota sp. 11	9	0.069	37	Ram_Dth	0	37	0	6	0
389	Inocybe	13	0.0776	49	NMBck_M	5	2	7	4	49
611	Tricholoma	9	0.0778	41	Ram_Bck	41	0	5	0	0
204	Cladosporium	8	0.0788	35	Ram_Bck	35	0	0	0	5
294	Helotiales sp. 10	7	0.083	31	Ram_Dth	0	31	2	0	2
639	Wilcoxina	14	0.0964	42	Ram_Dth	1	42	17	6	5
292	Helotiaceae sp. 9	8	0.1066	37	Ram_Dth	0	37	2	0	0
356	Herpotrichiellaceae sp. 8	7	0.1236	33	Ram_Dth	2	33	1	0	0
608	Tricholomaceae sp. 2	8	0.1286	29	NMDst_M	6	0	2	29	2
589	Syzygospora	6	0.1338	28	Ram_Bck	28	0	1	0	1
370	Hyaloscyphaceae sp. 7	7	0.1348	29	NMDst_M	2	0	0	29	6
496	Oidiodendron	20	0.1364	31	NMDst_M	15	27	16	31	11

Table 9: Indicator species analysis for soil fungi in the mineral horizon based on putative taxonomic affinities; blocked analysis was not possible due to uneven sampling. All treatments were analyzed together. "IV" stands for importance value based on relative abundance and relative frequency. A Monte Carlo randomization test based on 5,000 permutations was performed to identify statistically important genera; reported as "p *"; a high IV with a corresponding p-value < 0.05 is considered strong indication, $0.05 < p < 0.1$ is good indication and $0.1 < p < 0.l5$ is a reasonable indicator. High indicator values are colored green in each treatments respective column; the treatment with the highest IV is denoted in column "MaxGrp."

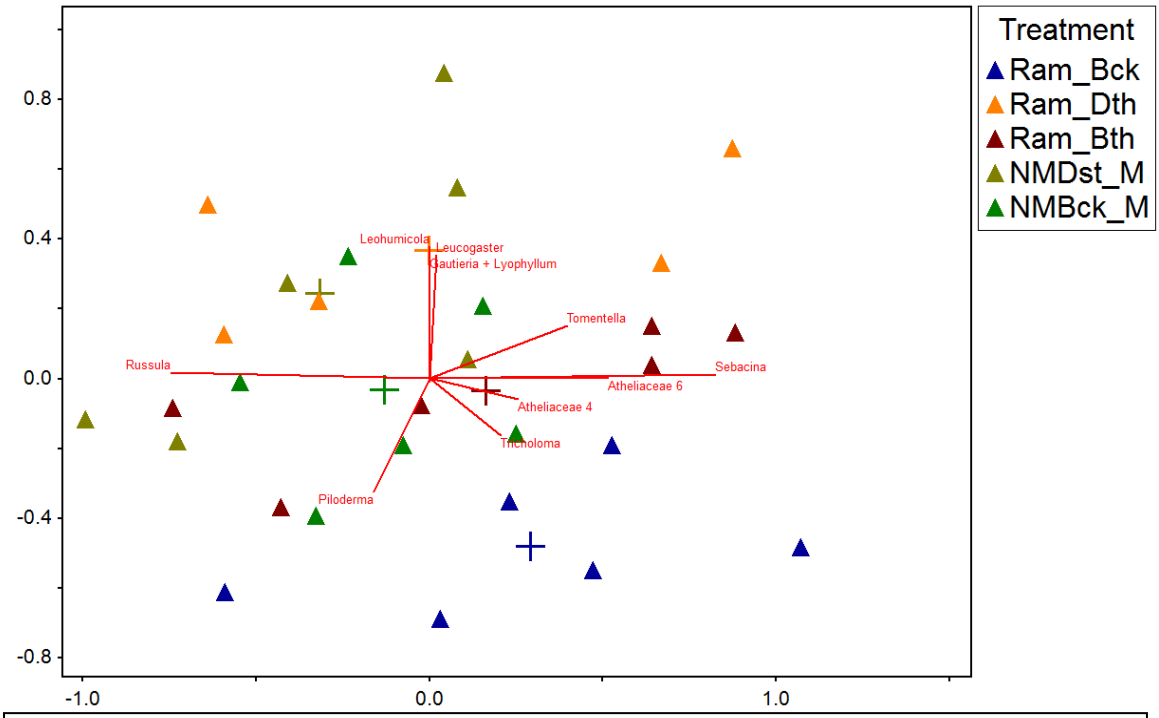


Figure 21: NMS ordination of mineral horizon soil cores (triangles) in soil EmF genus-space. Those genera whose ecologies were determined to be ectomycorrhizal were isolated and ordinated here. The figure represents the first two axes of a three-dimensional solution (total 81.4% of variation explained); axis-1 (x-axis) explained 46.2% and axis-2 (y-axis) explained 15.8% (stress = 13.13, $p = 0.004$; instability < 0.00001). Treatment centroids are expressed by tick marks of the same color. Spatial (site) variation was corrected for by translating site centroids to the origin. Joint plot vectors are scaled to 100% and displayed for $r^2 > 0.2$.

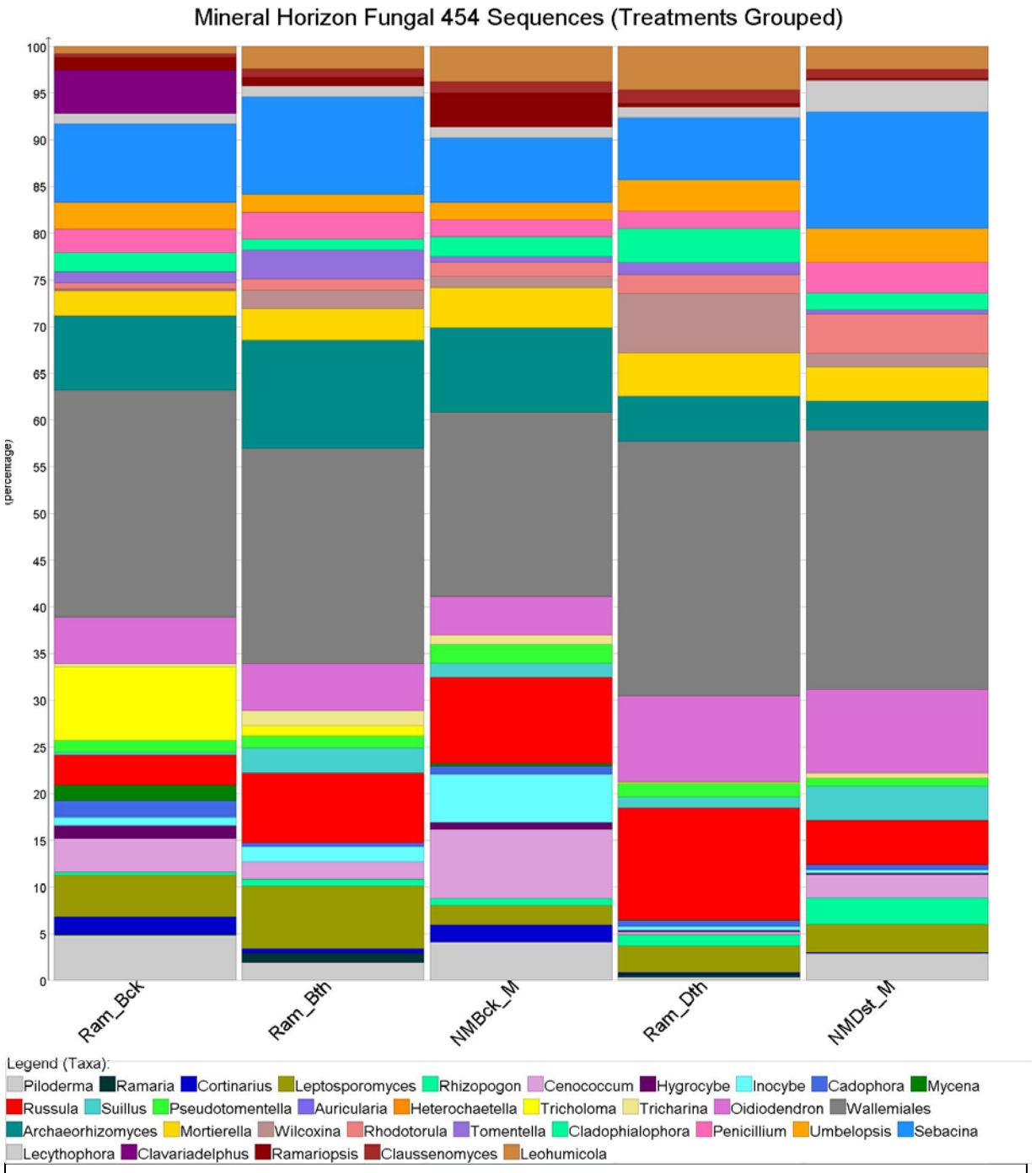


Figure 22: Mineral horizon soil fungal genera grouped by treatment. Each was relativized to the treatments total to account for uneven sampling depth. The 33 taxa above represent at least 90% of all encountered taxa in each treatment. The remaining genera occupy less than 10% of fungal abundance in each treatment.

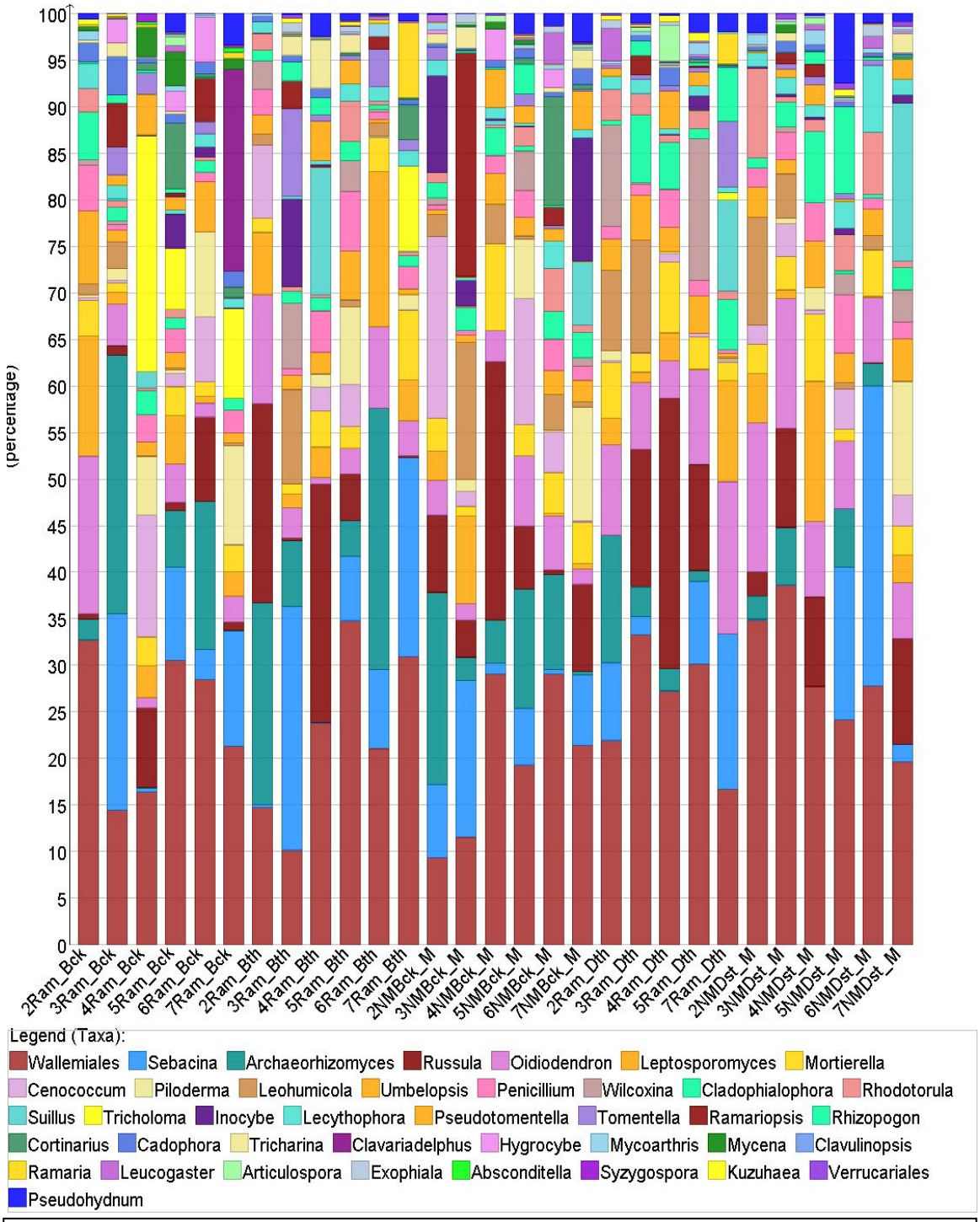


Figure 23: Mineral horizon soil fungal genera separated by individual sample. Each sample was relativized to its total to account for unequal sampling depth. Genera represented here are the 40 most abundant in the mineral horizon and account for over 80% of the sequence abundance for each respective sample.

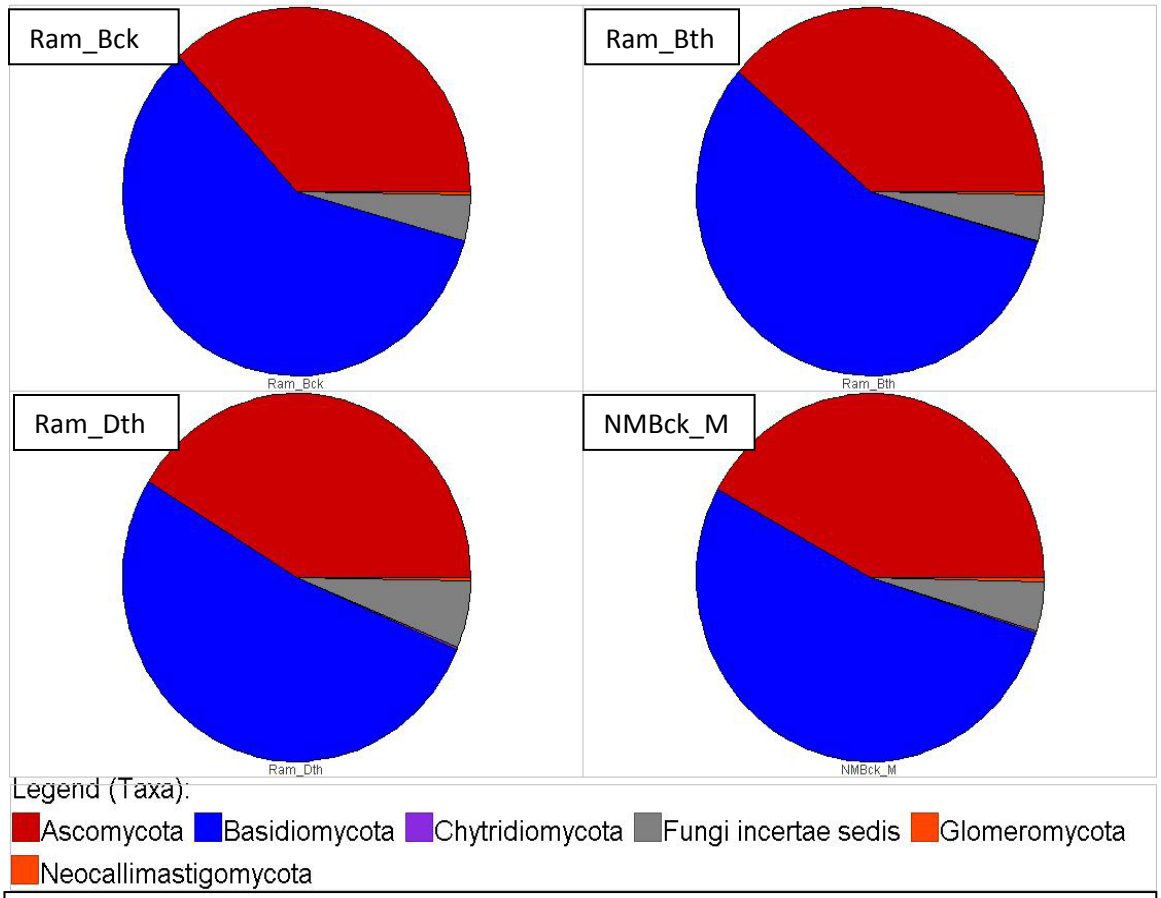


Figure 24: Soil fungal phyla in the mineral horizon grouped by treatment. Treatment NMDst M is omitted.

	Treatment	Richness	Evenness	Shannon's Diversity	Simpson's Diversity	% Empty Cells	Skewness	Kurtosis
Organic	Pil_Bck	63.20	0.785	3.246	0.9341	66.928	5.822	41.417
	Pil_Dth	73.00	0.808	3.460	0.9466	61.700	5.590	39.957
	Pil_Bth	59.20	0.779	3.159	0.9262	69.023	6.402	50.748
	NMDst_O	76.30	0.779	3.368	0.9374	60.035	6.323	49.629
	NMBck_O	77.50	0.786	3.412	0.9402	59.424	6.022	44.691
Mineral	Ram_Bck	57.00	0.802	3.233	0.9328	72.330	6.341	51.272
	Ram_Dth	83.20	0.772	3.402	0.9419	59.612	5.766	41.360
	Ram_Bth	76.30	0.793	3.421	0.9465	62.945	5.282	34.836
	NMDst_M	71.80	0.794	3.382	0.9431	65.129	5.714	40.508
	NMBck_M	83.20	0.783	3.450	0.9454	59.628	5.404	35.294

Table 10: Soil bacterial community (family and above) statistics grouped by horizon. Phylogenetic groupings here were the same as those used in NMS and MRPP comparisons of bacterial 454 data. Values are averages (n=6) from each treatment except Ram_Dth (n=5). The organic horizon included 191 families and the mineral horizon included 206 families (total for both horizons = 217).

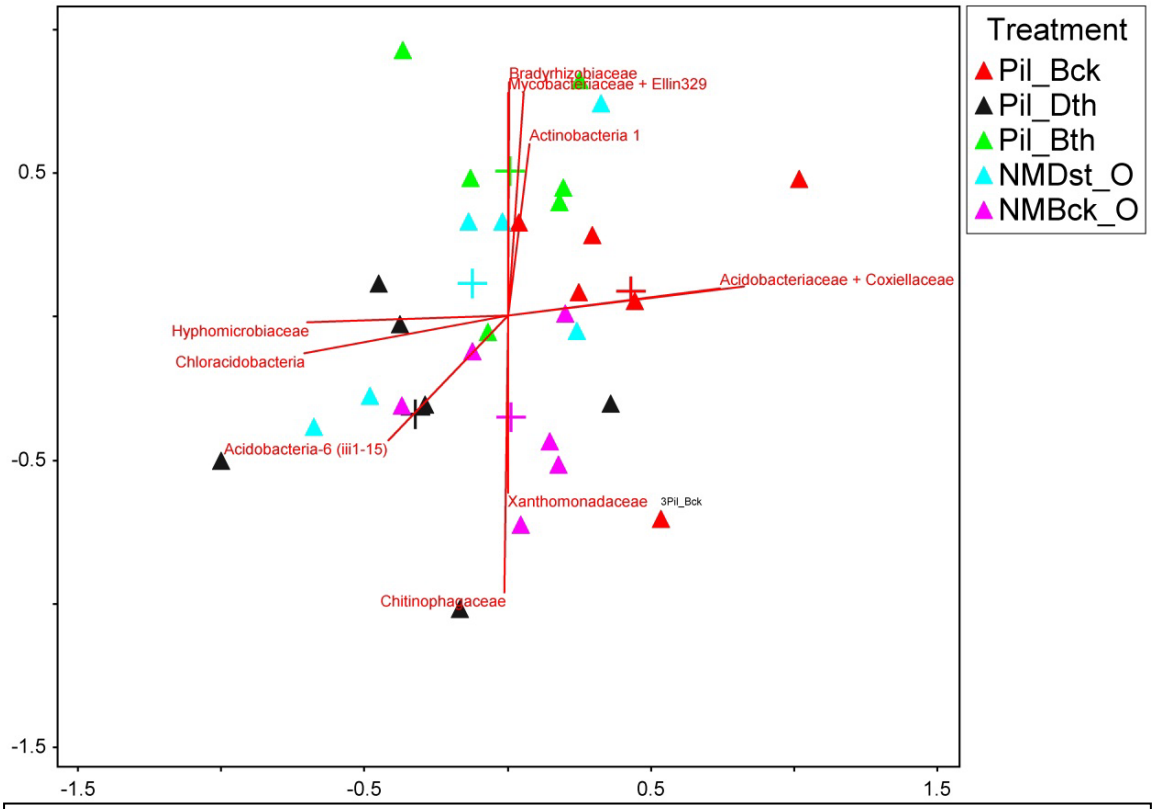


Figure 25: NMS ordination of organic horizon soil cores (triangles) in bacterial family-space. The figure represents the first two axes of a three-dimensional solution (total 93.3% of variation explained); axis-1 (x-axis) explained 49.3% and axis-2 (y-axis) explained 29.7% (stress = 8.58, p = 0.004; instability < 0.00001). Treatment centroids are expressed by tick marks of the same color. Spatial (site) variation was corrected for by translating site centroids to the origin; therefore axis scores are not reported in the graph. Joint plot vectors are scaled to 100% and displayed for $r^2 > 0.4$.

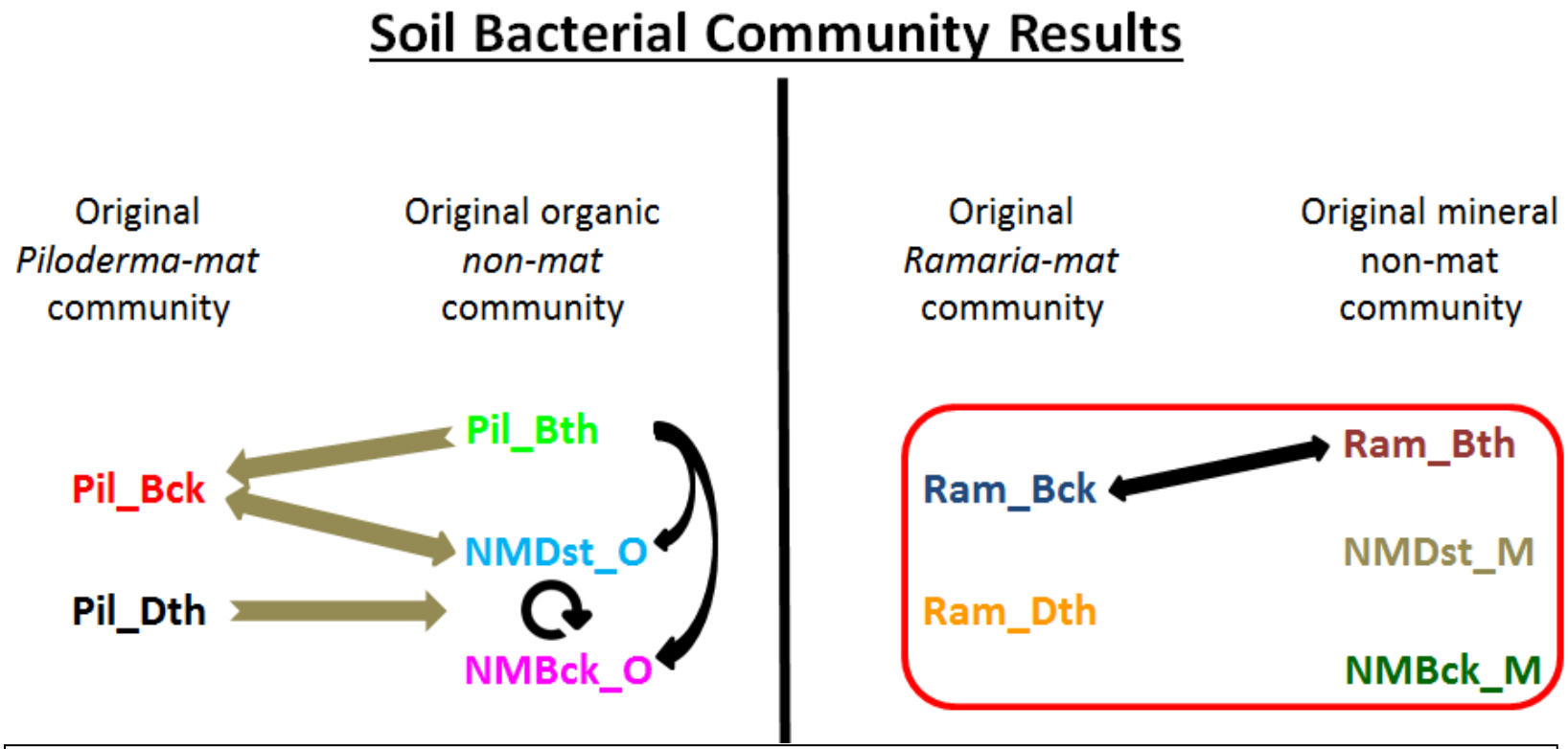


Figure 26: Schematic diagram of soil bacterial community results for the reciprocal soil transplant experiment. Each column represents soils that were originally hypothesized to possess similar communities. Arrows signify the trajectory by which communities remained unique or changed; black arrows indicate uniqueness, brown arrows indicate change. Red box around *Ramaria* indicates overall similarities between all treatments except for differences that remained between Ram_Bck and Ram_Bth. Compare to Figure 1, MRPP and NMS results. Refer to results text for written explanation.

Blocked Indicator Species Analysis for Soil Bacteria in the Organic Horizon - Vertical Order Based on Significance Test										
					Treatment	Pil_Bck	Pil_Dth	Pil_Bth	NMDst_O	NMBck_O
					n	6	6	6	6	6
OTU #	Taxon Name	Avg IV	p *	Maximum IV	MaxGrp					
88	FFCH4570	17	0.0008	50	Pil_Dth	5	50	11	3	15
20	SVA0725	16	0.003	57	Pil_Dth	0	57	6	1	18
149	PRR-10	20	0.004	28	Pil_Dth	14	28	20	22	15
45	Mycobacteriaceae	20	0.007	29	Pil_Bth	19	14	29	20	17
198	Coxiellaceae	19	0.0088	35	Pil_Bck	35	8	20	12	21
145	Ellin329	20	0.0122	24	Pil_Bth	19	19	24	21	18
70	Chitinophagaceae	20	0.0244	26	NMBck_O	18	22	17	17	26
10	CCU21	10	0.0326	43	NMDst_O	2	1	2	43	0
207	TM7-1	18	0.0334	38	NMBck_O	4	23	15	9	38
50	Sporichthyaceae	10	0.0402	50	NMDst_O	0	0	0	50	0
123	Nitrospiraceae	9	0.0406	46	Pil_Dth	0	46	0	1	0
61	Solirubrobacteraceae	9	0.0416	45	NMDst_O	0	0	0	45	2
7	Koribacteraceae	20	0.0418	30	Pil_Dth	19	30	18	16	17
31	Actinobacteria-7	12	0.044	39	NMBck_O	0	0	3	16	39
19	Solibacteraceae	20	0.056	25	Pil_Dth	22	25	14	20	21
41	Kineosporiaceae	8	0.0582	34	NMBck_O	2	2	0	1	34
206	SC3	12	0.0648	40	NMBck_O	6	1	0	13	40
172	Ellin6067	17	0.0736	34	NMBck_O	9	19	11	10	34
143	Caulobacterales	18	0.074	33	Pil_Dth	24	33	3	9	23
49	Pseudonocardiaceae	15	0.08	37	NMBck_O	4	4	7	23	37
28	C111	15	0.0836	40	NMDst_O	6	17	9	40	5
203	Sinobacteraceae	20	0.0952	27	Pil_Bck	27	17	18	19	18
113	Gemm-1	12	0.101	36	NMDst_O	0	19	6	36	0
25	Actinobacteria-1	20	0.1076	28	Pil_Bck	28	16	25	18	12
2	AD3	16	0.1122	35	Pil_Dth	12	35	17	13	6
204	Xanthomonadaceae	18	0.1144	36	Pil_Dth	15	36	2	11	28
209	EW055	9	0.1274	33	NMBck_O	11	0	0	0	33
171	Oxalobacteraceae	14	0.1304	36	Pil_Dth	2	36	19	8	4
43	Micrococcaceae	8	0.1318	32	Pil_Bck	32	0	0	2	4
157	Rhodobacteriaceae	8	0.1368	32	NMBck_O	0	2	0	4	32
126	ZB2	9	0.138	34	NMDst_O	0	11	0	34	0
159	Acetobacteraceae	20	0.142	27	Pil_Bth	24	15	27	24	9

Table 11: Blocked indicator species analysis for soil bacteria in the organic horizon based on putative taxonomic affinities. All treatments were analyzed together. "IV" stands for importance value based on relative abundance and relative frequency. A Monte Carlo randomization test based on 5,000 permutations was performed to identify statistically important genera; reported as "p *"; a high IV with a corresponding p-value < 0.05 is considered strong indication, $0.05 < p < 0.1$ is good indication and $0.1 < p < 0.l5$ is a reasonable indicator. High indicator values are colored green in each treatments respective column; the treatment with the highest IV is denoted in column "MaxGrp."

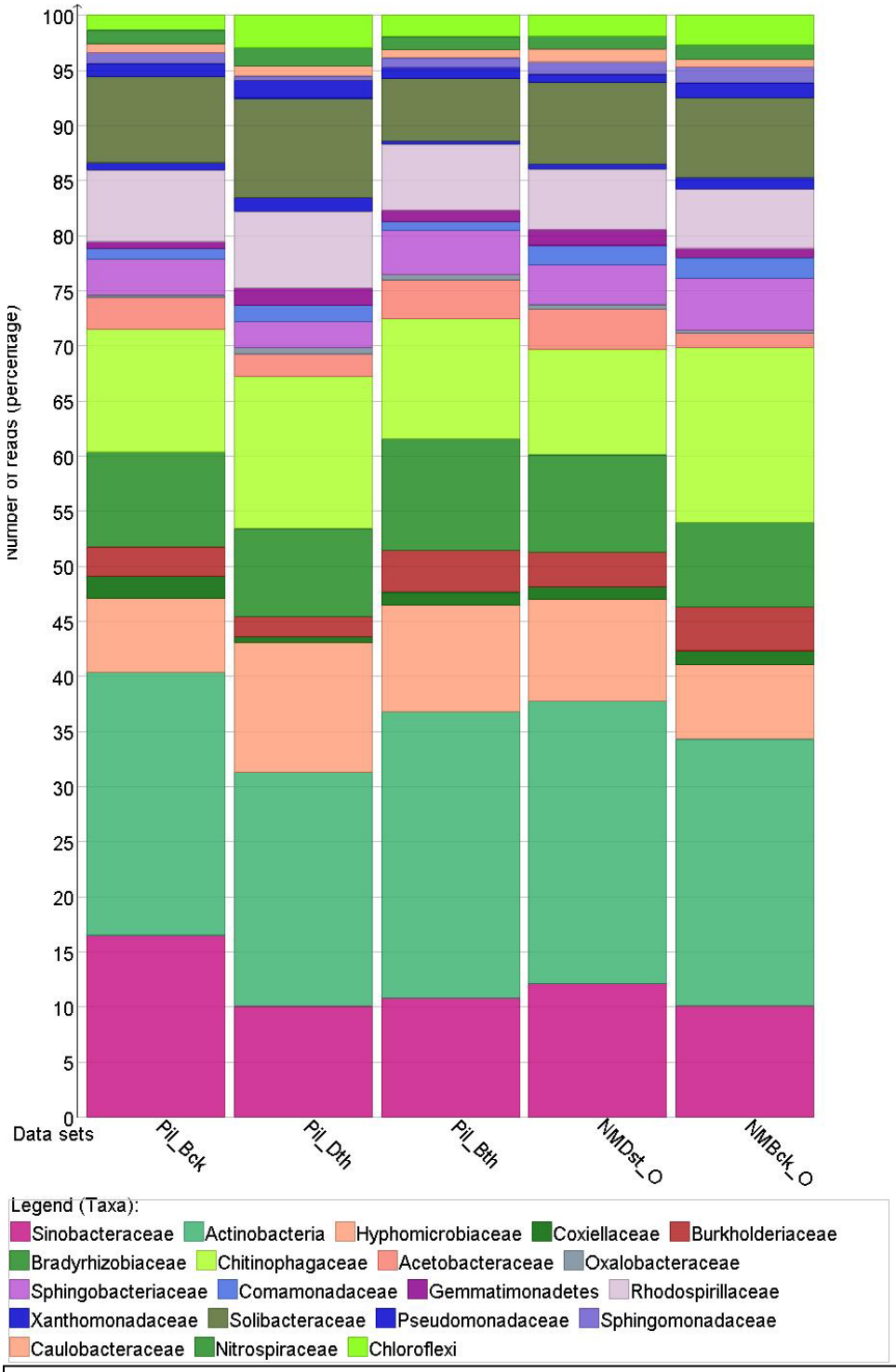


Figure 27: Organic horizon soil bacterial families grouped by treatment. Each was relativized to the treatments total to account for uneven sampling depth. The 20 taxa above represent at least 95% of all encountered families in each treatment; the remaining families occupy less than 5% of bacterial abundance.

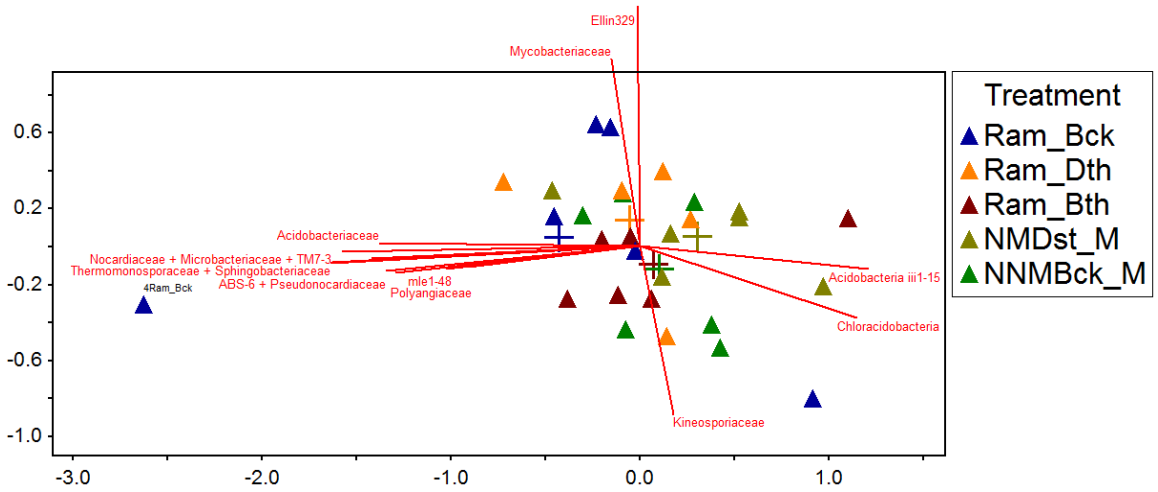


Figure 28: NMS ordination of mineral horizon soil cores (triangles) in bacterial family-space. The figure represents the only two axes of the solution (total 88.7% of variation explained); axis-1 (x-axis) explained 69.2% and axis-2 (y-axis) explained 19.5% (stress = 13.82, p = 0.004; instability < 0.00001). Treatment centroids are expressed by tick marks of the same color. Spatial (site) variation was corrected for by translating site centroids to the origin. Joint plot vectors are scaled to 100% and displayed for $r^2 > 0.4$.

Indicator Species Analysis for Soil Bacteria in the Mineral Horizon - Vertical Order Based on Significance Test										
					Treatment	Ram_Bck	Ram_Dth	Ram_Bth	NMDst_M	NMBck_M
					n	6	5	6	6	6
OTU #	Taxon Name	Avg IV	p *	Maximum IV	MaxGrp					
117	Ellin5290	12	0.0028	57	Ram_Dth	3	57	2	0	0
127	Class OP11-1	13	0.0032	67	NMDst_M	0	0	0	67	0
98	Order mle1-48	12	0.0084	51	Ram_Dth	0	51	7	1	1
101	Order MLE1-12	11	0.0114	48	Ram_Bth	3	2	48	1	1
185	Family 0319-6G20	8	0.0246	40	Ram_Dth	0	40	0	0	0
164	Betaproteobacteria	8	0.0264	40	Ram_Dth	0	40	0	0	0
83	Class Bljii12	12	0.0288	40	Ram_Dth	2	40	1	12	6
99	Thermobaculaceae	8	0.0302	38	NMBck_M	2	0	2	0	38
27	Family AKIW874	17	0.0338	39	Ram_Bth	10	11	39	5	19
20	Order Sva0725	19	0.0474	32	Ram_Bth	12	25	32	13	13
128	Planctomycetes	7	0.0484	35	Ram_Dth	0	35	0	0	2
159	Acetobacteraceae	7	0.0496	31	Ram_Dth	0	31	4	0	0
43	Micrococcaceae	17	0.0526	34	NMDst_M	7	26	9	34	11
105	Phylum FCPU426	9	0.0564	37	NMDst_M	0	3	1	37	4
2	Unk. Bacteria 2	20	0.0648	25	NMDst_M	15	19	21	25	20
144	Caulobacteraceae	8	0.0666	37	NMBck_M	0	0	0	4	37
107	Alicyclobacillaceae	8	0.069	33	Ram_Bth	0	0	33	2	7
68	Flavobacteriaceae	9	0.0968	35	Ram_Bth	3	7	35	1	0
44	Micromonosporaceae	15	0.1216	33	NMBck_M	7	7	12	16	33
35	Actinosynnemataceae	17	0.1288	32	NMBck_M	10	7	12	24	32
111	Peptostreptococcaceae	7	0.1324	29	NMBck_M	5	3	0	0	29
13	Family mb2424	20	0.142	26	Ram_Bck	26	17	21	21	15
108	Bacillaceae	8	0.1458	27	NMBck_M	15	0	0	0	27

Table 12: Indicator species analysis for soil bacteria in the mineral horizon based on putative taxonomic affinities; blocked analysis was not possible due to uneven sampling. All treatments were analyzed together. "IV" stands for importance value based on relative abundance and relative frequency. A Monte Carlo randomization test based on 5,000 permutations was performed to identify statistically important genera; reported as "p *"; a high IV with a corresponding p-value < 0.05 is considered strong indication, $0.05 < p < 0.1$ is good indication and $0.1 < p < 0.l5$ is a reasonable indicator. High indicator values are colored green in each treatments respective column; the treatment with the highest IV is denoted in column "MaxGrp."

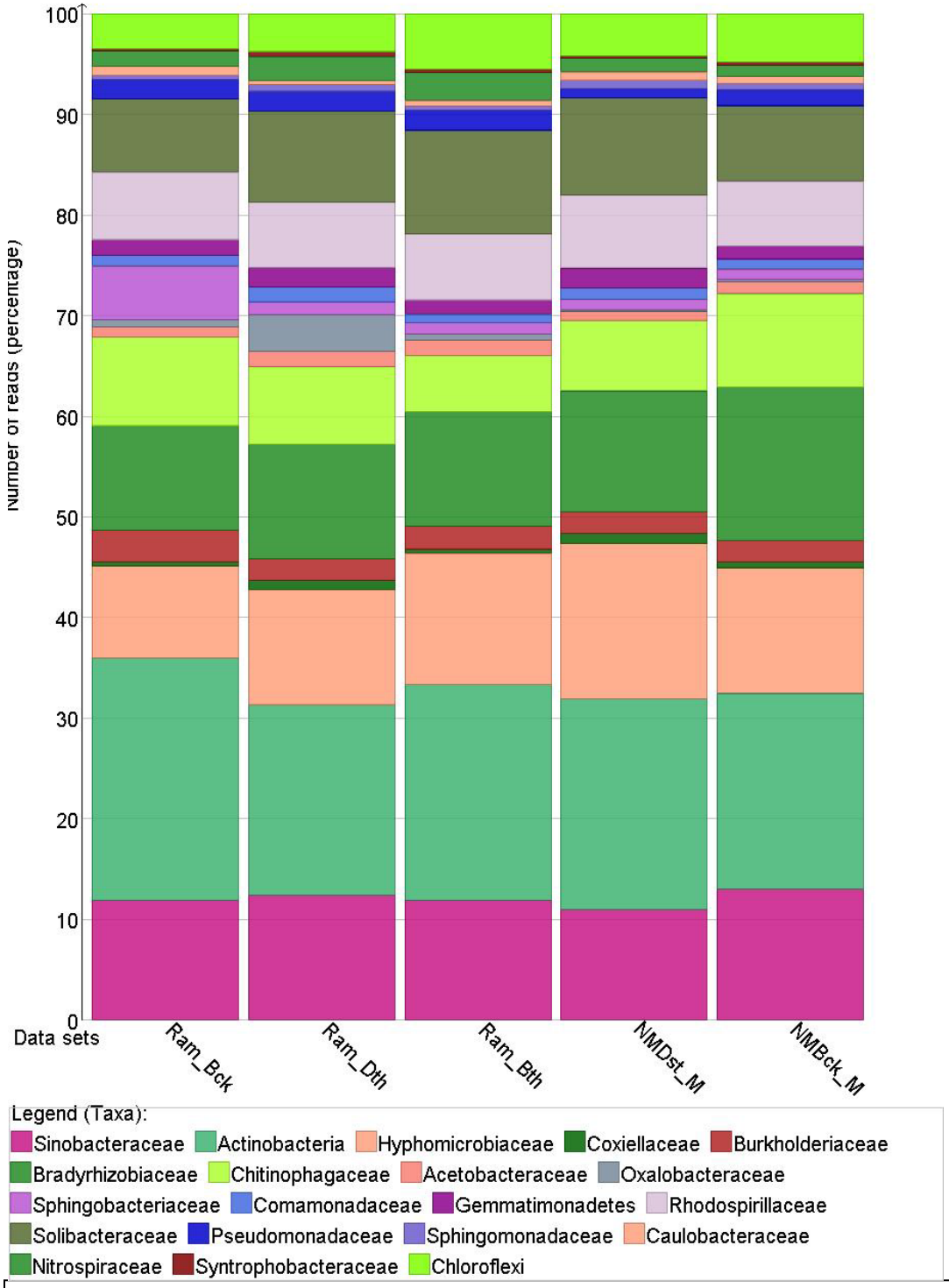


Figure 29: Mineral horizon soil bacterial families grouped by treatment. Each was relativized to the treatments total to account for uneven sampling depth. The 20 taxa above represent at least 95% of all encountered families in each treatment; the remaining families occupy less than 5% of bacterial abundance.

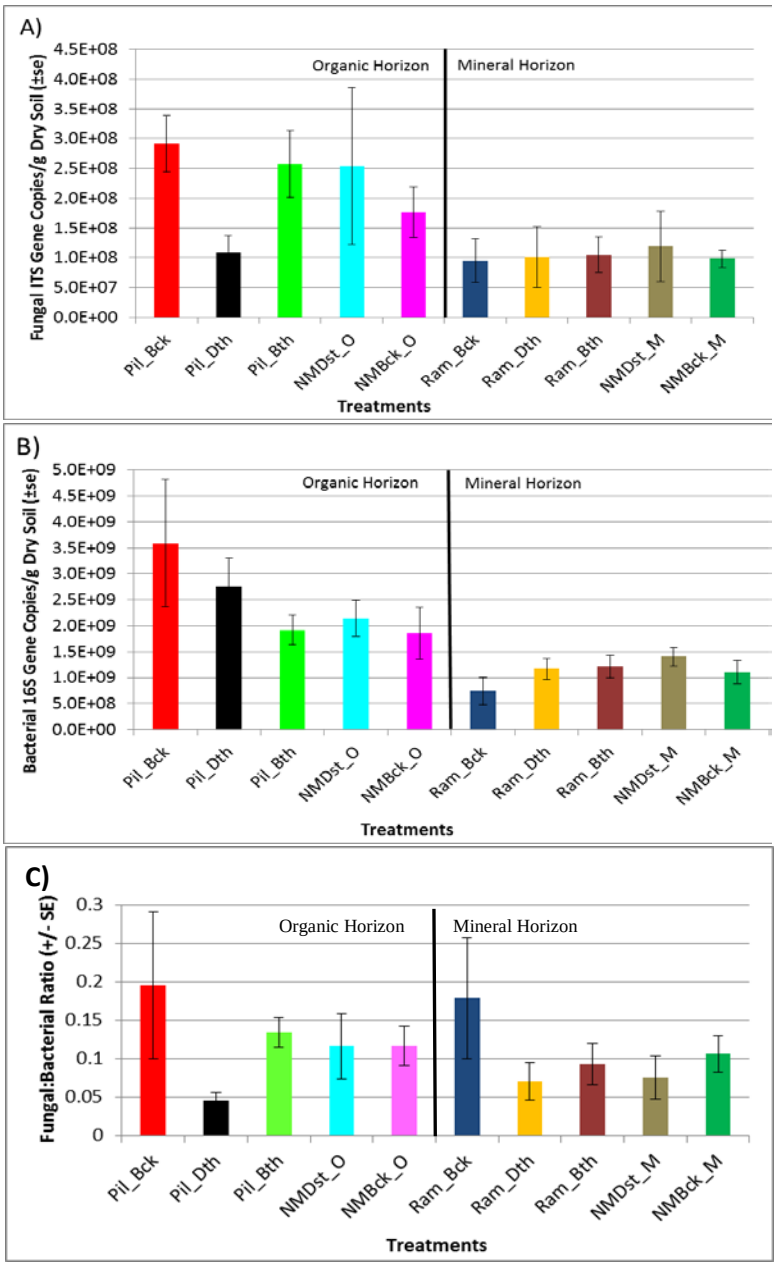


Figure 30: Soil fungal (A) and bacterial (B) gene copy numbers/g dry soil. Fungal:bacterial ratios (C) for each treatment, separated by horizon (+/- S.E.). Two-way ANOVA indicated no significant differences for any horizon comparison (n = 7; n = 6 for Ram_Bck, Ram_Dth and Ram_Bth); similarly, no treatment differences were found when correcting for multiple comparisons. Examination of only background treatments (Pil_Bck v. NMBck_O, Ram_Bck v. NMBck_M) yielded notable differences in for the organic horizon; Pil_Bck had significantly higher fungal rDNA copy numbers than NMBck_O ($p = 0.048$) and a trend toward higher bacterial rDNA copy numbers was observed ($p = 0.11$), while Ram_Bck tended to have lower bacterial rDNA copy numbers ($p = 0.15$). No significant differences were observed for fungal:bacterial ratios.

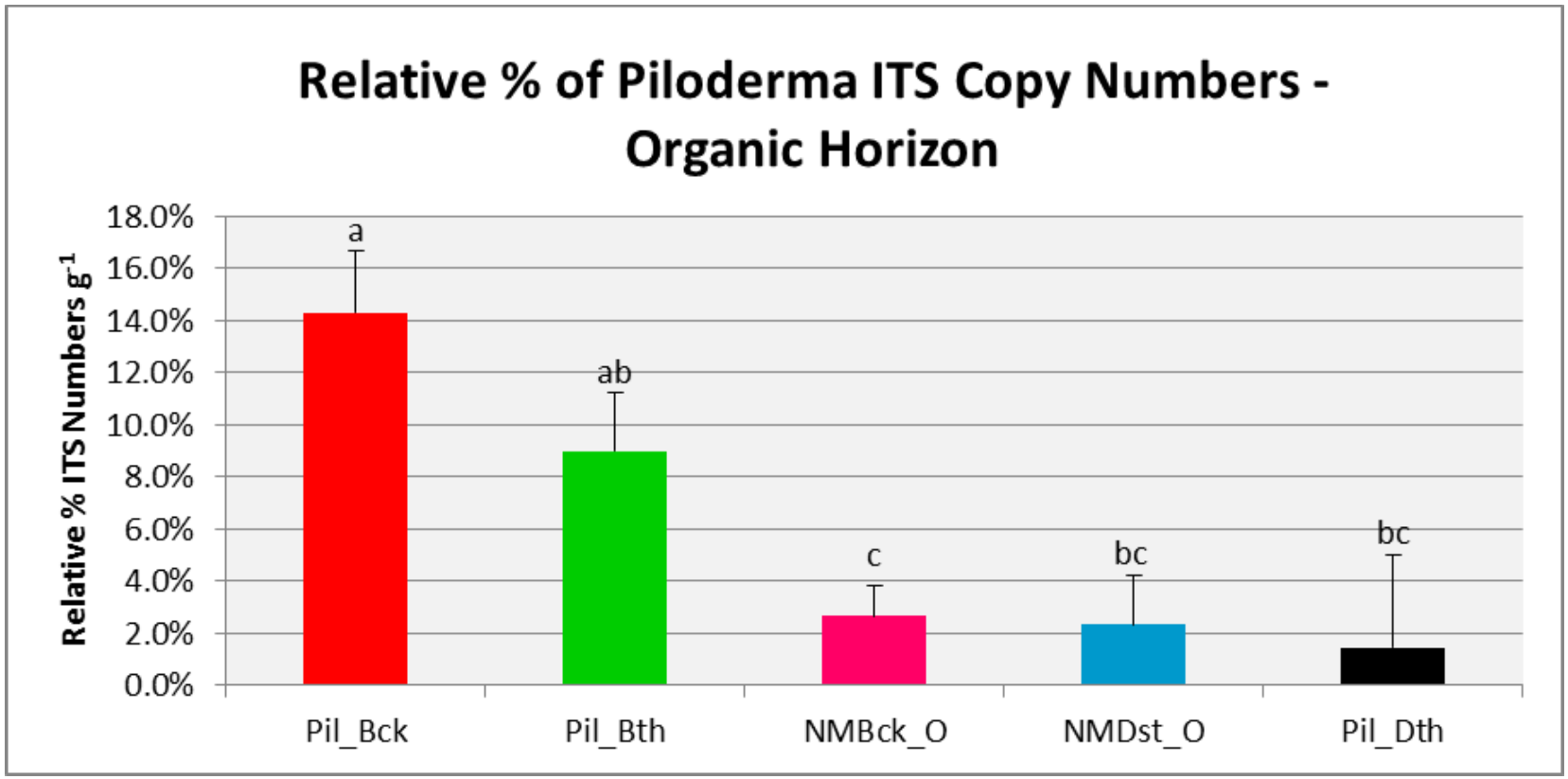


Figure 31: Means and standard errors of *Piloderma* ITS copy numbers for fungal 454 sequencing of the organic horizon, relativized by sample unit total and converted to percentage. Different letters denote statistical differences between treatments.

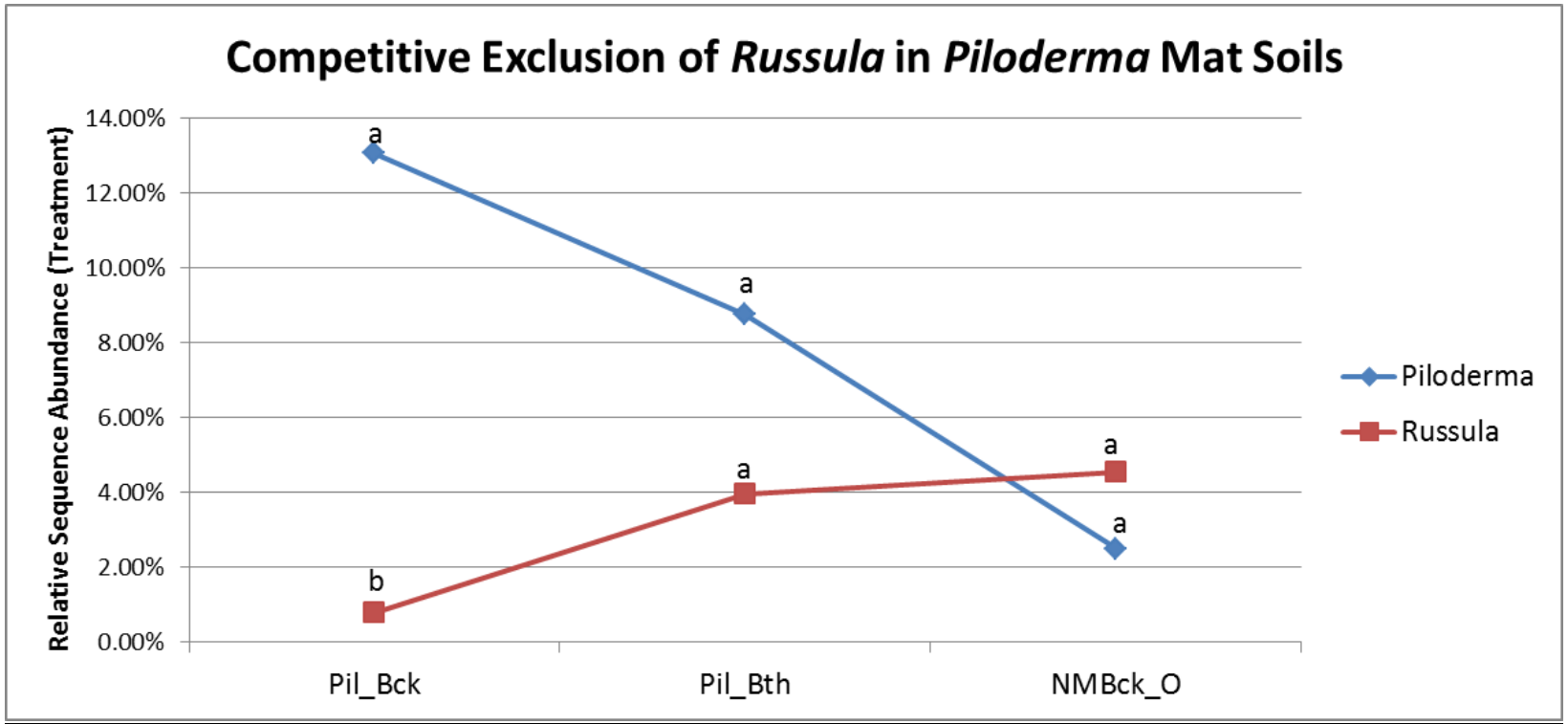


Figure 32: Relative abundance of *Piloderma* and *Russula* ITS copy numbers in O-horizon background and birth treatments. Where a two-way Friedman test indicated treatment differences, post-hoc means comparisons were made using a Kruskal-Wallis test. Comparisons were made between the relative abundance of each genus in each treatment (n = 6). Different letters indicate significant differences at $\alpha = 0.05$.

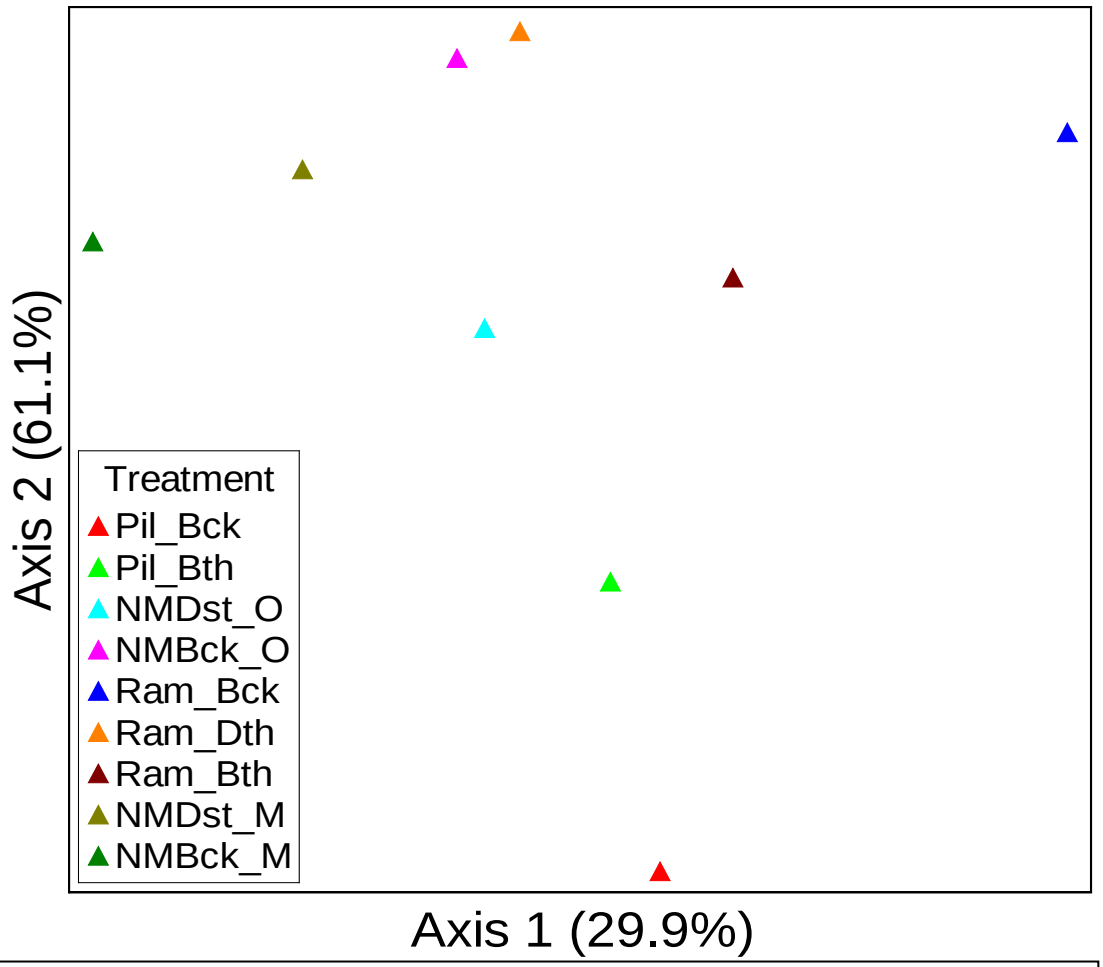
Appendices

Appendix 1:

Identity	Similarity (%)	Site	Horizon	*hinf* 1 cut site	LH-PCR length
Piloderma sp.	92	41	O		
Piloderma fallax	100	41	O	343	668
*Piloderma fallax**	100	82	O	343	668
*Piloderma fallax**	100	82	O	343	668
Piloderma sp.	99	116	O	343	664
*Piloderma fallax**	100	116	O	343	668
*Piloderma fallax**	100	120	O	343	668
Piloderma fallax	100	120	O	343	668
Piloderma fallax	100	125	O	343	668
Piloderma sp.	98	125	O	343	664
Piloderma fallax	100	137	O	343	668
*Piloderma fallax**	100	137	O	343	668
Piloderma fallax	100	147	O	343	668
*Piloderma fallax**	100	147	O	343	668
Piloderma sp.	90	41	M		
Ramaria claviramulata	98	41	M	328	666
Ramaria sp. SD-13.2	95	82	M	358	719
Ramaria sp. SD-13.2	96	82	M	358	719
Ramaria formosa	93	116	M	221	771
Ramaria sp. SD-13.2	96	116	M	358	719
Ramaria celerivirescens	99	120	M	322	660
Russula densifolia	81	120	M		
Hysterangium setchellii	99	125	M		
Ramaria formosa	93	125	M	221	771
Ramaria rubribrunnescens	99	137	M		
Ramaria celerivirescens	82	137	M	322	660
*Ramaria celerivirescens**	100	147	M	322	660
Ramaria celerivirescens	100	147	M	322	660

Appendix 1: Original identity of mats for each sampling site included in this study. Reproduced from Blanchard (2008). O = organic horizon; M = mineral horizon. * = Species identified by comparison of RFLP patterns to known species. N/A = The *hinf* 1 cut site and LH-PCR length was unable to be determined.

Appendix 2:



Appendix 2: NMS ordination of all grouped treatments in EmF species space. % variance explained by each axis represented is in parentheses.

Appendix 3:

Top 50 Fungal Genera								
OTU ID	Putative Taxonomic Affinity	Ecology	Total # Sequences	% of Total Sequences	# Soil Cores Present (out of 59)	Horizon Indicator	Organic Treatment Indicator	Mineral Treatment Indicator
638	Wallemia	SA	8339	12.28	59	Mineral***	Non-Mat**	All*
574	Sebacina	EcM/Endo	4138	6.10	57	-----	-----	-----
38	Archaeorhizomyces	SA/Enco	3311	4.88	45	-----	-----	-----
496	Oidiodendron	Endo	2626	3.87	59	-----	-----	-----
628	Venturia	Path	2529	3.73	58	Organic***	-----	NP
558	Russula	EcM	2486	3.66	48	Mineral*	-----	-----
529	Piloderma	EcM	2410	3.55	49	Organic***	Mat***	-----
218	Cryptococcus	SA	2062	3.04	59	-----	-----	-----
435	Leptosporomyces	SA	1997	2.94	44	-----	-----	-----
203	Cladophialophora	SA	1826	2.69	59	Organic***	-----	-----
476	Mortierella	SA	1801	2.65	59	-----	Non-Mat***	-----
624	Umbelopsis	Endo	1375	2.03	59	-----	-----	-----
500	Penicillium	SA	1343	1.98	59	Organic*	-----	-----
179	Cenococcum	EcM	1204	1.77	47	-----	-----	-----
639	Wilcoxina	EcM	1064	1.57	30	-----	-----	Death*
557	Rhodotorula	SA	844	1.24	54	-----	-----	Non-Mat*
594	Tomentella	EcM	813	1.20	46	-----	-----	-----
434	Leptodontidium	DSE	759	1.12	55	Organic***	-----	-----
567	Sagenomella	SA	757	1.12	41	Mineral***	-----	-----
524	Phialocephala	DSE	736	1.08	56	Mineral***	-----	-----
413	Leohumicola	EcM/ErM	729	1.07	41	Mineral***	Non-Mat (Bck)**	-----
588	Suillus	EcM	637	0.94	11	-----	-----	-----
412	Lecythophora	SA	601	0.89	58	Mineral**	Non-Mat***	Non-Mat (Dst)**
389	Inocybe	EcM	576	0.85	26	Mineral*	-----	Non-Mat (Bck)*
611	Tricholoma	EcM	565	0.83	10	-----	-----	Mat (Bck)*
158	Cadophora	EcM/ErM/Endo	534	0.79	53	-----	-----	Mat (Bck)***
554	Rhizopogon	EcM	525	0.77	36	-----	-----	-----
478	Mycena	SA	511	0.75	38	Organic***	-----	Mat (Bck)***
375	Hygrocybe	EcM	509	0.75	31	Organic**	-----	-----
215	Cortinarius	EcM	473	0.70	28	-----	-----	-----
430	Leotiomycetes OTU 7	EcM/Endo	460	0.68	39	-----	-----	-----
544	Pseudotomentella	EcM	459	0.68	34	-----	Non-Mat (Bck)***	-----
548	Ramariopsis	EcM	418	0.62	21	-----	-----	-----
541	Pseudeurotium	SA	356	0.52	30	Mineral***	Non-Mat (Dst)**	-----
605	Tricharina	EcM	336	0.49	22	-----	-----	-----
461	Mitosporic Helotiales OTU 10	SA	329	0.48	34	Organic***	-----	-----
97	Ascomycota OTU 8	Unk	325	0.48	3	-----	-----	-----
366	Hyaloscyphaceae OTU 3	SA	319	0.47	45	-----	-----	-----
547	Ramaria	EcM	278	0.41	11	-----	-----	-----
539	Porotheleum	SA	270	0.40	17	Organic***	-----	-----
205	Claussenomyces	SA	255	0.38	27	Mineral***	-----	-----
516	Pezizomycetes OTU 3	Unk	245	0.36	29	-----	Non-Mat (Dst)**	-----
107	Atheliaceae OTU 6	EcM	224	0.33	8	Mineral***	-----	-----
228	Dermateaceae OTU 5	SA	224	0.33	34	Mineral***	-----	-----
372	Hyaloscyphaceae OTU 9	SA	215	0.32	8	-----	-----	-----
231	Dermateaceae OTU 8	SA	211	0.31	30	Mineral***	-----	-----
207	Clavariadelphus	EcM	207	0.30	1	Mineral***	NP	-----
479	Mycoarthris	SA	203	0.30	36	-----	-----	-----
359	Heterochaetella	SA	198	0.29	4	-----	-----	-----
244	Exophiala	SA	189	0.28	40	-----	-----	-----

Appendix 3: List of the 50 most abundant fungal genera. The list includes putative taxonomies, ecologies (SA = Saprotroph; EcM = Ectomycorrhizal; Path = Pathogenic; Endo = Endophyte; DSE = Dark Septate Endophyte), abundance data and whether or not they were indicators of: (1) a particular soil horizon when all soils from the organic and mineral soil were considered together; (2) one of the organic-horizon treatments; and (3) one of the mineral-horizon treatments. These OTUs represent 79.3% of all sequences encountered among the 653 total fungal genera. A monte-carlo test for significance was reported as *$p < .10$; **$p < .05$; and ***$p < .01$. "-----" = not significant while "NP" = not present. A genus may have either; (1) indicated a soil horizon and had no treatment preference or (2) not indicated a soil horizon, but did indicate a treatment.

Appendix 4:

Top 50 Fungal 97% OTU's ("Species")					
Rank	Putative Taxonomic Affinity	Ecology	OTU ID	Total # Sequences	% of Total Sequences
1	Wallemia sp. 1	SA	994	7106	10.47%
2	Oidiodendron sp. 1	Endo	534	2024	2.98%
3	Umbelopsis sp. 1	Endo	1795	1303	1.92%
4	Cenococcum geophilum	EcM	1928	1101	1.62%
5	Archaeorhizomyces sp. 1	SA/Endo	533	1030	1.52%
6	Cryptococcus sp. 1	SA	531	972	1.43%
7	Cladophialophora sp. 1	SA	1327	962	1.42%
8	Russula sp. 1	EcM	224	954	1.41%
9	Venturia sp. 2	Path	129	947	1.40%
10	Archaeorhizomyces sp. 2	SA/Endo	44	890	1.31%
11	Piloderma olivaceum/fallax	EcM	1965	876	1.29%
12	Sebacina sp. 1	Endo	263	842	1.24%
13	Leptosporomyces sp. 1	SA	1137	733	1.08%
14	Phialocephala fortinii	DSE	1135	712	1.05%
15	Venturia sp. 3	Path	1446	638	0.94%
16	Suillus lakei	EcM	446	619	0.91%
17	Leptodontidium sp. 1	DSE	128	606	0.89%
18	Wallemia sp. 2	SA	1811	575	0.85%
19	Wilcoxina sp. 1	EcM	690	562	0.83%
20	Penicillium spinulosum	SA	1324	560	0.82%
21	Rhodotorula sp. 1	SA	1322	529	0.78%
22	Lecythophora mutabilis	SA	264	524	0.77%
23	Leptosporomyces sp. 2	SA	2033	509	0.75%
24	Sebacina sp. (ectomycorrhiza)	Endo	1790	492	0.72%
25	Oidiodendron sp. 2	Endo	689	481	0.71%
26	Wallemia sp. 3	SA	986	470	0.69%
27	Leotiomycetes sp. 1	EcM/Endo	693	460	0.68%
28	Mortierella sp. 1	SA	1331	460	0.68%
29	Sebacina sp. 2	Endo	1825	458	0.67%
30	Sagenomella diversispora	SA	532	441	0.65%
31	Cryptococcus terricola	SA	2130	439	0.65%
32	Leptosporomyces sp. 3	SA	1328	425	0.63%
33	Rhizopogon sp. 1	EcM	1823	420	0.62%
34	Leohumicola sp. 1	EcM	691	389	0.57%
35	Venturia hystrioides	Path	1838	379	0.56%
36	Mortierella sp. 2	SA	1139	337	0.50%
37	Wilcoxina sp. 2	EcM	948	336	0.49%
38	Tricharina sp. 1	EcM	1827	336	0.49%
39	Tricholoma saponaceum var. saponaceum	EcM	1826	334	0.49%
40	Mitosporic Helotiales 1	SA	2197	329	0.48%
41	Pseudeurotium sp. 1	SA	692	328	0.48%
42	Ascomycota sp. 1	Unk	688	325	0.48%
43	Piloderma aff. lanatum	EcM	51	320	0.47%
44	Hyaloscyphaceae sp. 1	SA	548	319	0.47%
45	Archaeorhizomyces sp. 3	SA/Endo	981	319	0.47%
46	Russula murrillii	EcM	1325	314	0.46%
47	Sebacina sp. 3	EcM	1326	309	0.46%
48	Cladophialophora sp. 3	SA	160	294	0.43%
49	Sebacina sp. 4	EcM	979	294	0.43%
50	Leptosporomyces sp. 4	SA	749	292	0.43%

Appendix 4: List of the 50 most abundant fungal 97% OTUs including putative taxonomies, ecologies, and abundance data (SA = Saprotroph; EcM = Ectomycorrhizal; Path = Pathogenic; Endo = Endophyte; DSE = Dark Septate Endophyte). These OTUs represent 52.5% of all sequences encountered among the 2235 total OTUs.

Appendix 5:

Relative Abundance of Fungal Functional Groups for Each Treatment								
Treatments	Arbuscular Mycorrhizal Fungi	Ectomycorrhizal Fungi	Root Endophytes	Ericoid Mycorrhizal Fungi	Mycoparasites	Plant Pathogens	Saprotrophic Fungi	Ectomycorrhizal: Saprotrophic Ratio
Pil_Bck	0.21%	34.56%	11.50%	5.09%	2.32%	11.05%	35.27%	0.9798
Pil_Dth	0.73%	17.92%	9.58%	4.01%	4.45%	12.45%	50.87%	0.3522
Pil_Bth	0.20%	28.24%	12.38%	6.14%	3.04%	3.63%	46.36%	0.6092
NMDst_O	0.25%	17.02%	12.20%	4.66%	5.79%	6.51%	53.58%	0.3176
NMBck_O	0.56%	25.39%	13.11%	2.10%	2.49%	11.47%	44.88%	0.5657
Ram_Bck	0.30%	31.84%	13.95%	4.38%	0.96%	4.25%	44.31%	0.7186
Ram_Dth	0.31%	27.75%	8.95%	7.26%	4.49%	2.06%	49.18%	0.5643
Ram_Bth	0.19%	29.45%	16.89%	4.17%	4.10%	1.65%	43.54%	0.6764
NMDst_M	0.24%	20.02%	9.88%	7.93%	2.90%	2.22%	56.81%	0.3525
NMBck_M	0.43%	36.69%	13.22%	3.07%	1.83%	3.65%	41.11%	0.8925

Appendix 5: Relative abundance of fungal ecological groupings by treatment and horizon.

Appendix 6:

Ectomycorrhizal Root-Tip Species List		
RFLP ID	**Genbank Taxonomic Identification**	**Identifier**
BH	Albatrellaceae sp. 1	Albatr_1
CU	Albatrellaceae sp. 2	Albatr_2
V	Alpova trappei	Alp_trap
BJ*	Cadophora finlandia	Cado_fin
A*	Cenococcum geophilum	Ceno_geo
DA	Ceratobasidiaceae sp. 1	Cerato_1
E*	Cortinarius sp. Daniel/variosimilis (sp. 1)	Cort_1
AF	Cortinarius caesiostramineus (sp. 2)	Cort_2
BD*	Cortinarius elutus/rufoolivaceus (sp. 3)	Cort_3
BQ	Cortinarius sp. 4 (casimiri/subsertipes/saturninus)	Cort_4
CL	Cortinarius velenovskyi (sp. 5)	Cort_5
CV	Cortinarius sp. 6 (rigens/obtusus/pseudocandelaris)	Cort_6
F*	Atheliaceae/Corticiaceae sp. 1	CortAc_1
DV	Corticiaceae sp. 2	CortAc_2
CX	Gomphaceae sp. 1 (Ramaria/Clavariadelphus)	Gomph_1
AG	Helvella lacunosa	Helv_lac
AU	Hydnum rufescens	Hyd_ruf
BE	Inocybe griseolilacina/pusio (sp. 1)	Inocyb_1
BM	Inocybe sp. 2 (flocculosa/nitidiuscula/pseudodestricta)	Inocyb_2
BZ	Inocybe sp. 3 (flocculosa/leiocephala/fuscidula)	Inocyb_3
U*	Lactarius rubrilacteus (sp. 1)	Lact_1
W	Lactarius aff. wenquanensis (sp. 2)	Lact_2
DD	Lactarius xanthogalactus (sp. 3)	Lact_3
M	Byssocorticum atrovirens	Leptos_1
H	Leucogaster rubescens	Leuc_rub
J*	Leucophleps spinispora	Lphl_spi
C	Otidea concinna	Otid_con
AE	Phialocephala sp. 1	Phialo_1
B*	Piloderma sp. 1	Pilod_1
D	Piloderma sp. 2	Pilod_2
I	Piloderma sp. 3	Pilod_3
O	Piloderma fallax (sp. 4)	Pilod_4
P*	Piloderma sp. A18 (sp. 5)	Pilod_5

Q	Piloderma byssinum (sp. 6)	Pilod_6
AJ*	Piloderma sp. 7	Pilod_7
BP*	Piloderma sp. 8	Pilod_8
BS	Piloderma sp. 9	Pilod_9
BY	Piloderma sp. 10	Pilod_10
CE*	Piloderma sp. 11 (olivaceum 1)	Pilod_11
CJ*	Piloderma sp. 12 (olivaceum 2)	Pilod_12
CZ	Piloderma sp. 13	Pilod_13
DG	Piloderma sp. 14	Pilod_14
AC	Pseudotomentella sp. 1	Pseudo_1
AY	Pseudotomentella sp. 2 (tristis)	Pseudo_2
N	Ramaria formosa (sp. 1)	Ram_form
CM*	Ramaria celerivirescens (sp. 2)	Ram_cel
CT	Ramaria sp. 3	Ram_3
G	Rhizopogon sp. 1	Rhizop_1
AB*	Rhizopogon sp. 2 (vesiculosus/vinicolor)	Rhizop_2
AW*	Rhizopogon sp. Luoma/parksii (sp. 3)	Rhizop_3
DK	Rhizopogon sp. 4 (villosulus/rudus)	Rhizop_4
K	Russula sp. 1 (tenuiceps)	Russ_1
AA*	Russula nigricans/acrifolia (sp. 2)	Russ_2
AI*	Russula cascadensis (sp. 3)	Russ_3
AV	Russula cuprea (sp. 4)	Russ_4
AX	Russula sp. 5	Russ_5
BI	Russula brevipes (sp. 6)	Russ_6
BV	Russula turci (sp. 7)	Russ_7
BW*	Russula aff. amethystina (sp. 8)	Russ_8
CK	Russula dissimulans (sp. 9)	Russ_9
CW	Russula sp. 10 (bicolor/raoultii)	Russ_10
X*	Sebacina sp. 1	Sebaci_1
AQ	Sebacina sp. 2	Sebaci_2
AS	Sebacina sp. 3	Sebaci_3
BK	Sebacina epigea (sp. 4)	Sebaci_4
DC*	Sebacina sp. 5	Sebaci_5
CD	Sistotrema sp. (muscicola)	Sistot_1
CR	Suillus lakei	Suil_lak
CC	Thelephoraceae sp. 1 (pseudotomentella)	ThelAc_1
AD	Tomentella sp. 1	Tomen_1
AH	Tomentella sp. 2 (bryophiila)	Tomen_2

AN	Tomentella sp. 3	Tomen_3
AO	Tomentella subclavigera (sp. 4)	Tomen_4
BL	Tomentella cinerascens (sp. 5)	Tomen_5
BN	Tomentella sp. 6	Tomen_6
CF	Tomentella sp. 7 (fuscocinerea)	Tomen_7
CS	Tomentella sp. 8	Tomen_8
DR	Tomentella sp. 9	Tomen_9
EB	Tomentella sp. 10 (sp. Nara)	Tomen_10
Y	Tomentellopsis echinospora	Tpsis_ech
DE	Tricharina sp. 1	Trichari
AL	Tricholoma vaccinum (sp. 1)	Tricho_1
BF*	Tricholoma saponaceum (sp. 2)	Tricho_2
S*	Truncocolumella citrina	Trun_cit
T	Wilcoxina rehmii	Wilc_reh
R	Unknown sp. 1	Unk_1
Z	Unknown sp. 2	Unk_2
AM	Unknown sp. 3	Unk_3
BC	Unknown sp. 4	Unk_4
BT	Unknown sp. 5	Unk_5
BX	Unknown sp. 6	Unk_6
CB*	Unknown sp. 7	Unk_7
CI	Unknown sp. 8	Unk_8
CP	Unknown sp. 9	Unk_9
DF	Unknown sp. 10	Unk_10
DI	Unknown sp. 11	Unk_11
DJ	Unknown sp. 12	Unk_12
DL	Unknown sp. 13	Unk_13
DM	Unknown sp. 14	Unk_14
DN	Unknown sp. 15	Unk_15
DQ	Unknown sp. 16	Unk_16
DT	Unknown sp. 17	Unk_17
DU	Unknown sp. 18	Unk_18
DW	Unknown sp. 19	Unk_19
EA	Unknown sp. 20	Unk_20

Appendix 7:

OTU #	Fungal 454 Taxonomic Identity Summarized at the Genus Level
1	Absconditella
2	Acaulospora
3	Acremonium
4	Agaricales sp. 1
5	Agaricales sp. 10
6	Agaricales sp. 2
7	Agaricales sp. 3
8	Agaricales sp. 4
9	Agaricales sp. 5
10	Agaricales sp. 6
11	Agaricales sp. 7
12	Agaricales sp. 8
13	Agaricales sp. 9
14	Agaricomycetes sp. 1
15	Agaricomycetes sp. 10
16	Agaricomycetes sp. 11
17	Agaricomycetes sp. 12
18	Agaricomycetes sp. 13
19	Agaricomycetes sp. 14
20	Agaricomycetes sp. 15
21	Agaricomycetes sp. 16
22	Agaricomycetes sp. 17
23	Agaricomycetes sp. 2
24	Agaricomycetes sp. 3
25	Agaricomycetes sp. 4
26	Agaricomycetes sp. 5
27	Agaricomycetes sp. 6
28	Agaricomycetes sp. 7
29	Agaricomycetes sp. 8
30	Agaricomycetes sp. 9
31	Agaricostilbales
32	Alatospora
33	Albatrellus
34	Alloclavaria
35	Amanita
36	Amorphotheca
37	Amphinema
38	Archaeorhizomyces
39	Arthrographis
40	Articulospora
41	Ascomycota sp. 1
42	Ascomycota sp. 10
43	Ascomycota sp. 11
44	Ascomycota sp. 12
45	Ascomycota sp. 13
46	Ascomycota sp. 14
47	Ascomycota sp. 15
48	Ascomycota sp. 16
49	Ascomycota sp. 17
50	Ascomycota sp. 18
51	Ascomycota sp. 19
52	Ascomycota sp. 2
53	Ascomycota sp. 20
54	Ascomycota sp. 21
55	Ascomycota sp. 22
56	Ascomycota sp. 23
57	Ascomycota sp. 24
58	Ascomycota sp. 25
59	Ascomycota sp. 26
60	Ascomycota sp. 27
61	Ascomycota sp. 28
62	Ascomycota sp. 29
63	Ascomycota sp. 3
64	Ascomycota sp. 30
65	Ascomycota sp. 31
66	Ascomycota sp. 32
67	Ascomycota sp. 33
68	Ascomycota sp. 34
69	Ascomycota sp. 35
70	Ascomycota sp. 36
71	Ascomycota sp. 37
72	Ascomycota sp. 38
73	Ascomycota sp. 39
74	Ascomycota sp. 4

75	Ascomycota sp. 40
76	Ascomycota sp. 41
77	Ascomycota sp. 42
78	Ascomycota sp. 43
79	Ascomycota sp. 44
80	Ascomycota sp. 45
81	Ascomycota sp. 46
82	Ascomycota sp. 47
83	Ascomycota sp. 48
84	Ascomycota sp. 49
85	Ascomycota sp. 5
86	Ascomycota sp. 50
87	Ascomycota sp. 51
88	Ascomycota sp. 52
89	Ascomycota sp. 53
90	Ascomycota sp. 54
91	Ascomycota sp. 55
92	Ascomycota sp. 56
93	Ascomycota sp. 57
94	Ascomycota sp. 58
95	Ascomycota sp. 6
96	Ascomycota sp. 7
97	Ascomycota sp. 8
98	Ascomycota sp. 9
99	Aspergillus
100	Astraeus
101	Athelia
102	Atheliaceae sp. 1
103	Atheliaceae sp. 2
104	Atheliaceae sp. 3
105	Atheliaceae sp. 4
106	Atheliaceae sp. 5
107	Atheliaceae sp. 6
108	Atheliaceae sp. 7
109	Atheliaceae sp. 8
110	Atheliaceae sp. 9
111	Auricularia
112	Auriculariales sp. 1
113	Auriculariales sp. 2
114	Basidioascus
115	Basidiobolus
116	Basidiodendron
117	Basidiomycota sp. 1
118	Basidiomycota sp. 10
119	Basidiomycota sp. 11
120	Basidiomycota sp. 12
121	Basidiomycota sp. 13
122	Basidiomycota sp. 14
123	Basidiomycota sp. 15
124	Basidiomycota sp. 16
125	Basidiomycota sp. 17
126	Basidiomycota sp. 18
127	Basidiomycota sp. 19
128	Basidiomycota sp. 2
129	Basidiomycota sp. 20
130	Basidiomycota sp. 21
131	Basidiomycota sp. 22
132	Basidiomycota sp. 23
133	Basidiomycota sp. 24
134	Basidiomycota sp. 25
135	Basidiomycota sp. 26
136	Basidiomycota sp. 27
137	Basidiomycota sp. 28
138	Basidiomycota sp. 29
139	Basidiomycota sp. 3
140	Basidiomycota sp. 30
141	Basidiomycota sp. 31
142	Basidiomycota sp. 32
143	Basidiomycota sp. 33
144	Basidiomycota sp. 34
145	Basidiomycota sp. 4
146	Basidiomycota sp. 5
147	Basidiomycota sp. 6
148	Basidiomycota sp. 7
149	Basidiomycota sp. 8
150	Basidiomycota sp. 9
151	Bionectria
152	Biscogniauxia

153	Boletales sp. 1
154	Boletus
155	Botryobasidium
156	Bullera
157	Byssonectria
158	Cadophora
159	Caloplaca
160	Camarophyllopsis
161	Candida
162	Cantharellales sp. 1
163	Cantharellales sp. 2
164	Capnobotryella
165	Capnodiales sp. 1
166	Capnodiales sp. 10
167	Capnodiales sp. 11
168	Capnodiales sp. 12
169	Capnodiales sp. 13
170	Capnodiales sp. 2
171	Capnodiales sp. 3
172	Capnodiales sp. 4
173	Capnodiales sp. 5
174	Capnodiales sp. 6
175	Capnodiales sp. 7
176	Capnodiales sp. 8
177	Capnodiales sp. 9
178	Capronia
179	Cenococcum
180	Ceratobasidiaceae sp. 1
181	Ceratobasidiaceae sp. 2
182	Ceratobasidiaceae sp. 3
183	Chaenotheca
184	Chaenothecopsis
185	Chaetosphaeria
186	Chaetothyriales sp. 1
187	Chaetothyriales sp. 2
188	Chaetothyriales sp. 3
189	Chaetothyriales sp. 4
190	Chalara
191	Chroogomphus
192	Chytridiales sp. 1
193	Chytridiomycota sp. 1
194	Chytridiomycota sp. 10
195	Chytridiomycota sp. 2
196	Chytridiomycota sp. 3
197	Chytridiomycota sp. 4
198	Chytridiomycota sp. 5
199	Chytridiomycota sp. 6
200	Chytridiomycota sp. 7
201	Chytridiomycota sp. 8
202	Chytridiomycota sp. 9
203	Cladophialophora
204	Cladosporium
205	Claussenomyces
206	Clavaria
207	Clavariadelphus
208	Clavicorona
209	Clavulina
210	Clavulinopsis
211	Clitocybe
212	Coniochaetales sp. 1
213	Coniochaetales sp. 2
214	Coniosporium
215	Cortinarius
216	Cosmospora
217	Cotylidia
218	Cryptococcus
219	Cryptosporosis
220	Cylindrosympodium
221	Dactylaria
222	Dactylella
223	Dendrophoma
224	Dermateaceae sp. 1
225	Dermateaceae sp. 2
226	Dermateaceae sp. 3
227	Dermateaceae sp. 4
228	Dermateaceae sp. 5
229	Dermateaceae sp. 6
230	Dermateaceae sp. 7

231	Dermateaceae sp. 8
232	Devriesia
233	Diplochytridium
234	Diplogelasinospora
235	Discocistella
236	Diversispora
237	Dothideomycetes sp. 1
238	Dothideomycetes sp. 2
239	Dothideomycetes sp. 3
240	Dothideomycetes sp. 4
241	Elaphomyces
242	Entoloma
243	Entrophospora
244	Exophiala
245	Fimetariella
246	Flagelloscypha
247	Fusidium
248	Galerina
249	Ganoderma
250	Gautieria
251	Geastrum
252	Geoglossum
253	Geomyces
254	Gibellulopsis
255	Globulicium
256	Glomeromycota sp. 1
257	Glomeromycota sp. 10
258	Glomeromycota sp. 11
259	Glomeromycota sp. 12
260	Glomeromycota sp. 2
261	Glomeromycota sp. 3
262	Glomeromycota sp. 4
263	Glomeromycota sp. 5
264	Glomeromycota sp. 6
265	Glomeromycota sp. 7
266	Glomeromycota sp. 8
267	Glomeromycota sp. 9
268	Glomus
269	Goidanichiella
270	Gomphidius
271	Gyalecta
272	Gymnomyces
273	Gymnopus
274	Gyoerffyella
275	Gyroporus
276	Handkea
277	Helotiaceae sp. 1
278	Helotiaceae sp. 10
279	Helotiaceae sp. 11
280	Helotiaceae sp. 12
281	Helotiaceae sp. 13
282	Helotiaceae sp. 14
283	Helotiaceae sp. 15
284	Helotiaceae sp. 16
285	Helotiaceae sp. 2
286	Helotiaceae sp. 3
287	Helotiaceae sp. 4
288	Helotiaceae sp. 5
289	Helotiaceae sp. 6
290	Helotiaceae sp. 7
291	Helotiaceae sp. 8
292	Helotiaceae sp. 9
293	Helotiales sp. 1
294	Helotiales sp. 10
295	Helotiales sp. 11
296	Helotiales sp. 12
297	Helotiales sp. 13
298	Helotiales sp. 14
299	Helotiales sp. 15
300	Helotiales sp. 16
301	Helotiales sp. 17
302	Helotiales sp. 18
303	Helotiales sp. 19
304	Helotiales sp. 2
305	Helotiales sp. 20
306	Helotiales sp. 21
307	Helotiales sp. 22
308	Helotiales sp. 23

309	Helotiales sp. 24
310	Helotiales sp. 25
311	Helotiales sp. 26
312	Helotiales sp. 27
313	Helotiales sp. 28
314	Helotiales sp. 29
315	Helotiales sp. 3
316	Helotiales sp. 30
317	Helotiales sp. 31
318	Helotiales sp. 32
319	Helotiales sp. 33
320	Helotiales sp. 34
321	Helotiales sp. 35
322	Helotiales sp. 36
323	Helotiales sp. 37
324	Helotiales sp. 38
325	Helotiales sp. 39
326	Helotiales sp. 4
327	Helotiales sp. 40
328	Helotiales sp. 41
329	Helotiales sp. 42
330	Helotiales sp. 43
331	Helotiales sp. 44
332	Helotiales sp. 45
333	Helotiales sp. 46
334	Helotiales sp. 47
335	Helotiales sp. 48
336	Helotiales sp. 49
337	Helotiales sp. 5
338	Helotiales sp. 50
339	Helotiales sp. 51
340	Helotiales sp. 6
341	Helotiales sp. 7
342	Helotiales sp. 8
343	Helotiales sp. 9
344	Herpotrichia
345	Herpotrichiellaceae sp. 1
346	Herpotrichiellaceae sp. 10
347	Herpotrichiellaceae sp. 11
348	Herpotrichiellaceae sp. 12
349	Herpotrichiellaceae sp. 13
350	Herpotrichiellaceae sp. 2
351	Herpotrichiellaceae sp. 3
352	Herpotrichiellaceae sp. 4
353	Herpotrichiellaceae sp. 5
354	Herpotrichiellaceae sp. 6
355	Herpotrichiellaceae sp. 7
356	Herpotrichiellaceae sp. 8
357	Herpotrichiellaceae sp. 9
358	Heterobasidion
359	Heterochaetella
360	Hirsutella
361	Hyalodendriella
362	Hyaloscypha
363	Hyaloscyphaceae sp. 1
364	Hyaloscyphaceae sp. 10
365	Hyaloscyphaceae sp. 2
366	Hyaloscyphaceae sp. 3
367	Hyaloscyphaceae sp. 4
368	Hyaloscyphaceae sp. 5
369	Hyaloscyphaceae sp. 6
370	Hyaloscyphaceae sp. 7
371	Hyaloscyphaceae sp. 8
372	Hyaloscyphaceae sp. 9
373	Hydnodontaceae sp. 1
374	Hydnum
375	Hygrocybe
376	Hygrophorus
377	Hymenochaetales sp. 1
378	Hymenochaetales sp. 2
379	Hymenoscyphus
380	Hyphodontiella
381	Hypholoma
382	Hypocrea
383	Hypocreaceae sp. 1
384	Hypocreales sp. 1
385	Hypocreales sp. 2
386	Hypocreales sp. 3

387	Hypocreopsis
388	Hypomyces
389	Inocybe
390	Isaria
391	Kendrickiella
392	Kockovaella
393	Kurtzmanomyces
394	Kuzuhaea
395	Laccaria
396	Lachnum
397	Lactarius
398	Lecanoromycetes sp. 1
399	Lecanoromycetes sp. 10
400	Lecanoromycetes sp. 11
401	Lecanoromycetes sp. 12
402	Lecanoromycetes sp. 13
403	Lecanoromycetes sp. 14
404	Lecanoromycetes sp. 2
405	Lecanoromycetes sp. 3
406	Lecanoromycetes sp. 4
407	Lecanoromycetes sp. 5
408	Lecanoromycetes sp. 6
409	Lecanoromycetes sp. 7
410	Lecanoromycetes sp. 8
411	Lecanoromycetes sp. 9
412	Lecythophora
413	Leohumicola
414	Leotiomycetes sp. 1
415	Leotiomycetes sp. 10
416	Leotiomycetes sp. 11
417	Leotiomycetes sp. 12
418	Leotiomycetes sp. 13
419	Leotiomycetes sp. 14
420	Leotiomycetes sp. 15
421	Leotiomycetes sp. 16
422	Leotiomycetes sp. 17
423	Leotiomycetes sp. 18
424	Leotiomycetes sp. 19
425	Leotiomycetes sp. 2
426	Leotiomycetes sp. 3
427	Leotiomycetes sp. 4
428	Leotiomycetes sp. 5
429	Leotiomycetes sp. 6
430	Leotiomycetes sp. 7
431	Leotiomycetes sp. 8
432	Leotiomycetes sp. 9
433	Lepiota
434	Leptodontidium
435	Leptosporomyces
436	Leucoagaricus
437	Leucogaster
438	Leucophleps
439	Libertella
440	Lycoperdon
441	Lyophyllum
442	Marasmius
443	Massarina
444	Mastigobasidium
445	Melanogaster
446	Melanopsamella
447	Meliniomyces
448	Meruliporia
449	Micarea
450	Microbotryomycetes sp. 1
451	Microbotryomycetes sp. 2
452	Microglossum
453	Mitosporic Ascomycota sp. 1
454	Mitosporic Ascomycota sp. 2
455	Mitosporic Ascomycota sp. 3
456	Mitosporic Ascomycota sp. 4
457	Mitosporic Ascomycota sp. 5
458	Mitosporic Ascomycota sp. 6
459	Mitosporic Dothioraceae sp. 1
460	Mitosporic Helotiales sp. 1
461	Mitosporic Helotiales sp. 10
462	Mitosporic Helotiales sp. 11
463	Mitosporic Helotiales sp. 12
464	Mitosporic Helotiales sp. 2

465	Mitosporic Helotiales sp. 3
466	Mitosporic Helotiales sp. 4
467	Mitosporic Helotiales sp. 5
468	Mitosporic Helotiales sp. 6
469	Mitosporic Helotiales sp. 7
470	Mitosporic Helotiales sp. 8
471	Mitosporic Helotiales sp. 9
472	Mitosporic Herpotrichiellaceae sp. 1
473	Mollisia
474	Monodictys
475	Morchella
476	Mortierella
477	Mortierellales sp. 1
478	Mycena
479	Mycoarthris
480	Mycosphaerella
481	Mytilinidion
482	Myxotrichaceae sp. 1
483	Myxotrichaceae sp. 2
484	Myxotrichaceae sp. 3
485	Myxotrichaceae sp. 4
486	Myxotrichaceae sp. 5
487	Myxotrichaceae sp. 6
488	Neoaleurodiscus
489	Neocallimastix
490	Neofabraea
491	Neonectria
492	Neuospora
493	Nidularia
494	Nigrospora
495	Nolanea
496	Oidiodendron
497	Oliveonia
498	Paecilomyces
499	Parmelia
500	Penicillium
501	Pezicula
502	Pezizales sp. 1
503	Pezizales sp. 2
504	Pezizomycetes sp. 1
505	Pezizomycetes sp. 10
506	Pezizomycetes sp. 11
507	Pezizomycetes sp. 12
508	Pezizomycetes sp. 13
509	Pezizomycetes sp. 14
510	Pezizomycetes sp. 15
511	Pezizomycetes sp. 16
512	Pezizomycetes sp. 17
513	Pezizomycetes sp. 18
514	Pezizomycetes sp. 19
515	Pezizomycetes sp. 2
516	Pezizomycetes sp. 3
517	Pezizomycetes sp. 4
518	Pezizomycetes sp. 5
519	Pezizomycetes sp. 6
520	Pezizomycetes sp. 7
521	Pezizomycetes sp. 8
522	Pezizomycetes sp. 9
523	Phellodon
524	Phialocephala
525	Phialophora
526	Phlebiella
527	Pholiota
528	Phoma
529	Piloderma
530	Placopsis
531	Plectania
532	Plectosphaerellaceae sp. 1
533	Pleosporales sp. 1
534	Pleosporales sp. 2
535	Pochonia
536	Podosordaria
537	Polyporales sp. 1
538	Polyporales sp. 2
539	Porotheleum
540	Preussia
541	Pseudeurotium
542	Pseudohydnum

543	Pseudopenidiella
544	Pseudotomentella
545	Pyrenochaeta
546	Pyronema
547	Ramaria
548	Ramariopsis
549	Rasamsonia
550	Rhinocladiella
551	Rhizoctonia
552	Rhizophagus
553	Rhizophydium
554	Rhizopogon
555	Rhizoscyphus
556	Rhizosphaera
557	Rhodotorula
558	Russula
559	Russulaceae sp. 1
560	Saccharomycetales sp. 1
561	Saccharomycetes sp. 1
562	Saccharomycetes sp. 2
563	Saccharomycetes sp. 3
564	Saccharomycetes sp. 4
565	Saccharomycetes sp. 5
566	Saccharomycetes sp. 6
567	Sagenomella
568	Sarcoleotia
569	Sarcosoma
570	Sarcosomataceae sp. 1
571	Scleroderma
572	Scutellospora
573	Scytalidum
574	Sebacina
575	Seimatosporium
576	Sistotrema
577	Sordariales sp. 1
578	Sordariales sp. 2
579	Sordariomycetes sp. 1
580	Sordariomycetes sp. 2
581	Spadicoides
582	Sphaerobolus
583	Sphaerophorus
584	Sphaerosporella
585	Sporidiobolales sp. 1
586	Sporobolomyces
587	Stibella
588	Suillus
589	Syzygospora
590	Talaromyces
591	Tetracladium
592	Thelephoraceae sp. 1
593	Thysanophora
594	Tomentella
595	Tomentellopsis
596	Trechispora
597	Trechisporaceae sp. 1
598	Trechisporales sp. 1
599	Tremellales sp. 1
600	Tremellales sp. 2
601	Tremellales sp. 3
602	Tremellales sp. 4
603	Tremellomycetes sp. 1
604	Tremellomycetes sp. 2
605	Tricharina
606	Trichocladium
607	Trichocomaceae sp. 1
608	Trichocomaceae sp. 2
609	Trichocomaceae sp. 3
610	Trichoderma
611	Tricholoma
612	Tricholomataceae sp. 1
613	Tricholomataceae sp. 2
614	Tricholomataceae sp. 3
615	Tricholomataceae sp. 4
616	Trichophaea
617	Trichosporon
618	Troposporella
619	Truncocolumella
620	Tuber

621	Tuckermannopsis
622	Tulasnella
623	Tylospora
624	Umbelopsis
625	Umbilicaria
626	Utharomyces
627	Varicosporium
628	Venturia
629	Verrucariales sp. 1
630	Verrucariales sp. 2
631	Verrucariales sp. 3
632	Verrucariales sp. 4
633	Verrucariales sp. 5
634	Verrucariales sp. 6
635	Verrucariales sp. 7
636	Verrucariales sp. 8
637	Verticillium
638	Wallemia
639	Wilcoxina
640	Xenopolyscytalum
641	Xerocomus
642	Xylaria
643	Xylariaceae sp. 1
644	Xylariales sp. 1
645	Zalerion
646	Zygomycota sp. 1
647	Zygomycota sp. 2
648	Zygomycota sp. 3
649	Zygomycota sp. 4
650	Zygomycota sp. 5
651	Zygomycota sp. 6
652	Zygomycota sp. 7
653	Zygomycota sp. 8

Appendix 8:

OTU #	Bacterial 454 Taxonomic Identity Summarized at the Family Level
1	k__Bacteria; p__; c__; o__; f__
2	k__Bacteria; p__AD3; c__; o__; f__
3	k__Bacteria; p__AD3; c__ABS-6; o__; f__
4	k__Bacteria; p__AD3; c__JG37-AG-4; o__; f__
5	k__Bacteria; p__Acidobacteria; c__; o__; f__
6	k__Bacteria; p__Acidobacteria; c__Acidobacteria; o__Acidobacteriales; f__Acidobacteriaceae
7	k__Bacteria; p__Acidobacteria; c__Acidobacteria; o__Acidobacteriales; f__Koribacteraceae
8	k__Bacteria; p__Acidobacteria; c__Acidobacteria-2; o__; f__
9	k__Bacteria; p__Acidobacteria; c__Acidobacteria-5; o__; f__
10	k__Bacteria; p__Acidobacteria; c__Acidobacteria-6; o__CCU21; f__
11	k__Bacteria; p__Acidobacteria; c__Acidobacteria-6; o__iii1-15; f__
12	k__Bacteria; p__Acidobacteria; c__Acidobacteria-6; o__iii1-15; f__RB40
13	k__Bacteria; p__Acidobacteria; c__Acidobacteria-6; o__iii1-15; f__mb2424
14	k__Bacteria; p__Acidobacteria; c__BPC102; o__; f__
15	k__Bacteria; p__Acidobacteria; c__Chloracidobacteria; o__; f__
16	k__Bacteria; p__Acidobacteria; c__Holophagae; o__Holophagales; f__Holophagaceae
17	k__Bacteria; p__Acidobacteria; c__PAUC37f; o__; f__
18	k__Bacteria; p__Acidobacteria; c__RB25; o__; f__
19	k__Bacteria; p__Acidobacteria; c__Solibacteres; o__Solibacterales; f__Solibacteraceae
20	k__Bacteria; p__Acidobacteria; c__Sva0725; o__Sva0725; f__
21	k__Bacteria; p__Acidobacteria; c__TM1; o__; f__
22	k__Bacteria; p__Acidobacteria; c__iii1-8; o__32-20; f__
23	k__Bacteria; p__Acidobacteria; c__iii1-8; o__DS-18; f__
24	k__Bacteria; p__Acidobacteria; c__iii1-8; o__SJA-36; f__
25	k__Bacteria; p__Actinobacteria; c__; o__; f__
26	k__Bacteria; p__Actinobacteria; c__Acidimicrobiia; o__Acidimicrobiales; f__
27	k__Bacteria; p__Actinobacteria; c__Acidimicrobiia; o__Acidimicrobiales; f__AKIW874
28	k__Bacteria; p__Actinobacteria; c__Acidimicrobiia; o__Acidimicrobiales; f__C111
29	k__Bacteria; p__Actinobacteria; c__Acidimicrobiia; o__Acidimicrobiales; f__EB1017
30	k__Bacteria; p__Actinobacteria; c__Acidimicrobiia; o__Acidimicrobiales; f__Microthrixaceae
31	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__; f__
32	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__
33	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__ACK-M1
34	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Actinospicaceae
35	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Actinosynnemataceae
36	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Catenulisporaceae
37	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Cellulomonadaceae
38	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Corynebacteriaceae
39	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Frankiaceae
40	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Gordoniaceae
41	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Kineosporiaceae
42	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Microbacteriaceae
43	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae
44	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micromonosporaceae
45	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Mycobacteriaceae

46	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Nakamurellaceae
47	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Nocardiaceae
48	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Nocardioidaceae
49	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Pseudonocardiaceae
50	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Sporichthyaceae
51	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Streptomycetaceae
52	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Streptosporangiaceae
53	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Thermomonosporaceae
54	k__Bacteria; p__Actinobacteria; c__MB-A2-108; o__; f__
55	k__Bacteria; p__Actinobacteria; c__MB-A2-108; o__0319-7L14; f__
56	k__Bacteria; p__Actinobacteria; c__Thermoleophilia; o__Gaiellales; f__
57	k__Bacteria; p__Actinobacteria; c__Thermoleophilia; o__Gaiellales; f__Gaiellaceae
58	k__Bacteria; p__Actinobacteria; c__Thermoleophilia; o__Solirubrobacterales; f__
59	k__Bacteria; p__Actinobacteria; c__Thermoleophilia; o__Solirubrobacterales; f__Conexibacteraceae
60	k__Bacteria; p__Actinobacteria; c__Thermoleophilia; o__Solirubrobacterales; f__Patulibacteraceae
61	k__Bacteria; p__Actinobacteria; c__Thermoleophilia; o__Solirubrobacterales; f__Solirubrobacteraceae
62	k__Bacteria; p__Armatimonadetes; c__Armatimonadia; o__Armatimonadales; f__Armatimonadaceae
63	k__Bacteria; p__Armatimonadetes; c__Armatimonadia; o__Armatimonadales; f__WD294
64	k__Bacteria; p__Armatimonadetes; c__Chthonomonadetes; o__Chthonomonadales; f__Chthonomonadaceae
65	k__Bacteria; p__Armatimonadetes; c__S1a-1H; o__; f__
66	k__Bacteria; p__BRC1; c__PRR-11; o__; f__
67	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__; f__
68	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae
69	k__Bacteria; p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__
70	k__Bacteria; p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__Chitinophagaceae
71	k__Bacteria; p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__Flammeovirgaceae
72	k__Bacteria; p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__Flexibacteraceae
73	k__Bacteria; p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__Saprospiraceae
74	k__Bacteria; p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__Sphingobacteriaceae
75	k__Bacteria; p__Chlorobi; c__Chlorobia; o__Chlorobiales; f__Chlorobiaceae
76	k__Bacteria; p__Chlorobi; c__Chlorobia; o__[Chloroherpetales]; f__SM1B02
77	k__Bacteria; p__Chlorobi; c__SJA-28; o__; f__
78	k__Bacteria; p__Chloroflexi; c__Anaerolineae; o__; f__
79	k__Bacteria; p__Chloroflexi; c__Anaerolineae; o__Caldilineales; f__Caldilineaceae
80	k__Bacteria; p__Chloroflexi; c__Anaerolineae; o__H39; f__
81	k__Bacteria; p__Chloroflexi; c__Anaerolineae; o__SBR1031; f__A4b
82	k__Bacteria; p__Chloroflexi; c__Anaerolineae; o__SBR1031; f__oc28
83	k__Bacteria; p__Chloroflexi; c__Bljii12; o__; f__
84	k__Bacteria; p__Chloroflexi; c__Bljii12; o__AKYG885; f__
85	k__Bacteria; p__Chloroflexi; c__Bljii12; o__AKYG885; f__5B-12
86	k__Bacteria; p__Chloroflexi; c__Bljii12; o__AKYG885; f__Dolo_23
87	k__Bacteria; p__Chloroflexi; c__Bljii12; o__B07_WMSP1; f__

88	k__Bacteria; p__Chloroflexi; c__Bljii12; o__B07_WMSP1; f__FFCH4570
89	k__Bacteria; p__Chloroflexi; c__Chloroflexi; o__Roseiflexales; f__Kouleothrixaceae
90	k__Bacteria; p__Chloroflexi; c__Ellin6529; o__; f__
91	k__Bacteria; p__Chloroflexi; c__Ktedonobacteria; o__; f__
92	k__Bacteria; p__Chloroflexi; c__Ktedonobacteria; o__JG30-KF-AS9; f__
93	k__Bacteria; p__Chloroflexi; c__Ktedonobacteria; o__Thermogemmatisporales; f__
94	k__Bacteria; p__Chloroflexi; c__Ktedonobacteria; o__Thermogemmatisporales; f__Thermogemmatisporaceae
95	k__Bacteria; p__Chloroflexi; c__RA13C7; o__; f__
96	k__Bacteria; p__Chloroflexi; c__TK17; o__; f__
97	k__Bacteria; p__Chloroflexi; c__TK17; o__S085; f__
98	k__Bacteria; p__Chloroflexi; c__TK17; o__mle1-48; f__
99	k__Bacteria; p__Chloroflexi; c__Thermobacula; o__Thermobaculales; f__Thermobaculaceae
100	k__Bacteria; p__Chloroflexi; c__Thermomicrobia; o__AKYG1722; f__
101	k__Bacteria; p__Cyanobacteria; c__4C0d-2; o__MLE1-12; f__
102	k__Bacteria; p__Cyanobacteria; c__Chloroplast; o__Streptophyta; f__
103	k__Bacteria; p__Elusimicrobia; c__Elusimicrobia; o__FAC88; f__
104	k__Bacteria; p__Elusimicrobia; c__Elusimicrobia; o__IIb; f__
105	k__Bacteria; p__FCPU426; c__; o__; f__
106	k__Bacteria; p__Fibrobacteres; c__Fibrobacteria; o__258ds10; f__
107	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Alicyclobacillaceae
108	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae
109	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Paenibacillaceae
110	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Thermoactinomycetaceae
111	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Peptostreptococcaceae
112	k__Bacteria; p__GN02; c__BD1-5; o__; f__
113	k__Bacteria; p__Gemmatimonadetes; c__Gemm-1; o__; f__
114	k__Bacteria; p__Gemmatimonadetes; c__Gemm-3; o__; f__
115	k__Bacteria; p__Gemmatimonadetes; c__Gemm-5; o__; f__
116	k__Bacteria; p__Gemmatimonadetes; c__Gemmatimonadetes; o__; f__
117	k__Bacteria; p__Gemmatimonadetes; c__Gemmatimonadetes; o__Ellin5290; f__
118	k__Bacteria; p__Gemmatimonadetes; c__Gemmatimonadetes; o__Gemmatimonadales; f__Ellin5301
119	k__Bacteria; p__Gemmatimonadetes; c__Gemmatimonadetes; o__Gemmatimonadales; f__Gemmatimonadaceae
120	k__Bacteria; p__Gemmatimonadetes; c__Gemmatimonadetes; o__KD8-87; f__
121	k__Bacteria; p__Gemmatimonadetes; c__Gemmatimonadetes; o__N1423WL; f__
122	k__Bacteria; p__Nitrospirae; c__Nitrospira; o__Nitrospirales; f__
123	k__Bacteria; p__Nitrospirae; c__Nitrospira; o__Nitrospirales; f__Nitrospiraceae
124	k__Bacteria; p__OD1; c__; o__; f__
125	k__Bacteria; p__OD1; c__SM2F11; o__; f__
126	k__Bacteria; p__OD1; c__ZB2; o__; f__
127	k__Bacteria; p__OP11; c__OP11-1; o__; f__
128	k__Bacteria; p__Planctomycetes; c__; o__; f__
129	k__Bacteria; p__Planctomycetes; c__BD7-11; o__; f__
130	k__Bacteria; p__Planctomycetes; c__OM190; o__; f__
131	k__Bacteria; p__Planctomycetes; c__OM190; o__agg27; f__
132	k__Bacteria; p__Planctomycetes; c__Phycisphaerae; o__; f__
133	k__Bacteria; p__Planctomycetes; c__Phycisphaerae; o__Phycisphaerales; f__
134	k__Bacteria; p__Planctomycetes; c__Phycisphaerae; o__Phycisphaerales; f__Phycisphaeraceae
135	k__Bacteria; p__Planctomycetes; c__Pla4; o__; f__
136	k__Bacteria; p__Planctomycetes; c__Planctomycetia; o__Gemmatales; f__Gemmataceae
137	k__Bacteria; p__Planctomycetes; c__Planctomycetia; o__Gemmatales; f__Isosphaeraceae
138	k__Bacteria; p__Planctomycetes; c__Planctomycetia; o__Planctomycetales; f__Planctomycetaceae
139	k__Bacteria; p__Planctomycetes; c__vadinHA49; o__DH61; f__

140	k__Bacteria; p__Proteobacteria; c__; o__; f__
141	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__; f__
142	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__BD7-3; f__
143	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Caulobacterales; f__
144	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Caulobacterales; f__Caulobacteraceae
145	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Ellin329; f__
146	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__
147	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Beijerinckiaceae
148	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Bradyrhizobiaceae
149	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Hyphomicrobiaceae
150	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Methylobacteriaceae
151	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Methylocystaceae
152	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Phyllobacteriaceae
153	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Rhizobiaceae
154	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Rhodobiaceae
155	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Xanthobacteraceae
156	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodobacterales; f__Hyphomonadaceae
157	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodobacterales; f__Rhodobacteraceae
158	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__
159	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__Acetobacteraceae
160	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria;
	o__Rhodospirillales; f__Rhodospirillaceae
161	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__
162	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Erythrobacteraceae
163	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae
164	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__; f__
165	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__A21b; f__EB1003
166	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__A21b; f__UD5
167	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__BVC71; f__
168	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Alcaligenaceae
169	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Burkholderiaceae
170	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Comamonadaceae
171	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae
172	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Ellin6067; f__
173	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Gallionellales; f__
174	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__IS-44; f__
175	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__MBT18; f__
176	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__MND1; f__
177	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Methylophilales; f__Methylophilaceae
178	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Rhodocyclales; f__Rhodocyclaceae
179	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__SC-I-84; f__
180	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__; f__
181	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Bdellovibrionales; f__

182	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Bdellovibrionales; f__Bdellovibrionaceae
183	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__MIZ46; f__
184	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Myxococcales; f__
185	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Myxococcales; f__0319-6G20
186	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Myxococcales; f__Haliangiaceae
187	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Myxococcales; f__Myxococcaceae
188	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Myxococcales; f__OM27
189	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Myxococcales; f__Polyangiaceae
190	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__NB1-j; f__MND4
191	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Spirobacillales; f__
192	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Syntrophobacterales; f__Syntrophaceae
193	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Syntrophobacterales; f__Syntrophobacteraceae
194	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Syntrophobacterales; f__Syntrophorhabdaceae
195	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__[Entotheonellales]; f__[Entotheonellaceae]
196	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Chromatiales; f__
197	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Legionellales; f__
198	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Legionellales; f__Coxiellaceae
199	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Legionellales; f__Legionellaceae
200	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Moraxellaceae
201	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae
202	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__
203	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Sinobacteraceae
204	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae
205	k__Bacteria; p__TM6; c__SJA-4; o__; f__
206	k__Bacteria; p__TM7; c__SC3; o__; f__
207	k__Bacteria; p__TM7; c__TM7-1; o__; f__
208	k__Bacteria; p__TM7; c__TM7-3; o__; f__
209	k__Bacteria; p__TM7; c__TM7-3; o__EW055; f__
210	k__Bacteria; p__Tenericutes; c__Mollicutes; o__Anaeroplasmatales; f__Anaeroplasmataceae
211	k__Bacteria; p__Thermi; c__Deinococci; o__Deinococcales; f__Deinococcaceae
212	k__Bacteria; p__Verrucomicrobia; c__[Spartobacteria]; o__[Chthoniobacterales]; f__[Chthoniobacteraceae]
213	k__Bacteria; p__WPS-2; c__; o__; f__
214	k__Bacteria; p__WS3; c__PRR-12; o__LD1-PA13; f__
215	k__Bacteria; p__WS3; c__PRR-12; o__Sediment-1; f__
216	k__Bacteria; p__WS3; c__PRR-12; o__Sediment-1; f__PRR-10
217	k__Bacteria; p__WS6; c__B142; o__; f__