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Abstract approved:

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Peter J. Bottomley

Ectomycorrhizal (ECM) fungi of the genera *Piloderma* and *Ramaria* form dense hyphal mats in the organic and upper mineral soil layers, respectively, in coniferous forest floors of the Pacific Northwest. Previous studies have determined that fungal mats change the chemical, physical, and biological properties of the soil within. Little information exists on the dynamics of development and decline of *Piloderma* and *Ramaria* mats, and the type of fungal and bacterial communities that are associated with the mats. To address these topics, a reciprocal soil core experiment was installed in *Piloderma* and *Ramaria* mats found in old-growth Douglas-fir (*Pseudotsuga menziesii)* stands in the H.J. Andrews Experimental Forest located in the Oregon Cascade Mountains. Samples from each mat type were severed from the host tree and transplanted in closed PVC cores, preventing new root and mat growth, to adjacent non-mat soil. In contrast, non-mat soil cores were transplanted into mats in open mesh netting, allowing tree root and mat development to occur. Cores were sampled at 10, 16, and 24 months after establishment, and changes in mat development and fungal and bacterial communities were monitored by measuring the number of ECM colonized root tips, fungal mat DNA fragment indicator species, and

fungal and bacterial community composition with Terminal Restriction Length Polymorphism (T-RFLP) and Length Heterogeneity PCR (LH-PCR) of ITS and 16S rDNA. DNA fragments representing mat-forming indicator species were detected in the development of *Piloderma* mats starting at 16 and continuing through 24 months post establishment. Both *Piloderma* and *Ramaria* mat fungal communities changed rapidly in cores that had been severed from the host but mat-associated fungal communities had not developed even two years post intimate contact by non-mat soil cores with mats. The richness of fungal and bacterial operational taxonomic units (OTU) in the *Piloderma* mats increased when they were severed from the host, but decreased when *Ramaria* fungal mats were severed from their host. Further study is required to determine the length of time required to develop mat-associated microbial communities from non-mat soil, as well as the identity and function of fungi and bacteria responsible for community changes.

Episodic Dynamics of Microbial Communities Associated with the Birth and Death of Ectomycorrhizal Mats in Old-growth Douglas-fir Stands

by
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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.

Joseph H. Blanchard, Author

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TABLE OF CONTENTS

Page

1. Introduction…… 1

2. Literature Review…….. 4

3. Materials and Methods……..…… 11

3.1. Site description……..... 11

3.2. Chemical Soil Properties… ……..... 11

3.3. Experimental design…….... 12

3.4. Field Sampling…….. 13

3.5. DNA extraction and purification……..... 14

3.6. PCR protocol…….… 14

3.7. Molecular characterization of mats……...… 15

3.8. LH-PCR and T-RFLP profiles……...… 16

3.9. Fungal ITS and bacterial 16s community data analysis…… 17

4. Results…….……...….. 22

4.1. Chemical analysis of soils associated with Piloderma and Ramaria mats relative to non-mat soil…….……..... 22

4.2. Microbial community shifts following the birth and death of *Piloderma* and *Ramaria* mats……. 22

4.2.1. Fungal community response to treatments……. 22
4.2.2. Bacterial community response to treatments……. 24

4.3. Fungal and bacterial OTUs following the birth and death of *Piloderma* and *Ramaria* mats……. 24

4.4. *Piloderma* and *Ramaria* mat dynamics……..... 26

TABLE OF CONTENTS (Continued)

Page

4.4.1. Colonized root tips……. 26

4.4.2. *Piloderma* indicators…….. 26

5. Discussion…….…….. 41

5.1. *Piloderma* mat indicators……. 42

5.2. Fungal community dynamics……. 43

5.3. Fungal and bacterial OTU richness…… 44

6. Conclusion……... 46

Bibliography……. 47

LIST OF FIGURES

Figure — Page

1. Diagram of the experimental design……... 20

2. Example of the Genotyper® output of a *Hinf* 1 restricted T-RFLP profile from a *Piloderma* mat sample ……. 21

3. Mean soil pH ± SE (n=21) for all three post treatment samplings, Spring 2007, Fall 2007, Spring 2008……..... 29

4. NMS ordination plots of fungal communities in soil associated with *Piloderma* mat, adjacent non-mat, and birth and death treatments based on LH-PCR and T-RFLP analysis of the ITS region of rDNA extracted from soil cores at three sampling times……..... 30

5. NMS ordination plots of fungal communities in soil associated with *Ramaria* mat, adjacent non-mat, and birth and death treatments based on LH-PCR and T-RFLP analysis of the ITS region of rDNA extracted from soil cores at three sampling times……. 31

6. NMS ordination plots of bacterial communities in soil associated with *Piloderma* mat, adjacent non-mat, and birth and death treatments based on T-RFLP analysis of the 16S rDNA gene sequence extracted from soil cores at three sampling times…….…….. 32

7. NMS ordination plots of bacterial communities in soil associated with *Ramaria* mat, adjacent non-mat, and birth and death treatments based on T-RFLP analysis of the 16S rDNA gene sequence extracted from soil cores at three sampling times…….…….. 33

8. Cumulative number of OTUs identified in the different soil treatments at installation (T0) as well as at three post-treatment sampling times (T1-T3). Points at T0 and T1 represent the sum of seven samples…..…….. 34

9. A histogram of the total number of occurrences of each OTU identified from *Piloderma* mat and death treatments from seven sites at three sampling times (T1-T3)…… ……. 35

10. A histogram of the total number of occurrences of each OTU identified from *Ramaria* mat and death treatments from seven sites at three sampling times (T1-T3)…….……. 36

LIST OF FIGURES (Continued)

Figure Page

11. A histogram of the 10% most frequently encountered fungal OTUs identified in mat and death treatments from seven sites at three sampling times (T1-T3)…… ……..…….. 37

12. Number of ECM colonized root tips recovered from cores of the different treatments at three sampling times (T1-T3)…… ……..... 38

13. Relative abundance of *Piloderma* indicator peaks found in *Piloderma* mat and adjacent non-mat soil cores taken at installation (T0) as well as from, *Piloderma* mat, adjacent non-mat, and birth and death treatments at three sampling times (T1-T3)…….... 39

14. Correlation between the numbers of ECM colonized root tips recovered from soil cores and Piloderma mat indicator peaks from *Piloderma* mat, adjacent non-mat, and birth and death treatments at three sampling times (T1-T3)……. 40

LIST OF TABLES

Table Page

1. Identity of selected ECM mats in this study …… 19

2. Oligonucleotide primers for fungal and bacterial community analysis …… 19

3. The levels of various elements in soil from *Piloderma* and *Ramaria* mats compared with adjacent non-mat soils …….. 27

Episodic Dynamics of Microbial Communities Associated with the Birth and Death of Ectomycorrhizal Mats in Old-growth Douglas-fir Stands

1. Introduction

Ectomycorrhizal (ECM) fungi form a symbiotic relationship with many tree species that facilitates tree growth through the exchange of soil nutrients for carbon (Smith and Read 2008). In some forests, including ones dominated by Douglas-fir (*Pseudotsuga menziesii)*, a specific type of ECM structure is common. This type of ECM fungal structure is generally referred to as a "mat" and has been studied extensively in forests of the Pacific Northwest. Mats have been recently described by Dunham et al. (2007) as dense profusions of rhizomorphs with obvious ECM root tips that aggregate soil and alter its appearance and are uniform in structure and appearance.

Fungal mats have been shown to change soil chemical properties and mineral nutrition in the soil rhizosphere (Entry et al. 1992; Griffiths et al. 1994). Soil surrounding fungal mats can be more acidic and contain higher concentrations of oxalic acid than adjacent non-mat soil. These factors may play a major role in mineral weathering and mineral nutrient availability in mat soils (Cromack et al. 1979; Malajczuk and Cromack 1982; Griffiths et al. 1994). Soil biological characteristics are also impacted in mats soils; increased microbial biomass (Cromack 1988; Entry et al. 1991) can result in increased numbers of soil animals including microarthropods and protozoans (Cromack et al. 1988). Along with increased biomass and activity, carbon (C) and nitrogen (N) cycling have been reported to be altered by fungal mats (Entry et al. 1991). It has been suggested that along with the

release of carbon-rich organic compounds into the mat soil, an observed increase in the C:N ratio in mats might also indicate that the fungal mat community is selectively removing organic nitrogen from the soil (Griffiths and Caldwell 1992).

Fungal mats can alter the soil environment and may effect change in the associated microbial composition both qualitatively and quantitatively. ECM colonization contributes to changes in the release of root exudates in the mycorrhizosphere. For example, high amounts of oxalate in the mat may increase the number of oxalate-degrading bacteria. Studies have shown a high incidence of oxalate-degrading streptomyces associated with mats in Douglas-fir stands in the Pacific Northwest (Knutson et al. 1980). The lower pH and reduced moisture content of the mat may favor fungi over bacteria (Griffiths and Caldwell 1992). Additionally, the turnover of ECM hyphal material may influence microbial community composition by favoring chitin degrading bacteria (Bending et al. 2002).

In a recent study, *Piloderma* and *Ramaria* species were found to be present in the mat-forming fungal communities in old-growth Douglas-fir stands in the H.J. Andrews Experimental Forest (Dunham et al. 2007). The stands used in the current study were representatives of those described by Dunham et al. (2007) and chosen because they contain the dominant ECM species; namely, *Piloderma* spp. in the organic horizon and *Ramaria* spp. in the mineral horizon. The objective of this study was to describe the structural changes of microbial communities during the development and decline of *Piloderma* and *Ramaria* ECM mats associated with old growth Douglas-fir stands. The ECM associated microbial community was tracked during the development of a new mat, and the decline of a mat when severed from its

host. I hypothesized that the birth of an ECM mat would result in gradual development of an associated microbial community similar to the mat, and that the death of the mat would contribute to the associated microbial community changing rapidly before reverting to a non-mat state.

2. Literature Review

The study of mycorrhizae provides a link between above-ground and below-ground ecosystem processes and aids in our understanding of ecosystems as a whole. Mycorrhizae refers to the symbiotic relationship between fungi and plant roots. Although while generally considered a mutualistic symbiotic relationship in which both plant and fungus benefit from the association, mycorrhizae can fall along the spectrum of parasitism to mutualism, depending on the specific plant and fungal genotypes and their abiotic and biotic environments (Jones and Smith 2004).

In this relationship the mycorrhizae receive carbon from the host plant and in exchange provide water and nutrients found in the soil including N, phosphorus (P), and potassium (K) to the host. Unlike saprotrophic fungi, mycorrhizal fungi are not restricted to living off the patchily distributed carbon resources in the soil and can explore for nutrients and water with extensive hyphael networks. In addition to gathering nutrients, these fungi also aid tree nutrition by mineral weathering (Landeweert et al. 2001) and mobilization of nutrients from organic matter (Read and Perez-Moreno 2003).

Several major mycorrhizal forms have been classified according to their morphology. ECM are distinguished from other forms by a mantel of hyphae which encloses the root, growth of hyphae between the epidermal and cortical cells called the Hartig net, and an outwardly growing system of hyphae that forms a connection with the soil. Between 5,000 and 6,000 species of fungi form ectomycorrhizae. The majority of these are basidiomycetes, but a significant number are ascomycetes (Smith and Read 1998).

Douglas-fir in Western North America may have as many as 2,000 different ECM fungal associates (Molina et al. 1992). These trees also have one of the largest assemblages of genus-specific fungi with an estimated 250 fungi specific to *Pseudotsuga* (Smith and Read 1997). Although while a relatively small number of plants are ECM compared to other forms of mycorrhizae, their coverage of terrestrial ecosystems and role in the production of coniferous trees for timber, is disproportionately large (Smith and Read 2008). Members of the predominantly ectomycorrhizal family Pinaceae, to which Douglas-fir belongs, dominate the vast boreal and temperate forests of the Northern Hemisphere. In some forests, including ones dominated by Douglas-fir, a specific type of ECM structure is common. This type of ECM fungal structure is generally referred to as a "mat" and has been studied extensively in forests of the Pacific Northwest.

Some species of ectomycorrhizal fungi form dense mats in the organic or mineral soil layers in many forested systems (Griffiths et al. 1991b). Mats have been recently described by Dunham et al. (2007) as dense profusions of rhizomorphs with obvious ECM root tips that aggregate soil and alter its appearance.

Fungal mats have been observed in a variety of ecosystems from subtropical zones in Australia to boreal forests as far north as Alaska, as well as Finland, and Canada.(Hintikka and Naykki 1967; Fisher 1972; Cromack et al. 1979; Castellano 1988; Griffiths and Caldwell 1992). Mats can cover up to 25% of the surface area of a forest floor (Cromack et al. 1979; Griffiths et al. 1996), but their distribution is not random. Mats are more common in mature and old-growth stands than young stands and are most likely to be found close to host trees (Hintikka and Naykki 1967;

Griffiths et al. 1996; Smith et al. 2000). Although several mat types can exist in a single stand, there is very little physical overlap between mat types, and the distance between mats commonly ranges from 10 to 20 cm in old-growth stands (Griffiths et al. 1996). They most commonly occupy the interface of the forest floor and mineral soil, but certain ECM mats show preferential colonization of the organic or mineral soil layers (Griffiths et al. 1990, 1996; Entry et al. 1992, Smith et al. 2000, 2002).

The definition of mat size is not established, but many studies have described mats as being anywhere between 0.5 to 1.5 m in diameter and 1 to 10 cm thick (Hintikka and Naykki 1967; Fisher 1972; Entry et al. 1991, Dunham et al. 2007). The dense profusion of fungal material dominates the soil environment in mats, with fungal material occupying up to 50% of the dry weight of soil (Ingham et al. 1991). The mycelium in mats has been reported to grow outwards at an estimated rate of 2 to 3 cm annually, and eventually die at the center (Hintikka and Naykki 1967). Although mats have been reported to persist up to two years following tree harvest (Griffiths et al. 1996), little other evidence has been reported on the rate of turnover of ECM mats.

The physical changes that occur in a mat are immediately apparent when surveying the forest floor. The dense clusters of ECM hyphae, rhizomorphs, and root tips are clearly visible and can be white, black, yellow, or green in color. The fungal material in the mats is so densely aggregated that there is apparently no space for other ECM species (Agerer 2006). Mats in the mineral layer can be ashy in appearance and appear drier than the surrounding soil. Hydrophobicity has been measured in several mat- forming species (Unestam and Sun 1995), reducing the

moisture content within and preventing downward movement of rainwater (Hintikka and Naykki 1967).

With as much as 50% of the dry wt of soil collected from mats comprised strictly of fungal biomass (Ingham et al. 1991), these mats can have a profound impact on soil processes. Fungal mats change soil chemical properties and mineral nutrition in the soil rhizosphere. Higher concentrations of dissolved organic carbon (DOC), phosphate (PO_4^{3-}), sulfate (SO_4^{2-}), hydrogen (H^+), aluminum (Al^{3+}), iron (Fe^{3+}), boron (B), K^+, copper (Cu^{2+}), manganese (Mn^{2+}), and zinc (Zn^{2+}) were found in mat soils (Entry et al. 1992; Griffiths et al. 1994). Lower pH and high concentrations of oxalic acid have been observed in fungal mats, which may be factors that play a role in mineral weathering and mineral nutrient availability in mat soils (Cromack et al. 1979; Malajczuk and Cromack 1982; Griffiths et al. 1994).

Biological characteristics of these soils are also influenced by fungal mats. For example, mats have been shown to have greater microbial biomass and soil respiration rates than surrounding non mat soil (Cromack et al. 1988; Entry et al. 1991). Studies show that mat biomass and respiration peak in the spring and fall when rainfall and temperature were optimal and are lowest during the summer (Griffiths et al. 1990; Ingham et al. 1991). This timeframe of activity coincides with high activity of Douglas-fir in the Pacific Northwest, suggesting a pairing of above and below ground biological activity. Biological impacts have been observed up the soil food web. An increase in the number of soil animals including microarthropods and protozoans has been observed, probably as a result of higher microbial biomass (Cromack et al. 1988).

Along with increased biomass and activity, changes in C and N cycling have been reported to be altered in fungal mats. The C:N ratio of mat soil is higher than non mat soil, suggesting that the fungal community is releasing organic C compounds with higher C:N ratios into the soil and/or the fungal mat community is selectively removing organic nitrogen from the soil (Griffiths and Caldwell 1992). The presence of high concentrations of oxalic acid in fungal mat soil provides one explanation for a strong N sink. Another one comes from the possibility that mats can processes complex detrital nitrogen. Physiologically, mat fungi are capable of using protein-N and polyphenol-complexed protein as N sources (Griffiths and Caldwell 1992), allowing access to an otherwise recalcitrant pool of detrital N (Perez-Moreno and Read 2000), and providing detrital N to the host tree without a need for microbial mineralization of N (Nasholm et al. 1998).

Most of the previous research done on ECM mat-forming fungi in Douglas-fir stands in the Pacific Northwest has identified mat forming fungal species by morphology and hypogeous fruiting bodies. Two main morphologies have been recognized in these ecosystems, those that dominate the organic layer with soil tightly aggregated by rhizomorphs and those that dominate the mineral layer with hydrophobic powdery structure (Griffiths et al. 1991a, 1996). The organic mat type has previously been associated with the genus *Hysterangium* (Cromack et al. 1979) and the mineral mat type has been associated with *Gautieria* (Griffiths et al. 1991a). In the first molecular based study of ECM fungal mats using sequence data from the rDNA ITS region, *Piloderma* and *Ramaria* species were found to dominate the mat forming fungal community in old-growth Douglas-fir stands in the PNW (Dunham et

al. 2007). The stands chosen for my study were those selected by Dunham et al. (2007) to contain the dominant ECM species; namely, *Piloderma* spp. in the organic horizon and *Ramaria* spp. in the mineral horizon.

The genus *Piloderma*, a basidiomycete belonging to the family *Atheliaceae*, dominated the communities of mat-forming fungi described in Dunham et al. (2007). Five *Piloderma* species were encountered, four of which formed mats, including species that are closely related to *P. fallax* and *P. byssinum* (Larsen et al. 1997). Dunham et al. (2007) suggests that species in this genus might have a role in N transport to ECM trees. In another recent study at the H.J. Andrews, a *P. fallax* species was found in over 50% of old-growth stands and was commonly associated with course woody debris (CWD) in advanced stages of decay (Smith et al 2002). A similar association with *P. fallax* and CWD was observed in a forest in Estonia, and the authors suggest this species has a role in the degradation of decayed wood and soil humic polymers to acquire extra carbon and other nutrients from polyaromatic complexes (Tedersoo et al. 2003).

Fungal mats exhibiting the powdery, hydrophobic morphology in upper mineral soils can be formed by several *Ramaria* species (Nouhra et al. 2005; Dunham et al. 2007). Species of the genus *Ramaria*, a basidiomycete belonging to the family Gomphaceae, were the most frequently encountered in mats exhibiting powdery, hydrophobic morphology in the upper mineral soil in Dunham et al. (2007). Species belonging to the genus *Ramaria* were also most commonly associated with old-growth stands (Smith et al. 2002; Dunham et al. 2007). *Ramaria* mats have similar morphologies and closely related phylogeny to the genus *Gautieria*, both of which are

in the family Gomphaceae, but it is yet to be seen if *Ramaria* mats affect soil chemistry and biology in ways similar to *Gautieria* mats (Griffiths et al. 1991b, 1996).

Cromack et al. (1988) pointed out in his study of soil animals in ECM mats, the relatively discrete nature of those species forming fungal mats affords opportunities to study processes associated with micro-organisms and soil animals as components of the mycorrhizosphere. The more homogeneous nature of mats compared to the rest of the forest soil environment and may simplify the interactions between microbes and their geochemical environment.

Fungal mats can alter the soil environment and may effect change in the associated microbial composition both qualitatively and quantitatively. ECM colonization contributes to changes in the release of root exudates in the mycorrhizosphere. For example, high amounts of oxalate in the mat may increase the number of oxalate-degrading bacteria. Oxalic acid decomposition occurs extremely rapidly in forest soil (Jones et al. 2001), and 50 to 85% of oxalate C added to organic horizons can be mineralized to CO_2 within six hours (van Hees et al. 2002). Studies have shown high incidences of oxalate-degrading streptomycetes associated with mats in Douglas-fir stands in the Pacific Northwest (Knutson et al. 1980). Isotopically labeled oxalate was incorporated into lipids of Gram-negative bacteria and actinomycetes in a recent study at the H.J. Andrews (Brant et al. 2006a). The higher pH and reduced moisture content of the mat may favor fungi over bacteria (Griffiths and Caldwell 1992). The turnover of ECM hyphal material may favor chitin degrading bacterial and fungal organisms.

3. Materials and Methods

3.1. Site description

The study was conducted at the H.J. Andrews Experimental Forest (44.2^{o} N, 122.2^{o} S), a 6400 ha research site located in the Cascade Mountains of Oregon, USA. This forest is typical of the maritime-influenced Pacific Northwest Mountain region with wet, mild winters and dry, cool summers. Mean monthly temperatures range from near 1 °C in January to 18 °C in July. Annual precipitation ranges from 230 cm at low elevations to 355 cm at high elevations, falling mainly in November through March. The bedrock is composed of Oligocene-lower Miocene volcanic rocks, andesite lava flows of Miocene age, and younger High Cascade rocks. The soil landscape is characterized by steep slopes caused by stream erosion, landslides, and glaciations. Soils across all sites are primarily Inceptisols (Dyrness 2005).

Seven sites containing *Piloderma* and *Ramaria* fungal mats were selected from a subset of 17 old-growth sites that were characterized by Dunham et al. (2007) in a survey of mat-forming ECM fungi at the H.J. Andrews LTER. Stands ranged from 696 m to 1155 m on north, south, and west facing slopes. All samples were taken from Douglas-fir dominated old-growth stands at least 400 years old.

3.2. Chemical Soil Properties

Soil pH was measured in all post treatment soil cores in a 1:2 soil to water dilution. Soil taken at the time of installation was air-dried and sieved (<4.75 mm). The concentrations of nitrate and ammonium (1N NH_4OAc pH= 7), total C and N (CNS 2000), and the extractable bases Ca, Mg, K, and Na (2M KCl) were determined

with standard methods of the Central Analytical Lab, Department of Crop and Soil Science, Oregon State University).

3.3. Experimental design

The experiment was installed in June 2006. Two *Piloderma* mats and two *Ramaria* mats were identified and selected to accommodate a number of temporal samples at each of the seven sites. *Piloderma* mats were identified in organic soil by the dense profusions of rhizomorphs associated with obvious ECM root tips. *Ramaria* mats were identified in mineral soil by their powdery, hydrophobic morphology associated with obvious ECM root tips. Selected mats of each type were ≥0.5 m in diameter. In most cases, the genus of each mat forming fungus was confirmed by molecular analysis of the fungal ITS region. Identification was accomplished by selecting colonized root tips with associated hyphal material from each mat. DNA was extracted, and direct sequencing (Table 1). Uncolonized soil adjacent to identified mats was flagged as non-mat soil.

Soil cores were 10 cm in diameter and 20 cm in length and included both organic and mineral soil layers. Cores from each mat type were transferred to non-mat soil and are referred to as *death* cores. The death cores were enclosed in PVC pipe to exclude root and hyphal penetration. Cores from non-mat soil were transferred into each mat type and are referred to as birth cores. A diagram of the experimental design is presented in Figure 1. The birth cores were enclosed in 2-mm size mesh barrier that allowed root and hyphal access. Fresh background cores were taken at the time of sampling, including one of each mat type and organic and mineral

non-mat soils. A disturbance control treatment was sampled at each time and represented non-mat soil cores that were sampled at initiation, enclosed in 2-mm mesh barrier and replaced back into non-mat soil.

3.4. Field Sampling

At the time of installation two replicate cores of each of organic non-mat, mineral non-mat, *Piloderma* mat, and *Ramaria* mat soil were taken at each of the seven sites. Sufficient cores were put in place to recover samples twice a year, in the spring after snowmelt and in the fall after wet-up. In actuality, cores were removed from each of the seven sites in April 2007, October 2007, and June 2008, 10, 16, and 24 months after establishment, respectively. At each site the following 10 samples were taken: (1) *Piloderma* death, (2) *Ramaria* death, (3) *Piloderma* birth, (4) *Ramaria* birth, (5) background *Piloderma* mat, (6) background *Ramari*a mat, (7) background organic non-mat, (8) background mineral non-mat, (9) disturbance control organic non-mat, and (10) disturbance control mineral non-mat. Background samples were fresh cores taken on each collection date. Organic and mineral soil was divided in the field by color and presence of partially decomposed needles and other organic material. Soil cores taken at the time of installation were taken back to the lab and frozen at -20°C for approximately six months before DNA was extracted. All other cores were brought back to the laboratory on the same day, stored overnight at 4° C, and sieved to <4.75 mm. Live root material was recovered from cores and stored at 4°C for 2 days until live and ECM colonized root tips could be counted. Using dissecting microscopes, roots tips were counted within one week of sampling

to evaluate root in-growth and ECM colonization according to Goodman et al. (1996). Samples with more than 100 live root tips or colonized root tips were given the score >100.

3.5. DNA extraction and purification

DNA was extracted from 0.25 g of sieved (<4.75 mm) and homogenized soil using the MOBio PowerSoil™ DNA isolation kit (MoBio Laboratories, Carlsbad, CA), according to manufacturer's instructions except that a Bio101 FastPrep instrument was used to lyse cells (Bio 101, Carlsbad, CA). The MOBio bead beating tubes were shaken for 45 s on the FastPrep (MP Biomedicals, Irvine, CA). DNA in extracts was quantified using a NanoDropTM ND-1000 UV-visible spectrophotometer (NanoDrop Technologies, Wilmington, DE) and diluted to 25 ng DNA•μl^{-1} for PCR amplification.

3.6. PCR protocol

DNA was amplified using fungal primers for the internal transcribed spacer region of rDNA using ITS1-F and ITS4 (Gardes and Bruns 1993; White et al. 1990), and bacterial primers 27F and 907R for 16S rDNA (Lane 1991; Muyzer et al. 1995) (Table 2). Primers were chosen to represent conserved DNA regions, about 1000 base pairs in length, that are commonly used in molecular systematics at the species level (Table 2). The forward primers were fluorescently labeled with 5'-6-FAM (6-carboxyfluorescein). Reaction mixtures (50 µl) contained soil DNA (100 ng), GoTaq™ DNA polymerase (2.5 U), GoTaq™ PCR Buffer (5x), $MgCl_2$ (2 mM),

deoxynucleoside triphosphates (0.2 mM each), forward and reverse primers (0.2 μM each), and bovine serum albumin (0.064 g ml^{-1}). Fungal community PCR amplification was conducted as follows using a PTC-100 hot bonnet thermocycler (MJ Research, Inc., Waltham, MA): 94° C for 5 min followed by 35 cycles of 94° C for 30 s, 55° C for 30 s, 72° C for 1 min. A final extension followed of 72° C for 7 min. Bacterial community PCR amplification was conducted as follows using a PTC-100 hot bonnet thermocycler (MJ Research, Inc., Waltham, MA): 94° C for 5 min followed by 30 cycles of 94° C for 30 s, 53° C for 1 min, 72° C for 30 s. A final extension followed for 72° C for 7 min. PCR products were visualized with electrophoresis on 1.1% agarose gels stained with ethidium bromide. PCR product was cleaned with a QIAquick™ PCR purification kit (QIAGEN sciences, Maryland) and quantified as described above.

3.7. Molecular characterization of mats

Roots tips and associated hyphal material from fungal mats were examined with stereomicroscopy to confirm colonization. A colonized root tip and associated fungal material was placed in cetyl trimethylammonium bromide (CTAB) buffer, frozen, then extracted using the MOBio PowerSoil™ DNA isolation kit, amplified, and cleaned as described. A mixture that contained 40 ng of DNA template and 12 pmol of ITS 1F primer was submitted to the Center for Genome Research and Biocomputing, Oregon State University, for sequencing on an ABI 3730 capillary sequence machine (Applied Biosystems, Inc., Foster City, CA). Using Bioedit, (v. 7.0.9) (Hall 1999) sequences were evaluated and truncated to reduce noise at the

beginning and the end of the sequence read. Sequences were identified to genus or species level by querying the GenBank database using a nucleotide query (BLASTN). Names were assigned to samples according to the best BLASTN matches and percent similarity to those matches was reported. Some species were identified by comparing RFLP patterns to those of known species (Table 1). Out of the 14 organic mats selected (2 mats x 7 sites) all of them were identified, at least to genus level, as *Piloderma* (Table 1). *Piloderma fallax* was the most commonly identified mat forming fungus in *Piloderma* selected sites. In the mineral soil, 11 out of the 14 mats were identified as *Ramaria*; five different species of *Ramaria* were identified as mat forming fungi in the mineral soil (Table 1).

The sequences from this study and those from other *Piloderma* and *Ramaria* mat forming fungi collected at the H.J. Andrews Experimental Forest were evaluated for the Hinf1 cut site and full length of the PCR product using Bioedit. The Hinf 1 cut sites and full lengths were reported for those mat-forming fungi with available sequence information and for those that matched other collected species found at the H.J. Andrews (Table 1).

3.8. LH-PCR and T-RFLP profiles

For LH-PCR profiles, PCR products were diluted to 1 ng DNA•μl^{-1} and submitted to OSU's Center for Genome Research and Biocomputing, to be analyzed on an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA). PCR products were electrophoresed along with XRhodamine MapMarker™ 1,000 internal lane size standard (BioVentures, Inc., Murfreesboro, TN) to determine

base pair length. In the case of T-RFLP profiles, clean PCR products were digested at 37° C for 3 hours followed by 15 min at 65° C using the restriction endonucleases Hinf1 for fungal samples, and AluI and MspI for bacterial samples (Promega Corporation, Madison, WI). Samples were then submitted to OSU Center for Genome Research and Biocomputing for column cleanup (Sephadex G-50, Sigma-Aldrich) and analysis. Samples taken in October 2007 and June 2008 were diluted three-fold before submitting to reduce the total fluorescence of the samples.

3.9. Fungal ITS and bacterial 16s community data analysis

Size and relative abundance of LH-PCR and T-RFLP fragments were quantified using GeneScan® version 3.5 and Genotyper® version 3.7 software (Applied Biosystems, Inc., Foster City, CA). An example of the Genotyper® output of a T-RFLP profile from a *Piloderma* mat sample is displayed (Figure 2). Data were downloaded into Microsoft Excel and manually binned. Fragments accounting for <1.5% of total fluorescence in each sample were discarded. LH-PCR and T-RFLP fragment distributions were then analyzed using PC-ORD Version 5.0 (MjM Software, Gleneden Beach, OR) Nonmetric multidimensional scaling (NMS) ordinations were constructed using the autopilot feature, "slow and thorough" with the Sørensen distance measure. Fungal LH-PCR and Hinf1 restriction data were combined for analysis, as were bacterial AluI and MspI restriction data. Some evidence suggests that more than one taxonomic group can have the same fragment length when digested with just one restriction endonuclease, leading to an underestimation of the community diversity (Thies 2007). In this study, multiple

restriction endonuclease digests were used along with variation in full amplicon sequence lengths, a strategy that allows increased ability to discriminate taxonomic groups (Rosch and Bothe 2005). Multivariate analysis of variance (MANOVA) was carried out on axis scores of the resulting ordination to test the strength of group association with a model that included site, date, and treatment using SAS version 9.1 (SAS Institute Inc., Cary, NC) (Brenner 2000). The Wilks' Lambda statistic was used to test differences between groups at the $p \leq 0.05$ level.

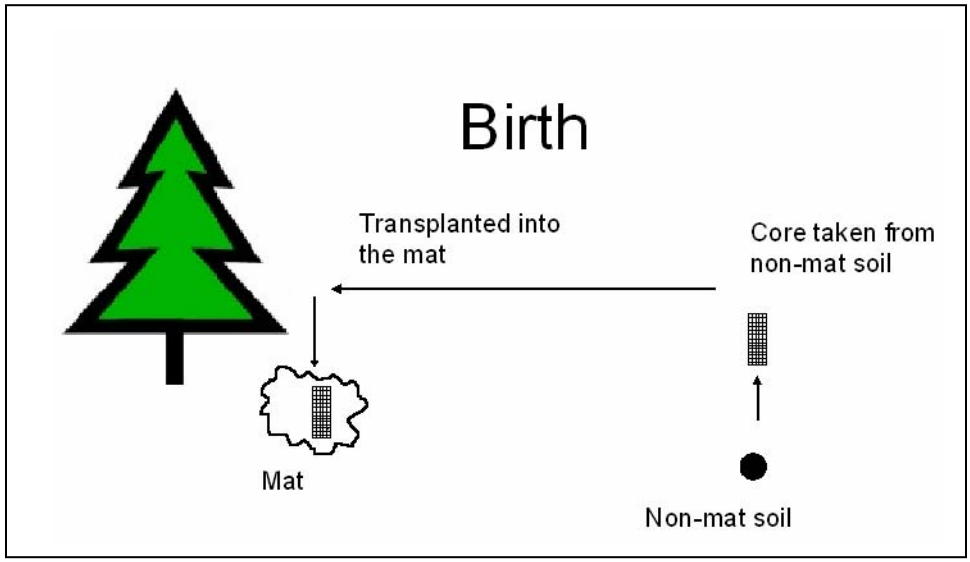
Significance of differences ($p \leq 0.05$) was determined for the background soil chemistry data by the Wilcoxon nonparametric test, carried out in S-PLUS (V. 6.1, TIBCO inc.). The post treatment differences were determined by an analysis of variance (ANOVA) using the Tukey method to test for significant differences ($p \leq 0.05$). The *Piloderma* indicator peak and root tip count data were log transformed to meet normality and constant variance assumptions in ANOVA.

Table 1. Identity of selected ECM mats in this study.

Identity	Similarity (%)	Site	Horizon	*hinf* 1 cut site	LH-PCR length
Piloderma sp.	92	41	O	N/A	N/A
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	N/A	N/A
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	N/A	N/A
Hysterangium setchellii	99	125	M	N/A	N/A
Ramaria formosa	93	125	M	221	771
Ramaria rubribrunnescens	99	137	M	N/A	N/A
Ramaria celerivirescens	82	137	M	322	660
*Ramaria celerivirescens**	100	147	M	322	660
Ramaria celerivirescens	100	147	M	322	660

*Species that were identified by comparing RFLP patterns to known species.
O is the organic soil horizon, M is the mineral soil horizon.
N/A=*hinf* 1 cut site and LH-PCR length could not be identified for some samples

a.



b.

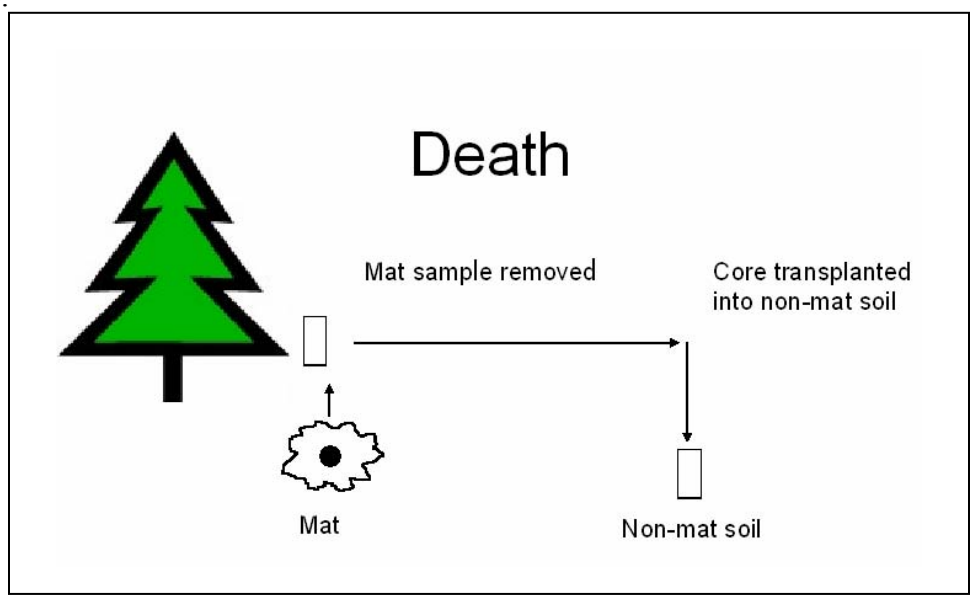


Figure 1. Diagram of the experimental design. a. Birth treatment cores were transplanted in wire mesh cores to allow mat development. b. Death treatment cores were transplanted in closed PVC cores to prevent mat development.

Table 2. Oligonucleotide primers for fungal and bacterial community analysis.

Primer name	Sequence	Reference
ITS1-F	5'-CTTGGTCATTTAGAGGAAGTAA-3'	Gardes and Bruns, 1993
ITS4	5'-TCCTCCGCTTATTGATATGC-3'	White et al., 1990
E8F	5'-AGAGTTTGATCCTGGCTCAG-3'	Lane 1991
907R	5'-CCGTCAATTCCTTTRAGTTT-3'	Muyzer et al., 1995

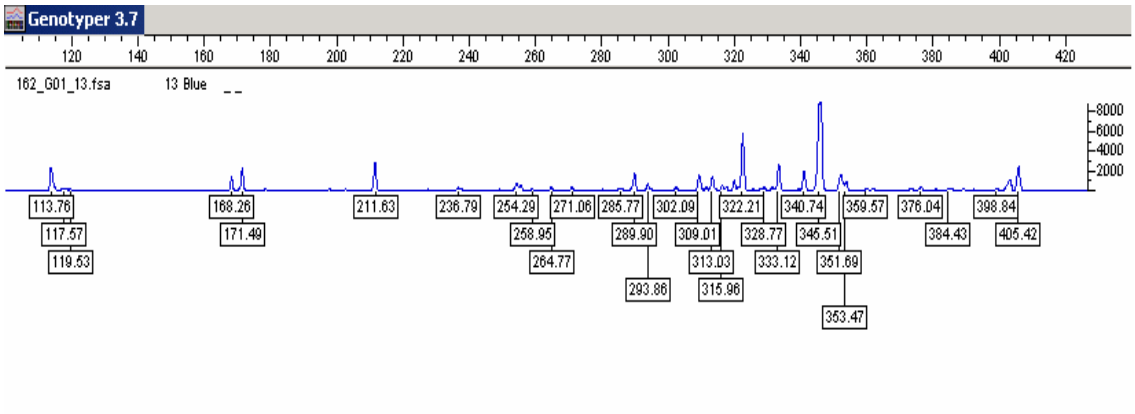


Figure 2. Example of the Genotyper® output of a *Hinf* 1 restricted T-RFLP profile from a *Piloderma* mat sample. The fragment length in base pairs is represented on the X axis and relative fluorescence is represented on the Y axis. The largest peak with a fragment length of 345.5 represents a *Piloderma* mat-forming species.

4. Results

4.1. Chemical analysis of soils associated with *Piloderma* and *Ramaria* mats relative to non-mat soil

Piloderma and *Ramaria* mats showed trends of increased levels of P, K^+, Mg^{2+}, Na^+, and NH_4^+ relative to organic and mineral non-mat soils, respectively, though not significant at the 0.05 level (Table 3). However, increased NH_4^+ in the *Ramaria* mats compared to mineral non-mat soils was significant at the 0.1 level ($p=0.06$). *Piloderma* and *Ramaria* mats did not have increased levels of Ca, NO_3^-, or total N compared to organic and mineral non-mat soils, respectively. *Ramaria* mats showed a significant increase in total C compared to mineral non-mat soils ($p=0.04$); *Piloderma* mats did not differ from organic non-mat soils. Though not statistically significant, the soil pH of mats and other treatments show some general trends (Figure 3). For example, both *Piloderma* and *Ramaria* mats had a lower pH than adjacent non-mat soil. However, the soil pH in the birth treatments of both mat types remained more similar to non-mat soil than mat soil. Interestingly, the pH of death treatments had risen marginally.

4.2. Microbial community shifts following the birth and death of *Piloderma* and *Ramaria* mats

4.2.1. Fungal community response to treatments

Organic soil treatments, including *Piloderma* mat samples, were analyzed separately from mineral soil and *Ramaria* mat treatments. NMS ordinations of the fungal community yielded a three-dimensional solution for each mat type. An ordination of the fungal communities associated with the *Piloderma* mat type shows a

clear separation of mat communities from the group of birth, death, and non-mat communities (Figure 4). MANOVA of the three axis scores revealed that the fungal community structures of *Piloderma* mat and organic non-mat soil differed significantly ($p=0.0001$). Furthermore, the community structure of the death treatment also differed from *Piloderma* mat soil at all sample times ($p<0.0001$). At the first two sampling times, the fungal communities of the mat death treatment differed from non-mat communities, but difference could not be detected at the last sampling (Spring 2008). The birth treatment communities remained more different from the *Piloderma* mat communities throughout ($p<0.003$), and tended to be similar to non-mat soil ($p=0.3842$) Removing the mat-forming *Piloderma* peaks from the analysis did not significantly change the fungal community ordination (data not shown).

An ordination of the fungal communities associated with the *Ramaria* mat type shows a separation of the mat communities from the group of birth, death, and non-mat communities (Figure 5). MANOVA of all three axis scores revealed that the fungal community structure differed somewhat between *Ramaria* mat and mineral non-mat soil ($p=0.0591$). The mat death treatment differed from *Ramaria* mat soil at all sample times ($p=0.0017$). As with the *Piloderma* mat community, there was a trend for death samples to become increasingly similar to non-mat community as time progressed. Again, there was no difference between communities in mineral non-mat and birth treatment soils, and only a slight difference between birth and *Ramaria* mat communities ($p=0.0719$). Removing the mat-forming *Ramaria* peaks from the

analysis did not significantly change the fungal community ordination (data not shown).

4.2.2. Bacterial community response to treatments

Organic soil, including *Piloderma* mat treatments, were analyzed separately from mineral soil and *Ramaria* mat treatments. NMS ordinations of the bacterial community yielded a three-dimensional solution for each mat type. Ordinations of the bacterial communities associated with the *Piloderma* and *Ramaria* mat types show a clear clustering based on time of sampling (Figures 6 and 7). Bacterial communities in these ordinations did not separate by treatment, except in the spring of 2007 where *Piloderma* mat differed from non-mat soil. There is an exceptionally large difference between bacterial communities in the spring 2007 and the other two sample times. This may be due to the change in the dilution factor before bacterial DNA was submitted for sequencing between spring 2007 and the later two sample times. This change was made after the spring 2007 sample to reduce the level of fluorescence in each sample to levels that were identified as producing more accurate results.

4.3. Fungal and bacterial OTUs following the birth and death of *Piloderma* and *Ramaria* mats

The sum of unique fungal ITS and bacterial 16S rDNA fragment lengths were counted for each treatment at all three samples times to represent a cumulative number of operational taxonomic units (OTUs) for each treatment. The *Piloderma* mat soils consistently showed a lower cumulative number of fungal and bacterial

OTUs than non-mat soils (Figures 8a and 8b, respectively). Death treatment soils showed similar counts to non-mat soils (Figures 8a and 8b, respectively). With the exception of the spring of 2007 fungal sampling, death treatments showed the highest number of fungal and bacterial OTUs. The elevated number of OTUs in the fungal and bacteria *Piloderma* death samples is mostly a result of more singletons (Figures 9a, 9b, 9c, and 9d).

Although *Ramaria* mat soils consistently showed a lower cumulative number of fungal OTUs than non-mat soils, they possessed a greater or equal number of bacterial OTUs (Figures 8c and 8d, respectively). Contrary to *Piloderma* mats, both the fungal and bacterial OTUs in the *Ramaria* death treatments were less numerous than those found in mat or non-mat soils (Figures 8c and 8d, respectively). The lower number of fungal OTUs in the *Ramaria* death treatment contributed to a reduction of OTUs occurring four or fewer times (Figures 10a and 10c). The reduction in the number of OTUs in the bacteria *Ramaria* death treatment is mostly a result of fewer singletons than compared to bacteria in *Ramaria* mats (Figures 10b and 10d). Although the *Ramaria* birth treatments had similar numbers of fungal and bacterial OTUs to *Ramaria* mat soils, in the case of fungi the number of OTUs declined with time, whereas, in the case of bacteria, the number of OTUs increased with time (Figures 8c and 8d, respectively).

Among the most frequently occurring OTUs, *Piloderma* mat indicators 345H and 669L constitute a major part of the *Piloderma* mat community that are greatly reduced in death samples (Figure 11a). Many of the frequently occurring *Piloderma*

and *Ramaria* mat OTUs are elevated in death treatments (Figures 11a and 11b, respectively).

4.4. *Piloderma* and *Ramaria* mat dynamics

4.4.1 Colonized root tips

ECM colonized root tips were enumerated across all treatments to assess the status of mycorrhizal presence in roots during the two years of the experiment (Figures 12a and 12b). At year one (spring of 2007), there was no evidence of ECM root tip colonization of roots recovered from birth samples in either *Piloderma* or *Ramaria* mats. By the second sampling (fall of 2007), birth samples showed evidence of ECM colonized roots in both mat types, though not significantly different from the non-mat soil (Figure 12a). This trend continued for both mat types at the third sampling (spring of 2008), with the *Piloderma* birth samples not differing in the amount of ECM colonization from the *Piloderma* mat sample. At all sample times the ECM root tip count of death treatments was not significantly different than of roots in non-mat soil, though ECM root tips did begin to appear in the *Ramaria* death samples in the spring 2008 (Figures 12a and 12b).

4.4.2 *Piloderma* indicators

Piloderma specific Hinf1 restriction fragment lengths and full sequence lengths were identified (Table 1), and the relative abundances of the indicators were followed during the two years of the experiment (Figures 13a and 13b, respectively). In agreement with the root tip data, there was no evidence of *Piloderma* colonization

of birth samples in the spring of 2007. In the fall of 2007, however, the *Piloderma* birth samples continued the same relative abundance of *Piloderma* indicator peaks as mat samples. A similar trend occurred in the spring of 2008, although the birth samples were not significantly different from non-mat due to the high amount of variation in the data. The relative abundance of *Piloderma* indicator peaks dramatically declined over the first year in the *Piloderma* death samples (Figures 13a and 13b). At all time points, the *Piloderma* death cores were not significantly different than the non-mat soil samples. In the spring of 2008, the indicator peak of the Hinf1 restriction fragment appeared in the *Piloderma* death sample, though it is not significantly different than the non-mat sample (Figure 13a).

The relative amount of *Piloderma* indicator fragments was positively correlated with the number of ECM colonized root tips across all treatments and sample times ($r^2 = 0.76$, $p= 0.0002$) (Figure 14). Although the same analysis was attempted with *Ramaria* mat treatments, no differences were found in the relative abundance of fragments of a known size to be from *Ramaria* species in any treatments (data not shown).

Table 3. The levels of various elements in soil from *Piloderma* and *Ramaria* mats compared with adjacent non-mat soils.

	Bray-P ppm (SE)	K ppm (SE)	Mg ppm (SE)	Na ppm (SE)	Ca ppm (SE)	NO3-N ppm (SE)	NH4-N ppm (SE)	C % (SE)	N % (SE)
Piloderma mat	81 (14)	610 (36)	296 (25)	33 (2.6)	4184 (420)	0.5 (0.1)	44 (6)	27 (2)	0.7 (0.1)
Organic non mat	65 (6)	530 (54)	270 (28)	32 (2.5)	4170 (691)	0.8 (0.2)	38 (3)	27 (3)	0.7 (0.1)
Ramaria mat	19 (4)	338 (51)	223 (89)	30 (2.1)	1615 (547)	0.4 (0.1)	9 (1)	*7 (1)	0.2 (0.0)
Mineral non mat	16 (5)	289 (58)	198 (48)	26 (2.0)	1869 (397)	0.3 (0.0)	8 (1)	*5 (1)	0.2 (0.0)

*Significant at the 0.05 level

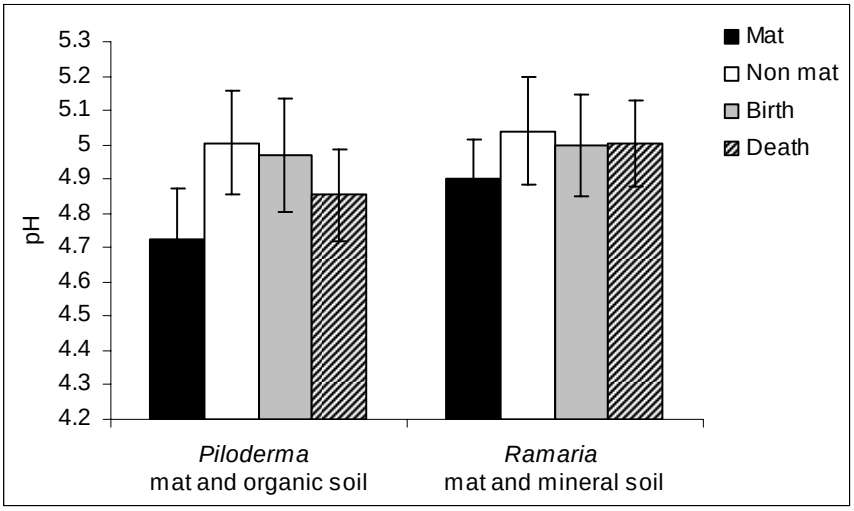


Figure. 3. Mean soil pH ± SE (n=21) for all three post treatment samplings, Spring 2007, Fall 2007, Spring 2008.

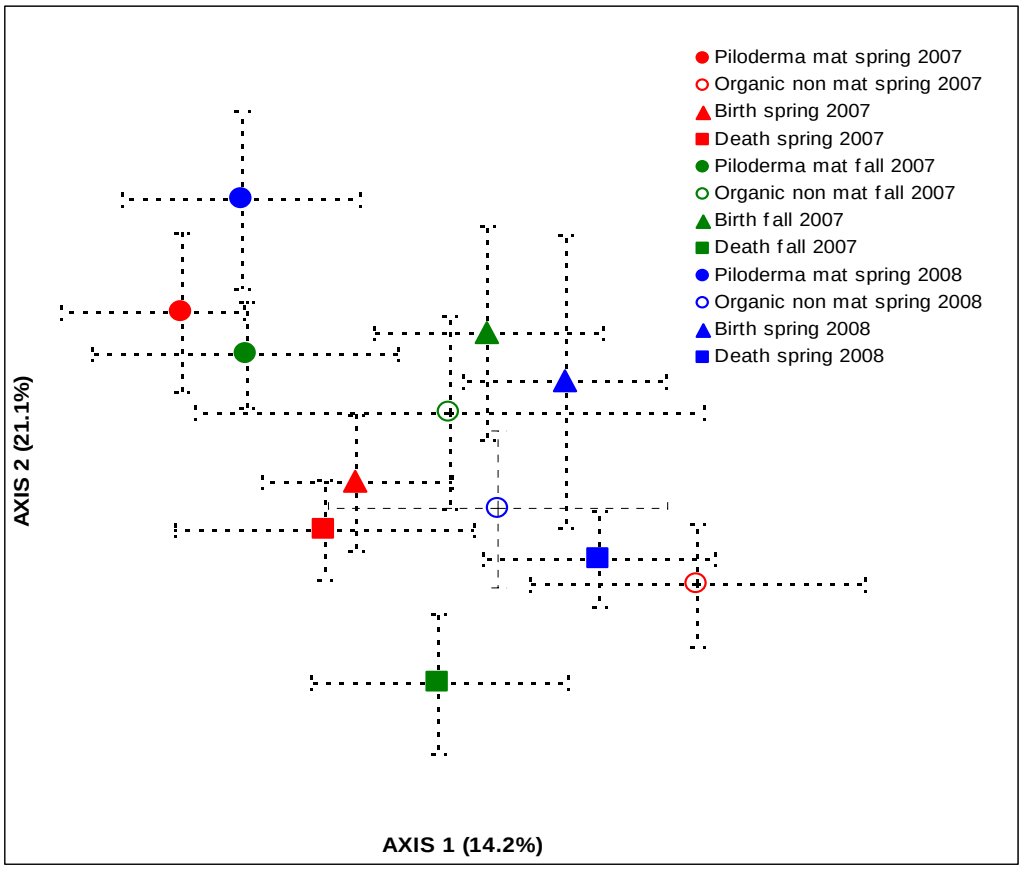


Figure. 4. NMS ordination plots of fungal communities in soil associated with *Piloderma* mat, adjacent non-mat, and birth and death treatments based on LH-PCR and T-RFLP analysis of the ITS region of rDNA extracted from soil cores at three sampling times. Legends of symbols affiliated with each treatment are presented in the figure. Two axes of a three-dimensional solution are shown with values in parentheses representing the variance explained by each axis. The variance of the third axis was 21.1% (not shown). Each point represents the mean ± SE (n=7).

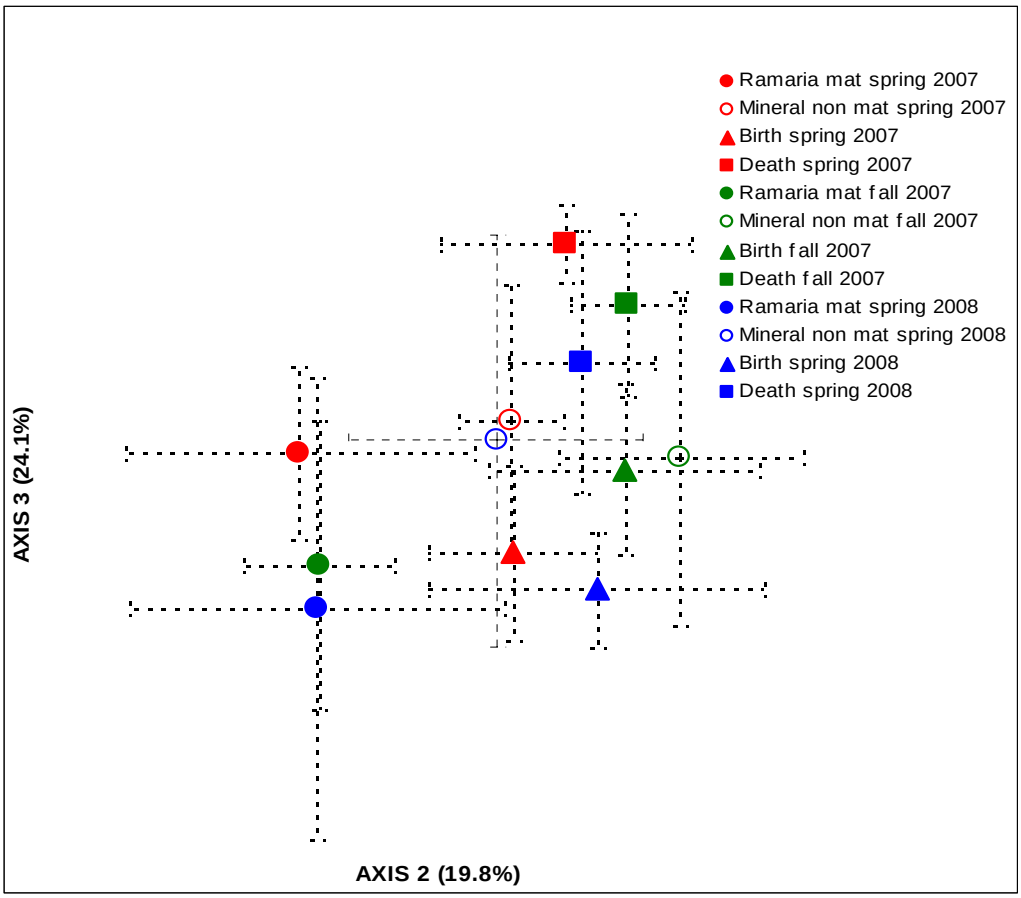


Figure. 5. NMS ordination plots of fungal communities in soil associated with *Ramaria* mat, adjacent non-mat, and birth and death treatments based on LH-PCR and T-RFLP analysis of the ITS region of rDNA extracted from soil cores at three sampling times. Legends of symbols affiliated with each treatment are presented in the figure. Two axes of a three-dimensional solution are shown with values in parentheses representing the variance explained by each axis. The variance of the third axis was 22.5% (not shown). Each point represents the mean ± SE (n=7).

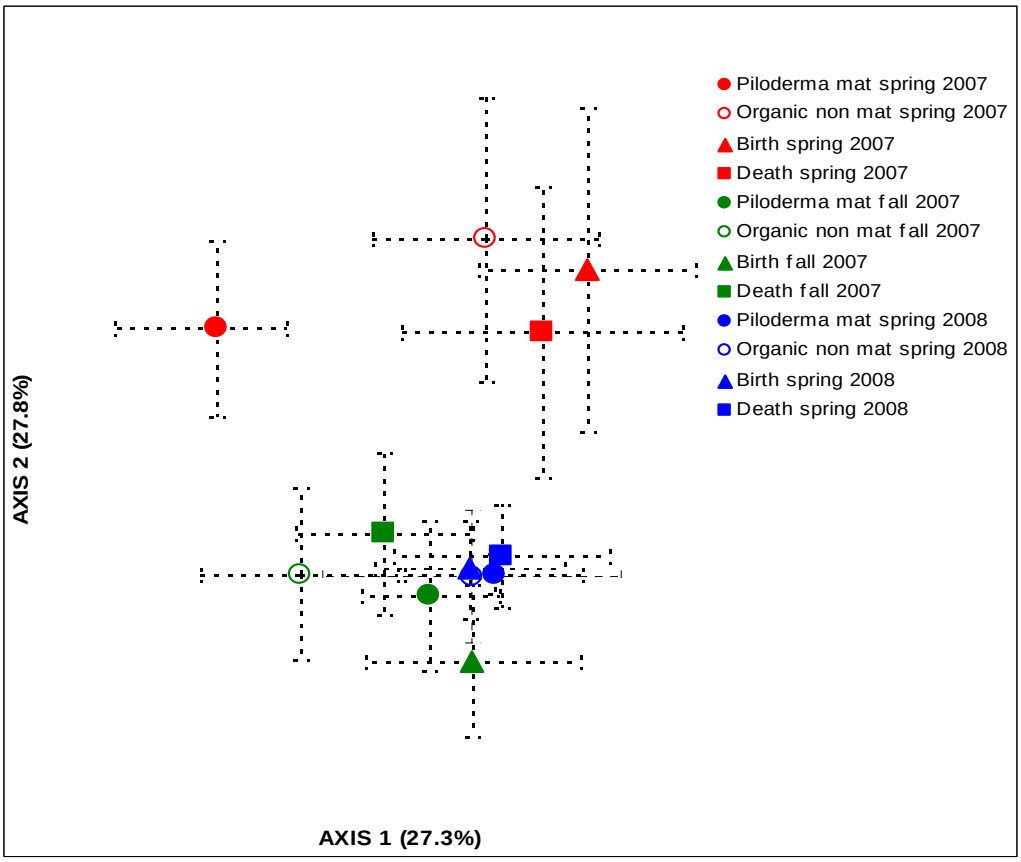


Figure. 6. NMS ordination plots of bacterial communities in soil associated with *Piloderma* mat, adjacent non-mat, and birth and death treatments based on T-RFLP analysis of the 16S rDNA gene sequence extracted from soil cores at three sampling times. A legend of symbols affiliated with each treatment is presented in the figure. Two axes of a three-dimensional solution are shown with values in parentheses representing the variance explained by each axis. The variance of the third axis was 33.6% (not shown). Each point represents the mean ± SE (n=7).

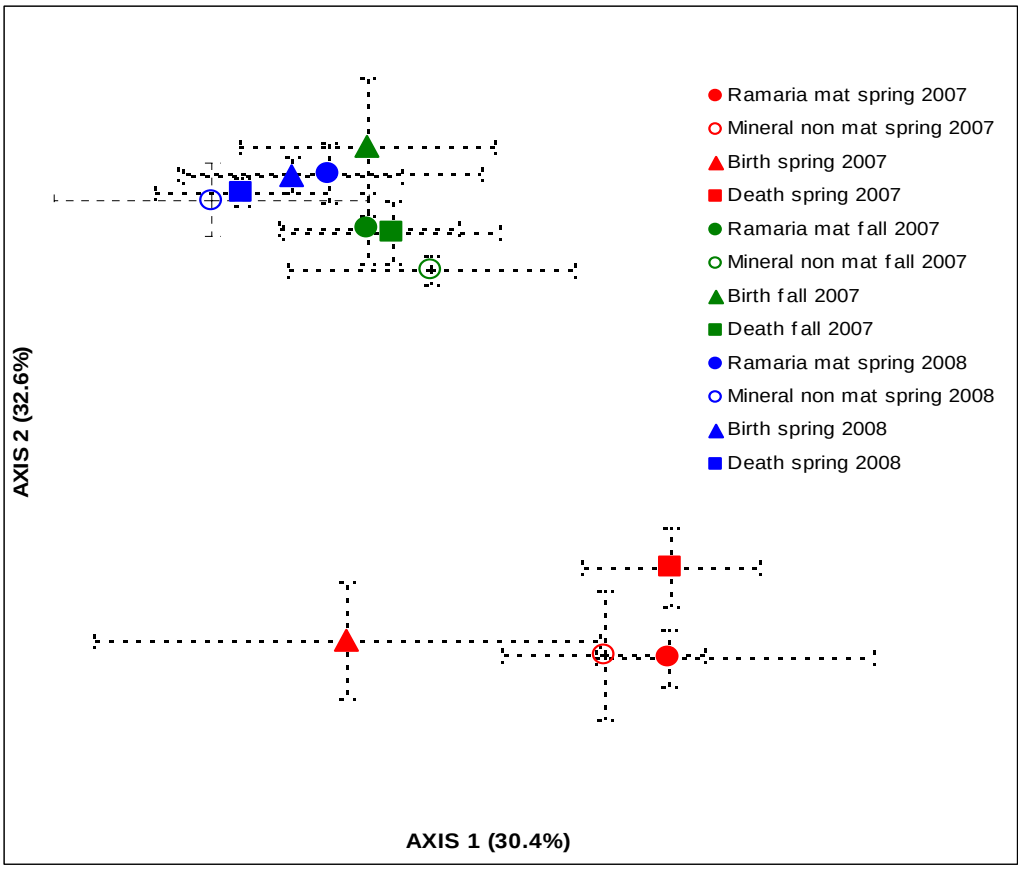


Figure. 7. NMS ordination plots of bacterial communities in soil associated with *Ramaria* mat, adjacent non-mat, and birth and death treatments based on T-RFLP analysis of the 16S rDNA gene sequence extracted from soil cores at three sampling times. A legend of symbols affiliated with each treatment is presented in the figure. Two axes of a three-dimensional solution are shown with values in parentheses representing the variance explained by each axis. The variance of the third axis was 21.8% (not shown). Each point represents the mean ± SE (n=7).

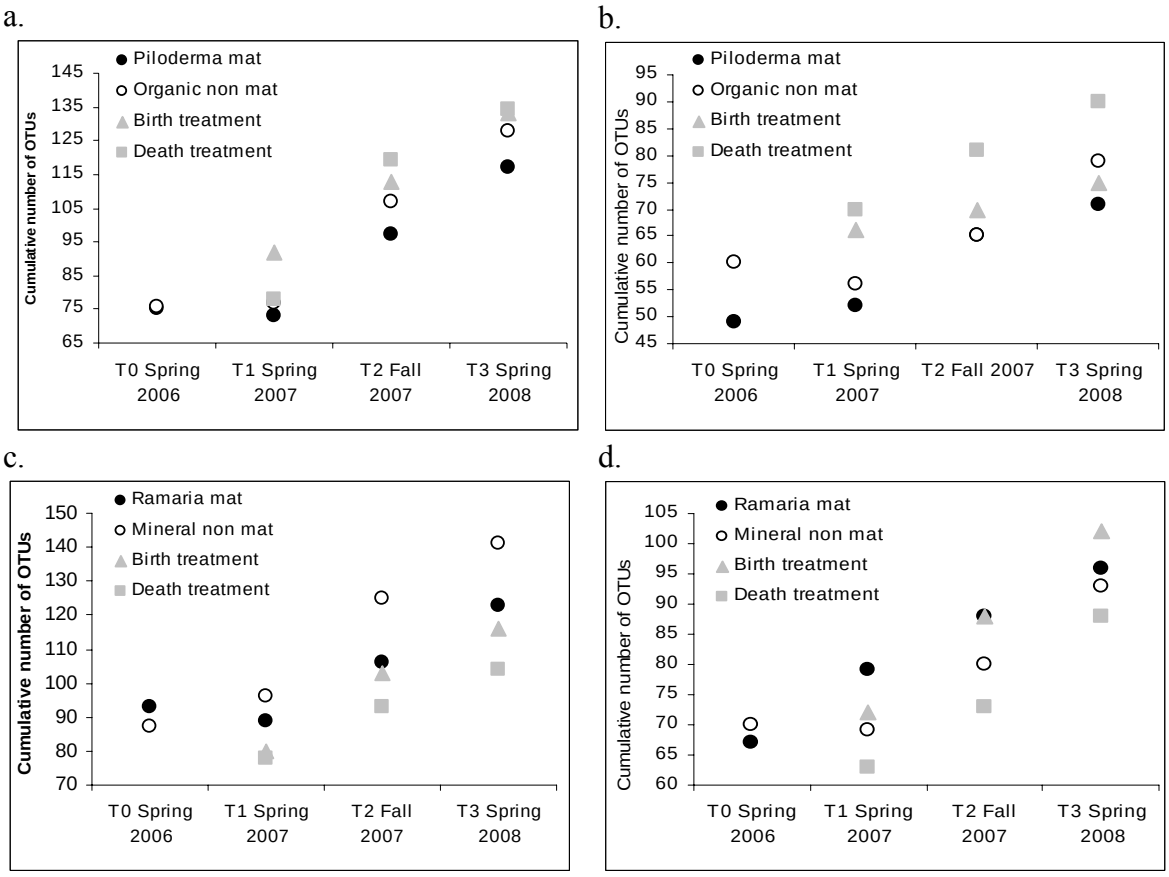


Figure. 8. Cumulative number of OTUs identified in the different soil treatments at installation (T0) as well as at three post-treatment sampling times (T1-T3). Points at T0 and T1 represent the sum of seven samples. Points at T2 and T3 represent the sum of 14 and 21 samples, respectively. The fungal OTU count was determined by the sum of unique LH-PCR full lengths and Hinf 1 restriction fragments. Bacteria OTU count determined by the number of unique MspI and AluI restriction fragments a. Histogram of the fungal OTUs detected in *Piloderma* mat, organic non-mat, and birth and death treatments. b. Histogram of the bacterial OTUs detected in *Piloderma* mat, organic non-mat, and birth and death treatments. c. Histogram of the fungal OTUs in *Ramaria* mat, mineral non-mat, and birth and death treatments. d. Histogram of the bacterial OTUs in *Ramaria* mat, mineral non-mat, and birth and death treatments.

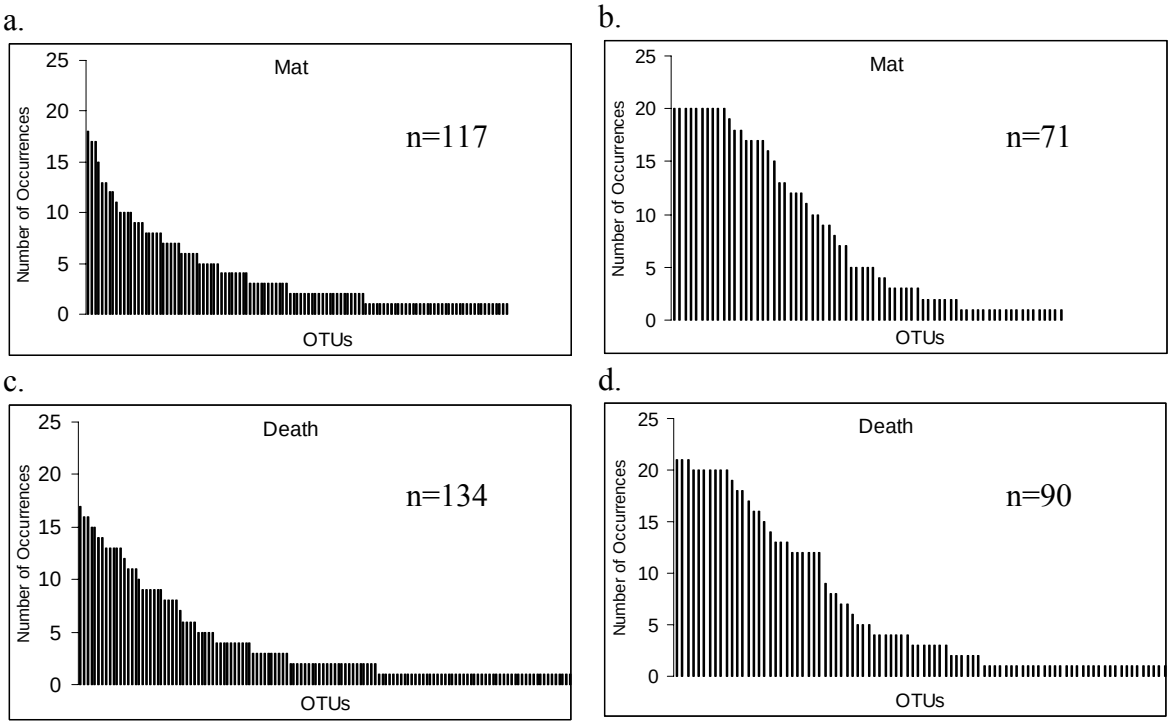


Figure 9. A histogram of the total number of occurrences of each OTU identified from *Piloderma* mat and death treatments from seven sites at three sampling times (T1-T3). The highest possible number of occurrences of a single OTU is 21. Fungal OTU counts were determined from the sum of unique LH-PCR full lengths and Hinf 1 restriction fragments. Bacteria OTU counts represents the number of unique MspI and AluI restriction fragments. a. Histogram of the fungal OTUs detected in *Piloderma* mats. b. Histogram of the bacterial OTUs detected in *Piloderma* mats. c. Histogram of the fungal OTUs detected in *Piloderma* death treatments. d. Histogram of the bacterial OTUs detected in *Piloderma* death treatments.

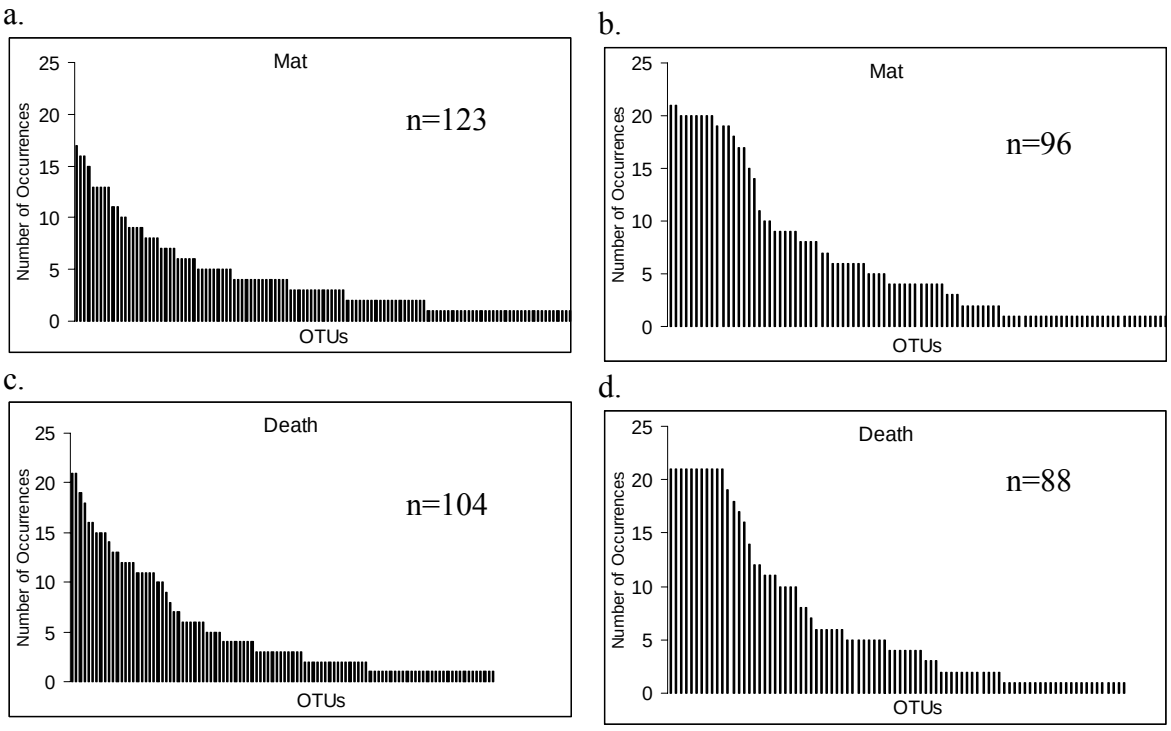
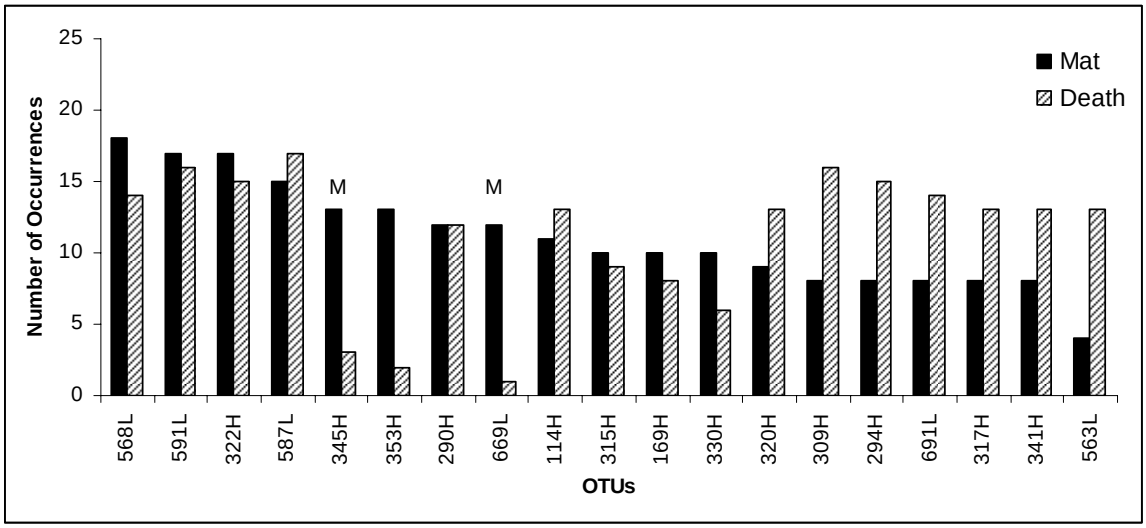


Figure 10. A histogram of the total number of occurrences of each OTU identified from *Ramaria* mat and death treatments from seven sites at three sampling times (T1-T3). The highest possible number of occurrences of a single OTU is 21. Fungal OTU counts were determined from the sum of unique LH-PCR full lengths and Hinf 1 restriction fragments. Bacteria OTU counts represents the number of unique MspI and AluI restriction fragments. a. Histogram of the fungal OTUs detected in *Ramaria* mats. b. Histogram of the bacterial OTUs detected in *Ramaria* mats. c. Histogram of the fungal OTUs detected in *Ramaria* death treatments. d. Histogram of the bacterial OTUs detected in *Ramaria* death treatments.

a.



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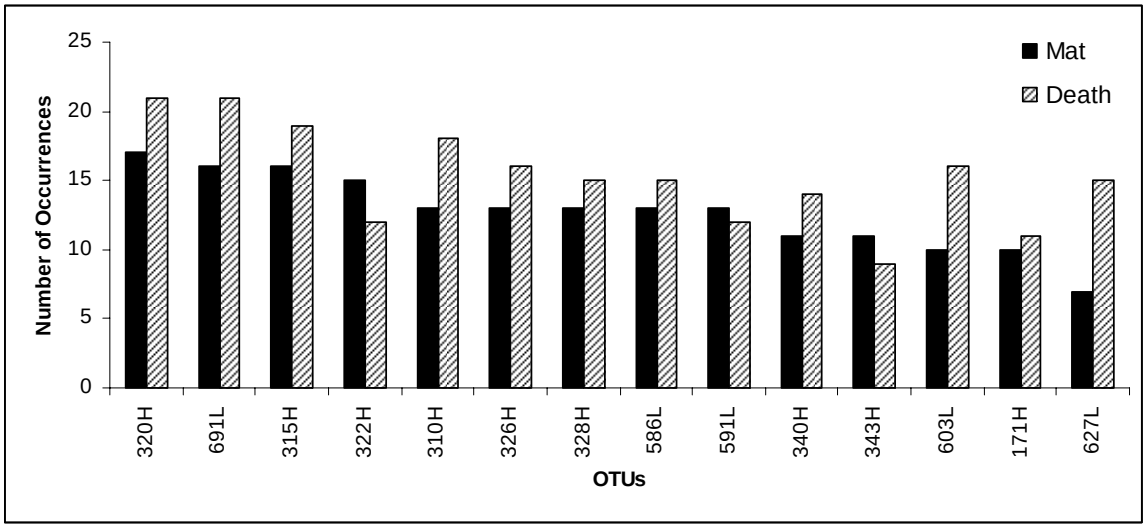
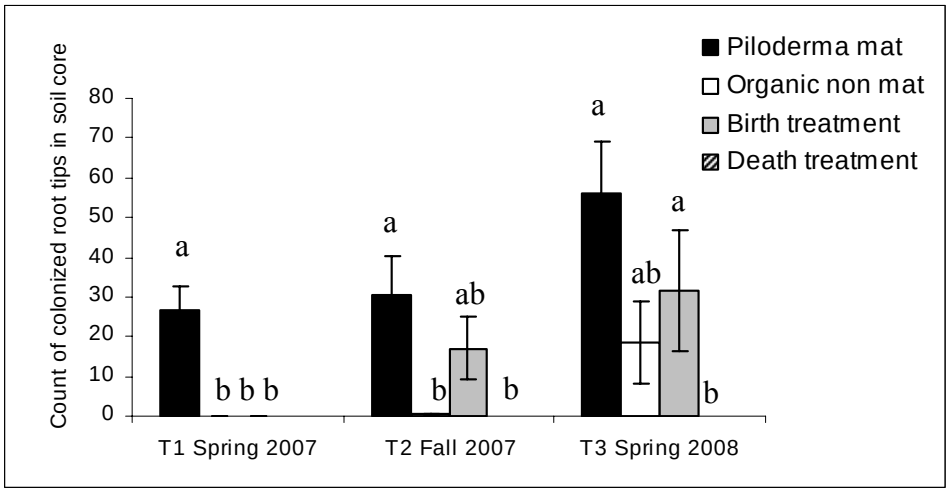


Figure 11. A histogram of the 10% most frequently encountered fungal OTUs identified in mat and death treatments from seven sites at three sampling times (T1-T3). The numbers represent the fragment length of the DNA fragment in base pairs; the "H" refers to Hinf 1 restriction, the "L" refers to LH-PCR, and the "M" indicates fungal mat marker. The highest possible number of occurrence of a single OTU is 21. Fungal OTU count determined by the number of unique LH-PCR full lengths and Hinf 1 restriction fragments. a. Histogram of the Fungi in the *Piloderma* mat. b. Histogram of the fungi in the *Ramaria* mat.

a.



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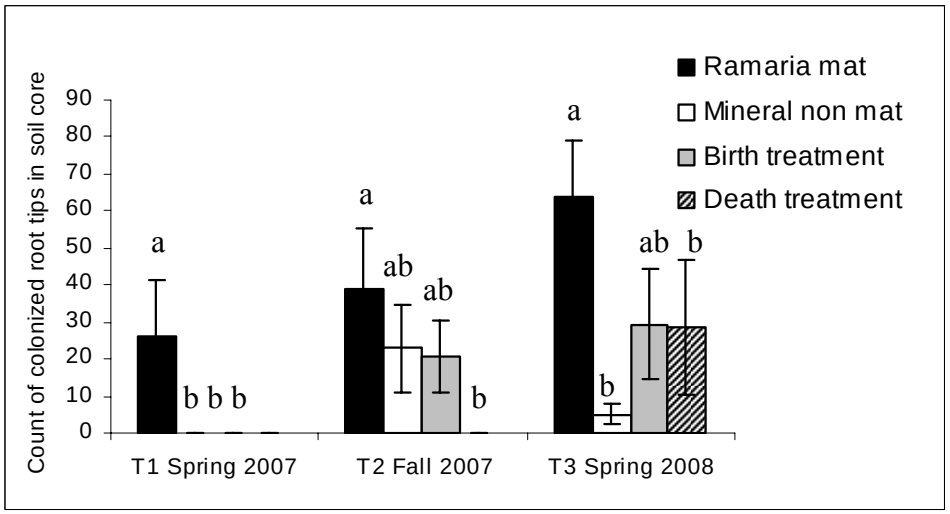
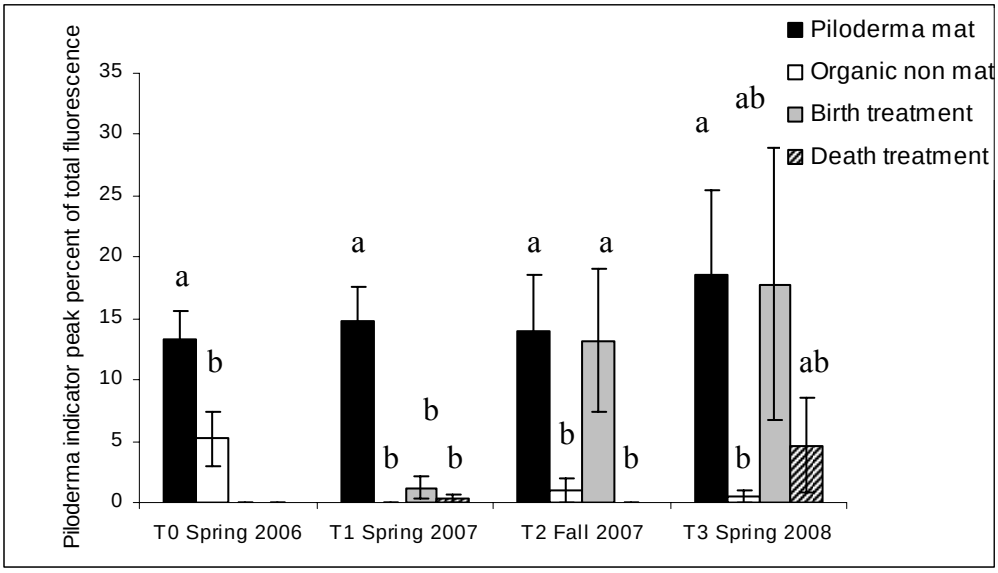


Figure 12. Number of ECM colonized root tips recovered from cores of the different treatments at three sampling times (T1-T3). Data represents the mean ± SE (n=7). Absent bars represent treatments that had zero ECM colonized root tips. Letters a and b in the figure represent significant difference ($p<0.05$), while ab represents treatments without significant differences ($p>0.05$). a. Histogram of the *Piloderma* mat, adjacent non-mat, and birth and death treatments. b. Histogram of the *Ramaria* mat, adjacent non-mat, and birth and death treatments.

a.



b.

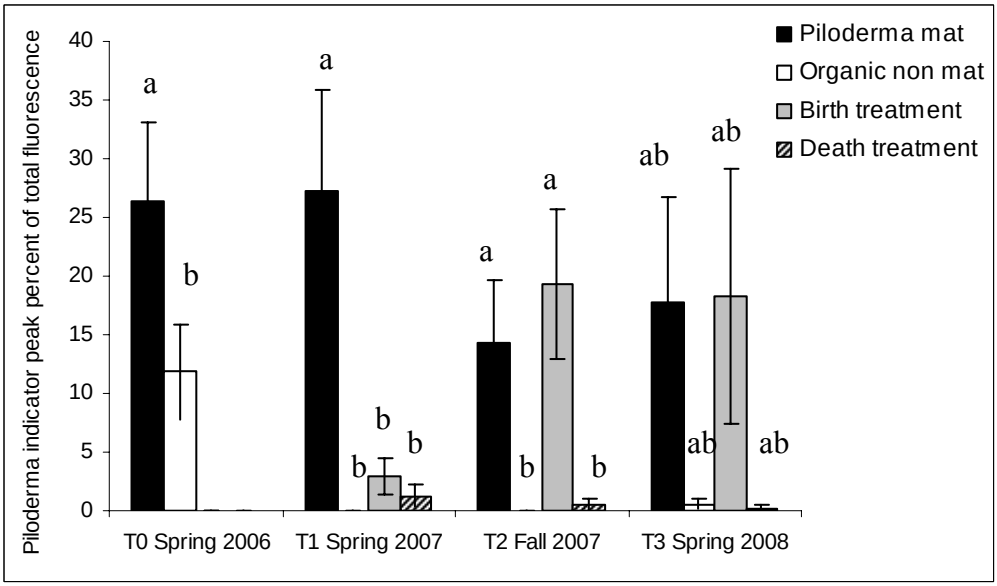


Figure 13. Relative abundance of *Piloderma* indicator peaks found in *Piloderma* mat and adjacent non-mat soil cores taken at installation (T0) as well as from, *Piloderma* mat, adjacent non-mat, and birth and death treatments at three sampling times (T1-T3). Data represents the mean ± SE (n=7). Absent bars represent treatments that had no *Pilderma* indicator peaks. Letters a and b represent significant difference ($p<0.05$), while ab represent treatments without significant differences ($p>0.05$). a. Histogram of indicator peaks representing fragment size 345 base pairs in length from T-RFLP analysis. b. Histogram of indicator peaks representing fragment sizes 664 and 669 base pairs in length from LH-PCR analysis.

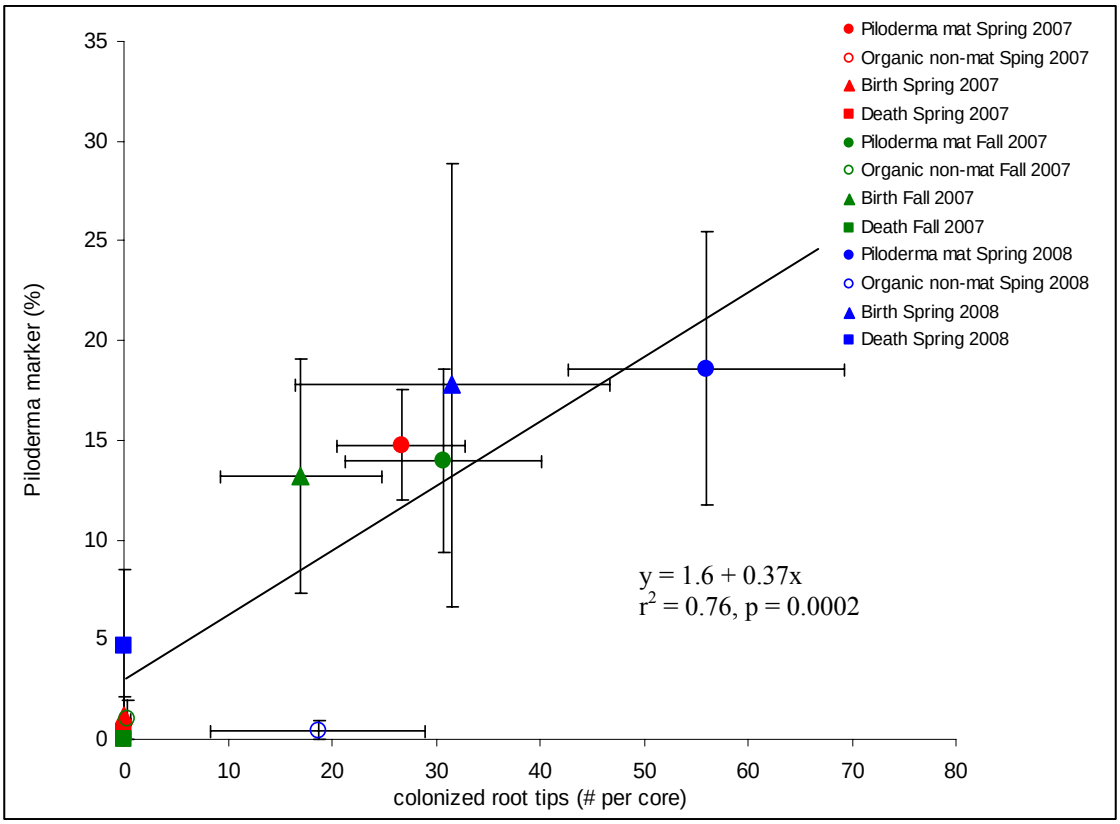


Figure 14. Correlation between the numbers of ECM colonized root tips recovered from soil cores and *Piloderma* mat indicator peaks from *Piloderma* mat, adjacent non-mat, and birth and death treatments at three sampling times (T1-T3). Data represents the mean ± SE (n=7).

5. Discussion

Piloderma and *Ramaria* mats provide unique soil habitats for specialized fungal and bacterial communities. My data clearly demonstrate that soil found in the organic layer of *Piloderma* mats and the mineral layer of *Ramaria* mats under old-growth conifers had different fungal communities than adjacent organic and mineral non-mat soils, respectively. Similarly, the ECM system of a deciduous tree forest in Central France was shown to exert selective pressure on microbial communities including non-mycorrhizal fungi in both organic and mineral soil horizons (Calvaruso et al. 2007). These authors concluded that ECM symbiosis not only has a direct positive effect on plant growth via the nutrient competencies of the ECM fungal associate, but also has an indirect effect on the ectomycorrhizosphere microbial community (Calvaruso et al. 2007). Although the significance of many of these mat associated microbes is unknown, but previous work has described some of the interactions between ECM fungi and associated microbial communities. Non-pathogenic microfungi in the rhizosphere of mycorrhizal spruce and beech may have antagonistic interactions with pathogenic fungi thereby, reducing root disease (Qian et al. 1998). Saprotrophic fungi in the ectomycorrhizosphere may be a direct source of nutrients as ECM fungi are thought to digest chitin from living or dead fungal tissue (Buee et al. 2007). Bacteria known as mycorrhiza helper bacteria are thought to enhance mycorrhizal development (Garbaye and Bowen 1989; Garbaye 1994), increase nutrient mobilization from soil minerals, fix atmospheric nitrogen, and protect plants against root pathogens (Frey-Klett et al. 2007).

5.1. *Piloderma* mat indicators

Appearance of T-RFs identified as being derived from mat forming *Piloderma* species occurred simultaneously with the appearance of ECM root tips in the birth soil treatment. *Piloderma* mat and non-mat soil were differentiated by the presence of mat-forming *Piloderma* species markers in both LH-PCR and T-RFLP data. The presence of these indicators was used to track birth and death treatments through time. The presence of *Piloderma* indicators one and a half years after initiation confirmed that a soil population of *Piloderma* was developing at the same time as colonized root tips were observed in the birth cores. *Piloderma* spp. have been observed to form associations with at least nine different host tree species, mainly conifers (Dunham et al 2007). *Piloderma fallax*, the most commonly encountered mat species in the latter study, has been previously associated with old-growth Douglas-fir in the Pacific Northwest (Smith et al. 2000). The method of tracking *Piloderma* mat-forming fungi in forest soils may be beneficial in futures studies of this widespread mat forming fungi.

In contrast, *Ramaria* spp. markers could not be used to track the treatments, probably because there were many *Ramaria* mat-forming species, all with unique TRFs, compared to the few *Piloderma* mat-forming species that had similar TRFs. Nouhra et al. (2005) also noted a high variation in the ITS sequences of *Ramaria* mat-forming species. Another factor contributing to the lack of *Ramaria* indicator TRFs could be that *Ramaria* mats might consist of a large amount of senescent fungi. In a study of *Ramaria* mat fungi formed in Douglas-fir stands in Oregon, Nouhra et al. (2005) noticed “numerous root tips that appeared senescent, with some mycorrhizae

that were brownish olive to dark brown to almost black, probably indicating that they were old and inactive". These old-looking mycorrhizae failed to PCR amplify in the latter study, strongly suggesting they were dead. Some of *Ramaria* spp. are able to colonize mineral soil in the absence of roots; isotopic evidence has indicated that some species are competent saprotrophs (Hobbie et al. 2001).

5.2. Fungal community dynamics

The specific dynamics of the unique habitats provided by ECM mats were further investigated with the birth and death treatments. As hypothesized, the community changes associated with mat death occurred faster than with mat birth. When severed from the host, the fungal community changed within one year, whereas even after two years of non-mat soils being introduced to the mat, the fungal community composition did not become more mat-like even though colonized roots were present, and there was evidence of *Piloderma* in the non-mat soil cores introduced into *Piloderma* mats. In a study of soil microbial communities associated with viable and decaying ECM root tips, Burke et al. (2006) found that some fungal phylotypes were only observed in decaying ECM tips. In this current study, the dynamics in the death treatment differed between mat types, as *Piloderma* death microbial community gained richness while the *Ramaria* death community lost richness. This finding raises questions about the relationship between ECM and their associated fungi. For example, decreased richness might imply there has been a selective expansion of a few members of the fungal community in response to

nutrient release from ECM senescence. Alternatively, death of the ECM might alter competitiveness and result in a loss of ECM-dependent types.

What was unexpected in the current study was the lack of progression of the birth sample communities toward mat communities. This was not due to a lack of root in-growth into the birth cores, since, in the case of *Piloderma* and *Ramaria* birth treatments, the colonized root tips were detected as early as a year and a half after initiation. What seems to be the case is that the conditions favorable for a mat-like soil microbial community had not developed after two years. Upon visual inspection, the birth cores did not have the physical characteristics normally associated with either *Piloderma* or *Ramaria* mats, such as tightly clustered white or yellow rhizomorphs in the case of *Piloderma*, or a powdery, hydrophobic appearance in the case of *Ramaria*. Because no previous studies in the literature have measured the growth rates of new ECM mats, we may conclude that it is a slow process compared to mat death.

5.3. Fungal and bacterial OTU richness

Both fungal and bacterial OTU richness decreased when mineral layer *Ramaria* mats were severed from their hosts, but increased when organic layer *Piloderma* mats were severed from their host. This difference reflects recent observations that soil microbial biomass, composition, and activity of microbial communities are influenced by the soil horizon in which the community is located (Dickie et al. 2002; Fierer et al. 2003; Rosling et al. 2003). When mats are severed from the host, carbon exudates would cease to influence the microbial community.

Root carbon inputs have been shown to influence the microbial community composition as determined by PLFA analysis of soil under old-growth conifers at the H.J. Andrews Experimental Forest (Brant et al. 2006b). In the *Ramaria* death treatment, the reduced richness may be due to the loss of carbon and nitrogen inputs, a known driver of soil microbial populations (Wardle 1992). Indeed, the chemical analysis of mats in this study has shown that *Ramaria* mats elevate the amount of carbon in the surrounding soil, likely due to the release of simple carbon rich compounds such as oxalic acid (Malajczuk and Cromack 1982; Griffiths et al. 1994).

It is reasonable to think that because the organic layer contains a higher level of background carbon and nutrients compared to the mineral layer, severing the host roots from *Piloderma* mats would be less detrimental to mat-associated fungal and bacterial richness than in the mineral soil layer. The difference may also be attributed to the recent findings that the upper litter layer has a more active saprotrophic fungal community while the vast majority of DNA in the mineral layer is derived from mycorrhizal fungi (Lindahl et al. 2007). The bacterial community richness was also elevated in the *Piloderma* death treatments implying that the diversity of bacterial communities was negatively affected by active ECM. A study of the bacteria communities associated with ECM root tips of loblolly pine in North Carolina also found that bacterial richness, as determined by the number of TRFs, was higher in soil associated with decaying versus viable ECM tips in organic soil (Burke et al. 2006).

6. Conclusion

Piloderma mats in the organic soil and *Ramaria* mats in the mineral soil influenced microbial communities in the surrounding soil environment. Both mat types fostered fungal communities that were dependent on connectivity with the host tree. These mat fungal communities did not develop after two years when non-mat soil was placed in intimate contact with mats. The fungal and bacterial OTU richness in the *Piloderma* mats increased when severed from their host but decreased when *Ramaria* fungal mats were severed from their hosts.

These finding lend themselves to questions that can be addressed with further analysis. For example, how long does it take for a mat fungal community to fully develop? What species are responsible for community shifts? What are the quantitative changes in soil microbial communities? To answer these questions the current study can be followed up by taking additional samples beyond two years after initiation. At these samplings, chemical analysis of the birth treatment soils can be done to determine if mat characteristic soil environments have developed. The ECM colonized root tips in the birth treatment cores should be phylotyped to determine if the species of mat former in which the core was initially placed is responsible for its colonization. Cloning and sequencing of major T-RFs will identify which organisms are contributing to the shifts in community composition and may also provide insight into the function of microbial communities in mat soils. Q-PCR could be used to determine if fungal or bacterial populations change quantitatively when mats develop or are severed from their host.

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