AN ABSTRACT OF THE DISSERTATION OF

Stacie A. Kageyama for the degree of Doctor of Philosophy in Forest Science presented on October 13, 2005.

Title: Effects of Vegetation and Disturbance on Fungal Communities in the Western Cascades of Oregon.

Abstract approved:

Signature redacted for privacy.

David D. Myrold

Signature redacted for privacy.

Randolph J. Molina

The use of high throughput molecular methods that allow for the study of bacterial communities in environmental samples is commonplace in microbial ecology. Until recently, fungal community ecology has focused on isolation, collection of sporocarps, or collection of ectomycorrhizal roots. The techniques used to extract and amplify DNA from environmental samples are relatively new to the world of fungal community ecology. Many fungi are difficult to isolate. Collection and identification of sporocarps and root tips is time-consuming. Sporocarp production may vary from year-to-year. Here, we present three studies using length heterogeneity PCR (LH-PCR) with the general fungal primers ITS1-F and ITS4 for the internal transcribed spacer region of rRNA to

examine changes in fungal community composition in response to changes in vegetation, and disturbance events. In the first study, we used LH-PCR to examine differences in fungal community composition along transects from the forest to the meadow sites at two sites in the western Cascades of Oregon. We found dramatic differences in fungal community composition due to vegetation type and found an intermediate community in the transition zone between meadow and forest. In the second and third studies, we used LH-PCR to identify treatment effects in two manipulative experiments. In both studies, LH-PCR allowed us to observe the changes in community dynamics and identify some of the factors involved in community changes following disturbance. In the second study, we found that fungal succession occurred rapidly when cores were transferred to new environments. We also found evidence that succession occurred more slowly when roots were excluded. In the third study, we used bacterial primers in addition to the fungal ITS primers. We found that presence of live roots rather than either aboveground litter inputs or seasonal differences in sampling times were the greatest determinant of both fungal and bacterial communities at the Detritus Input and Removal Treatment plots 6 years after initiation.

Effects of Vegetation and Disturbance on Fungal Communities in the Western Cascades of Oregon

By

Stacie A. Kageyama

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented October 13, 2005

Commencement June 2006

ACKNOWLEDGEMENTS

I would like to thank my co-major professors, Dave Myrold and Randy Molina, for their guidance and encouragement, and for sharing their collective knowledge of forest soil ecology. Dave invited me to join his lab and encouraged my interest in fungal ecology although it was not his area of expertise. Dave battled a recurrence of non-Hodgkin's lymphoma during my final year of graduate school, yet he chose to continue as my co-major professor. He read drafts of my dissertation while recovering from rounds of chemotherapy. I am grateful for his input and feedback. Randy has encouraged my interest in studying mycorrhizas since I first met him in Boulder, Colorado where I was an undergraduate. I would also like to thank the rest of my committee: minor professors in Botany and Plant Pathology Joey Spatafora and Bob Linderman, Kermit Cromack, Jr., and grad rep Jeff Morrell. With expertise ranging from fungal systematics to the role of fungi in nutrient cycling, they exhibit the strength of Oregon State University in mycology.

The projects described in chapters 2 and 3 were funded by the NSF-Microbial Observatory Program (MCB-9977933). The H. J. Andrews LTER funded the analyses for chapter 4. The College of Forestry awarded me the Richardson Fellowship. The Oregon State University Graduate School awarded me the Minority Pipeline Fellowship. The department of Crop and Soil Science provided me with a teaching assistantship for one term.

Numerous individuals were part of the H. J. Andrews MO crew through the years, and assisted with sampling and processing the

numerous soil samples. Nancy Ritchie Posavatz and Sarah Shaffar Jones extracted DNA for chapter 3. I would like to thank Phil Sollins, Kate Lajtha, Elizabeth Sulzman, and Bruce Caldwell for allowing me to be a part of the Detritus Input and Removal Treatment (DIRT) story at the H. J. Andrews. Justin Brant, Susan Crow, Heath Keirstead, Sarah Beldin, and Cameron Bergen were fellow grad students at the DIRT plots and were all a pleasure to work with. Many other individuals participated on the HJA DIRT crew through the years. I would also like to thank the USDA Forest Service and the H. J. Andrews Experimental Forest. Caprice Rosato and Alex Krupkin at the Central Services Laboratory at Oregon State University ran the many LH-PCR samples for fragment analysis.

I would like to thank Bruce McCune for answering my questions regarding the program PC-ORD and community analysis. Kentaro Hosaka and Gi-Ho Sung patiently answered my question regarding PCR. Jennifer Hooke, Rebecca Huot, and James Djibo Zanzot assisted with the collection and identification of plants at Carpenter and Lookout. Heath Keirstead assisted with the collection and identification of plants at Carpenter and Lookout.

Finally, I would like to thank friends and family. Steve Schmidt, Lori Carris, and Joyce Gellhorn have been mentors as well as good friends. I would like to thank Heath Keirstead, Sheila Slevin, Laurel Kluber, Julia Kerrigan, Teresa Matteson, Sarah Beldin, Jennifer Hooke, Justin Brant, Jess, Butler, Lisa Brutcher, Sunny Jones, and Zach Kayler for their encouragement throughout the process of completing my Ph.D. Gordon Stipe has been neighbor, mechanic, counselor, and emotional support

system for the last three years. My dog Taiga has waited patiently at home (and occasionally wreaking havoc) or in the truck for me on countless evenings as I worked late nights either analyzing data or writing. My parents have been extremely patient as I progressed from undergraduate to graduate school. I would like to thank them for everything.

CONTRIBUTION OF AUTHORS

Peter Bottomley and Kermit Cromack, Jr. were co-PIs on the National Science Foundation Microbial Observatory grant and provided feedback on the data presented in this dissertation. They participated in the setup and sampling of the transect and reciprocal transplant experiments, and the processing of numerous soil samples.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

Effects of Vegetation and Disturbance on Fungal Communities in the Western Cascades of Oregon

Chapter 1: Introduction To The Dissertation

Large scale natural and anthropogenic disturbances can have enormous impacts on belowground fungal communities as they decrease heterogeneity in the landscape (Zak 1992). Changes in belowground inputs or roots may have the greatest impact on fungal communities. A great deal of research effort has focused on aboveground litter as carbon inputs in soil ecosystems (Heilmann-Clausen and Christensen 2004; Spears et al. 2003; Spears and Lajtha 2004; Yano et al. 2005). Recently, phospholipid fatty acid data indicated that belowground carbon inputs rather than aboveground inputs control microbial community composition in forest soils in the western Cascades of Oregon (Brant et al. 2005).

Fungi play key roles in nutrient cycling and ecosystem function. Saprotrophic fungi break down recalcitrant organic compounds for use by other organisms (Trappe and Luoma 1992). Saprophytic fungi contribute to soil organic matter formation through decomposition of plant litter (Beare et al. 1992; Miller and Lodge 1997; Swift et al. 1979). Other fungi produce polysaccharides in soil that contribute to soil stabilization (Chenu 1989; Miller and Lodge 1997). Mycorrhizal fungi are an important carbon sink for primary production (Allen 1991; Vogt et al. 1982). Vogt et al. (1982) estimated that 15% of primary production was allocated to ectomycorrhizal fungi in western Washington forests. However, this 15% only included sporocarps, sclerotia, and mycorrhizal sheaths. This percentage would be much higher if extradical mycelium were included. In many forest systems, ectomycorrhizal

fungi are an important carbon link between the photosynthetic overstory and the soil. Photosynthate is transferred from plants hosts to their ectomycorrhizal partners in exchange for nutrients. According to Högberg and Högberg (2002), ectomycorrhizal mycelium alone comprises at least one-third of the microbial biomass in forest soils. The ectomycorrhizal mycelium also contributes a large percentage of the dissolved organic carbon to forests soils (Högberg and Högberg 2002). Supporting the fungi comes at a cost to host plants but the benefits appear to be worth the cost.

Many fungi are adapted to disturbance in natural ecosystems. Some of the first colonizers of the new tephra following the eruption of Mount St. Helens were phoenicoid fungi (Carpenter et al. 1987). Sporocarps of these fungi provided refuge for photosynthetic nonvascular plants. Natural disturbance is often patchy and leaves islands of vegetation that are sources of fungi for recolonization and succession. However, the rate of recolonization following large scale disturbance is dependent on the availability of fungal inoculum (Friese et al. 1997).

Our knowledge of the effects of small scale disturbances on fungal communities is limited (Denslow 1985). Size of fungal individuals is important as the effect of disturbance on fungi is relative to the scale of disturbance (Friese et al. 1997). Small-scale disturbances such as digging by animals may only serve to fragment or disrupt the fungal individuals. The persistence of some meadows is dependent on small-scale disturbance such as animal burrowing (Franklin and Halpern 2000). It is likely that fungi in these systems are adapted to these types of disturbance.

Studying belowground fungal communities

Until the development of molecular techniques, researchers had no choice but to collect root tips for morphotyping or to wait for fungi to produce sporocarps in order to monitor changes in ectomycorrhizal fungal communities. Both morphotyping and collecting sporocarps yield useful information, but are time-consuming methods. Although morphotyping was often inaccurate, it did yield valuable insight into the diversity of ectomycorrhizal fungi on roots. Extracting and sequencing DNA from ectomycorrhizal root tips is a better method but a large sample size is required in order to get a complete picture of the belowground community. Sporocarps may yield an incomplete picture of the belowground community as the fungi fruiting may not be the dominant ectomycorrhizae belowground (Horton and Bruns 2001). Some fungi rarely produce sporocarps or produce only inconspicuous sporocarps. Collections are often biased towards those that fruit abundantly or those that are fruiting at the time of collection.

Sporocarp surveys (Bills et al. 1986; Bruns 1995; Chen and Cairney 2002; Murakami 1987; Peter et al. 2001; Richardson 1970) have been used to examine diversity in ectomycorrhizal communities. However, many fungi may not fruit every year due to variable environmental conditions such as precipitation so surveys must be conducted over a period of several years. Richardson's (1970) survey spanned 5 years and found a total of 13 fungi. Most studies are 2 to 3 years in duration. Another problem is that the most abundant fungi fruiting aboveground may not be the most abundant on root tips. Many fungi may also produce inconspicuous sporocarps or not fruit at all (Peter et al. 2001). However, sporocarp data still yields useful information.

Peter et al. (2001) compared numbers of sporocarps species, morphotypes from root tips, and restriction fragment length polymorphism (RFLP) types from root tips. They had on average 51 sporocarp species per site, 17 morphotypes, and 19 RFLP types. The discrepancy between sporocarps and morphotypes and RFLPs may be due to limited sampling of root tips.

Early studies of fungal communities relied heavily on the collection of sporocarps. For example, Nantel and Neumann (1992) conducted a study of ectomycorrhizal basidiomycetes along a vegetation gradient using sporocarp collections. They found that fungal symbionts followed host trees for only a part of the gradient that the trees occurred on. In another study, Schmit et al. (2005) used cluster analysis to examine data from 25 earlier studies and found tree species to be good indicators of macrofungal species present. However, they found that even when sites clustered together based on tree species they did not always cluster together based on ectomycorrhizal species. Many ectomycorrhizal species are reported from a greater range than tree species, e. g. Europe and North America. Their explanation for the difference in range is that trees are more dispersal limited than fungi.

Many ectomycorrhizal and saprophytic fungi occurring in forests produce large identifiable sporocarps. However, the most abundant ectomycorrhizal sporocarps are not always the abundant fungi found on root tips (Peter et al. 2001). In Switzerland, Peter et al. (2001) found that only 22% of ectomycorrhizal fungal sporocarps found were present on *Picea abies* root tips. Fungal taxa that either produce inconspicuous or no sporocarps at all were the most abundant on root tips. Although recent studies have shown that sporocarp abundance may not be truly indicative in terms of relative

abundance belowground, sporocarp studies are still useful because they do give an indication of which fungi are present in a community at a given time.

Prior to the development of PCR-based techniques, identification of root tips and fungal isolates was often problematic. In the late 1980s and early 1990s, the development of internal transcribed spacer (ITS) primers made it possible to match DNA extracted from ectomycorrhizal root tips to DNA from sporocarps (Gardes and Bruns 1993). The ability to match DNA sequences or RFLPs to fungi colonizing ectomycorrhizal root tips yielded valuable insight into belowground communities. Root tip DNA data made it clear that the most abundant fungi aboveground are not necessarily the most dominant belowground.

More recently, the ability to extract DNA from soils and other environmental sources has added a new dimension to the study of fungal communities. Studies of soil communities, such as Schadt et al. (2003) and O'Brien et al. (2005), are changing the way that we view belowground soil fungal communities. Molecular techniques have evolved from being the exclusive tools of systematists to being used for the study of soil microbial communities. The use of PCR-based techniques has proven useful for studying organisms that cannot be cultured by current techniques and have been widely used by those studying bacterial communities. They have only recently been adopted for the study of fungal communities. These tools include cloning and sequencing (Jumpponen 2003; Landeweert et al. 2003a; O'Brien et al. 2005; Schadt et al. 2003), and "quick and dirty" methods such as denaturing gel gradient electrophoresis (DGGE) (Landeweert et al. 2005; van Elsas et al. 2000), amplified rDNA restriction analysis (ARDRA),

terminal fragment length polymorphisms (T-RFLP) (Dickie et al. 2002) and length heterogeneity PCR (LH-PCR).

The ability to extract DNA from soils has given researchers a new tool to use in monitoring changes in the fungal community (Kuyper and Landeweert 2002; Landeweert et al. 2003a; Landeweert et al. 2003b; Rosling et al. 2003). Molecular techniques such as LH-PCR (Ritchie et al. 2000) and T-RFLP (Dickie et al. 2002) are relatively simple methods that can be used to track changes in ectomycorrhizal fungal communities. The advantage of these techniques is that a single PCR reaction can yield an entire community profile. The disadvantages are that the fungi amplified may not all be ectomycorrhizal and that some fungi may not amplify at all. Several databases are available for matching T-RFLP patterns so that fungi can be identified (Dickie et al. 2003).

We chose to use length heterogeneity PCR (LH-PCR) with fungal-specific internal transcribed spacer (ITS) region rRNA primers with DNA extracted from soil. Length heterogeneity PCR is similar to fungal-automated rRNA intergenic spacer analysis (F-ARISA) which also uses a fluorescently tagged primer (Ranjard et al. 2001). The diffence is that F-ARISA measures only the length of the intergenic spacer region (ITS1, 5.8S, and ITS2). The general fungal primers ITS1-F and ITS4 that we have used in this study include parts of the small and large subunits, in addition to the spacer region. The ITS is a rapidly evolving region that varies in length among species within genera. Differences in the length of the spacer region results from insertions and deletions (Horton and Bruns 2001). The natural variation in length makes the region ideal for use with fragment analysis techniques

such as LH-PCR and T-RFLP. The ITS primers have been used in numerous studies to amplify fungal DNA from mixed DNA samples such as those extracted from ectomycorrhizal root tips.

My objectives were to:

1. Examine differences in belowground fungal community profiles in adjacent montane forest and meadow sites using length heterogeneity PCR (LH-PCR).
2. Examine shifts in belowground fungal community profiles over a two year period in adjacent montane forest and meadow sites following disturbance events and reciprocal transfer of soil cores using LH-PCR.
3. Examine the effects of varying aboveground and belowground carbon inputs on bacterial and fungal communities using LH-PCR.

Conclusions

Fungi play important roles in ecosystem carbon cycling. Ectomycorrhizae are extremely important in facilitating the establishment of both natural and artificially regenerated seedlings following disturbance events (Perry et al. 1982; Perry et al. 1987). Other fungi play important roles in decomposition of litter and woody debris and are necessary for the formation of soil organic matter. The belowground fungal community is an important characteristic of ecosytems and efforts should be made so that the fungi are carried over into future generations.

Chapter 2:

Fungal Communities Across Meadow/Forest Transects in the Western Cascades of Oregon

Stacie A. Kageyama, David D. Myrold, Peter J. Bottomley,

and Kermit Cromack, Jr.

Molecular Ecology

Blackwell Publishing

In Preparation

INTRODUCTION

Edge effects and habitat fragmentation have been widely studied in relation to wildlife and plants but little is known about their effects on microbial communities. Ninety-five percent of the central western Cascades of Oregon is forested (Hickman 1976). Much of the remaining 5% of non-forested land is composed of meadows. According to Hickman (1976), up to 85% of the plant diversity occurs in these non-forested areas. Historically, meadows have been relatively stable features on the landscape, although the boundaries between mesic meadows and forests in the Pacific Northwest are quite dynamic (Hickman 1976). Many of the meadows have been persistent features and some are more than 1000 years old (Hickman 1968; Hickman 1976). Recently, conifer invasion of meadows due to various factors such as the cessation of sheep-grazing has become a frequent occurrence in the Pacific Northwest (Griffiths et al. 2005; Hickman 1976; Miller and Halpern 1998). Soil properties have been observed to change as conifers invade meadows: Griffiths et al. (2005) found that conifer invasion into meadows is changing nitrogen availability and cycling in the transition zones to being more like the forests.

As part of the NSF Microbial Observatory program at the H. J. Andrews Experimental Forest, earlier work along the meadow-to-forest transects focused on deciphering the processes and understanding the organisms involved in nitrogen cycling. Previous work along these transects examined rates of nitrification and denitrification and used PCR-amplification of functional genes to examine their corresponding bacterial communities. Mintie and colleagues (2003) examined changes in ammonia-

oxidizing bacteria, while Rich and colleagues (2003) examined denitrifying bacteria along the meadow-to-forest transects. Both studies found higher rates of nitrification and denitrification in the meadows. In addition, shifts in ammonia-oxidizing and denitrifying bacterial communities were quite abrupt as the meadows transitioned in forests. Neither of these studies observed any edge effects. Although this earlier work yielded a greater understanding of the structure and dynamics of bacterial communities, the question of how fungal communities behave as vegetation changes remains.

Edge effects with fungi are of particular interest because saprotrophic, pathogenic, and mycorrhizal communities change with vegetation. Shifts in saprophytic fungal communities occur with changes in litter quality as meadow plant communities transition into forests (Griffiths et al. 2005). Soilborne fungal pathogens are also specific to the plants and other organisms. Although boundaries between different vegetation types can be abrupt, roots and their associated fungi may extend beyond the edge (Dickie and Reich 2005). Fungal community structure in these areas can influence succession and nutrient cycling. In particular, the presence of mycorrhizal fungi in transition-zones on the landscape can have implications for plant succession. The presence of ectomycorrhizal mycelium at forest edges may be a very important factor in facilitating tree seedling colonization and the invasion of trees into open habitat (Dickie and Reich 2005).

Our goal for this study was to link the belowground communities to those aboveground by examining the corresponding changes in total fungal community with changes in vegetation across the landscape in the western Cascade Mountains of Oregon. The meadow vegetation at our sites is

dominated by plants that are known to be associated with arbuscular mycorrhizal fungi, whereas the forests are dominated by ectomycorrhizal conifers with ericaceous understory plants such as *Vaccinium* spp. Litter quality also changes as meadows transition into forests and results in different saprotrophic communities. In order to study the changes in fungal communities with vegetation type and in transition areas at two sites, we set up three transects that began in the meadows and ended in the forests. We chose to use a method that would allow us to examine the fungal communities on both sides of the meadow/forest boundary. In order to compare fungi associated with both meadow and forest vegetation including the transition zone, we chose to use a PCR-based technique with the general fungal primers, ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990), to amplify fungal DNA fragments from soil in order to obtain as complete a view of the fungal community as possible.

METHODS

Site characteristics and sampling. Study sites were located at the H. J. Andrews Experimental Forest Long Term Ecological Research site (44.2°N, 122.2°W) in the Cascade Mountain Range of Oregon, USA. Two high montane sites, designated Lookout and Carpenter, were selected at the H. J. Andrews Experimental Forest (Mintie et al. 2003; Rich 2004; Rich et al. 2003) (Figures 1 and 2). Both sites had adjacent meadow and forest communities. Carpenter meadow had been clearcut in the early part of the twentieth century. Lookout meadow appears to be a natural meadow. Dominant

meadow vegetation at both sites included *Bromus carinatus* Hook. & Arn., *Elymus glaucus* Buckl., *Pteridium aquilinum* (L.) Kuhn, *Eriophyllum lanatum* (Pursh.) Forbes, and *Anaphalis margaritacea* (L.) Benth. & Hook. The Carpenter site also included *Rudbeckia occidentalis* Nutt. Vegetation at the Lookout site was more diverse than at the Carpenter and included *Solidago canadensis* (Piper) M. E. Jones and *Hydrophyllum capitatum* Dougl. which were not found at Carpenter. Dominant forest trees at both sites included *Pseudotsuga menziesii* (Mirb.) Franco, *Abies amabilis* Douglas ex Forbes, *A. grandis* (Dougl. ex D. Don) Lindl., and *Tsuga mertensiana* (Bong.) Carr. Carpenter meadow appeared have more soil disturbance due to animal activity than Lookout meadow. We saw several large burrows (> 30 cm in diameter) and other evidence of animal activity. We also observed large stumps, decayed wood, and charcoal scattered across the Carpenter meadow site, which may indicate that the site had once been forested. In addition, Carpenter forest had a more developed understory than Lookout forest.

Three parallel transects were established at each site perpendicular to the meadow/forest boundary (Figure 2.3). Transects were spaced 20 m apart and sampled at eight evenly spaced points along each transect. Three sampling points along each transect were in the meadow, two in the meadow-to-forest transition zone, and the final three were in the forest. Sampling points were 20 m apart at the Carpenter site and 10 m apart at the Lookout site in order to account for the wider transition zone and greater forest canopy height at Carpenter. We collected five soil cores (6-cm inner diameter, 15-cm depth) in a 0.5-m radius of each sampling point. Litter and humus were removed from all samples. The remaining mineral soil from the

cores was pooled into a composite sample for each sampling point. There were 48 samples in total. Samples were stored on ice following collection in the field. They were then refrigerated at 4°C in the laboratory and sieved (4.75 mm) within 24 h after sampling. Aliquots of soil for DNA extraction were frozen at -20°C immediately after sieving and remained frozen until DNA extraction occurred in 2004.

DNA extraction and length heterogeneity PCR. DNA was extracted from 0.5 g of soil (fresh weight) using the FastDNA® kit (Bio 101, Inc., Irvine, CA) according to the manufacturer's directions. Extracted DNA was checked by running 10 μl of extract on an agarose gel (1%) stained with ethidium bromide (0.5 μg ml^{-1}). DNA was quantified on a BioSpec-1601 DNA/Protein/Enzyme Analyzer (Shimadzu Corporation, Kyoto, Japan). Extracts were diluted to 25 ng of DNA per μl for PCR amplification. DNA was amplified using primers for the internal transcribed spacer region of rDNA: ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). Reaction mixtures (50 μl) contained soil DNA (100 ng), AmpliTaq DNA polymerase (2.5 U), GeneAmp PCR buffer (1x), $MgCl_2$ (2 mM), deoxynucleoside triphosphates (0.2mM each), forward and reverse primers (0.2 μM each), and bovine serum albumin (0.064 g ml^{-1}). The forward primer, ITS1-F, was labeled with 6-FAM (6-carboxyfluorescein) for length heterogeneity PCR (LH-PCR). PCR amplification was conducted as follows using a PTC-100 hot bonnet thermocycler (MJ Research, Inc., Waltham, MA): 94° for 2 min followed by 35 cycles of 94° for 30 s, 55° for 30 s, 72° for 1 min. A final

extension followed for 72° for 2 min. PCR products were visualized with electrophoresis on 1% agarose gels stained with ethidium bromide. Samples were diluted to 1 ng μl^{-1}. One µl of PCR product containing 1 ng of DNA was submitted for capillary sequencing on an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA) in the Central Analytical Laboratory, Oregon State University. PCR products were run on the Genetic Analyzer along with X-Rhodamine MapMarker™ 1000 internal lane size standard (BioVentures, Inc., Murfreesboro, TN).

Data analysis. Size and relative abundance of LH-PCR fragments were quantified using GeneScan® v. 3.5 software and Genotyper® v. 2.5 software (Applied Biosystems, Inc., Foster City, CA). Sequence length and peak area data were obtained using Genotyper®. Data were downloaded into Microsoft® Excel and manually binned. Community data were analyzed with PC-ORD Version 4.36 (MJM Software, Gleneden, OR).

Species richness and beta diversity

For this study, species richness is defined as the number of fungal ITS fragments per sample. We calculated the beta diversity of the fragment data for each of the meadows, meadow-forest transition zones, and forests using PC-ORD Version 4.36 (MJM Software, Gleneden, OR). The program PC-ORD calculates beta diversity without reference to specific gradients. Beta diversity was calculated by using the equation:

$$\beta = \frac{\lambda}{\overline{\alpha}}$$

where β = beta diversity or the amount of compositional change in a sample, λ = the overall diversity in a collection of sample units, and $\overline{\alpha}$ = the diversity in individual sample units or number of ITS fragments per sample (McCune and Grace 2002).

Ordinations of community data

Ordinations of LH-PCR fragment data were run in PC-ORD Version 4.36 (MJM Software, Gleneden, OR) using nonmetric multidimensional scaling (NMS) using the "Slow and thorough" autopilot feature with the Sørensen distance measure. We chose to use NMS because it avoids the assumption of linear relationships among variables, and allows the use of any relativization method (McCune and Grace 2002). Analyses were run on the entire data set (both Lookout and Carpenter), by individual site (Lookout only or Carpenter only), and then by site excluding samples collected in the transition zones. For the dataset containing all sites, we deleted columns (LH-PCR fragments) containing fewer than three non-zero values. The dataset was then relativized by row totals (samples) to adjust for differences in fluorescence levels of our amplified fragments.

Multi-response permutation procedures

Multi-response permutation procedures (MRPP) were used to test fungal community composition between vegetation types and different sites.

MRPP is a nonparametric method that is used to test for group differences (McCune and Grace 2002). We used MRPP in the statistics package PC-ORD Version 4.36 (MJM Software, Gleneden, OR). We used the Sørensen (Bray-Curtis) distance measure. Groups were defined by site.

Indicator species analyses

Indicator species analyses were conducted using PC-ORD Version 4.36 (MJM Software, Gleneden, OR) by comparing either meadow sites to forest sites or similar vegetation types with 1000 randomizations in the Monte Carlo test. According to Dufrêne and Legendre (Dufrêne and Legendre 1997), a perfect indicator is always present in a particular group and is exclusive to that group. PC-ORD employs Dufrêne and Legendre's (Dufrêne and Legendre 1997) indicator species analysis method to produce indicator values for each species (LH-PCR fragment) for each group (site).

RESULTS

We examined a range of LH-PCR internal transcribed spacer fragments ranging from 400 to 900 base pairs in length (Figure 2.2). We analyzed a total of 97 fragments for the complete dataset. Total numbers of fragments varied by site but were consistent for each vegetation type (Table 2.1). The highest numbers of fragments were found in the meadows with 76 fragments for Carpenter meadow and 75 fragments for Lookout meadow. The forest sites had the fewest number of fragments with 62 fragments each for both Carpenter and Lookout forests. The meadow-forest transition zones

were intermediate between the meadow and forest sites, with Carpenter having 68 fragments and Lookout having 65 fragments. The relative fluorescence of indicator fragments was highest in Carpenter and Lookout meadows (Figure 2.5).

Nonmetric multidimensional scaling ordinations of fungal community data yielded 3-dimesional solutions for the combined transects (Figure 2.6), and individual site comparisons (Figures 2.7 and 2.8). The ordination of the combined transects indicated that fungal communities from Lookout and Carpenter meadows were similar. Forest and meadow sites separated along Axis 1 which explained 38.2% of the variation, while Axis 2 explained only 16.0% (Figure 2.6). The two meadow sites grouped together in the NMS ordination, although the Lookout site was more variable. Fungal community profiles in the forest samples exhibited greater heterogeneity and grouped less tightly in the ordination. The samples from both Lookout and Carpenter forests also grouped together but not as tightly as the meadow samples. Samples collected in the meadow-forest transition zone fell between the meadow and forest samples, although the Lookout transition samples were more scattered than the Carpenter transition samples in the ordination.

Most of the variation in the Carpenter NMS ordination was on Axis 1 with 50.4% (Figure 2.7). Forest, transition zone, and meadow samples separated along Axis 1. Transition zone samples separated from the meadow and forest sides separated distinctly along Axis 1. In the Lookout NMS ordination, variation was divided between Axis 1 with 35.1 % and Axis 2 with 25.7%. Again, forest, transition zone, and meadow samples separated

along Axis 1 (Figure 2.8). However, both meadow and forest transition zones samples were much more scattered.

Calculations of *A*-statistics and *p*-values with MRPP indicated that meadow fungal communities differed from the forest communities (Table 2.2). However, MRPP indicated that Carpenter and Lookout meadow samples were similar in their composition of ITS fragments. Carpenter and Lookout forest samples were also similar in their composition of ITS fragments.

A similar pattern was found for the indicator species analysis of the two meadows (Figure 2.5, Table 2.3). Indicator species analysis found several indicator fragments in the meadows but only three for Carpenter forest and one for Lookout forest. There were two indicators for the comparison between Carpenter forest and Carpenter transition, but no indicators in the comparison between Lookout forest and the Lookout transition.

Beta diversity was low overall for all of the fungal ITS fragments in the transect samples (Table 2.1). However, beta diversity was higher for the fungal ITS fragments in samples collected in the forest than the meadows due to the heterogeneity of fragments in the forest samples. Beta diversity was 2.5 for Carpenter and 2.4 for Lookout forests. It was lowest in the meadows at 1.9 for Carpenter and 2.0 for Lookout. Again, the transition zones were intermediate between the meadows and forests at 2.0 for Carpenter and 2.4 for Lookout.

DISCUSSION

Although the total number of fungal ITS fragments was lower, there was greater overall ITS fragment diversity in the forest samples than in the meadow samples. Heterogeneity of ITS fragments in samples increased in the transition zones and became much more pronounced in the forests. We believe this is due to greater belowground spatial heterogeneity of fungi in the forests. The diversity and spatial distribution of fungi in our sites appears to be analogous to that of the vegetation on the sites. Meadow vegetation in the Cascades is more diverse than in forests (Hickman 1976). Although the forests were dense, they were nowhere near the density of herbaceous plants in the meadows. Tree roots in our forest sites were dispersed throughout the soil, while herbaceous roots in the meadows formed dense thick mats comprised of several different taxa.

In the meadows, ITS fragment profiles were more similar in spite of the greater number of fragments. We found 76 LH-PCR fragments at Carpenter meadow and 75 at Lookout Meadow. The number of fragments found in the meadows is comparable to the number of 18S clones found in meadows in the United Kingdom (Hunt et al. 2004). In comparison, only a third of the number of fragments were found in a terminal restriction fragment length polymorphism study in grassland soils in Ireland (Brodie et al. 2003). We found 62 LH-PCR fragments at both forest sites. Dickie et al. (2002) found 40 terminal restriction fragments in a *Pinus resinosa* forest in Pennsylvania.

Nonmetric multidimensional scaling ordinations of meadow, forest, and transition samples separated along axis 1. Meadow samples were grouped closer together than forest samples. We believe that is due to their overall homogeneity. Forest samples were much more scattered in the ordination. Between the two sites, Lookout exhibited the greatest amount of variation in the meadow, transition zone, and forest samples. This pattern may be a reflection of the greater plant diversity at Lookout than at Carpenter.

Transition zone samples were intermediate between the meadow and forest samples in terms of species richness and β-diversity. In NMS ordinations of ITS fragment data, transition samples fell between forest and meadow samples. Previous studies utilized the same transect samples used in this study to examine changes in rates of nitrogen cycling and their corresponding bacterial communities (Mintie et al. 2003; Rich et al. 2003). These studies found sharp demarcations between the meadows and forests. Neither nitrification and denitrification rate or ammonia-oxidizing or denitrifying bacterial community data showed a transition or edge effect. Nitrification and denitrification rates in transition samples next to the meadow and transition samples next to the forest were not significantly different from those sites. We believe that we observed more of an edge effect due to the presence of roots from both meadow and forest vegetation as well as edge specialists in the transition zone. Recently, phospholipid fatty acid data indicated that belowground carbon inputs or "root effects" rather than aboveground inputs control microbial community composition in forest soils in the western Cascades of Oregon (Brant et al. 2005).

Distance from sources of ectomycorrhizal inoculum makes a difference in the establishment of seedlings. Earlier studies of ectomycorrhizal root tips and sporocarps have observed changes in ecotomycorrhizas in relation to vegetation gradients, distance from the edge, or distance from a host. Nantel and Neumann (1992) conducted a study of ectomycorrhizal basidiomycetes along a vegetation gradient using sporocarp collections. They found that fungal symbionts followed host trees for only a part of the gradient that the trees occurred on. Dickie and Reich (Dickie and Reich 2005) found that *Quercus macrocarpa* seedlings at a forest edge were ectomycorrhizal 0 to 8 m from the edge but seedlings at 16 to 20 m remained uninfected after 2 years. In a study of seedlings planted near mature Douglas-fir, Cline et al. (2005) found that seedlings near mature trees had higher species richness and diversity than seedlings growing more than 16 m from the mature trees. The seedlings that were more than 16 m away from the mature trees had ectomycorrhizas similar to those in the glasshouse study.

It is likely that many of the ITS fragments in this study are linked to either mycorrhizal or saprotrophic fungi. However, a significant drawback with techniques like LH-PCR is that it is extremely difficult to identify fragments based solely on their sequence length. Although databases containing restriction fragment length polymorphism (RFLP) or sequence length information do exist for many ectomycorrhizal fungi, they are often for fungi in specific ecosystems. Because of the high levels of variation in the ITS region, it would be a mistake to attempt to identify fragments using a database from a different ecosystem (Horton and Bruns 2001). The ITS region

is highly variable and that makes it perfect for techniques like LH-PCR and T-RFLP. Unfortunately, the high levels of variation makes the ITS region very difficult to use for identification based on sequence length using databases. In fact, both interspecific and intraspecific variation in the ITS region often exists (Horton 2002; Horton and Bruns 2001; Kårén et al. 1997).

In spite of the shortcomings with techniques like LH-PCR, it is useful technique for studying community dynamics. Length heterogeneity PCR uses a fluorescently tagged primer, like T-RFLP, but omits the process of restriction digestion. The method requires that resulting PCR products vary in sequence length. The resulting data is the comprised of fragment lengths. We found the ITS primers to be ideal for this technique. The technique has previously been used with bacterial 16S primers (Bernhard et al. 2005; Ritchie et al. 2000) but has not been used with fungal primers. The ITS region is composed of two highly variable non-coding spacer regions. The conserved small (18S) and large (28S) subunits flank the region. The 5.8S is nested between ITS1 and ITS2. The fungal-specific primer ITS1-F (Gardes and Bruns 1993) and the "universal primer" ITS4 (White et al. 1990) typically yield PCR products that are between 400 to 900 base pairs. The method has some major advantages over T-RFLP, as omitting the restriction digest steps makes it faster and less expensive. In addition, LH-PCR yields good results for landscape-level community studies.

Meadows are dynamic areas in the Pacific Northwest. Conifer invasion has been a frequent occurrence at many of these sites (Miller and Halpern 1998). The Carpenter meadow site used in our study was clearcut in the early twentieth century. There are a few scattered trees in the meadow,

but for the most part natural regeneration has failed to occur. Although it is likely that many factors are involved in the invasion of meadows by conifers, the presence or absence of ectomycorhizal inoculum may be an important factor in the establishment and persistence of conifer seedlings at the meadow/forest boundary.

Use of LH-PCR allowed us to observe the changes in fungal community composition across transects spanning from forest to meadow. In addition, we were able to identify some edge effects in the samples collected in the transition zone between the forest and the meadows. Although the technique did not allow us to identify our ITS fragments, we gained valuable insight into fungal community dynamics.

LITERATURE CITED

Bernhard AE, Colbert D, McManus J, Field KG (2005) Microbial community dynamics based on 16S rRNA gene profiles in a Pacific Northwest estuary and its tributaries. FEMS Microbiology Ecology 52:115-128

Brant JB, Myrold DD, Sulzman EW (2005) Root controls on soil microbial community structure in forest soils. Oecologia in review

Brodie E, Edwards S, Clipson N (2003) Soil fungal community structure in a temperate upland grassland soil. FEMS Microbiology Ecology 45:105-114

Cline, ET, Ammirati JF, and Edmonds RL (2005) Does proximity to mature trees influence ectomycorrhizal fungus communities of Douglas-fir seedlings? New Phytologist 166: 993-1009.

Dickie IA, Reich PB (2005) Ectomycorrhizal fungal communities at forest edges. Journal of Ecology 93:244-255

Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible approach. Ecological Monographs 67:345-366

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

Griffiths R, Madritch M, Swanson A (2005) Conifer invasion of forest meadows transforms soil characteristics in the Pacific Northwest. Forest Ecology and Management 208:347-358

Hickman JC (1968) Disjunction and Endemism in the Flora of the Central Western Cascades of Oregon: An Historical and Ecological Approach to Plant Distributions. Ph.D dissertation. University of Oregon, Eugene

Hickman JC (1976) Non-forest vegetation of the western Cascade Mountains of Oregon. Northwest Science 50:145-155

Horton TR (2002) Molecular approaches to ectomycorrhizal diversity studies: variation in ITS at a local scale. Plant and Soil 244:29-39

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

Hunt J, Boddy L, Randerson PF, Rogers HJ (2004) An evaluation of 18S rDNA approaches for the study of fungal diversity in grassland soils. Microbial Ecology 47:385-395

Kårén O, Högberg N, Dahlberg A, Jonsson L, Nylund J-E (1997) Inter- and intraspecific variation in the ITS region of rDNA of ectomycorrhizal fungi in Fennoscandia as detected by endonuclease analysis. New Phytologist 136:313-325

McCune B, Grace JB (2002) Analysis of Ecological Communities. MJM Software Design, Gleneden Beach

Miller EA, Halpern CB (1998) Effects of environment and grazing disturbance on tree establishment in meadows of the central Cascade Range, Oregon, USA. Journal of Vegetation Science 9:265-282

Mintie AT, Heichen RS, Cromack K, Jr. , Myrold DD, Bottomley PJ (2003) Ammonia-oxidizing bacteria along meadow-to-forest transects in the Oregon Cascade Mountains. Applied and Environmental Microbiology 69:3129-3136

Nantel P, and Neumann P (1992) Ecology of ectomycorrhizal-basidiomycete communities on a local vegetation gradient. Ecology 73:99-117.

Rich JJ (2004) Community Composition and Activities of Denitrifying Bacteria in Soils. In: Crop and Soil Science. Oregon State University, Corvallis, p 102

Rich JJ, Heichen RS, Bottomley PJ, Cromack K, Jr., Myrold DD (2003) Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. Applied and Environmental Microbiology 69:5974-5982

Ritchie NJ, Schutter ME, Dick RP, Myrold DD (2000) Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterize microbial communities in soil. Applied and Environmental Microbiology 66:1668-1675

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninksky JJ, White TJ (eds) PCR Protocols: a Guide to Methods and Applications. Academic Press, New York, pp 315-322

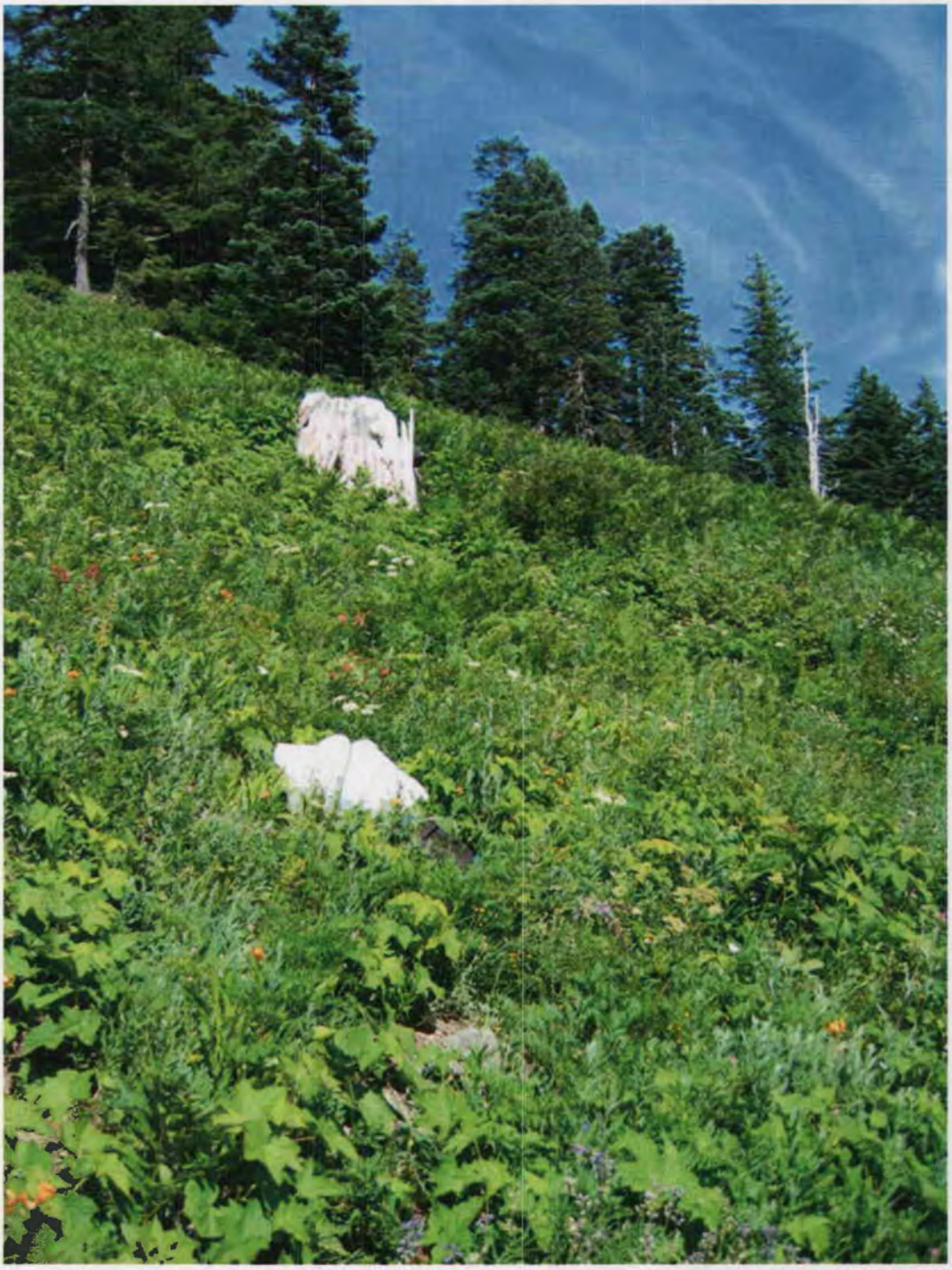

Figure 2.1. Carpenter site at the H. J. Andrews Experimental Forest.

Figure 2.2. Lookout site at the H. J. Andrews Experimental Forest.

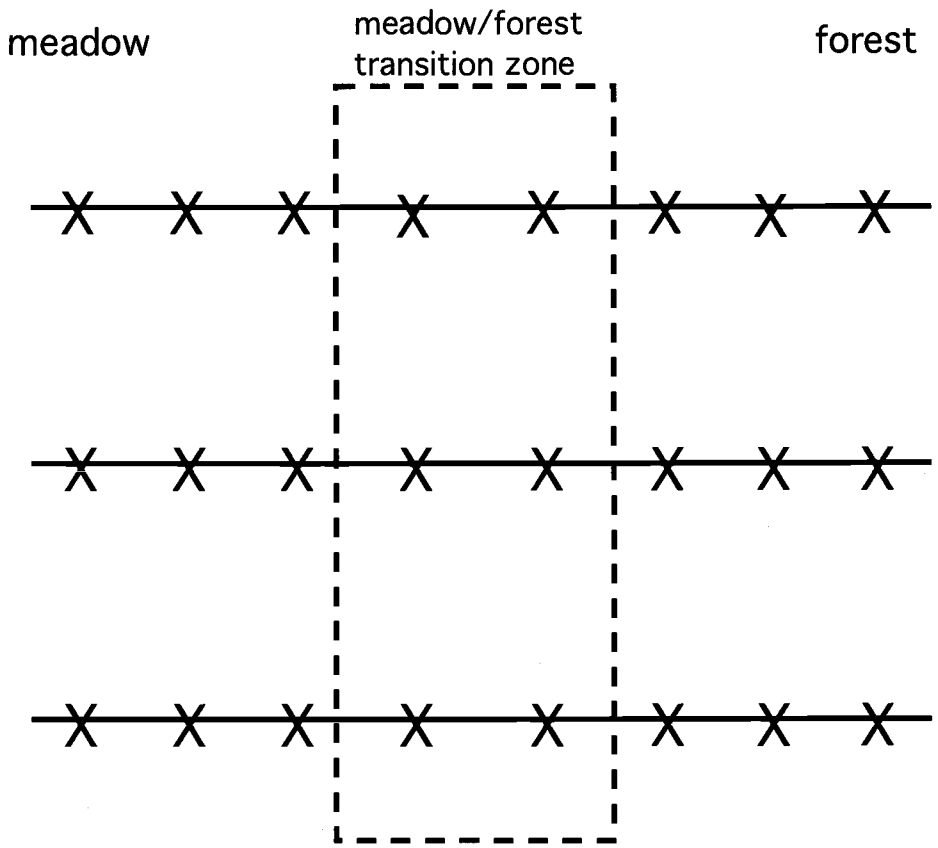

Figure 2.3. Sampling diagram of meadow, transition zone, and forest.

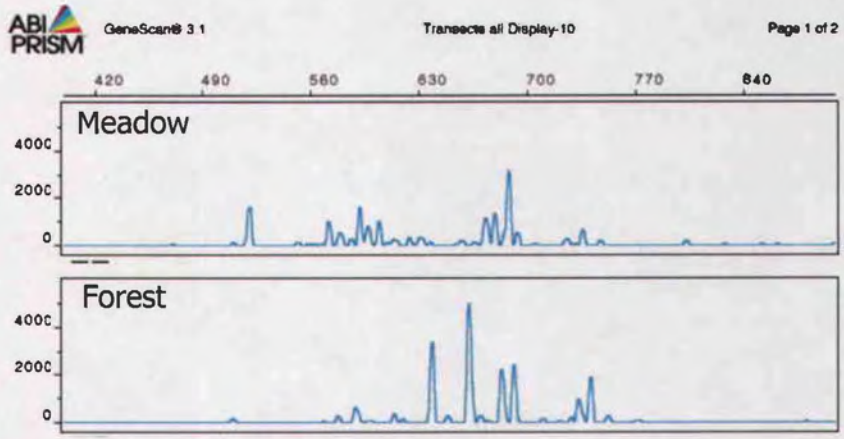

Figure 2.4. Examples of meadow and forest electropherograms with base pairs on the x-axis and relative fluorescence on the y-axis.

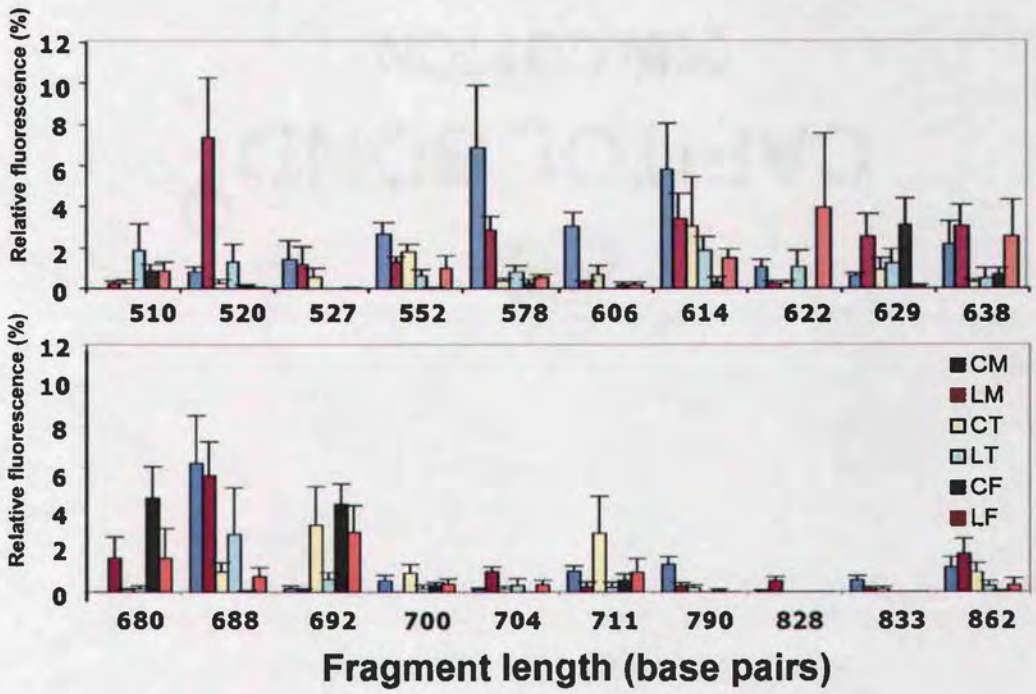

Figure 2.5. Distribution of indicator fragments along transects. CM = Carpenter meadow; LM = Lookout meadow; CT = Carpenter transition; LT = Lookout transition; CF = Carpenter forest; LF = Lookout forest.

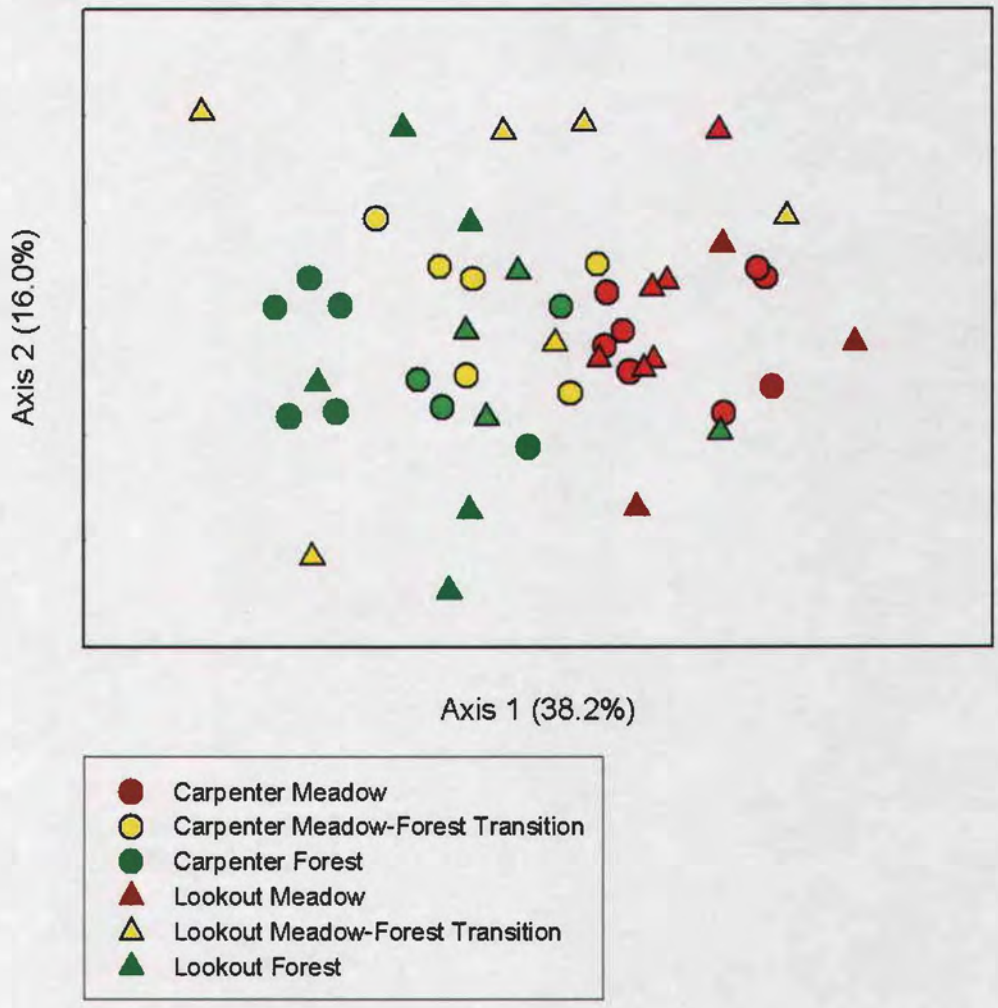

Figure 2.6. Nonmetric multidimensional scaling ordination of ITS fragment data at both Carpenter and Lookout transects.

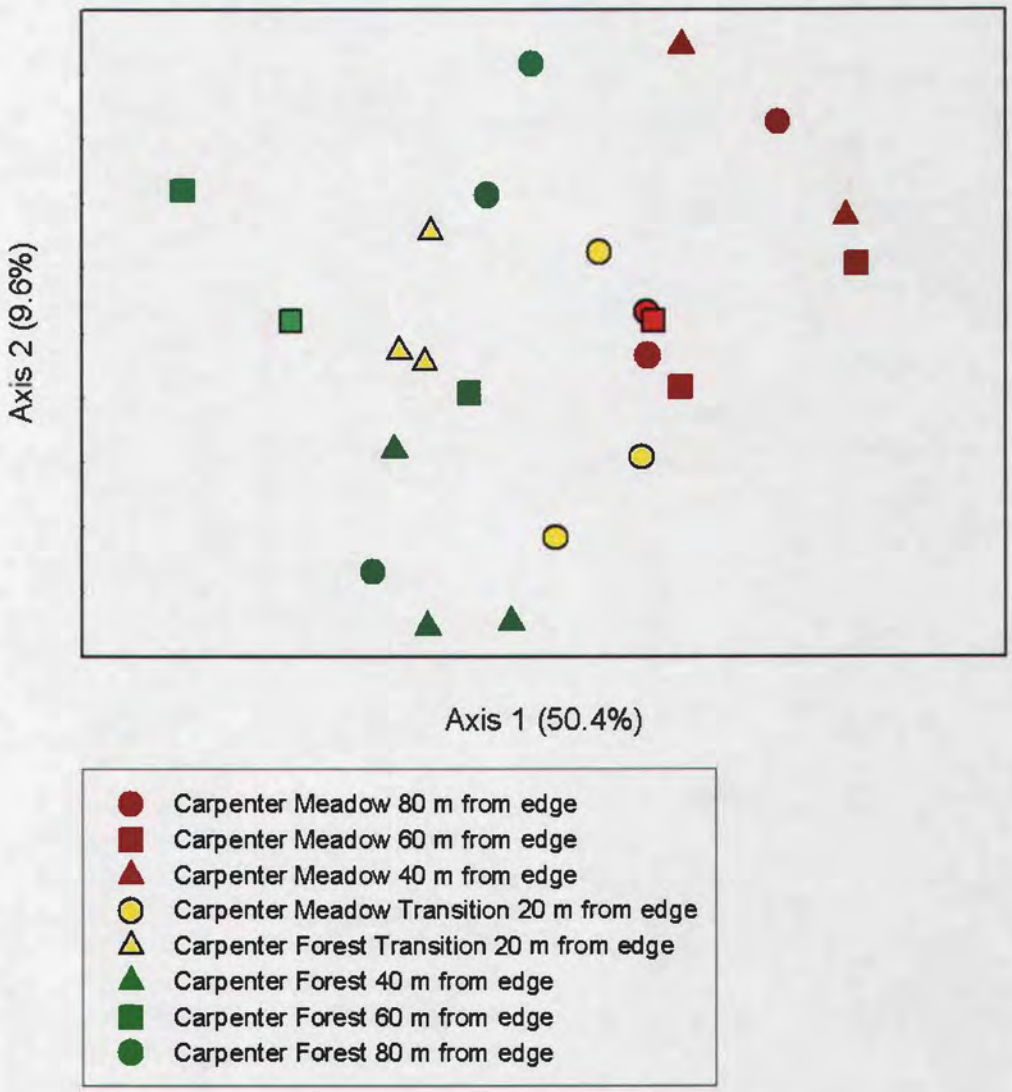

Figure 2.7. Nonmetric multidimensional scaling ordination of fungal ITS samples at Carpenter according to the distance from the meadow/forest boundary.

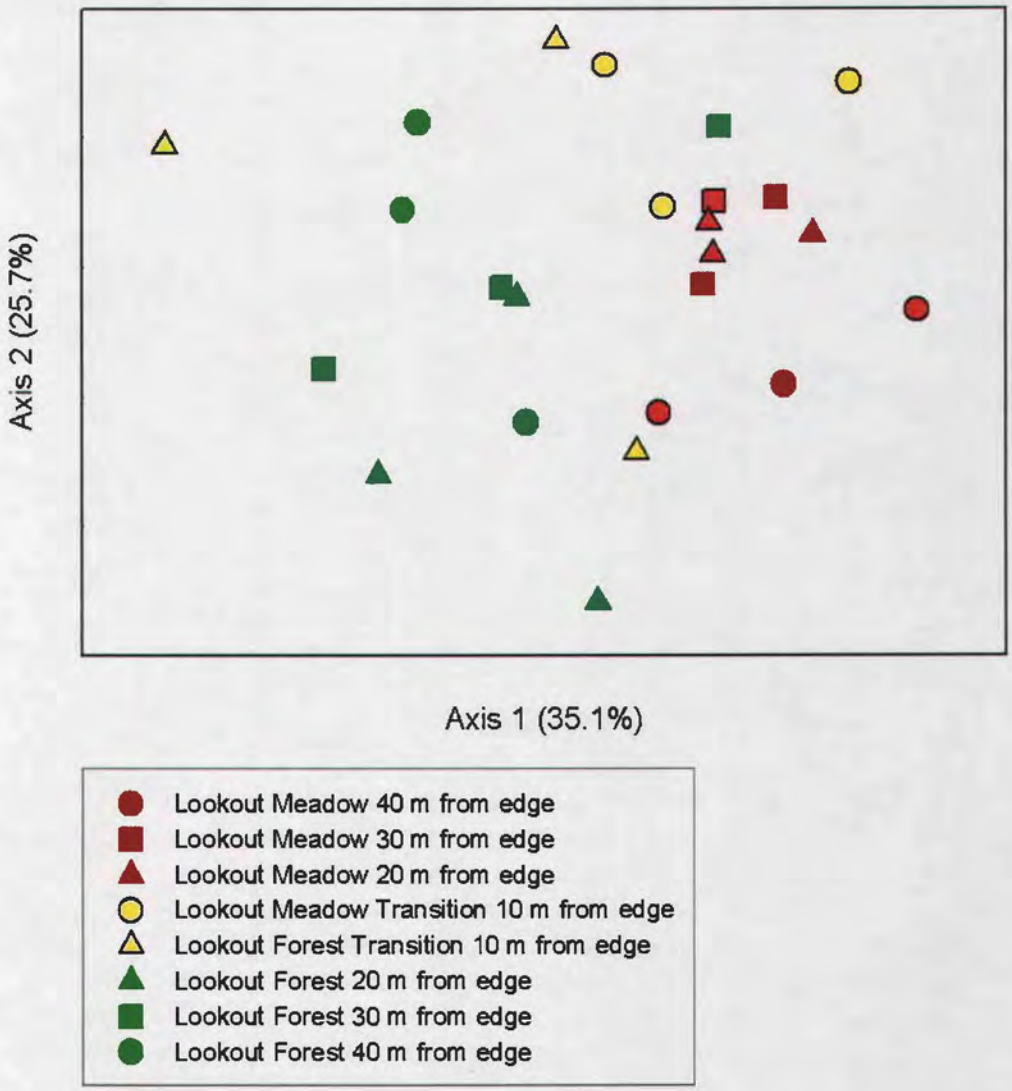

Figure 2.8. Nonmetric multidimensional scaling ordination of fungal ITS samples at Lookout according to the distance from the meadow/forest boundary.

Table 2.1. Species richness, beta-diversity, and average distance of data matrix.

	Carpenter site			Lookout site		
Measurement	Meadow	Transition	Forest	Meadow	Transition	Forest
Number of samples	8	6	9	9	6	9
Number of ITS fragments (species richness)	76	68	62	75	65	62
Beta diversity	1.9	2.0	2.5	2.0	2.4	2.4

Table 2.2. Multi-Response Permutation Procedures table by site and by vegetation type.

Test	*A*-statistic[1]	*p*-value
Difference by site:		
Carpenter meadow vs. Lookout meadow	0.052	< 0.0001
Carpenter transition vs. Lookout transition	0.005	0.317
Carpenter forest vs. Lookout forest	0.0076	0.10
Difference by vegetation type:		
Carpenter meadow vs. Carpenter forest	0.055	<0.001
Carpenter meadow vs. Carpenter transition zone	0.028	0.007
Carpenter forest vs. Carpenter transition zone	0.015	0.079
Lookout meadow vs. Lookout forest	0.104	<0.0001
Lookout meadow vs. Lookout transition zone	0.068	0.002
Lookout forest vs. Lookout transition zone	0.027	0.015

[1]A = 1 – (observed delta/expected delta); A = 0 when heterogeneity within groups equals expectation by chance; A < 0 with more heterogeneity within groups than expected by chance

Table 2.3. Indicator species analysis by vegetation type, by site, and across all sites.

Test	Site[1]	Fragment	Indicator value	p-value[2]
Indicators across all sites:				
	CM	520	66.3	0.002
	CT	606	61.9	0.002
	LM	704	45.8	0.001
	CM	790	60.9	0.001
	LM	828	71.7	0.001
Indicators by site:				
Carpenter meadow vs. Lookout meadow	CM	700	75	0.004
	LM	704	79.2	0.003
	CM	711	70.4	0.009
	LM	828	71.7	0.008
Carpenter transition vs. Lookout transition		none	n.a.	n.a.
Carpenter forest vs. Lookout forest		none	n.a.	n.a.
Indicators by vegetation type:				
Carpenter meadow vs. Carpenter forest	CF	510	77.8	0.003
	CM	552	98.9	0.001
	CM	578	84.5	0.003
	CM	606	81.8	0.002
	CM	614	95	0.001
	CM	622	75	0.003
	CF	680	66.7	0.009
	CF	692	85.2	0.003
	CM	790	83	0.001
	CM	833	62.5	0.007
	CM	862	85.3	0.001
Carpenter meadow vs. Carpenter transition zone		none	n.a.	n.a.
Carpenter forest vs. Carpenter transition zone	CT	552	98.4	0.001
	CT	614	90.8	0.006
Lookout meadow vs. Lookout forest	LM	520	88.5	0.002
	LM	629	96.6	0.001
	LM	688	78.4	0.008
	LF	692	75.3	0.009
	LM	828	77.8	0.003
Lookout meadow vs. Lookout transition zone	LM	638	85.3	0.008
Lookout forest vs. Lookout transition zone		none	n.a.	n.a.

[1]CM = Carpenter meadow; CT = Carpenter transition; CF = Carpenter forest; LM = Lookout meadow; LT = Lookout transition; LF = Lookout forest.
[2]proportion of randomized trials with indicator value equal to or exceeding the observed indicator value. p = (1 + number of runs ≥ observed)/(1 + number of randomized runs).

Chapter 3:

Effects of Disturbance on Fungal Communities in the Western Cascades of Oregon

Stacie A. Kageyama, David D. Myrold, Peter J. Bottomley,

and Kermit Cromack, Jr.

Oecologia

Springer-Verlag GmbH

In Preparation

INTRODUCTION

According to Friese et al. (1997), "Disturbance may be the single most important process regulating the structure and functioning of fungal communities because of the unique physiology, morphology and reproductive biology of fungi." Disturbances range in scale from large-scale catastrophic to small-scale. They are a natural component of ecosystems. However, some types of disturbance may test ecosystem resilience, the degree to which an ecosystem can be disturbed and return to function as before (Holling 1973).

Fungi play key roles nutrient cycling and ecosystem function. Mycorrhizal fungi serve as important carbon sinks in soil (Vogt et al. 1982). Saprotrophic fungi break down recalcitrant organic compounds for use by other organisms (Trappe and Luoma 1992). Saprophytic fungi contribute to soil organic matter formation through decomposition of plant litter (Beare et al. 1992; Miller and Lodge 1997; Swift et al. 1979). Other fungi produce polysaccharides in soil that contribute to soil stabilization (Chenu 1989; Miller and Lodge 1997).

Many fungi are adapted to disturbance in natural ecosystems. Some of the first colonizers of the new tephra following the eruption of Mount St. Helens were phoenicoid fungi (Carpenter et al. 1987). Sporocarps of these fungi provided refuge for photosynthetic nonvascular plants. Natural disturbance is often patchy and leaves islands of vegetation that are sources of fungi for recolonization and succession. However, the rate of recolonization following large scale disturbance is dependent on the availability of fungal inoculum (Friese et al. 1997).

Fungi range in size from single-celled yeasts to larger multicellular individuals whose mycelium can extend across relatively large patches. For example, an *Armillaria bulbosa* clone in Michigan was found to cover at least 15 hectares (Smith and Bruhn 1992). In the Pacific Northwest, genet size of *Cantharellus formosus* was determined using microsatellite markers and found to have a maximum width of 3.2 ± 3.6 m (Dunham et al. 2003). Large-scale disturbances such as volcanic eruptions or fire can result in the loss of the mycelium of an entire fungal individual. In contrast, small-scale disturbances such as digging by animals may only serve to fragment or disrupt the individual. Size of fungal individuals is as important as the effect of disturbance on fungi is relative to the scale of disturbance (Friese et al. 1997).

Forest ecosystems in the Pacific Northwest have evolved with a history of disturbance ranging in scale from infrequent catastrophic (Agee 1993) to frequent small-scale disturbances. The infrequent catastrophic disturbances include wildfire and volcanic eruptions. Natural fire intervals in the Pacific Northwest are several hundred years (Franklin and Halpern 2000). Volcanic eruptions are extremely rare occurrences but are still very much a part of the natural disturbance regime. Although the catastrophic disturbances can result in dramatic changes in the landscape, the smaller scale disturbances may play a greater role in vegetation dynamics and ecosystem function. Processes such as litter fall from the canopy, and root growth and turnover can also be considered disturbances as these processes open up new habitat for colonization (Zak 1992).

Meadows are natural features that are dispersed throughout upper montane and subalpine forests in the Cascade Range (Franklin and Halpern 2000). Many of the meadows in the Cascades have been persistent features on the landscape, some for more than 1000 years (Hickman 1976). Numerous factors have contributed to the creation and maintenance of these features on the landscape (Franklin and Halpern 2000; Miller and Halpern 1998). Some meadows have remained in place due to the presence of shallow rocky soils on extremely steep slopes (Hickman 1976), while others are maintained by small-scale disturbance such as animal burrowing (Franklin and Halpern 2000). It is likely that fungi in these systems are adapted to small-scale disturbances.

In 1999, we established a Microbial Observatory at the H. J. Andrews Experimental Forest in the central Cascade Mountains of Oregon to study the bacterial and fungal communities that are critical to biogeochemical processes. A goal of our Microbial Observatory was to gain a better understanding of how bacterial and fungal communities respond to disturbance events. In 2000, we initiated a reciprocal transplant soil core experiment in adjacent forest and meadow sites and tracked the changes in microbial community composition over the next two years. The goal of the experiment was to address disturbance on two different scales. The first part of the experiment addressed small-scale disturbance and the changes in microbial communities that occur when plants roots and hyphal connections are severed. We hypothesized that severing roots and hyphal connections would result in short-term changes that would disappear once soil cores

were recolonized by roots and hyphae. We hypothesized that would see a sampling effect, a change in community composition due to disturbance occurring during sampling. We also hypothesized that we would see a decomposition effect as roots and hyphae decompose. Finally, we hypothesized that we would see a root effect. The second part of the study examined disturbance on a larger scale and the changes in microbial community that occur when plant communities drastically change. We hypothesized that we would see more dramatic changes once soil cores were transferred to new environments as fungal communities from their original communities would not persist. For this study, we examined the changes in fungal community that occurred during the two years following disturbance. Our goals for this study were to gain a better understanding of how fungal communities respond to disturbance and how quickly fungal succession occurs after disturbance. We expected to see differences in fungal community composition between the forest and meadow sites (Kageyama et al. 2005). We also expected to see rapid colonization of transferred cores by fungi in their new environments. However, we did not expect mycorrhizal fungi in transferred cores to persist in their new environments in the absence of their plant hosts.

METHODS

Site characteristics and sampling. Study sites were located at the H. J. Andrews Experimental Forest Long Term Ecological Research site (44.2°N, 122.2°W) in the Cascade Mountain Range of Oregon, USA. Two high

montane sites (1500 m), designated Lookout and Carpenter, were selected at the H. J. Andrews Experimental Forest (Rich 2004; Rich et al. 2003). Both sites had adjacent meadow and forest communities. Dominant meadow vegetation at both sites included *Bromus carinatus* Hook. & Arn., *Elymus glaucus* Buckl., *Pteridium aquilinum* (L.) Kuhn, and *Anaphalis margaritacea* (L.) Benth. & Hook. Dominant forest trees at both sites included *Pseudotsuga menziesii* (Mirb.) Franco, *Abies amabilis* Douglas ex Forbes, *A. grandis* (Dougl. ex D. Don.) Lindl., and *Tsuga mertensiana* (Bong.) Carr. Large stumps and charcoal at the Carpenter meadow site indicate that the site was clearcut in the past.

Reciprocal transplant study design. A 35 x 35 m grid was installed at each meadow and forest site at Carpenter and Lookout in September 2000. Each grid had 64 sampling points that were 5 m apart. Soil cores were collected by pounding 6 x 15 cm PVC pipe into soil. The litter layer from removed from the soil surface before sampling. Roots were left intact in the soil cores.

There were a total of five treatments: background, open remaining, closed remaining, open transfer, and closed transfer. There were six cores per treatment per year (Figure 1). The background cores were not manipulated. The open remaining and open transfer cores were placed in mesh bags that allowed root growth into the cores. The closed remaining and closed transfer cores were placed in mesh bags and also enclosed in PVC pipe in order to exclude roots. Open and closed remaining cores were replaced on site. Transfer cores were transplanted from the meadow and moved to the adjacent forest and vice versa at both the Carpenter and Lookout sites.

Background cores were collected at the time of installation in 2000, and at the times of harvesting in 2001 and 2002. The open remaining, closed remaining, open transfer, and closed transfer were incubated *in situ* for one and two years, and harvested in September of 2001 and 2002. Two cores of each treatment were pooled into a composite sample and homogenized through a 4 mm sieve. There were three composite cores for each treatment. Soil aliquots were then frozen at -20°C for DNA extraction.

DNA extraction and length heterogeneity PCR. DNA was extracted from 0.5 g of soil (fresh weight) using the FastDNA® kit (Bio 101, Inc. ., Irvine, CA) according to the manufacturer's directions. Extracted DNA was checked by running 10 μl of extract on an agarose gel (1%) stained with ethidium bromide (0.5 μg ml^{-1}). DNA was quantified on a BioSpec-1601 DNA/Protein/Enzyme Analyzer (Shimadzu Corporation, Kyoto, Japan). Extracts were diluted to 25 ng of DNA per μl for PCR amplification. DNA was amplified using 6-FAM labeled forward primers for Length Heterogeneity PCR (LH-PCR). Primers for the internal transcribed spacer region of rDNA, ITS1F and ITS4 (Gardes and Bruns 1993), were used for LH-PCR. Reaction mixtures (50 μl) contained soil DNA (100 ng), AmpliTaq DNA polymerase (2.5 U), GeneAmp PCR buffer (1x), $MgCl_2$ (2 mM), deoxynucleoside triphosphates (0.2mM each), forward and reverse primers (0.2 μM each), and bovine serum albumin (0.064 g ml^{-1}). The forward primer, ITS1F, was labeled with 6-FAM (6-carboxyfluorescein). PCR amplification was conducted as follows using a PTC-100 hot bonnet thermocycler (MJ

Research, Inc., Waltham, MA): 94° for 2 min followed by 35 cycles of 94° for 30 s, 55° for 30 s, 72° for 1 min. A final extension followed for 72° for 2 min. PCR products were visualized with electrophoresis on 1% agarose gels stained with ethidium bromide. One μl of PCR product containing 1 ng of DNA was submitted for capillary sequencing on an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA) in the Central Analytical Laboratory, Oregon State University. PCR products were run on the Genetic Analyzer along with X-Rhodamine MapMarker™ 1000 internal lane size standard (BioVentures, Inc., Murfreesboro, TN).

Data analysis. Size and relative abundance of LH-PCR fragments was quantified using GeneScan® v. 3.5 software and Genotyper® v. 2.5 software (Applied Biosystems, Inc., Foster City, CA). Sequence length and peak area data was obtained using Genotyper. Data was downloaded into Microsoft® Excel v. X for Mac and manually binned. We excluded fragments that were below 1.5% of the total peak area for each sample. We also excluded rare peaks that occurred in less than 5% of our samples. The dataset was then relativized by row totals (samples) to adjust for differences in fluorescence levels of our amplified fragments.

Community data was analyzed with PC-ORD Version 4.36 (MJM Software, Gleneden, OR). Ordinations of LH-PCR fragment data were run in PC-ORD using nonmetric multidimensional scaling (NMS) using the autopilot feature, "Slow and thorough" with the Sørensen distance measure. We chose to use NMS because it avoids the assumption of linear

relationships among variables, and allows the use of any relativization method (McCune and Grace 2002). Analyses were run on the entire data set (both Lookout and Carpenter), background samples only for 2000-2002, and then by year, 2001 or 2002. For the dataset containing all sites, we deleted columns (LH-PCR fragments) containing fewer than three non-zero values.

Multi-response permutation procedures (MRPP) were used to test fungal community composition between vegetation types and different sites. MRPP is a nonparametric method that is used to test for group differences (McCune and Grace 2002). We used MRPP in PC-ORD Version 4.36 (MJM Software, Gleneden, OR). We used the Sørensen similarity coefficient and defined our groups by site.

Indicator species analyses were conducted using PC-ORD Version 4.36 (MJM Software, Gleneden, OR) by comparing either meadow sites to forest sites or similar vegetation types with 1000 randomizations in the Monte Carlo test. According to Dufrêne and Legendre (Dufrêne and Legendre 1997), a perfect indicator is always present in a particular group and is exclusive to that group. PC-ORD employs Dufrêne and Legendre's (Dufrêne and Legendre 1997) indicator species analysis method to produce indicator values for each species (LH-PCR fragment) for each group (site).

RESULTS

Nonmetric multidimensional scaling ordination indicated that fungal ITS LH-PCR fragments in background cores exhibited some temporal variation for the three years sampled but this was minor compared to vegetation and site effects (Figure 3.2). Meadow and forest samples clearly

separated in the NMS ordination. There was also some separation between the Carpenter Meadow and Lookout Meadow samples. Meadow samples also grouped more tightly within themselves than forest samples in the ordination.

Multi-Response Permutation Procedures also indicated that there were some temporal differences among the years (Table 3.1). Multi-Response Permutation Procedures also indicated that there were some differences in the background samples between the two meadows for both 2001 and 2002, whereas there were no differences between the two forests (Table 3.2). For the 2001 NMS ordination, background samples continued to show a separation between meadows and forests (Figure 3.3). The separation is supported by the MRPP analysis. The meadow samples grouped more closely than the forest samples. For the 2002 NMS ordination, background fungal ITS LH-PCR data indicated a separation between the communities in the forest and meadow sites (Figure 3.4).

Remaining cores

2001

Carpenter Meadow background and both open and closed remaining cores remained distinct from cores from Lookout Meadow in the NMS ordination (Figure 3.3). The separation between the Carpenter Meadow background and remaining cores and the Lookout Meadow background and remaining cores is supported by MRPP analysis (Tables 3.3 and 3.4). The open remaining cores in the forests grouped with the forest background samples in the NMS ordination, while the closed remaining cores were

intermediate between the background and open remaining cores and the cores transferred into the forest. However, with a few exceptions these differences are not supported by MRPP analysis (Tables 3.3, 3.4, and 3.5). There were no significant sampling effects as indicated by comparisons of background and open remaining cores by MRPP (Table 3.3). There were also no significant decomposition effects when background and open remaining cores were compared by MRPP (Table 3.4).

2002

Background and closed and open remaining meadow cores show a separation from the forest background and Carpenter Forest open remaining cores in the NMS ordination (Figure 3.4). There continued to be a distinct separation between Carpenter and Lookout Meadows. These differences are supported by MRPP (Table 3.2). Some of the closed remaining forest samples grouped with the meadows in the ordination. However, only the Lookout Forest background vs. Lookout Forest closed remaining difference is supported by MRPP (Table 3.4). There were no significant sampling effects as indicated by comparisons of background and open remaining cores by MRPP (Table 3.3). The only significant decomposition effect occurred with Lookout Forest background vs. remaining (Table 3.4). Root effects were significant for open/closed transfers except Carpenter Forest (Table 3.5).

Transplanted cores

2001

For cores incubated for one year and harvested in 2001, NMS ordination indicated that all of the transplanted cores had shifted away from their sites of origin with the exception of some of the closed transferred meadow cores (Figure 3.2). There were also some open/closed treatment effects for the cores transferred in 2001 in the NMS ordination. However, with the exception of Carpenter Meadow closed vs. open transfer, open/closed treatment effects were not supported by MRPP (Table 3.5).

2002

Transferred soil cores harvested in 2002 (incubated for two years) exhibited clear shifts in fungal communities according to NMS ordination (Figure 3.4). By 2002, NMS ordination indicated that the fungal community fingerprints of transferred cores had shifted from that of their sites of origin over to their sites of incubation with the exception of some of the closed meadow cores transferred to the forest. MRPP generally supported these results (Table 3.6). Both open and closed cores that had originated in the forest and had been transferred to the meadows had developed fungal community profiles similar to background meadow cores. The same was true for open meadow cores transferred to the forests. Open/closed core effects were seen for all transferred cores except for Carpenter Forest (Table 3.5).

Indicator species analysis

Background samples

We compared all background years by vegetation and site. We found several indicator fragments from the meadow sites when meadow vs. forest samples were analyzed (Table 3.7). However, we found only one indicator fragment from the forest. Several indicators were found when Carpenter Meadow was compared to Lookout Meadow (Table 3.7). However, no indicators were found when Carpenter Forest was compared to Lookout Forest. Several indicators were found when Carpenter Meadow was compared to Carpenter Forest and Lookout Meadow was compared to Lookout Forest (Table 3.7). All of the indicator fragments in the meadow vs. forest comparisons were from meadow sites.

2001

Background, remaining, and transfer samples were compared by site. Closed and open cores were grouped together because there were few differences between them in 2001. Several indicators were found when we compared backgrounds to remaining, backgrounds to transfers, and remaining to transfers (Table 3.8). The forest indicator fragment 719 appears in transfers when Carpenter Meadow backgrounds and remaining are compared to transfers, cores that were moved form meadow to forest. The meadow indicator 614 appears in transfers when Lookout Forest remaining are compared to transfers, cores that were moved form forest to meadow.

2002

Background, remaining, and transfer samples were compared by site. Several good indicators (indicator value = 100) were found in the Carpenter Meadow background and remaining to transfer comparisons (Table 3.9). The fragment 684 frequently appears as an indicator for disturbed cores, regardless of vegetation type or whether the cores were transferred or not.

DISCUSSION

We observed distinct separations between fungal communities in the meadows and forests in the NMS ordination of the 2000-2002 background samples. We saw similar results with an earlier transect study at the same sites (Kageyama et al. 2005). In addition, the tighter grouping and separation of the Carpenter Meadow and Lookout Meadow sites was also observed in the earlier study. Out of all the sites, Carpenter Forest showed the most variation over the three years.

One year after disturbance, fungal communities in the closed and open cores in the Carpenter and Lookout Forests separated in the NMS ordination. For cores harvested in 2001, differences between background samples and treatments seem to indicate a disturbance effect. Some of the differences may be accounted for by more rapid colonization of the open cores by roots and fungi. Fungi in the open remaining cores in the forests may have also been able to reestablish hyphal connections that had been severed in the disturbance. An increase in the abundance of saprophytic fungi associated with the severed roots and hyphae may have contributed to

the separation of communities between the closed remaining cores from the forest sites and the background cores. After one year, open cores transferred from the meadow sites to the forests had clearly differentiated from the meadow background cores.

Two years after disturbance, NMS ordination indicated that fungal communities in both the closed and open transfer cores from the forest had shifted in similarity to those of the meadow background and remaining cores. All of the open transfer cores and one each of the closed transfer cores from the meadows had become more similar to the forests. In addition, there were significant root effects for all closed/open transfers except for Carpenter Forest. By the 2002, root exclusion had become a greater factor influencing fungal communities. Curiously, two out of the three closed remaining cores from each of the forests had become more similar to those meadows. Several studies of root activity and ectomycorrhizae after clearcutting have shown significantly decreased root activity and active root tips (Elmqvist et al. 2003; Hagerman et al. 1999; Harvey et al. 1980a; Harvey et al. 1980b). It is likely that ectomycorrhizal fungi did not persist after roots and hyphal connections were severed. It is also likely that those closed cores were not recolonized by ectomycorrhizal fungi in the forest soils. In addition, the shift in the ordination to the meadows may be accounted for by an increase in saprophytic fungi. Some ECM fungal mycelium may persist for a short time after clearcuts (Hagerman et al. 1999; Harvey et al. 1980a; Perry et al. 1987) but only a few ECM fungi have the ability to produce long-term survival structures in the soil (Jones et al. 2003).

Indicator species analysis supports the differences found between meadows and forests in the ordinations. For 2001, the indicator fragment 719 found in cores transferred from Carpenter Meadow to Carpenter Forest suggests that transferred cores are being colonized by fungi in their new environment. By 2002, differences between Carpenter Meadow backgrounds and remaining and transfers are more dramatic with several good indicators appearing between cores in the meadow and cores transferred to the forest. Patterns are less clear for Carpenter Forest and Lookout Meadow and Forest. However, the fragment 684 appears in some comparisons in 2001 and most comparisons in 2002 and appears to be an indicator of disturbance as it appears as an indicator only in remaining or transferred cores.

The goal of the remaining treatment was to examine the effects of small-scale disturbance on fungal communities. Fungal communities in the forest were more sensitive to disturbance although they were replaced on site. Root exclusion in closed remaining cores also had an impact as several of the closed remaining cores from the forest became more similar to the meadows. This shift in fungal community may have been due to the loss of ECM and an increase in saprophytic fungi. Our results are consistent with earlier root exclusion studies. Brant et al. (2005) found that fungal communities were particularly sensitive to root exclusion and that fungal biomass decrease when roots were excluded. Wallander et al. (2001) used root ingrowth bags inside and outside trenched plots to estimate the amount of ectomycorrhizal fungal biomass. They found that the fungal marker 18:2ω6,9 was significantly reduced inside the plots which they concluded was due to a decrease in ectomycorrhizal mycelium.

The goal of the transfer treatment was to mimic a large-scale disturbance such as clearcutting. In fact, fungi from the forest did not persist in cores transferred to meadows. Root exclusion affected fungal communities in the closed transfer meadow cores moved to the forest. Two years after transfer, those communities were still more like the meadow communities than those in the forests. Our fungal ITS transfer data are consistent with Harvey et al. (1980) and Hagerman et al. (1999) who found that the abundance of ECM root tips declined following clearcutting. Some ECM fungal mycelium are capable of persisting for a few years after clearcutting (Hagerman et al. 1999; Harvey et al. 1980a; Perry et al. 1987). Most ECM mycelium declines rapidly in the absence of a plant host.

Previous studies that examine the effect of disturbance and reciprocal transfer using soil cores from this experiment found little change in bacterial communities associated with ammonia oxidization or denitrification activity (Bottomley et al. 2004; Boyle et al. 2005). It is likely that we observed a rapid response to disturbance and transfer with the fungal communities due to the intimate association of mycorrhizal and saprophytic fungi with plants and the exploratory nature of fungal hyphae.

Disturbance events open up new habitat for colonization and result in increased heterogeneity in the system (Denslow 1985). Even catastrophic disturbances such as the Yellowstone Fires of 1988 result in a mosaic pattern on the landscape (Platt and Connell 2003; Turner et al. 1998). Residuals or biotic legacies are organisms that survive disturbances either intact, or as seeds or other propagules (Turner et al. 1998). The composition of the fungal community following large-scale disturbance depends on the ability of

individuals to survive as vegetative hyphae, the germination of new individuals, and on competitive interactions between survivors and immigrants (Friese et al. 1997). Our results show that forest fungal communities are particularly sensitive to disturbance and the extent of disturbance in forests may have implications for succession.

LITERATURE CITED

Agee JK (1993) Fire Ecology of Pacific Northwest Forests. Island Press, Washington, D. C.

Beare MH, Parmelee RW, Hendrix PF, Cheng W, Coleman DC, Crossley DA (1992) Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. Ecological Monographs 62:569-591

Bottomley PJ et al. (2004) Responses of nitrification and ammonia-oxidizing bacteria to reciprocal transfers of soil between adjacent coniferous forest and meadow vegetation in the Cascade Mountains of Oregon. Microbial Ecology 48:500-508

Boyle SA, Rich JJ, Bottomley PJ, Cromack Jr. K, Myrold DD (2005) Disturbance effects on denitrifying community composition and activity at forest and meadow sites in the Cascade Mountains of Oregon. in preparation

Carpenter SE, Trappe JM, Ammirati J, J. (1987) Observations of fungal succession in the Mount St. Helens devastation zone, 1980-1983. Canadian Journal of Botany 65:716-728

Chenu C (1989) Influence of a fungal polysaccharide, scleroglucan on clay microstructure. Soil Biology & Biochemistry 21:299-305

Denslow JS (1985) Disturbance-mediated coexistence of species. In: Pickett STA, White PS (eds) The Ecology of Natural Disturbances and Patch Dynamics. Academic Press, Inc., New York, pp 307-323

Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible approach. Ecological Monographs 67:345-366

Dunham SM, Kretzer A, Pfrender ME (2003) Characterization of Pacific golden chanterelle (Cantharellus formosus) genet size using co-dominant microsatellite markers. Molecular Ecology 12:1607-1618

Elmqvist T et al. (2003) Response diversity, ecosystem change, and resilience. Frontiers in Ecology and Environment 1:488-494

Franklin JF, Halpern CB (2000) Pacific Northwest Forests. In: Barbour MG, Billings WD (eds) North American Terrestrial Vegetation, 2nd edn. Cambridge University Press, Cambridge, pp 123-159

Friese CF, Morris SF, Allen MF (1997) Disturbance in natural ecosystems: Scaling from fungal diversity to ecosystem functioning. In: Wicklow DT, Söderström BE (eds) The Mycota IV: Environmental and Microbial Relationships, vol IV. Springer-Verlag, Berlin, pp 47-63

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

Hagerman SM, Jones MD, Bradfield GE, Gillespie M, Durall DM (1999) Effects of clear-cut logging on the diversity and persistence of ectomycorrhizae at a subalpine forest. Canadian Journal of Forest Research 29:124-134

Harvey AE, Jurgensen MF, Larsen MJ (1980a) Clearcut harvesting and ectomycorrhizae: survival of activity on residual roots and influence on a bordering forest stand in Western Montana. Canadian Journal of Forest Research 10:300-303

Harvey AE, Jurgensen MF, Larsen MJ (1980b) Clearcut harvesting and ectomycorrhizae:survival of activity on residual roots and influence of bordering forest stand in western Montana. Canadian Journal of Forest Research 10:300-303

Hickman JC (1976) Non-forest vegetation of the western Cascade Mountains of Oregon. Northwest Science 50:145-155

Holling CS (1973) Resilience and stability of ecological systems. Annual Review of Ecology and Systematics 4:1-23

Jones MD, Durall DM, Cairney JWG (2003) Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytologist 157:399-422

Kageyama SA, Myrold DD, Bottomley PJ, Cromack K, Jr. (2005) Chapter 2: Fungal communities across meadow/forest transects in the western Cascades of Oregon. In: Effects of vegetation and disturbance on fungal communities in the western Cascades of Oregon, Ph.D. dissertation. Oregon State University

McCune B, Grace JB (2002) Analysis of Ecological Communities. MJM Software Design, Gleneden Beach

Miller EA, Halpern CB (1998) Effects of environment and grazing disturbance on tree establishment in meadows of the central Cascade Range, Oregon, USA. Journal of Vegetation Science 9:265-282

Miller RM, Lodge DJ (1997) Fungal responses to disturbance: Agriculture and forestry. In: Wicklow DT, Söderström BE (eds) The Mycota IV: Environmental and Microbial Relationships, vol IV. Springer-Verlag, Berlin, pp 65-84

Perry DA, Molina R, Amaranthus MP (1987) Mycorrhizae, mycorrhizospheres, and reforestation: current knowledge and research needs. Canadian Journal of Forest Research 17:929-940

Platt WJ, Connell JH (2003) Natural disturbances and directional replacement of species. Ecological Monographs 73:507-522

Rich JJ (2004) Community Composition and Activities of Denitrifying Bacteria in Soils. In: Crop and Soil Science. Oregon State University, Corvallis, p 102

Rich JJ, Heichen RS, Bottomley PJ, Cromack K, Jr., Myrold DD (2003) Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. Applied and Environmental Microbiology 69:5974-5982

Smith ML, Bruhn JN (1992) The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature 356:428

Swift MJ, Heal OW, Anderson JM (1979) Decomposition in terrestrial ecosystems. Blackwell, Oxford

Trappe JM, Luoma DL (1992) The Ties That Bind: Fungi in Ecosystems. In: Carroll GC, Wicklow DT (eds) The Fungal Community: Its Organization and Role in the Ecosystem. Marcel Dekker, Inc., New York, pp 17-27

Turner MG, Baker WL, Peterson CJ, Peet RK (1998) Factors influencing succession: Lessons from large, infrequent natural disturbances. Ecosystems 1:511-523

Vogt KA, Grier CC, E. M, Edmonds RL (1982) Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* ecosystems in western Washington. Ecology 63:370-380

Zak JC (1992) Response of Soil Fungal Communities to Disturbance. In: Carroll GC, Wicklow DT (eds) The Fungal Community: Its Organization and Role in the Ecosystem, 2nd edn. Marcel Dekker, Inc., New York, pp 403-425

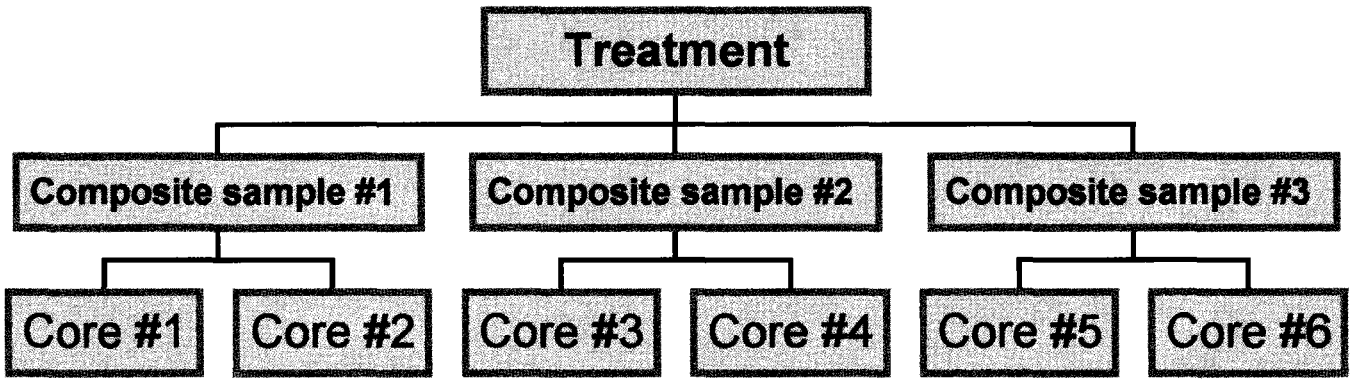

Figure 3.1. Number of samples per treatment.

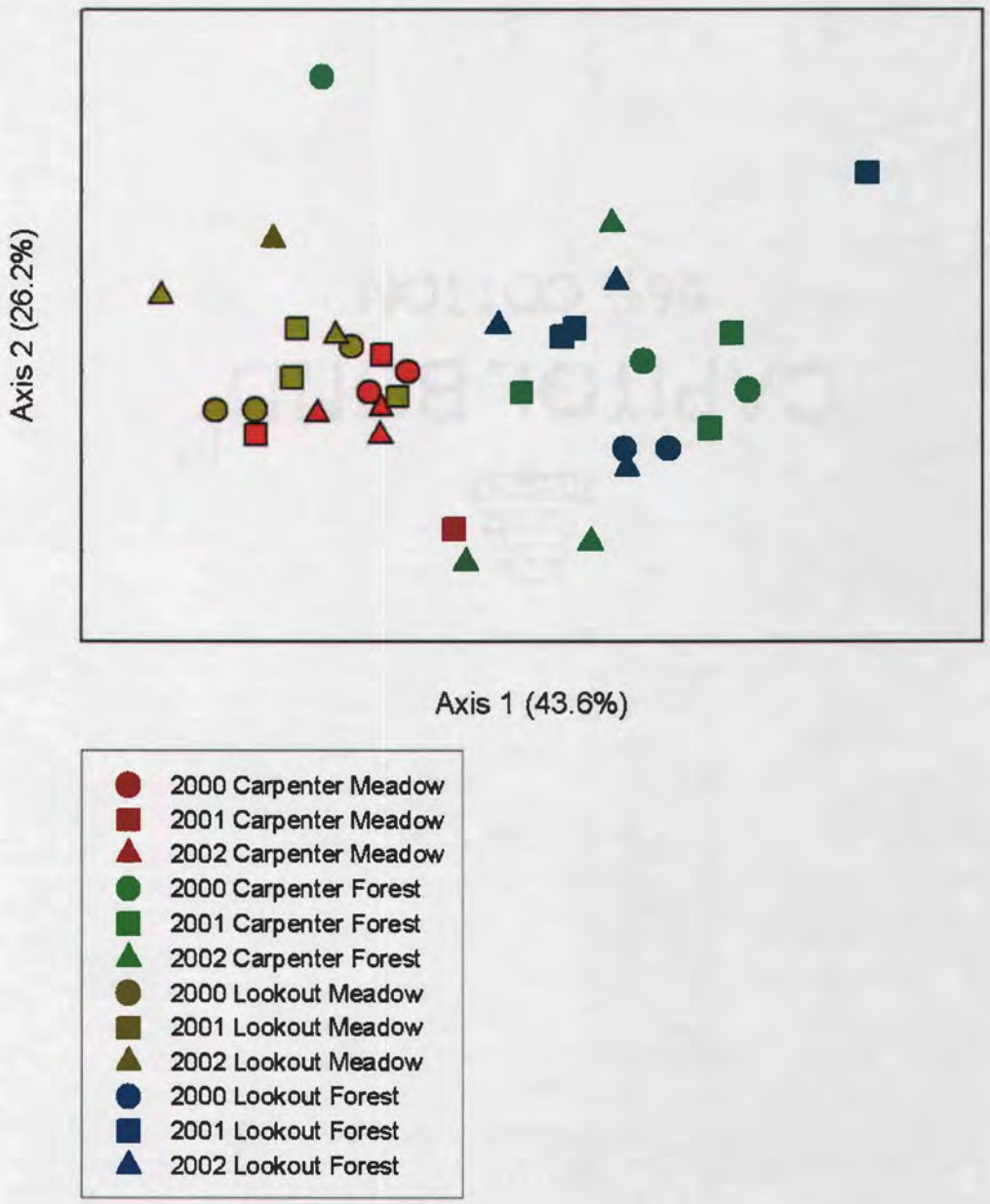

Figure 3.2. Nonmetric multidimensional scaling ordination of ITS fragment data from background samples collected from 2000-2002.

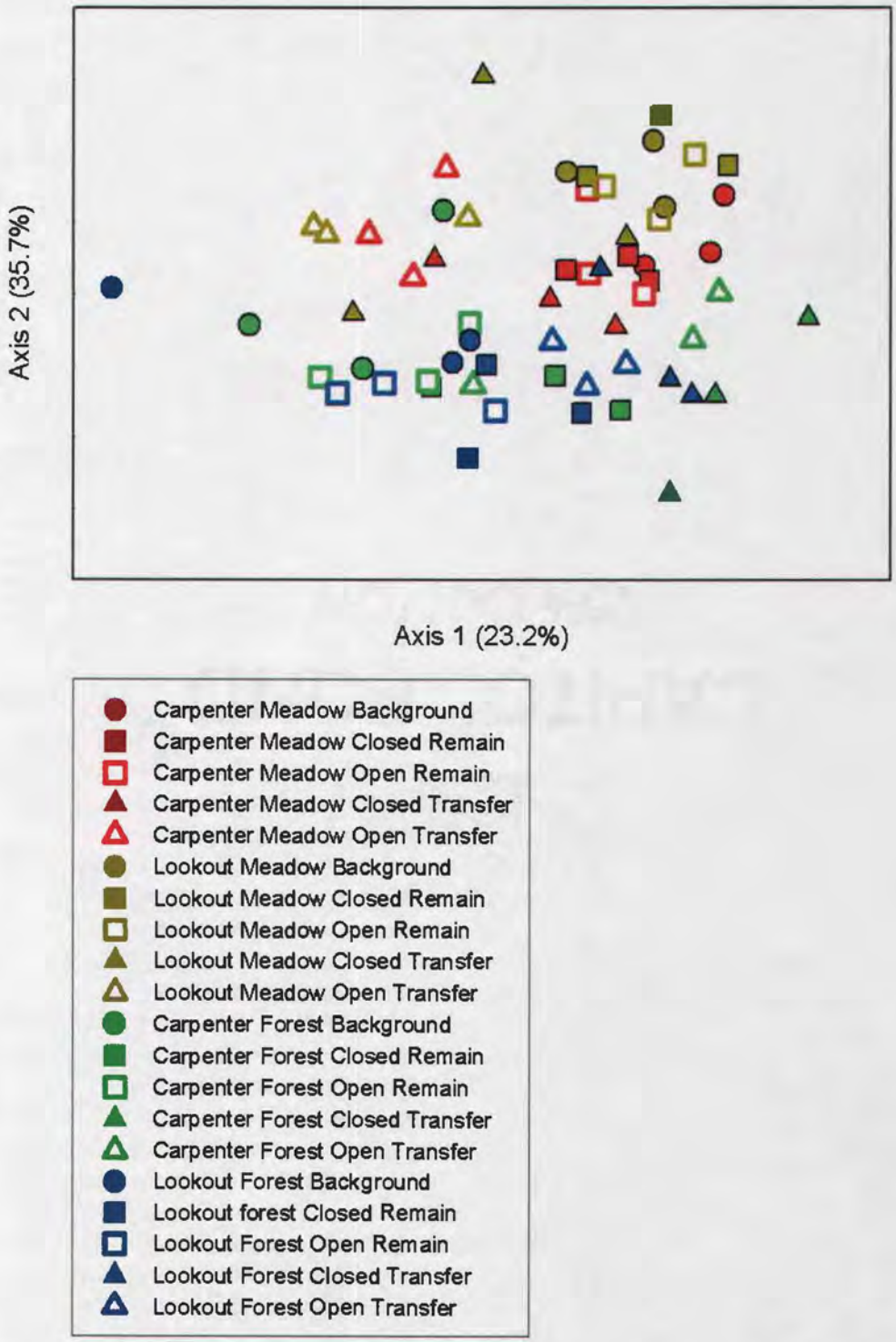

Figure 3.3. Nonmetric multidimensional scaling ordination of ITS fragment data from background and reciprocal transplant cores harvested in 2001.

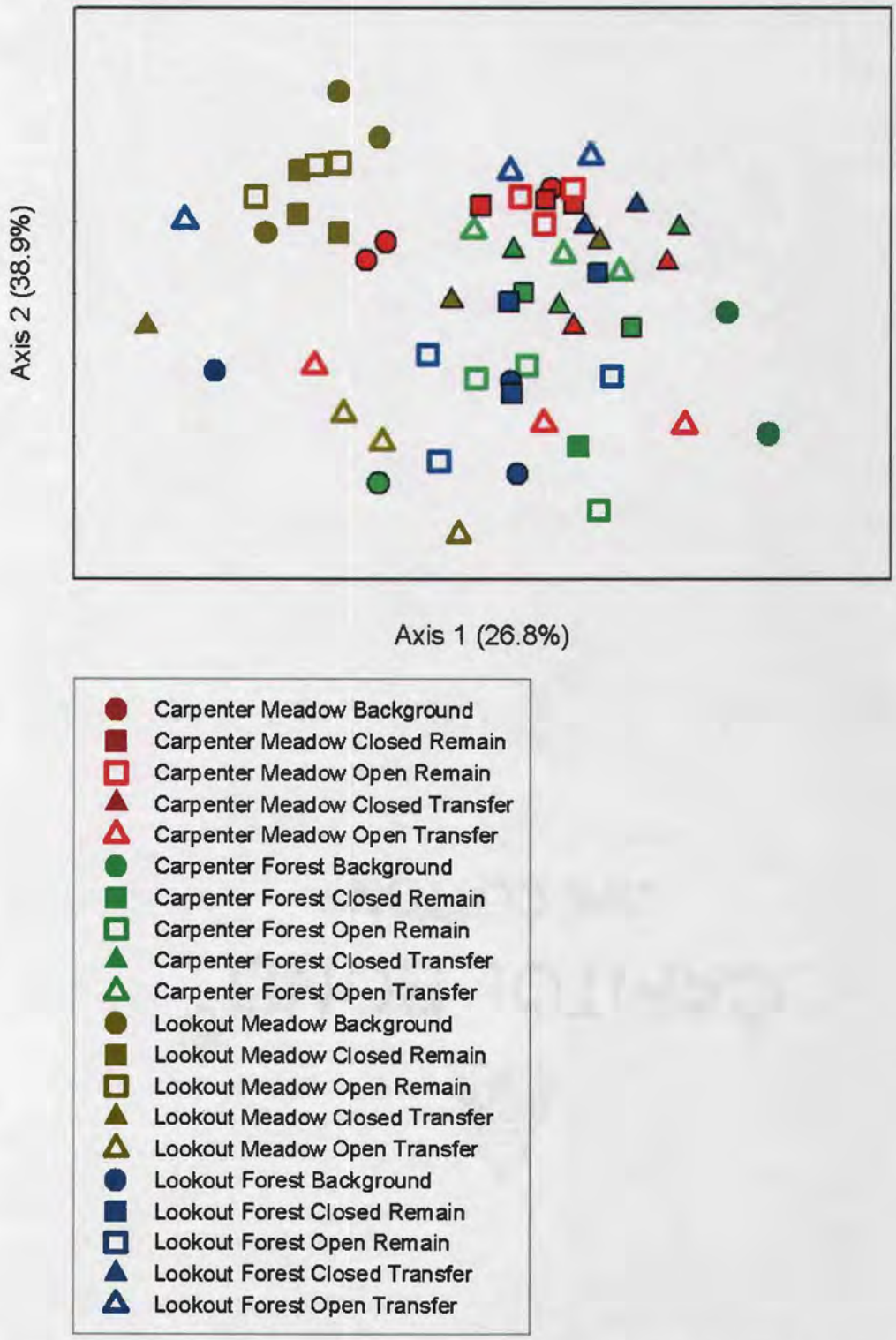

Figure 3.4. Nonmetric multidimensional scaling ordination of ITS fragment data from background and reciprocal transplant cores harvested in 2002.

Table 3.1. Multi-Response Permutation Procedures table for background samples.

Test	*A*-statistic[1]	*p*-value
Difference by site and year:		
Carpenter meadow 2000 vs. 2001	0.035	<0.001
Carpenter meadow 2000 vs. 2002	0.105	<0.001
Carpenter meadow 2001 vs. 2002	0.001	0.488
Carpenter forest 2000 vs. 2001	-0.035	0.844
Carpenter forest 2000 vs. 2002	0.044	0.068
Carpenter forest 2001 vs. 2002	0.021	0.274
Lookout meadow 2000 vs. 2001	0.004	0.373
Lookout meadow 2000 vs. 2002	0.102	0.023
Lookout meadow 2001 vs. 2002	0.011	0.318
Lookout forest 2000 vs. 2001	0.053	<0.001
Lookout forest 2000 vs. 2002	-0.017	1.000
Lookout forest 2001 vs. 2002	0.017	0.290

[1]A = 1 – (observed delta/expected delta); A = 0 when heterogeneity within groups equals expectation by chance; A < 0 with more heterogeneity within groups than expected by chance

Table 3.2. Multi-Response Permutation Procedures table for 2001 and 2002 reciprocal transplant background samples.

	2001		2002	
Test	*A*-statistic[2]	*p*-value	*A*-statistic[2]	*p*-value
Difference by vegetation:				
Meadow vs. Forest	0.087	0.001	0.074	<0.001
Difference by site:				
CM vs. CF[1]	0.119	0.028	0.117	0.035
LM vs. LF	0.103	0.024	0.112	0.027
CM vs. LM	0.068	0.038	0.144	0.031
CF vs. LF	0.003	0.540	-0.036	0.870
CM vs. LF	0.079	0.025	0.098	0.049
LM vs. CF	0.163	0.026	0.104	0.023

[1]CM = Carpenter meadow; CF = Carpenter forest; LM = Lookout meadow; LF = Lookout forest.

[2]A = 1 – (observed delta / expected delta); A = 0 when heterogeneity within groups equals expectation by chance; A < 0 with more heterogeneity within groups than expected by chance

Table 3.3. Multi-Response Permutation Procedures table indicating sampling effects for 2001 and 2002.

Test	2001		2002	
	A-statistic[1]	*p*-value	*A*-statistic[1]	*p*-value
CM background vs. CM open remain	0.011	0.345	0.150	0.071
CF background vs. CF open remain	0.024	0.229	0.034	0.080
LM background vs. LM open remain	-0.028	0.730	0.051	0.071
LF background vs. LF open remain	0.047	0.107	-0.052	0.905

[1]A = 1 – (observed delta/expected delta); A = 0 when heterogeneity within groups equals expectation by chance; A < 0 with more heterogeneity within groups than expected by chance

Table 3.4. Multi-Response Permutation Procedures table indicating decomposition effects for 2001 and 2002.

Test	2001		2002	
	A-statistic[1]	*p*-value	*A*-statistic[1]	*p*-value
CM background vs. CM closed remain	0.006	0.399	0.102	0.143
CF background vs. CF closed remain	0.024	0.229	0.034	0.080
LM background vs. LM closed remain	-0.006	0.602	0.045	0.115
LF background vs. LF closed remain	0.039	0.096	0.089	**0.032**

[1]A = 1 – (observed delta/expected delta); A = 0 when heterogeneity within groups equals expectation by chance; A < 0 with more heterogeneity within groups than expected by chance

Table 3.5. Multi-Response Permutation Procedures table of root effects for 2001 and 2002 reciprocal transplant treatment samples.

Test	2001		2002	
	A-statistic[1]	*p*-value	*A*-statistic[1]	*p*-value
CM closed remain vs. CM open remain	-0.016	0.624	-0.004	0.541
CF closed remain vs. CF open remain	0.013	0.302	-0.001	0.491
LM closed remain vs. LM open remain	0.001	0.432	-0.005	0.597
LF closed remain vs. LF open remain	0.039	0.183	0.017	0.348
CF closed transfer vs. CF open transfer (incubated in CM)	0.002	0.422	-0.042	0.861
CM closed transfer vs. CM open transfer (incubated in CF)	0.103	**0.036**	0.051	**<0.001**
LF closed transfer vs. LF open transfer (incubated in LM)	0.044	0.135	0.016	**<0.001**
LM closed transfer vs. LM open transfer (incubated in LF)	0.020	0.164	0.084	**0.029**

[1]A = 1 – (observed delta/expected delta); A = 0 when heterogeneity within groups equals expectation by chance; A < 0 with more heterogeneity within groups than expected by chance

Table 3.6. Multi-Response Permutation Procedures table for 2001 and 2002 reciprocal transplant treatment samples.

Test	2001		2002	
	A-statistic[1]	*p*-value	*A*-statistic[1]	*p*-value
Carpenter Meadow				
CM closed remain vs. CF closed transfer (soil core from CF, incubated in CM)	0.175	**0.025**	0.211	**0.023**
CM closed remain vs. CM closed transfer (soil core from CM, incubated in CF)	0.104	**0.031**	0.193	**<0.001**
CM open remain vs. CF open transfer (soil core from CF, incubated in CM)	0.071	0.069	0.104	0.054
CM open remain vs. CM open transfer (soil core from CM, incubated in CF)	0.153	**0.023**	0.211	**0.022**
Carpenter Forest				
CF closed remain vs. CM closed transfer (soil core from CM, incubated in CF)	0.137	**0.025**	0.043	**<0.001**
CF closed remain vs. CF closed transfer (soil core from CF, incubated in CM)	0.089	**0.035**	0.021	0.283
CF open remain vs. CM open transfer (soil core from CM, incubated in CF)	0.054	0.082	0.030	0.190
CF open remain vs. CF open transfer (soil core from CF, incubated in CM)	0.017	0.290	0.125	**0.028**
Lookout Meadow				
LM closed remain vs. LF closed transfer (soil core from LF, incubated in LM)	0.074	**0.023**	0.066	**0.032**
LM closed remain vs. LM closed transfer (soil core from LM, incubated in LF)	0.197	**0.023**	0.068	0.058
LM open remain vs. LF open transfer (soil core from LF, incubated in LM)	0.190	**0.022**	0.077	**0.032**
LM open remain vs. LM open transfer (soil core from LM, incubated in LF)	0.125	**0.032**	0.231	**0.023**

[1]A = 1 – (observed delta/expected delta); A = 0 when heterogeneity within groups equals expectation by chance; A < 0 with more heterogeneity within groups than expected by chance

Table 3.6 (continued).

Test	2001		2002	
	A-statistic[1]	*p*-value	*A*-statistic[1]	*p*-value
Lookout Forest				
LF closed remain vs. LM closed transfer (soil core from LM, incubated in LF)	0.099	**0.029**	-0.038	0.766
LF closed remain vs. LF closed transfer (soil core from LF, incubated in LM)	0.064	0.068	0.126	**<0.001**
LF open remain vs. LM open transfer (soil core from LM, incubated in LF)	0.118	**0.025**	0.027	0.285
LF open remain vs. LF open transfer (soil core from LF, incubated in LM)	0.081	0.083	0.080	**0.030**

[1]A = 1 – (observed delta/expected delta); A = 0 when heterogeneity within groups equals expectation by chance; A < 0 with more heterogeneity within groups than expected by chance

Table 3.7. Indicator species analysis for background samples from 2000-2002 by vegetation and by site.

Test	Site[1]	Fragment	Indicator value	p-value[2]
Indicators by vegetation:				
Meadow vs. forest	M	520	62.3	0.001
	M	570	42.2	0.008
	M	577	88.9	0.001
	M	586	50.0	0.003
	M	592	77.3	0.002
	M	614	88.9	0.001
	F	719	68.5	0.002
Indicators by site:				
Carpenter meadow vs. Lookout meadow	CM	552	62.5	0.003
	CM	608	100.0	0.001
	CM	684	74.2	0.002
	LM	726	76.6	0.006
Carpenter meadow vs. Carpenter forest	CM	570	71.7	0.008
	CM	577	87.5	0.003
	CM	592	84.6	0.002
	CM	608	94.7	0.001
	CM	614	100.0	0.001
Carpenter forest vs. Lookout forest		none	n. a.	n. a.
Lookout meadow vs. Lookout forest	LM	520	100.0	0.001
	LM	577	100.0	0.001
	LM	605	88.9	0.001
	LM	614	88.9	0.001

[1]M = meadow; F = forest; CM = Carpenter meadow; LM = Lookout meadow.
[2]proportion of randomized trials with indicator value equal to or exceeding the observed indicator value. p = (1 + number of runs ≥ observed) / (1 + number of randomized runs).

Table 3.8. Indicator species analysis by treatment for background, remaining, and transfer samples for 2001.

Test	Treatment	Fragment	Indicator value	p-value[1]
Indicators by treatment:				
Carpenter meadow background vs. remaining	remain	520	80.9	0.016
Carpenter meadow background vs. transfer	transfer	719	100.0	0.011
Carpenter meadow remaining vs. transfer	remain	520	93.0	0.004
	remain	552	70.6	0.035
	remain	592	65.1	0.023
	remain	673	65.4	0.020
	transfer	719	82.9	0.016
Carpenter forest background vs. remaining	remain	684	66.4	0.028
Carpenter forest background vs. transfer	background	636	92.2	0.013
	transfer	684	74.5	0.024
	background	719	84.7	0.042
	background	733	93.4	0.025
Carpenter forest remaining vs. transfer	transfer	597	83.3	0.029
	transfer	631	100.0	0.004
	remain	743	75.0	0.041
Lookout meadow background vs. remaining	remain.	673	75.6	0.050
Lookout meadow background vs. transfer	background	577	83.9	0.034
	background	592	76.6	0.043
	background	597	90.3	0.015
	background	631	100.0	0.015
	background	688	84.8	0.033
Lookout meadow remaining vs. transfer	transfer	510	64.1	0.013
	remain	517	75.0	0.017
	remain	620	62.5	0.035
Lookout forest background vs. remaining	background	520	92.8	0.029
	background	597	90.2	0.030
	remain	684	88.2	0.019

[1]proportion of randomized trials with indicator value equal to or exceeding the observed indicator value. p = (1 + number of runs ≥ observed) / (1 + number of randomized runs).

Table 3.8 (continued)

Test	Treatment	Fragment	Indicator value	p-value[1]
Lookout forest background vs. transfer	background	597	100.0	0.014
	transfer	684	89.7	0.014
	background	738	93.1	0.024
Lookout forest remaining vs. transfer	transfer	592	92.8	0.005
	transfer	614	83.3	0.023

[1]proportion of randomized trials with indicator value equal to or exceeding the observed indicator value. p = (1 + number of runs ≥ observed)/(1 + number of randomized runs).

Table 3.9. Indicator species analysis by treatment for background, remaining, and transfer samples for 2002.

Test	Treatment	Fragment	Indicator value	p-value[1]
Indicators by treatment:				
Carpenter meadow background vs. remaining	remain	552	79.9	0.024
	background	570	90.5	0.016
	remain	673	78.5	0.016
	remain	684	66.78	0.040
Carpenter meadow background vs. transfer	background	586	100.0	0.021
	background	608	100.0	0.021
	transfer	654	100.0	0.021
	transfer	684	70.5	0.021
Carpenter meadow remaining vs. transfer	transfer	510	80.0	0.011
	transfer	570	68.5	0.047
	remain	592	76.1	0.002
	remain	608	100.0	0.002
	transfer	614	70.9	0.035
	transfer	654	100.0	0.002
Carpenter forest background vs. remaining	background	688	88.7	0.009
Carpenter forest background vs. transfer	background	577	90.2	0.017
	background	614	90.7	0.044
	transfer	684	77.7	0.017
	transfer	695	93.2	0.017
	background	726	100.0	0.017
Carpenter forest remaining vs. transfer	remain	614	74.7	0.036
	remain	666	70.3	0.043
	transfer	684	77.8	0.004
	remain	688	87.1	0.003
	transfer	695	89.5	0.001
Lookout meadow background vs. remaining	remain	636	86.2	0.042
	remain	684	82.5	0.024
Lookout meadow background vs. transfer	background	658	91.2	0.014
Lookout meadow remaining vs. transfer	transfer	666	76.7	0.023

[1]proportion of randomized trials with indicator value equal to or exceeding the observed indicator value. p = (1 + number of runs ≥ observed)/(1 + number of randomized runs).

Table 3.9 (continued).

Test	Treatment	Fragment	Indicator value	p-value[1]
Lookout forest background vs. remaining	remain	636	83.4	0.025
	remain	684	76.6	0.026
Lookout forest background vs. transfer	n.a.	none	n.a	n.a
Lookout forest remaining vs. transfer	remain	684	74.6	0.014

[1]proportion of randomized trials with indicator value equal to or exceeding the observed indicator value. p = (1 + number of runs ≥ observed)/(1 + number of randomized runs).

Chapter 4:

Effects of Carbon Input Manipulations on Microbial Communities in the Western Cascades of Oregon

Stacie A. Kageyama and David D. Myrold

New Phytologist

New Phytologist Trust

In preparation

INTRODUCTION

Changes in aboveground and belowground carbon inputs due to natural or anthropogenic disturbance in forests could potentially alter the structure of belowground microbial communities (Brant et al. 2005). In Pacific Northwest forests, the majority of aboveground carbon inputs occur in the form of coniferous needles and woody detritus. Belowground carbon inputs are "new carbon" or carbon derived from photosynthesis. Brant et al. (2005) used phospholipid fatty acid (PLFA) biomarkers to explore the effects of aboveground vs. belowground carbon inputs on microbial communities at three Detritus Input and Removal Treatment sites (DIRT) in the US and Europe. At the H. J. Andrews DIRT site in the western Cascades of Oregon, PLFA data indicated that belowground carbon inputs control microbial community composition in forest soils. In addition, they observed seasonal variation in the PLFA biomarkers.

Shifts in microbial community (Niemelä and Sundman 1977) and decreases in microbial biomass occur following disturbance in forests (Bååth 1980; Bååth and Söderström 1982; Pietikäinen and Fritze 1995; Siira-Pietikainen et al. 2001b). Several studies have indicated that the changes in microbial community and biomass are due to "root effects" or loss of live roots and their associated microorganisms. Niemelä and Sundman (1977) observed shifts in bacterial populations up to seven years after clearcutting. In addition, they found that bacterial populations in mineral soil had changed more than those in litter in the clearcut areas due to the loss of live roots. Reduction in microbial biomass after harvesting forests has been attributed to a decrease in fungal biomass associated with roots (Bååth 1980;

Bååth et al. 1995; Siira-Pietikainen et al. 2001a). Although overall PLFA microbial biomass was not significantly different with treatments at the H. J. Andrews DIRT plots, percentages of fungal biomarkers exhibited significant differences between plots with and without live roots (Brant et al. 2005). Numbers of active ECM root tips rapidly decline in abundance after clearcutting although some may persist for nearly a year (Harvey et al. 1980) to up to three years (Hagerman et al. 1999). Decreases in soil respiration following disturbance have been attributed to either decreases in ECM fungal biomass alone (Högberg and Högberg 2002; Högberg et al. 2001) or to decreases in heterotrophic respiration (Boone et al. 1998; Fisk and Fahey 2001; Sulzman et al. 2005).

Disturbance of aboveground litter can also result in shifts in fungal communities (Amaranthus and Perry 1989; Bååth 1981; Harvey et al. 1980; Perry et al. 1982). However, bacterial communities appear to be more resistant to shifts due to changes in aboveground carbon inputs than fungal communities (Chow et al. 2002). Spears et al. (2003) found that additions of coarse woody debris did change microbial biomass in mineral soil in coniferous forests. Bååth (1981) found that removal of slash following clearcutting resulted in shifts in the populations of microfungi recovered by isolation in organic soil layers in Sweden. Litter and humus layers in Pacific Northwest forests often contain spores of both arbuscular mycorrhizal (AM) and ECM fungi (Parke et al. 1983). Removal of aboveground litter through site preparation has been found to reduce numbers of ECM root tips (Harvey et al. 1997; Perry et al. 1982; Pietikäinen and Fritze 1995) and ECM biomass (Harvey et al. 1996), as well as reducing ECM diversity and richness (Harvey

et al. 1997; Simard et al. 2003). Burning of slash piles in ponderosa pine (*Pinus ponderosa*) stands in Arizona was found to reduce the numbers of AM propagules and seeds of native forbs (Korb et al. 2004). The chronic removal of coarse woody debris can also potentially impact ECM as more ECM root tips are often found in decayed wood or decayed wood that has been incorporated into the soil than in mineral soils (Harvey et al. 1997).

We utilized an existing long-term experiment, the DIRT Plots, in order to better understand the influence of different litter and detritus pools on fungal and bacterial communities coniferous forests in the Pacific Northwest. The existing plot treatments allowed us to make comparisons between control plots and plots that had no live roots and/or no aboveground litter, double litter, and double wood treatments. We hypothesized that we would see changes in both the fungal and bacterial community with root disturbance and changes in aboveground litter inputs. We also hypothesized that we would see temporal changes in fungal and bacterial communities. Our goals for this study were (1) to use molecular techniques to gain insight into the effects of manipulating both above- and belowground carbon inputs on fungal and bacterial communities and (2) to understand the temporal community shifts in fungal and bacterial populations over the course of one year. In order to accomplish these goals, we used PCR-based molecular techniques to study changes in fungal and bacterial communities at the DIRT Plots at the H. J. Andrews Experimental Forest in the western Cascade Mountains of Oregon.

METHODS

Study site

The H. J. Andrews DIRT Plots are located at 531 m above sea level at the H. J. Andrews Experimental Forest (44°15′ N, 122°10′ W). The mean annual temperature at the H. J. Andrews headquarters is 8.7 °C (1973-2002) and the mean annual precipitation over the same period is 2370 mm, which falls mostly as rain. Over 70% of the precipitation falls between November and March. The DIRT plots were established in 1997 in a mature Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and western hemlock stand (*Tsuga heterophylla* (Raf.) Sarg.). Other important tree and shrub species include *Thuja plicata* D. Don, *Rhododendron macrophyllum* D. Don ex G. Don, and *Acer circinatum* Pursh. Both *P. menziesii* and *T. heterophylla* are ECM taxa, whereas *T. plicata* and *A. circinatum* are AM. Soils at the site are classified as coarse loamy mixed mesic Typic Hapludands (Dixon 2003). Plot treatments are replicated three times and are 10 m x 15 m (Table 4.1) (Brant et al. 2005; Sulzman et al. 2005). Plots that exclude aboveground litter are covered with nylon mesh and are swept on a regular basis. Trees were girdled on the root-excluded plots. Plots that exclude roots were then trenched when the plots were installed. Root-impermeable plastic barriers were installed in the trenches to exclude roots from growing in from vegetation outside the boundaries of the plots. Roots were left to decompose in the No Live Root and No Input plots were left in the plots following girdling and trenching. Shrubs and understory vegetation were also removed. Litter additions are made several times a year to the Double Litter plots. Wood is added to the

Double Wood plots every other year.

Sampling

We collected seven soil cores using a 2.5-cm corer from each plot in July and November 2003, and April 2004. The same samples were used for PLFA analysis and determination of gravimetric water content in Brant et al. (2005). Cores were composited and sieved through a 2-mm sieve. Aliquots of soil for DNA extraction were frozen at -20°C immediately after sieving.

DNA extraction.

Test of soil sample size. Tests of soil sample size for DNA extraction in agricultural systems have indicated that at least 1 g of soil is necessary in order to capture the full spectrum of bacterial and fungal diversity in a sample (Ranjard et al. 2003). To date, no one has tested the minimum sample size required for DNA extraction for Pacific Northwest forest soils. We tested soil sample size for PCR amplification using fungal ITS primers. DNA was extracted from six separate 0.5 g (fresh weight) soil samples from each of the Control plots at the HJA DIRT site using the Fast DNA kit (Bio 101, Inc., Irvine, CA) according to the manufacturer's directions. Extracted DNA was checked by running 10 μl of extract on an agarose gel (1%) stained with ethidium bromide (0.5 μg ml^{-1}). DNA was quantified on a BioSpec-1601 DNA/Protein/Enzyme Analyzer (Shimadzu Corporation, Kyoto, Japan). Extracts were diluted to 25 ng of DNA per μl for PCR amplification. We combined samples in pairs so that we now had three pooled samples from a

total of 1 g of soil. We also combined the samples in sets of three so that we had two pooled samples from 1.5 g of soil. We then used the general fungal primers ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) for Length Heterogeneity PCR (LH-PCR). The resulting data was then analyzed using Nonmetric Multidimensional Scaling in PC-ORD Version 4.36 (MJM Software, Gleneden, OR).

DNA extraction for complete study. DNA was extracted from 0.5 g of soil using the Fast DNA kit (Bio 101, Inc., Irvine, CA) from all plots at all sampling times. DNA was quantified as stated above. Length Heterogeneity PCR (LH-PCR) was used to examine bacterial and fungal community profiles. Primers used were for the bacterial 16S rDNA gene region (Brant et al. 2005; Ritchie et al. 2000) and the fungal primers ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). Forward primers were fluorescently labeled with 6-FAM (6-carboxyfluorescein). Reaction mixtures (50 μl) contained soil DNA (100 ng), AmpliTaq DNA polymerase (2.5 U), GeneAmp PCR buffer (1×), MgCl2 (2 mM), deoxynucleoside triphosphates (0.2 mM each), forward and reverse primers (0.2 M each), and bovine serum albumin (0.064 g ml^{-1}). PCR amplification was conducted as follows using a PTC-100 hot bonnet thermocycler (MJ Research, Inc., Waltham, MA): 94° for 2 min followed by 35 cycles of 94° for 30 s, 55° for 30 s, 72° for 1 min. A final extension followed for 72° for 2 min. PCR products were visualized with electrophoresis on 1% agarose gels stained with ethidium bromide. One μl of PCR product containing 1 ng of DNA was submitted for capillary sequencing on an ABI

Prism® 3100 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA) in the Central Analytical Laboratory, Oregon State University. PCR products were run on the Genetic Analyzer along with X-Rhodamine MapMarker™ 1000 internal lane size standard (BioVentures, Inc., Murfreesboro, TN).

Data Analysis

Size and relative abundance of LH-PCR fragments were quantified using GeneScan® v. 3.5 software and Genotyper® v. 2.5 software (Applied Biosystems, Inc., Foster City, CA). Sequence length and peak area data were obtained using Genotyper. Data were downloaded into Microsoft® Excel v. X for Mac and manually binned. Community data were analyzed with PC-ORD Version 4.36 (MJM Software, Gleneden, OR).

Ordinations of LH-PCR fragment data were run in PC-ORD Version 4.36 (MJM Software, Gleneden, OR) using nonmetric multidimensional scaling (NMS) using the autopilot feature, "Slow and thorough" with the Sørensen distance measure. We chose to use NMS because it avoids the assumption of linear relationships among variables, and allows the use of any relativization (McCune and Grace 2002). Analyses were run on the entire data set (all sampling times) and by individual sampling times for each of the fungal ITS and bacterial 16S datasets. For the dataset containing all sampling times, we deleted columns (LH-PCR fragments) containing fewer than three non-zero values. The dataset was then relativized by row totals (samples) to adjust for differences in fluorescence levels of our amplified fragments.

Multi-response permutation procedures (MRPP) were used to test fungal and bacterial community composition between plot treatments and different sampling times. MRPP is a nonparametric method that is used to test for group differences (McCune and Grace 2002). We used MRPP in the statistics package PC-ORD Version 4.36 (MJM Software, Gleneden, OR). We used the Sørensen similarity coefficient and defined our groups by treatment.

Indicator species analyses were conducted using PC-ORD Version 4.36 (MJM Software, Gleneden, OR) by comparing either treatments or sampling times with 1000 randomizations in the Monte Carlo test. According to Dufrêne and Legendre (Dufrêne and Legendre 1997), a perfect indicator is always present in a particular group and is exclusive to that group. PC-ORD employs Dufrêne and Legendre's (Dufrêne and Legendre 1997) indicator species analysis method to produce indicator values for each species (LH-PCR fragment) for each group.

RESULTS

Test of soil sample size

For each plot, DNA extracts were pooled so that there were six DNA extracts derived from 0.5 g of soil, three extracts from 1.0 g of soil, and two extracts 1.5 g of soil. Nonmetric multidimensional scaling analysis indicated that there were no detectable differences between 0.5, 1.0, and 1.5 g of soil from the three Control plots. We chose to use 0.5 g of soil for the DNA extractions for the complete study.

Effects of above- and belowground litter manipulation

Length heterogeneity PCR resulted in 100 fungal ITS fragments and 24 16S fragments. Nonmetric multidimensional scaling analysis of fungal community data (entire dataset, all sampling times) indicated that the greatest difference occurred between plots with and without roots (Table 4.2). Bacterial community data also exhibited a difference between plots with and without live roots, although the difference was not as great as with the fungal data. In the NMS ordination of the entire fungal ITS dataset, plots with live roots (CO, DL, DW, and NL) separated from plots without live roots (NR and NI) (Figure 4.1). An exception was the November NI plots which grouped with the plots with live roots. For July 2003 samples, plots with and without live roots separated in the NMS ordination (Figure 4.2). For November 2003, NR plots separated from plots with live roots (CO, DL, DW, and NL) whereas the NI plots grouped with live root plots in the ordination. For April 2004, plots with and without live roots separated in the NMS ordination.

In the NMS ordination of the entire 16S dataset, there were some treatment effects with the control plots separating from most of the other treatments (Figure 4.3). In addition, there were some temporal shifts between the different sampling times. November 2003 samples clearly separated from April 2004 samples. July 2003 samples fell between November and April. Treatment effects were less clear for the NMS ordinations of the individual sampling times (Figure 4.4).

Treatment effects were apparent when fungal ITS data were analyzed using MRPP. For the complete fungal ITS dataset, MRPP indicated statistically significant ($p < 0.05$) treatment effects between the control plots and all other treatments (Table 4.2). The greatest difference occurred between the control plots and the no live root plots. There were also significant treatment effects between double litter vs. no input plots, double litter vs. no live root plots, double wood vs. no input plots, double wood vs. no live root plots, no aboveground litter vs. no input plots, and no aboveground litter vs. no live root plots. For the July sampling, MRPP indicated that there were significant differences between control vs. no input plots, control vs. no live root plots, double litter vs. no input plots, double litter vs. no live root plots, double wood vs. no live root plots, and no aboveground litter vs. no live root plots. For the November sampling, MRPP indicated that there were significant differences between control vs. no live root plots, double litter vs. no live root plots, and double wood vs. no live root plots. For the April sampling, MRPP indicated that there were significant differences between control vs. double wood plots, control vs. no live root plots, double wood vs. no live root plots, no aboveground litter vs. no input plots, and no aboveground litter vs. no live root plots.

For the 16S complete dataset, MRPP analyses found fewer significant ($p < 0.05$) treatment effects than with the fungal ITS complete dataset. However, there is still evidence of a root effect (Table 4.2). The only significant treatment effects occurred between plots with and without live roots. For the July sampling, MRPP analyses indicated that there were significant differences between control vs. double wood plots, control vs. no

aboveground litter plots, and control vs. no input plots. There were no significant treatment effects for the November sampling and only control vs. no input plots for April.

Temporal changes in fungal and bacterial communities

Fungal communities in the no live root plots exhibited differences between the July sampling and the November and April samplings in the NMS ordination (Figure 4.5). Bacterial 16S communities indicated some temporal differences between November 2003 and April 2004 samples in the NMS ordinations (Figure 4.3). Bacterial 16S treatments exhibited differences among all sampling times (Figure 4.6). MRPP analyses also found few temporal differences per treatment for either the fungal ITS or the bacterial 16S datasets (Table 4.3). MRPP supported the differences found in ordinations of data. July ITS samples in the no live root plots were different from both November and April ($p < 0.05$); however, there was no difference between November and April. July and April 16S samples were also different in the no aboveground litter plots ($p < 0.05$).

Indicator species analysis

Indicator species analysis of the fungal ITS datasets found no indicators when treatments within individual sampling times were compared. However, several indicators were found for the entire dataset when control plots were compared to plots with live roots (DL, DW, and NL) and plots without live roots (NR and NI) and plots with live roots compared to plots without live roots (Table 4.4). Results for the 16S datasets showed

similar patterns with no indicators when treatments within individual sampling times were compared (Table 4.5). The majority of the indicators occurred between control vs. no live root plots (NR and NI) and plots with live roots vs. plots without live roots when the entire dataset was examined.

DISCUSSION

Loss of live roots at the DIRT plots impacted fungal and bacterial communities to a greater degree than either removal or additions of aboveground litter. Brant et al. (2005) examined the same soil samples used in this study with PLFAs. They found lower percentages of PLFA fungal biomarkers and lower fungal:bacterial ratios in the plots without live roots suggesting that fungal biomass has decreased in the plots without live roots. We observed shifts in both bacterial and fungal communities using LH-PCR. We believe that the shift in fungal communities is due to the loss of ECM fungal root tips and extramatrical mycelium. There may have been an increase in saprophytic fungi colonizing the dead roots in the no live root and no input plots 6 years after trenching and girdling. However, the PLFA data indicates that the increase in biomass was small, or at least did not match the fungal biomass in the live root plots. Length-heterogeneity PCR data indicates that bacterial communities were also affected by the loss of live roots and organisms associated with roots. Brant et al. (2005) found that percentages of PLFA actinomycete biomarkers had increased in the plots without live roots.

Indicator species analysis contributed additional support to the conclusion that fungal communities had shifted in the absence of live roots. Three fungal ITS fragments, 553, 629, and 659, were indicators for the no live root plots vs. both the control and the live root plots. Three fragments, 714, 723, and 732, were indicators for control and live root plots vs. the no live root plots. In addition, fungal indicator fragments were found between the control and live root plots suggesting a disturbance effect. A girdling and litter manipulation study in a *Picea abies* stand in Germany found similar shifts in fungal species composition (Subke et al. 2004).

Although several arbuscular mycorrhizal (AM) plant taxa are present at the DIRT plots, it is unlikely that AM fungi were amplified consistently with our primers. Very few studies have reported consistent success with amplifying AM fungal DNA directly from soil. Glomeromycota sequences composed only 0.5% of ITS and 1% of small subunit sequences recovered in a large-scale cloning study at Duke Forest (O'Brien et al. 2005). Therefore, we do not believe that changes in AM communities were responsible for the differences in our root treatments.

Root effects were observed with the 16S bacterial data in the HJA DIRT plots but were not as distinct as with the fungal ITS data. Earlier PLFA data at the HJA DIRT plots using the same soil samples used in this study found different PLFA community profiles in plots with and without live roots (Brant et al. 2005). Indicator species analysis of 16S fragments supports the conclusion that bacterial communities have shifted in plots without live roots. Two fragments, 310 and 312, were indicators of plots without live roots. We also see evidence of a disturbance effect as the 348 and 359

fragments are indicators of the control plots vs. treatments. Niemelä and Sundman (1977) found greater changes in bacterial populations in mineral soil than in organic soil layers after clearcutting. They concluded that the mineral soil populations are more dependent on roots than the organic soil populations. A study of 16S rDNA clones in clearcut treatments vs. an unlogged reference stand found a shift in bacterial species composition in the clearcut vs. uncut stand 2-4 years after harvesting (Axelrood et al. 2002). Eight years after clearcutting, Donegan et al. (2001) found that *Pseudomonas* community fingerprints had shifted in a forested vs. clearcut sites in the Oregon Cascades.

Overall, the bacterial and fungal communities appear to be stable seasonally. Temporal effects seemed apparent with the 16S bacterial NMS ordination, but were not supported by MRPP. The only temporal difference observed with the fungi occurred in the no live root plots where July samples group separately in the NMS ordination than November and April. Brant et al. (2005) found that gravimetric water content was lowest in July 2003 of the three sampling dates, but that soil moisture was highest in the plots without live roots at that time. Soil moisture may have been a factor in the absence of roots. Dramatic seasonal differences in fungal communities were found in the Colorado alpine where entirely different winter and summer clades have been discovered (Schadt et al. 2003). The lack of seasonal variation in microbial communities at the HJA DIRT site is surprising given that there are large differences in precipitation in winter vs. summer. However, the majority of fungi and bacteria are associated to some degree with conifers that remain physiologically active to some degree throughout the winter.

Removal of large tracts of forest through practices such as clearcutting or natural disturbances such as fire or wind can result in long-term changes in the microbial community. Our bacterial 16S data is consistent with the results found in by Niemelä and Sundman (1977) who found that bacteria isolated from mineral soil were different in clearcut and intact forests. Our fungal ITS data are consistent with Harvey et al. (1980) and Hagerman et al. (1999) who found that the abundance of ECM root tips declined following clearcutting. Some ECM fungal mycelium may persist for a short time after clearcuts (Hagerman et al. 1999; Harvey et al. 1980; Perry et al. 1987). However, loss of ECM fungal taxa at forest sites translates to a loss of potential inoculum for future generations of trees on the sites (Perry et al. 1982; Perry et al. 1987).

Disruptions in the flow photosynthate due to disturbance have been shown to have immediate effects on microbial communities (Hernesmaa et al. 2005). ECM fungal communities respond rapidly to disruptions of the flow of photosynthate to roots and mycorrhizal fungi belowground (Cullings et al. 2001; Högberg and Högberg 2002; Högberg et al. 2001). Defoliating *Pinus contorta* caused shifts in the ECM of undefoliated *Picea engelmanii* in a mixed conifer forest in Yellowstone National Park (Cullings et al. 2001). Changes have also been observed in microfungi isolated from the rhizospheres of live and dead oaks (Kwasna 2004). Bacterial and saprophytic fungal biomass including chitin degraders have been observed to increase in the rhizospheres of cut trees soon after harvesting (Hernesmaa et al. 2005).

Manipulations of litter inputs have affected fungal communities to a greater extent than bacterial communities at the DIRT plots. However, both

removal and doubling of litter and wood have contributed to both fungal and bacterial community changes, although not as great as the effect of manipulating live root inputs. Coarse woody debris did not affect microbial biomass in the Oregon Cascades (Spears et al. 2003). Litters additions increased ECM infection and resulted in species shifts in a young *P. contorta* stand that became established after a stand-replacing fire in 1988 in Yellowstone National Park (Cullings et al. 2003).

Although subtle, biological changes appear to be occurring faster than soil chemical changes due to above- and belowground litter manipulations at the DIRT plots (Spears and Lajtha 2004; Yano et al. 2005); it may take longer for the effects of litter removals and additions to become apparent in mineral soils due to the length of time involved in the decomposition of conifer needles and coarse woody debris. In fact, roots rather than aboveground litter inputs have been found to produce most of the dissolved organic nitrogen at the site (Yano et al. 2005). Yano et al. (2005) proposed that there is a lag in dissolved organic matter production in the doubled litter treatments. We have found that at 6 years after the DIRT plots were installed that the greatest influence on both soil fungal and bacterial communities is the presence or absence of live roots. It would be important to revisit the sites in 10 or 20 when the continuing doubled litter and wood treatments have been incorporated into mineral soil.

LITERATURE CITED

Amaranthus MP, Perry DA (1989) Interaction effects of vegetation type and Pacific madrone soil inocula on survival, growth, and mycorrhiza formation of Douglas-fir. Canadian Journal of Forest Research 19: 550-556

Axelrood PE, Chow ML, Radomski CC, McDermott JM, Davies J (2002) Molecular characterization of bacterial diversity from British Columbia forest soils subjected to disturbance. Canadian Journal of Microbiology 48: 655-674

Bååth E (1980) Soil fungal biomass after clear-cutting of a pine forest in central Sweden. Soil Biology & Biochemistry 12: 495-500

Bååth E (1981) Microfungi in a clear-cut pine forest soil in central Sweden. Canadian Journal of Botany 59: 1331-1337

Bååth E, Frostegård Å, Pennanen T, Fritze H (1995) Microbial commuunity structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. Soil Biology & Biochemistry 27: 229-240

Bååth E, Söderström B (1982) Seasonal and spatial variation in fungal biomass in a forest soil. Soil Biology & Biochemistry 14: 353-358

Boone RD, Nadelhoffer KJ, Canary JD, Kaye J (1998) Roots exert a strong influence on the temperature sensitivity of soil respiration. Nature 396: 570-572

Brant JB, Myrold DD, Sulzman EW (2005) Root controls on soil microbial community structure in forest soils. Oecologia in review

Chow ML, Radomski CC, McDermott JM, Davies J, Axelrood PE (2002) Molecular characterization of bacterial diversity in Lodgepole pine (*Pinus contorta*) rhizosphere soils from British Columbia forest soils differing in disturbance and geographic source. FEMS Microbiology Ecology 42: 347-357

Cullings KW, New MH, Makhija S, Parker VT (2003) Effects of litter addition on ectomycorrhizal associates of a lodgepole pine (*Pinus contorta*) stand in Yellowstone National Park. Applied and Environmental Microbiology 69: 3772-3776

Cullings KW, Vogler DR, Parker VT, Makhija S (2001) Defoliation effects on the ectomycorrhizal community of a mixed *Pinus contorta/Picea engelmannii* stand in Yellowstone Park. Oecologia 127: 533-539

Dixon JJ (2003) Applying GIS to soil-geomorphic landscape mapping in the Lookout Creek valley, Western Cascades, Oregon. In: Department of Crop and Soil Science. Oregon State University, Corvallis

Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible approach. Ecological Monographs 67: 345-366

Fisk MC, Fahey TJ (2001) Microbial biomass and nitrogen cycling responses to fertilization and litter removal in young northern hardwood forests. Biogeochemistry 53: 201-223

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

Hagerman SM, Jones MD, Bradfield GE, Gillespie M, Durall DM (1999) Effects of clear-cut logging on the diversity and persistence of ectomycorrhizae at a subalpine forest. Canadian Journal of Forest Research 29: 124-134

Harvey AE, Jurgensen MF, Larsen MJ (1980) Clearcut harvesting and ectomycorrhizae: survival of activity on residual roots and influence on a bordering forest stand in Western Montana. Canadian Journal of Forest Research 10: 300-303

Harvey AE, PageDumroese DS, Jurgensen MF, Graham RT, Tonn JR (1996) Site preparation alters biomass, root and ectomycorrhizal development of outplanted western white pine and Douglas-fir. New Forests 11: 255-270

Harvey AE, PageDumroese DS, Jurgensen MF, Graham RT, Tonn JR (1997) Site preparation alters soil distribution of roots and ectomycorrhizae on outplanted western white pine and Douglas-fir. Plant and Soil 188: 107-117

Hernesmaa A, Björklöf K, Kiikilä O, Fritze H, Haahtela K, Romantschuk M (2005) Structure and function of microbial communities in the rhizosphere of Scots pine after tree-felling. Soil Biology & Biochemistry 37: 777-785

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

Högberg P et al. (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411: 789-792

Korb JE, Johnson NC, Covington WW (2004) Slash pile burning effects on soil biotic and chemical properties and plant establishment: Recommendations for amelioration. Restoration Ecology 12: 52-62

Kwasna H (2004) Natural shifts in communities of rhizosphere fungi of common oak after felling. Plant and Soil 264:209-218

McCune B, Grace JB (2002) Analysis of Ecological Communities. MJM Software Design, Gleneden Beach

Niemelä S, Sundman V (1977) Effects of clear-cutting on the composition of bacterial populations of northern spruce forest soil. Canadian Journal of Microbiology 23: 131-138

O'Brien HE, Parrent JL, Jackson JA, Moncalvo J-M, Vilgalys R (2005) Fungal community analysis by large scale sequencing of environmental samples. Applied and Environmental Microbiology 71: 5544-5550

Parke JL, Linderman RG, Trappe JM (1983) Effects of forest litter on mycorrhiza development and growth of Douglas-fir and western red cedar seedlings. Canadian Journal of Forest Research 13: 666-671

Perry DA, Meyer MM, Egeland D, Rose SL, Pilz D (1982) Seedling growth and mycorrhizal formation in clearcut and adjacent, undisturbed soils in Montana: A greenhouse bioassay. Forest Ecology and Management 4: 261-273

Perry DA, Molina R, Amaranthus MP (1987) Mycorrhizae, mycorrhizospheres, and reforestation: current knowledge and research needs. Canadian Journal of Forest Research 17: 929-940

Pietikäinen J, Fritze H (1995) Clear-cutting and prescribed burning in coniferous forest: Comparison of effects on soil fungal and total microbial biomass, respiration activity and nitrification. Soil Biology & Biochemistry 27:101-109

Ranjard L, Lejon DPH, Mougel C, Schehrer L, Merdinoglu D, Chaussod R (2003) Sampling strategy in molecular microbial ecology: influence of soil sample size on DNA fingerprinting analysis of fungal and bacterial communities. Environmental Microbiology 5: 1111-1120

Ritchie NJ, Schutter ME, Dick RP, Myrold DD (2000) Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterize microbial communities in soil. Applied and Environmental Microbiology 66: 1668-1675

Schadt CW, Martin AP, Lipson DA, Schmidt SK (2003) Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301: 1359-1361

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

Siira-Pietikainen A, Pietikainen J, Fritze H, Haimi J (2001b) Short-term responses of soil decomposer communities to forest management: clear felling versus alternative forest harvesting methods. Canadian Journal of Forest Research 31: 88-99

Simard SW et al. (2003) Chemical and mechanical site preparation: effects on *Pinus contorta* growth, physiology, and microsite quality on grassy, steep forest sites in British Columbia. Canadian Journal of Forest Research 33: 1495-1515

Spears JDH, Holub SM, Harmon ME, Lajtha K (2003) The influence of decomposing logs on soil biology and nutrient cycling in an old-growth mixed coniferous forest in Oregon, U.S.A. Canadian Journal of Forest Research 33: 2193-2201

Spears JDH, Lajtha K (2004) The imprint of coarse woody debris on soil chemistry in the western Oregon Cascades. Biogeochemistry 71: 163-175

Subke J-A, Hahn V, Battipaglia G, Linder S, Buchmann N, Cotrufo MF (2004) Feedback interactions between needle litter decomposition and rhizosphere activity. Oecologia 139: 551-559

Sulzman EW, Brant JB, Bowden RD, Lajtha K (2005) Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. Biogeochemistry 73: 231-256

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninksky JJ, White TJ (eds) PCR Protocols: a Guide to Methods and Applications. Academic Press, New York, pp 315-322

Yano Y, Lajtha K, Sollins P, Caldwell BA (2005) Chemistry and dynamics of dissolved organic matter in a temperate coniferous forest on andic soils: Effects of litter quality. Ecosystems 8: 286-300

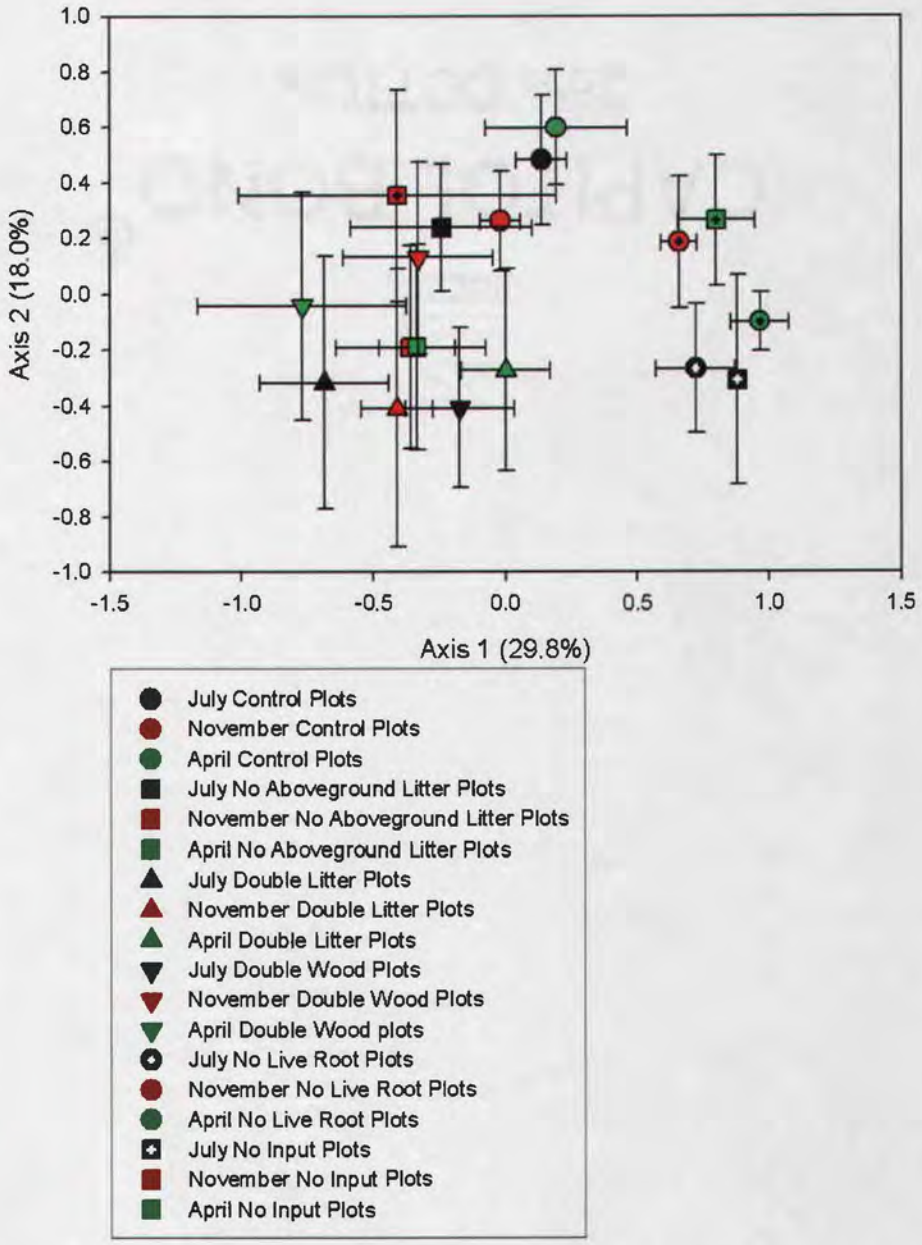

Figure 4.1. Nonmetric multidimensional scaling ordination of fungal ITS LH-PCR data of all sampling times and treatments.

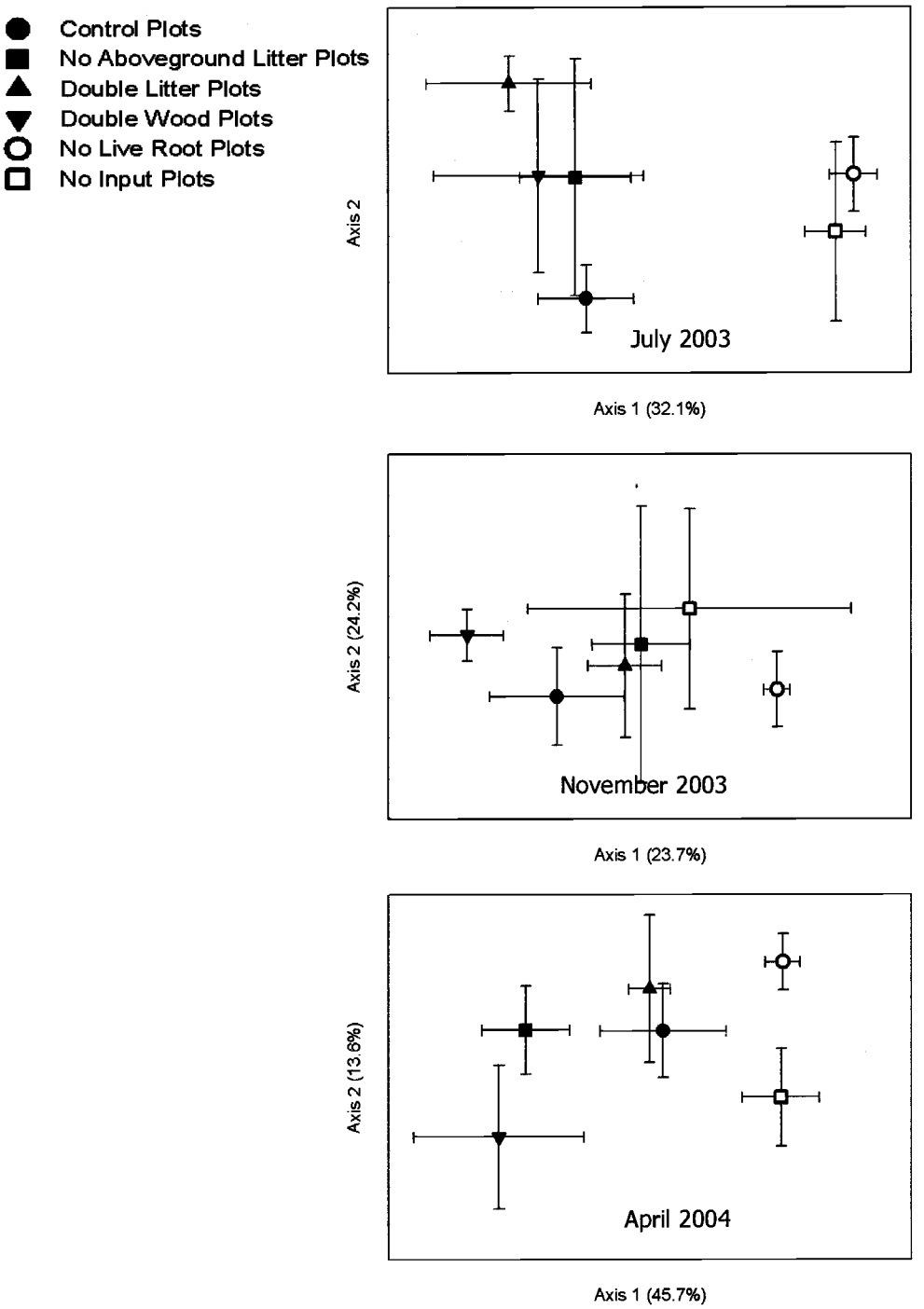

Figure 4.2. Nonmetric multidimensional scaling ordination of fungal ITS LH-PCR data of July 2003, November 2003, and April 2004.

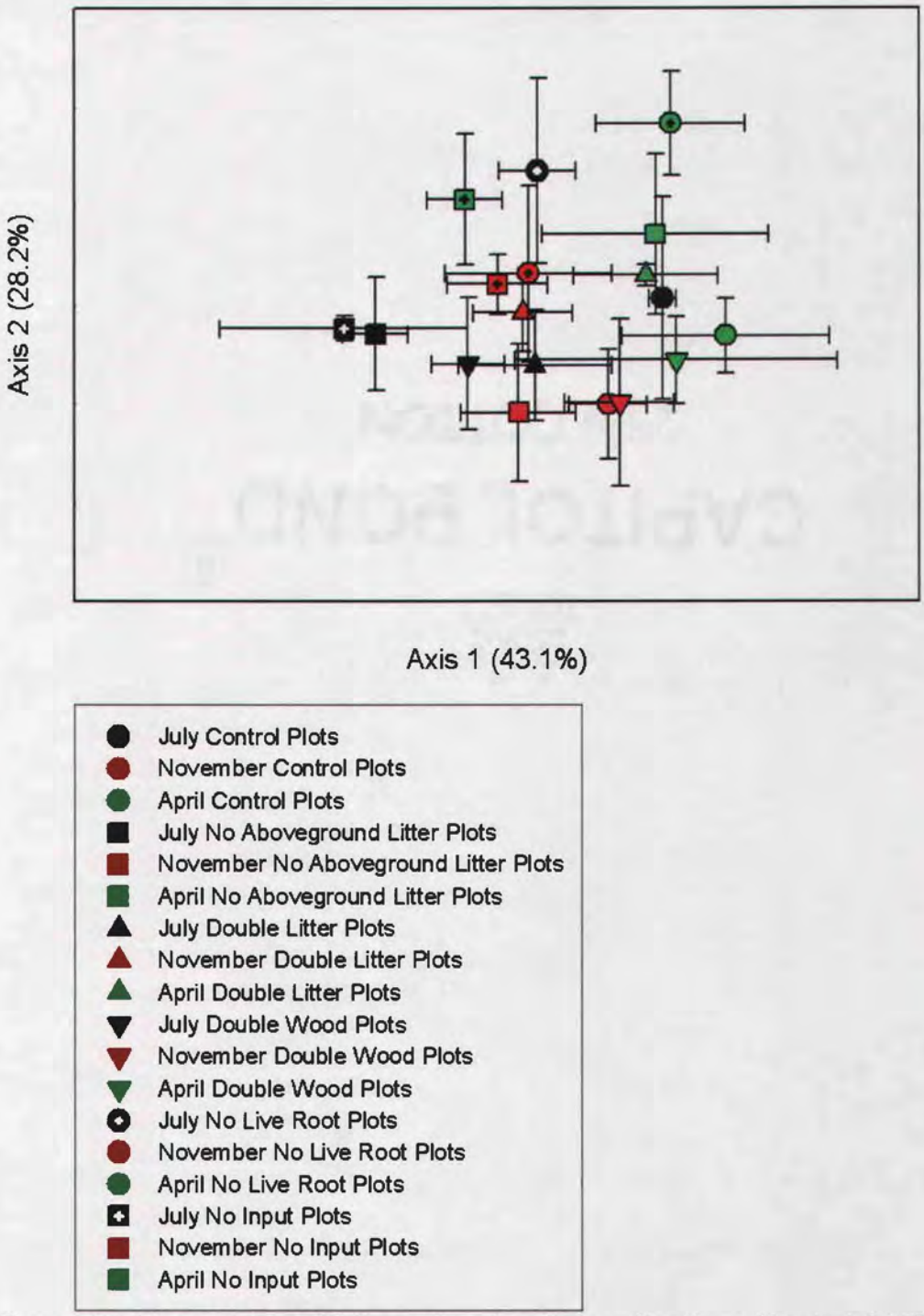

Figure 4.3. Nonmetric multidimensional scaling ordination of bacterial 16S LH-PCR data of all sampling times and treatments.

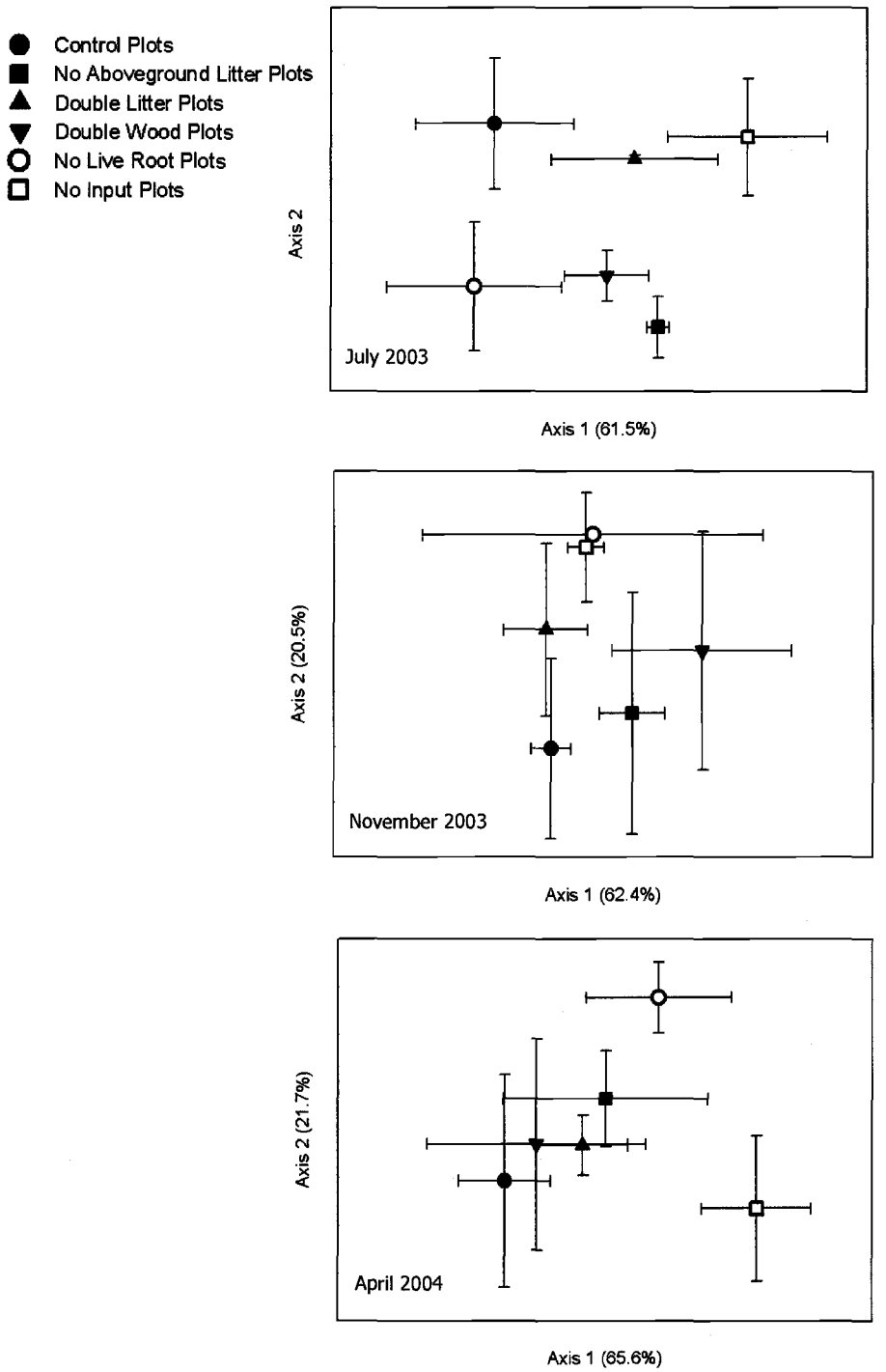

Figure 4.4. Nonmetric multidimensional scaling ordination of bacterial 16S LH-PCR data of July 2003, November 2003, and April 2004.

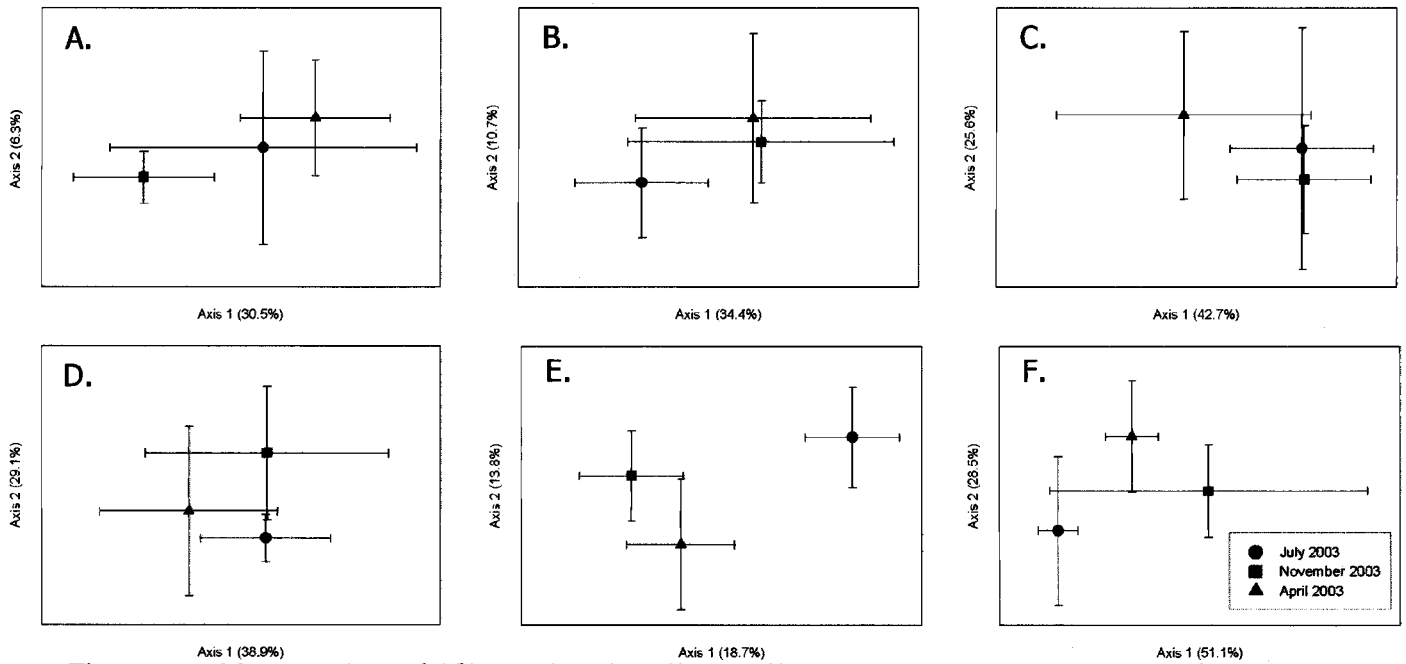

Figure 4.5. Nonmetric multidimensional scaling ordination of fungal ITS LH-PCR data for (A.) control plots, (B.) double litter plots, (C.) double wood plots, (D.) no aboveground litter plots, (E.) no live root plots, and (F.) no input plots.

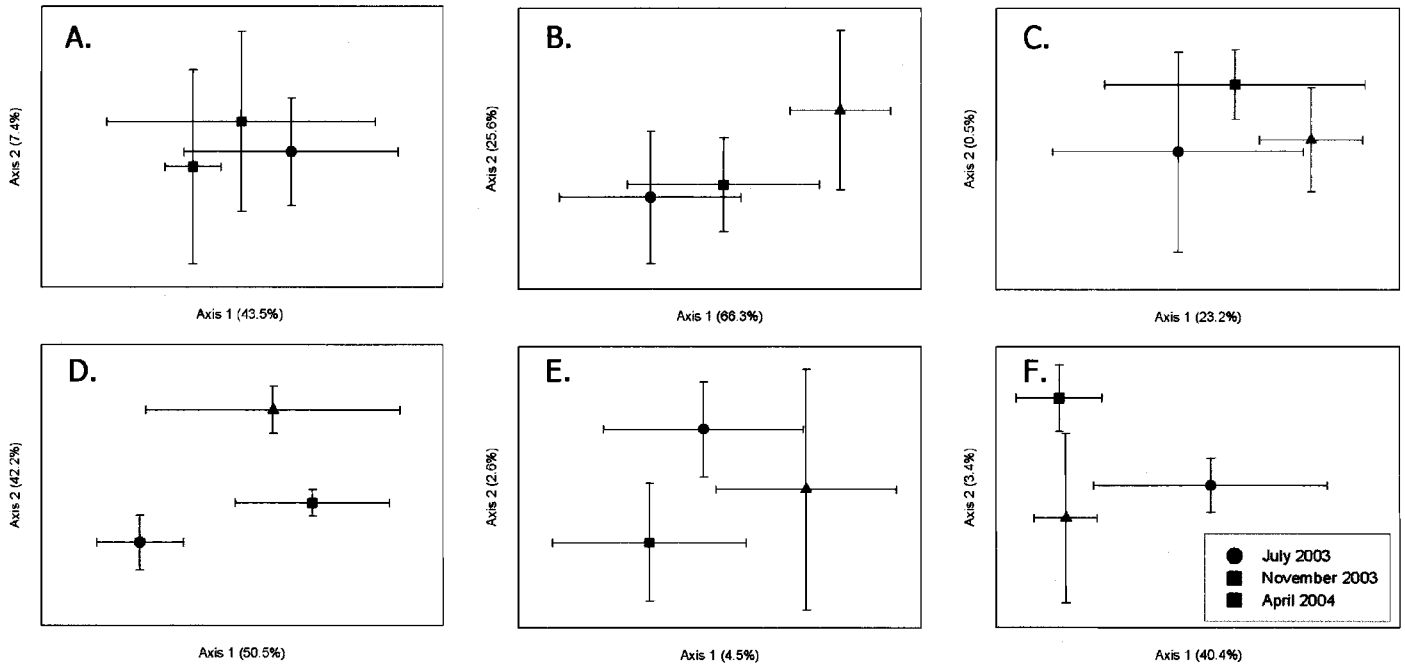

Figure 4.6. Nonmetric multidimensional scaling ordination of bacterial 16S LH-PCR data for (A.) control plots, (B.) double litter plots, (C.) double wood plots, (D.) no aboveground litter plots, (E.) no live root plots, and (F.) no input plots.

Table 4.1. Treatments at the H. J. Andrews DIRT plots.

Treatment		Method
Controls	CO	Normal litter inputs
No aboveground litter	NL	Plots are screened and swept to exclude aboveground litter
Double litter	DL	Aboveground leaves/needles are doubled by adding litter from No Aboveground Litter plots
Double Wood	DW	Aboveground CWD inputs are doubled by adding shredded CWD
No Live Roots	NR	Live roots are excluded by inserting barriers around plots in backfilled trenches
No Inputs	NI	Plot are trenched, trees girdled, live roots are excluded, Litter is excluded

Table 4.2. Multi-Response Permutation Procedures table for DIRT fungal ITS and bacterial 16S fragments by treatment.

	Fungi		*Bacterial 16S*	
Test	*A*-statistic[1]	*p*-value	*A*-statistic[1]	*p*-value
Difference by treatment:				
All sampling times:				
Control vs. Double Litter	0.046	**<0.001**	0.018	0.154
Control vs. Double Wood	0.032	**<0.001**	0.030	0.059
Control vs. No Aboveground Litter	0.019	**<0.050**	0.029	0.076
Control vs. No Inputs	0.045	**<0.001**	0.092	**<0.0001**
Control vs. No Live Roots	0.087	**<0.0001**	0.077	**0.012**
Double Litter vs. Double Wood	0	0.433	0.005	0.312
Double Litter vs. No Aboveground Litter	0.015	0.086	-0.012	0.694
Double Litter vs. No Inputs	0.041	**0.002**	0.019	0.152
Double Litter vs. No Live Roots	0.085	**<0.0001**	0.046	**0.044**
Double Wood vs. No Litter	0.009	0.176	-0.001	0.447
Double Wood vs. No Inputs	0.0266	0.018	0.057	**0.006**
Double Wood vs. No Live Roots	0.072	**<0.0001**	0.053	**0.022**
No Aboveground Litter vs. No Inputs	0.037	**0.004**	-0.001	0.461
No Aboveground Litter vs. No Live Roots	0.078	**<0.0001**	0.038	0.060
No Inputs vs. No Live Roots	0.004	0.328	0.029	0.101

[1]A = 1 – (observed delta/expected delta); A = 0 when heterogeneity within groups equals expectation by chance; A < 0 with more heterogeneity within groups than expected by chance

Table 4.2 (continued).

	Fungi		*Bacterial 16S*	
Test	*A*-statistic[1]	*p*-value	*A*-statistic[1]	*p*-value
July				
Control vs. Double Litter	0.025	0.236	0	0.435
Control vs. Double Wood	-0.026	0.870	0.080	**0.037**
Control vs. No Aboveground Litter	-0.035	0.791	0.138	**0.024**
Control vs. No Inputs	0.050	**0.026**	0.098	**0.023**
Control vs. No Live Roots	0.093	**0.023**	0.010	0.324
Double Litter vs. Double Wood	-0.045	0.916	0.027	0.303
Double Litter vs. No Aboveground Litter	-0.014	0.619	0.049	0.108
Double Litter vs. No Inputs	0.086	**0.037**	-0.034	0.852
Double Litter vs. No Live Roots	0.109	**0.024**	0.068	0.251
Double Wood vs. No Litter	-0.018	0.635	-0.027	0.581
Double Wood vs. No Inputs	0.008	0.390	0.051	0.135
Double Wood vs. No Live Roots	0.053	**0.026**	0.056	0.251
No Aboveground Litter vs. No Inputs	0.076	0.066	0.043	0.172
No Aboveground Litter vs. No Live Roots	0.084	**0.039**	0.103	0.170
No Inputs vs. No Live Roots	0.032	0.242	0.064	0.252

[1]A = 1 – (observed delta/expected delta); A = 0 when heterogeneity within groups equals expectation by chance; A < 0 with more heterogeneity within groups than expected by chance

Table 4.2 (continued).

	Fungi		*Bacterial 16S*	
Test	*A*-statistic[1]	*p*-value	*A*-statistic[1]	*p*-value
November				
Control vs. Double Litter	0.061	0.069	-0.009	0.532
Control vs. Double Wood	0.033	0.059	-0.015	0.552
Control vs. No Aboveground Litter	0.027	0.104	0.009	0.443
Control vs. No Inputs	0.020	0.152	0.115	0.066
Control vs. No Live Roots	0.130	0.023	0.005	0.427
Double Litter vs. Double Wood	0.037	0.189	0.003	0.433
Double Litter vs. No Aboveground Litter	0.007	0.372	-0.034	0.682
Double Litter vs. No Inputs	-0.028	0.805	-0.059	0.839
Double Litter vs. No Live Roots	0.094	0.027	-0.067	0.925
Double Wood vs. No Aboveground Litter	0.019	0.187	-0.013	0.567
Double Wood vs. No Inputs	-0.034	0.738	0.075	0.096
Double Wood vs. No Live Roots	0.108	**0.031**	-0.074	0.790
No Aboveground Litter vs. No Inputs	-0.033	0.918	0.005	0.387
No Aboveground Litter vs. No Live Roots	0.051	0.080	-0.027	0.715
No Inputs vs. No Live Roots	-0.003	0.460	-0.050	0.877

[1]A = 1 – (observed delta/expected delta); A = 0 when heterogeneity within groups equals expectation by chance; A < 0 with more heterogeneity within groups than expected by chance

Table 4.2 (continued).

	Fungi		*Bacterial 16S*	
Test	*A*-statistic[1]	*p*-value	*A*-statistic[1]	*p*-value
April				
Control vs. Double Litter	0.030	0.067	-0.018	0.615
Control vs. Double Wood	0.063	**0.023**	-0.066	0.765
Control vs. No Aboveground Litter	0.021	0.169	-0.033	0.648
Control vs. No Inputs	0.074	0.055	0.142	**0.048**
Control vs. No Live Roots	0.129	0.026	0.121	0.104
Double Litter vs. Double Wood	0.002	0.452	-0.055	0.835
Double Litter vs. No Aboveground Litter	-0.035	0.808	-0.071	0.869
Double Litter vs. No Inputs	0.032	0.223	0.092	0.074
Double Litter vs. No Live Roots	0.047	0.104	0.047	0.317
Double Wood vs. No Aboveground Litter	-0.014	0.736	-0.053	0.699
Double Wood vs. No Inputs	0.067	0.068	0.083	0.179
Double Wood vs. No Live Roots	0.138	**0.023**	0.072	0.123
No Aboveground Litter vs. No Inputs	0.069	**0.042**	0.034	0.250
No Aboveground Litter vs. No Live Roots	0.123	**0.024**	-0.011	0.520
No Inputs vs. No Live Roots	0.043	0.110	0.063	0.151

[1]A = 1 – (observed delta/expected delta); A = 0 when heterogeneity within groups equals expectation by chance; A < 0 with more heterogeneity within groups than expected by chance

Table 4.3. Multi-Response Permutation Procedures table for DIRT fungal ITS and bacterial 16S fragments by sampling time.

	Fungal ITS		*Bacterial 16S*	
Test	*A*-statistic[1]	*p*-value	*A*-statistic[1]	*p*-value
Difference by sampling time:				
Control plots				
July vs. November	-0.032	0.751	-0.031	0.691
July vs. April	-0.019	0.726	-0.033	0.723
November vs. April	0.029	0.096	-0.009	0.418
Double Litter plots				
July vs. November	-0.015	0.680	-0.068	0.755
July vs. April	-0.016	0.598	0.059	0.260
November vs. April	-0.017	0.601	-0.035	0.680
Double Wood plots				
July vs. November	-0.044	0.837	0.029	0.278
July vs. April	-0.029	0.857	-0.004	0.479
November vs. April	0.025	0.210	-0.082	0.964
No Aboveground Litter plots				
July vs. November	-0.024	0.731	0.062	0.098
July vs. April	-0.018	0.696	0.127	**0.029**
November vs. April	-0.059	0.803	0.036	0.220

[1]A = 1 – (observed delta / expected delta); A = 0 when heterogeneity within groups equals expectation by chance; A < 0 with more heterogeneity within groups than expected by chance

Table 4.3 (continued).

	Fungal ITS		*Bacterial 16S*	
Test	*A*-statistic[1]	*p*-value	*A*-statistic[1]	*p*-value
No Input plots				
July vs. November	0.004	0.400	0.040	0.150
July vs. April	-0.019	0.697	0.070	0.173
November vs. April	-0.007	0.539	0.021	0.384
No Live Root plots				
July vs. November	0.070	**0.020**	-0.055	0.717
July vs. April	0.097	**0.030**	-0.024	0.584
November vs. April	-0.030	0.701	0.003	0.484

[1]A = 1 – (observed delta/expected delta); A = 0 when heterogeneity within groups equals expectation by chance; A < 0 with more heterogeneity within groups than expected by chance

Table 4.4. Fungal ITS indicator species analysis by root treatment for the July 2003, November 2003, and April 2004 samplings.

Test	Treatment[1]	Fragment	Indicator value	*p*-value[2]
Indicators across all sampling times:				
Control vs. Root plots (DL, DW, NL)	CO	602	65.5	0.009
	CO	664	52.8	0.043
	DL, DW, NL	670	58.6	0.032
	CO	691	70.4	0.011
	DL, DW, NL	742	78.1	0.004
	DL, DW, NL	780	40.7	0.050
Control vs. No Live Root plots (NR, NI)	NR, NI	553	69.7	0.006
	NR, NI	583	44.4	0.028
	NR, NI	590	60.2	0.055
	NR, NI	629	63.9	0.027
	NR, NI	636	66.1	0.014
	CO	657	52.7	0.003
	NR, NI	659	73.0	0.019
	CO	664	65.3	0.003
	CO	714	72.4	0.005
	CO	723	88.5	0.001
	CO	732	84.5	0.002
	NR, NI	742	69.2	0.021
	NR, NI	780	55.6	0.033
Root plots (DL, DW, NL) vs. No Live Root plots (NR, NI)	NR, NI	481	22.2	0.021
	NR, NI	514	45.0	0.006
	NR, NI	553	71.9	0.001
	NR, NI	558	29.7	0.003
	DL, DW, NL	566	61.8	0.001
	NR, NI	578	27.8	0.006
	NR, NI	602	65.2	0.003
	NR, NI	625	67.7	0.004
	NR, NI	629	73.9	0.001
	NR, NI	631	59.1	0.008
	NR, NI	659	71.3	0.002
	DL, DW, NL	670	53.8	0.018
	NR, NI	683	47.0	0.015
	DL, DW, NL	714	66.3	0.006
	DL, DW, NL	723	78.6	0.001
	DL, DW, NL	732	66.0	0.005
	NR, NI	740	30.6	0.019
	NR, NI	775	44.4	0.033

[1]CO = control plots; DL = double litter plots; DW = double wood plots; NL = no aboveground litter plots; NI = no input plots; NR = no live root plots.
[2]proportion of randomized trials with indicator value equal to or exceeding the observed indicator value. p = (1 + number of runs ≥ observed) / (1 + number of randomized runs).

Table 4.5. Bacterial 16S indicator species analysis by root treatment for the July 2003, November 2003, and April 2004 samplings.

Test	Treatment[1]	Fragment	Indicator value	*p*-value[2]
Indicators across all sampling times:				
Control vs. Root plots (DL, DW, NL)	CO	348	61.1	0.044
	CO	359	62.9	0.003
Control vs. No Live Root plots (NR, NI)	NR, NI	310	63.1	0.005
	NR, NI	312	64.2	0.023
	CO	342	67.2	0.031
	CO	348	68.2	0.016
	CO	359	62.1	0.008
Root plots (DL, DW, NL) vs. No Live Root plots (NR, NI)	NR, NI	310	60.2	0.001
	NR, NI	312	67.7	0.002
	DL, DW, NL	329	60.9	0.005

[1]CO = control plots; DL = double litter plots; DW = double wood plots; NL = no aboveground litter plots; NI = no input plots; NR = no live root plots.
[2]proportion of randomized trials with indicator value equal to or exceeding the observed indicator value. p = (1 + number of runs ≥ observed)/(1 + number of randomized runs).

Using Molecular Techniques To Understand Fungal Communities

Chapter 5: Conclusion To The Dissertation

Rapid methods such as LH-PCR and terminal fragment length polymorphisms (T-RFLPs) are gaining in popularity for studying fungal communities in soil. In all three studies (Chapter 2, 3, and 4), we found length heterogeneity PCR (LH-PCR) to be a useful tool for examining changes in fungal communities. In Chapter 2, we found that the technique allowed us to examine differences in fungal community composition along transects from the forest to the meadow sites at two sites in the western Cascades of Oregon. We also observed edge effects in the samples collected in the transition zone between the forest and the meadows. Edge effects were not observed in earlier studies of ammonia oxidizing and denitrifying bacteria along the same transects (Mintie *et al.*, 2003; Rich *et al.*, 2003).

In addition to identifying observational effects of vegetation and edges on fungal communities, we found that LH-PCR is useful for identifying treatment effects in manipulative experiments. In Chapters 3 and 4, LH-PCR allowed us to observe the changes in community dynamics and identify some of the factors involved in community changes following disturbance. In Chapter 3, we found that fungal succession occurred rapidly when cores were transferred to new environments. We also saw evidence that succession occurred more slowly when roots were excluded. In contrast, Boyle *et al.* (2005) found that temporal differences were greater than transfer effects in denitrifying bacterial communities. In fact, temporal differences were slight with fungal communities. In Chapter 4, we found that live roots rather than either aboveground litter

inputs or seasonal differences in sampling times were the greatest determinant of both fungal and bacterial communities at the Detritus Input and Removal Treatment (DIRT) plots 6 years after initiation. Our results here are supported by Brant et al.'s (2005) results at the DIRT plots using the same samples.

High throughput methods such as LH-PCR and T-RFLP are fast and allow for the rapid collection of data and have the advantage of giving researchers access to organisms that cannot be cultures with existing methods. Methods such as LH-PCR have drawbacks, however. The biggest drawback is that it is difficult to identify organisms without the additional steps of cloning and sequencing. It is nearly impossible to assign function to organisms without identification. Fortunately, manipulative studies such as DIRT allow for the elimination of some variables. Although LH-PCR did not allow us to identify our ITS fragments, we gained valuable insight into fungal community dynamics. A logical next step for the studies presented in this dissertation is to clone and sequence the dominant LH-PCR fragments. Identifying fragments would yield a better understanding of how function relates to communities, and whether or not changes in community composition are due changes in the abundance of mycorrhizal or saprotrophic fungi.

Molecular techniques such as LH-PCR (Ritchie *et al.*, 2000) and T-RFLP (Dickie *et al.*, 2002) are relatively simple methods that can be used to track changes in fungal communities. These techniques are commonly used to study bacterial communities but are still rather new to the study of fungal communities. Although there are some shortcomings, high throughput molecular methods have the potential to be valuable tools for understanding how fungal communities respond to disturbance. Our work presented here and

by earlier studies (Harvey *et al.*, 1980; Jones *et al.*, 2003; Perry *et al.*, 1982) indicates that fungal communities in forests are particularly sensitive to disturbance.

Ecosystem resilience is defined as the amount of disturbance that a system can absorb and remain in the same state or domain of attraction (Elmqvist *et al.*, 2003; Holling, 1973). The definition of resilience also includes the ability of an ecosystem that is subject to disturbance to reorganize and renew itself (Elmqvist *et al.*, 2003). Ecosystems in the Pacific Northwest have evolved with a history of disturbance ranging from small-scale to catastrophic, yet are surprisingly resilient. Consider the eruption of Mount St. Helens in May 1980. Only three years after the eruption, 230 plant species or 90% of those in the pre-eruption communities were found in the blast area (Franklin *et al.*, 1985). Despite this very high level of resilience, occasionally ecosystems in the Pacific Northwest are pushed beyond their capacities to renew themselves.

The strength of high throughput molecular techniques is that they have the potential to help us to understand fungal communities on a finer scale than was possible in the past. These techniques make it possible to track the everyday changes that occur seasonally, or with changes in precipitation and plant phenology. They can also help us to better understand the thresholds involved in ecosystem resilience and succession. However, it is still necessary to understand the day-to-day changes as systems can be very resilient but still exhibit a great deal of fluctuation (Holling, 1973). We will gain a better understanding of fungal communities, fungal succession, and the response of fungi to disturbance as more studies are completed.

BIBLIOGRAPHY

Agee JK (1993) Fire Ecology of Pacific Northwest Forests. Island Press, Washington, D. C.

Allen MF (1991) The Ecology of Mycorrhizae. Cambridge, Cambridge University Press

Amaranthus MP, Perry DA (1989) Interaction effects of vegetation type and Pacific madrone soil inocula on survival, growth, and mycorrhiza formation of Douglas-fir. Canadian Journal of Forest Research 19: 550-556

Axelrood PE, Chow ML, Radomski CC, McDermott JM, Davies J (2002) Molecular characterization of bacterial diversity from British Columbia forest soils subjected to disturbance. Canadian Journal of Microbiology 48: 655-674

Bååth E (1980) Soil fungal biomass after clear-cutting of a pine forest in central Sweden. Soil Biology & Biochemistry 12: 495-500

Bååth E (1981) Microfungi in a clear-cut pine forest soil in central Sweden. Canadian Journal of Botany 59: 1331-1337

Bååth E,. Frostegård Å, Pennanen T, Fritze H (1995) Microbial commuunity structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. Soil Biology & Biochemistry 27: 229-240

Bååth E, Söderström B (1982) Seasonal and spatial variation in fungal biomass in a forest soil. Soil Biology & Biochemistry 14: 353-358

Beare, M. H., R. W. Parmelee, P. F. Hendrix, W. Cheng, D. C. Coleman, and D. A. Crossley. 1992. Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. Ecological Monographs 62: 569-591

Bernhard AE, Colbert D, McManus J, Field KG (2005) Microbial community dynamics based on 16S rRNA gene profiles in a Pacific Northwest estuary and its tributaries. FEMS Microbiology Ecology 52: 115-128

Bills GF, Holtzman GI, Miller OK Jr (1986) Comparison of the ectomycorrhizal-basidiomycete communities in red spruce versus northern hardwood forests of West Virginia. Canadian Journal of Botany 64: 760-768

Boone RD, Nadelhoffer KJ, Canary JD, Kaye J (1998) Roots exert a strong influence on the temperature sensitivity of soil respiration. Nature 396: 570-572

Bottomley PJ, Taylor AE, Boyle SA, McMahon SK, Rich JJ, Cromack K Jr, Myrold DD (2004) Responses of nitrification and ammonia-oxidizing bacteria to reciprocal transfers of soil between adjacent coniferous forest and meadow vegetation in the Cascade Mountains of Oregon. Microbial Ecology 48: 500-508

Boyle SA, Rich JJ, Bottomley PJ, Cromack K Jr, Myrold DD (2005) Disturbance effects on denitrifying community composition and activity at forest and meadow sites in the Cascade Mountains of Oregon. Soil Biology and Biochemistry in press

Brant JB, Myrold DD, Sulzman EW (2005) Root controls on soil microbial community structure in forest soils. Oecologia in review

Brodie E, Edwards S, Clipson N (2003) Soil fungal community structure in a temperate upland grassland soil. FEMS Microbiology Ecology 45: 105-114

Bruns T (1995) Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. Plant and Soil 170: 63-73

Carpenter SE, Trappe JM, Ammirati J Jr (1987) Observations of fungal succession in the Mount St. Helens devastation zone, 1980-1983. Canadian Journal of Botany 65: 716-728

Chen DM, Cairney JWG (2002) Investigation of the influence of prescribed burning on ITS profiles of ectomycorrhizal and other soil fungi at three Australian sclerophyll forest sites. Mycological Research 106: 532-540

Chenu C (1989) Influence of a fungal polysaccharide, scleroglucan on clay microstructure. Soil Biology & Biochemistry 21: 299-305

Chow ML, Radomski CC, McDermott JM, Davies J, Axelrood PE (2002) Molecular characterization of bacterial diversity in Lodgepole pine (*Pinus contorta*) rhizosphere soils from British Columbia forest soils differing in disturbance and geographic source. FEMS Microbiology Ecology 42: 347-357

Cline ET, Ammirati JF, Edmonds RL (2005) Does proximity to mature trees influence ectomycorrhizal fungus communities of Douglas-fir seedlings? New Phytologist 166: 993-1009

Cullings KW, New MH, Makhija S, and Parker VT (2003) Effects of litter addition on ectomycorrhizal associates of a lodgepole pine (*Pinus contorta*) stand in Yellowstone National Park. Applied and Environmental Microbiology 69: 3772-3776

Cullings KW, Vogler DR, Parker VT, Makhija S (2001) Defoliation effects on the ectomycorrhizal community of a mixed *Pinus contorta / Picea engelmannii* stand in Yellowstone Park. Oecologia 127: 533-539

Denslow JS (1985) Disturbance-mediated coexistence of species. Pages 307-323 *in* S. T. A. Pickett and P. S. White, editors. The Ecology of Natural Disturbances and Patch Dynamics. Academic Press, Inc., New York

Dickie IA, Avis PG, McLaughlin DJ, Reich PB (2003) Good-Enough RFLP Matcher (GERM) program. Mycorrhiza 13: 171-172

Dickie IA, Koide RT, Steiner KC (2002a) Influences of established trees on mycorrhizas, nutrition, and growth of *Quercus rubra* seedlings. Ecological Monographs 72: 502-521

Dickie IA, Reich PB (2005) Ectomycorrhizal fungal communities at forest edges. Journal of Ecology 93: 244-255

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

Dixon JJ (2003) Applying GIS to soil-geomorphic landscape mapping in the Lookout Creek valley, Western Cascades, Oregon. M. Sc. Oregon State University, Corvallis

Donegan KK, Watrud LS, Seidler RJ, Maggard SP, Shiroyama T, Porteous LA (2001) Soil and litter organisms in Pacific northwest forests under different management practices. Applied Soil Ecology 18: 159-175

Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible approach. Ecological Monographs 67: 345-366

Dunham SM, Kretzer A, Pfrender ME (2003) Characterization of Pacific golden chanterelle (*Cantharellus formosus*) genet size using co-dominant microsatellite markers. Molecular Ecology 12: 1607-1618

Elmqvist T, Folke C, Nyström M, Peterson G, Bengtsson J, Walker B, Norberg J (2003) Response diversity, ecosystem change, and resilience. Frontiers in Ecology and Environment 1: 488-494

Fisk MC, Fahey TJ (2001) Microbial biomass and nitrogen cycling responses to fertilization and litter removal in young northern hardwood forests. Biogeochemistry 53: 201-223

Franklin JF, MacMahon JA, Swanson FJ, Sedell JR (1985) Ecosystem responses to the eruption of Mount St. Helens. National Geographic Research 1: 198-216

Franklin JF, Halpern CB (2000) Pacific Northwest Forests. Pages 123-159 *in* M. G. Barbour and W. D. Billings, editors. North American Terrestrial Vegetation. Cambridge University Press, Cambridge.

Friese CF, Morris SF, Allen MF (1997) Disturbance in natural ecosystems: Scaling from fungal diversity to ecosystem functioning. Pages 47-63 *in* D. T. Wicklow and B. E. Söderström, editors. The Mycota IV: Environmental and Microbial Relationships. Springer-Verlag, Berlin

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

Griffiths R, Madritch M, Swanson A (2005) Conifer invasion of forest meadows transforms soil characteristics in the Pacific Northwest. Forest Ecology and Management 208: 347-358

Hagerman SM, Jones MD, Bradfield GE, Gillespie M, Durall DM (1999) Effects of clear-cut logging on the diversity and persistence of ectomycorrhizae at a subalpine forest. Canadian Journal of Forest Research 29: 124-134

Harvey AE, Jurgensen MF, Larsen MJ (1980) Clearcut harvesting and ectomycorrhizae: survival of activity on residual roots and influence on a bordering forest stand in Western Montana. Canadian Journal of Forest Research 10: 300-303

Harvey AE, PageDumroese DS, Jurgensen MF, Graham RT, Tonn JR (1996) Site preparation alters biomass, root and ectomycorrhizal development of outplanted western white pine and Douglas-fir. New Forests 11: 255-270

Harvey AE, PageDumroese DS, Jurgensen MF, Graham RT, Tonn JR (1997) Site preparation alters soil distribution of roots and ectomycorrhizae on outplanted western white pine and Douglas-fir. Plant and Soil 188: 107-117

Hernesmaa A, Björklöf K, Kiikilä O, Fritze H, Haahtela K, Romantschuk M (2005) Structure and function of microbial communities in the rhizosphere of Scots pine after tree-felling. Soil Biology & Biochemistry 37: 777-785

Heilmann-Clausen J, Christensen M (2004) Does size matter? On the importance of various dead wood fractions for fungal diversity in Danish beech forests. Forest Ecology and Management 201: 105-117

Hickman JC (1968) Disjunction and Endemism in the Flora of the Central Western Cascades of Oregon: An Historical and Ecological Approach to Plant Distributions. Ph.D dissertation. University of Oregon, Eugene

Hickman JC (1976) Non-forest vegetation of the western Cascade Mountains of Oregon. Northwest Science 50: 145-155

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

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Lofvenius M, Read DJ (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411: 789-792

Holling CS (1973) Resilience and stability of ecological systems. Annual Review of Ecology and Systematics 4: 1-23

Horton TR (2002) Molecular approaches to ectomycorrhizal diversity studies: variation in ITS at a local scale. Plant and Soil 244: 29-39

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

Hunt J, Boddy L, Randerson PF, Rogers HJ (2004) An evaluation of 18S rDNA approaches for the study of fungal diversity in grassland soils. Microbial Ecology 47: 385-395

Jones MD, Durall DM, Cairney JWG (2003) Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytologist 157: 399-422

Jumpponen A (2003) Soil fungal community assembly in a primary successional glacier forefront ecosystem as inferred from rDNA sequence analyses. New Phytologist 158: 569-578

Kageyama SA, Myrold DD, Bottomley PJ, Cromack K Jr. (2005) Chapter 2: Fungal communities across meadow/forest transects in the western Cascades of Oregon Effects of vegetation and disturbance on fungal communities in the western Cascades of Oregon, Ph.D. dissertation. Oregon State University

Kårén O, Högberg N, Dahlberg A, Jonsson L, Nylund J-E (1997) Inter- and intraspecific variation in the ITS region of rDNA of ectomycorrhizal fungi in Fennoscandia as detected by endonuclease analysis. New Phytologist 136: 313-325

Korb JE, Johnson NC, Covington WW (2004) Slash pile burning effects on soil biotic and chemical properties and plant establishment: Recommendations for amelioration. Restoration Ecology 12: 52-62

Kuyper TW, Landeweert R (2002) Vertical niche differentiation by hyphae of ectomycorrhizal fungi in soil. New Phytologist 156: 323-325

Kwasna H (2004) Natural shifts in communities of rhizosphere fungi of common oak after felling. Plant and Soil 264: 209-218

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

Landeweert R, Leeflang P, Smit E, Kuyper T (2005) Diversity of an ectomycorrhizal fungal community studied by a root tip and total soil DNA approach. Mycorrhiza 15: 1-6

Landeweert R, Veenman, Kuyper TW, Fritze H, Wernars K, Smit E (2003b) Quantification of ectomycorrhizal mycelium in soil by real-time PCR compared to conventional quantification techniques. FEMS Microbiology Ecology 45: 283-292

McCune B, Grace JB (2002) Analysis of Ecological Communities. MJM Software Design, Gleneden Beach.

Miller EA, Halpern CB (1998) Effects of environment and grazing disturbance on tree establishment in meadows of the central Cascade Range, Oregon, USA. Journal of Vegetation Science 9: 265-282.

Miller RM, Lodge DJ (1997) Fungal responses to disturbance: Agriculture and forestry. Pages 65-84 *in* D. T. Wicklow and B. E. Söderström, editors. The Mycota IV: Environmental and Microbial Relationships. Springer-Verlag, Berlin

Mintie AT, Heichen RS, Cromack K Jr, Myrold DD, Bottomley PJ (2003) Ammonia-oxidizing bacteria along meadow-to-forest transects in the Oregon Cascade Mountains. Applied and Environmental Microbiology 69: 3129-3136

Murakami Y (1987) Spatial distribution of *Russula* species in *Castanopsis cuspidata* forest. Transactions of the British Mycological Society 89: 187-193

Nantel P, Neumann P (1992) Ecology of ectomycorrhizal-basidiomycete communities on a local vegetation gradient. Ecology 73: 99-117

Niemelä S, Sundman V (1977) Effects of clear-cutting on the composition of bacterial populations of northern spruce forest soil. Canadian Journal of Microbiology 23: 131-138

O'Brien HE, Parrent JL, Jackson JA, Moncalvo J-M, Vilgalys R (2005) Fungal community analysis by large scale sequencing of environmental samples. Applied and Environmental Microbiology 71: 5544-55

Parke JL, Linderman RG, Trappe JM (1983) Effects of forest litter on mycorrhiza development and growth of Douglas-fir and western red cedar seedlings. Canadian Journal of Forest Research 13: 666-671

Perry DA, Meyer MM, Egeland D, Rose SL, Pilz D (1982) Seedling growth and mycorrhizal formation in clearcut and adjacent, undisturbed soils in Montana: A greenhouse bioassay. Forest Ecology and Management 4: 261-273

Perry DA, Molina R, Amaranthus MP (1987) Mycorrhizae, mycorrhizospheres, and reforestation: current knowledge and research needs. Canadian Journal of Forest Research 17: 929-940

Peter M, Ayer F, Egli S, Honegger R (2001) Above- and below-ground community structure of ectomycorrhizal fungi in three Norway spruce (*Picea abies*) stands in Switzerland. Canadian Journal of Botany 79: 1134-1151

Pietikäinen J, Fritze H (1995) Clear-cutting and prescribed burning in coniferous forest: Comparison of effects on soil fungal and total microbial biomass, respiration activity and nitrification. Soil Biology & Biochemistry 27: 101-109

Platt WJ, Connell JH (2003) Natural disturbances and directional replacement of species. Ecological Monographs 73: 507-522

Ranjard L, Lejon DPH, Mougel C, Schehrer L, Merdinoglu D, Chaussod R (2003) Sampling strategy in molecular microbial ecology: influence of soil sample size on DNA fingerprinting analysis of fungal and bacterial communities. Environmental Microbiology 5: 1111-1120

Ranjard L, Poly F, Lata JC, Mougel C, Thioulouse J, Nazaret S (2001) Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: Biological and methodological variability. Applied and Environmental Microbiology 67: 4479-4487.

Rich JJ (2004) Community Composition and Activities of Denitrifying Bacteria in Soils. Ph.D. Dissertation. Oregon State University, Corvallis

Rich JJ, Heichen RS, Bottomley PJ, Cromack K Jr, Myrold DD (2003) Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. Applied and Environmental Microbiology 69: 5974-5982

Richardson MJ (1970) Studies on *Russula emetica* and other agarics in a scots pine plantation. Transactions of the British Mycological Society 55: 217-229

Ritchie NJ, Schutter ME, Dick RP, Myrold DD (2000) Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterize microbial communities in soil. Applied and Environmental Microbiology 66: 1668-1675.

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: 775-783

Schadt CW, Martin AP, Lipson DA, Schmidt SK (2003) Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301: 1359-1361

Schmit JP, Mueller GM, Leacock PR, Mata JL, Wu Q, Huang Y (2005) Assesment of tree species richness as a surrogate for macrofungal species richness. Biological Conservation 121: 99-110

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

Siira-Pietikainen A, Pietikainen J, Fritze H, Haimi J (2001b) Short-term responses of soil decomposer communities to forest management: clear felling versus alternative forest harvesting methods. Canadian Journal of Forest Research 31: 88-99

Simard SW, Jones MD, Durall DM, Hope GD, Stathers RJ, Sorensen NS, Zimonick BJ (2003) Chemical and mechanical site preparation: effects on *Pinus contorta* growth, physiology, and microsite quality on grassy, steep forest sites in British Columbia. Canadian Journal of Forest Research 33: 1495-1515

Smith ML, Bruhn JN (1992) The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature 356: 428

Spears JDH, Holub SM, Harmon ME, Lajtha K (2003) The influence of decomposing logs on soil biology and nutrient cycling in an old-growth mixed coniferous forest in Oregon, U.S.A. Canadian Journal of Forest Research 33: 2193-2201.

Spears JDH, Lajtha K (2004) The imprint of coarse woody debris on soil chemistry in the western Oregon Cascades. Biogeochemistry 71: 163-175.

Subke J-A, Hahn V, Battipaglia G, Linder S, Buchmann N, Cotrufo MF (2004) Feedback interactions between needle litter decomposition and rhizosphere activity. Oecologia 139: 551-559

Sulzman EW, Brant JB, Bowden RD, Lajtha K (2005) Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. Biogeochemistry 73: 231-256

Swift MJ, O. W. Heal OW, Anderson JM (1979) Decomposition in terrestrial ecosystems. Blackwell, Oxford University Press

Trappe JM, Luoma DL (1992) The Ties That Bind: Fungi in Ecosystems. Pages 17-27 *in* G. C. Carroll and D. T. Wicklow, editors. The Fungal Community: Its Organization and Role in the Ecosystem. Marcel Dekker, Inc., New York

Turner MG, Baker WL, Peterson CJ, Peet RK (1998) Factors influencing succession: Lessons from large, infrequent natural disturbances. Ecosystems 1: 511-523.

van Elsas JD, G. D. Duarte GD, Keijzer-Wolters A, Smit E (2000) Analysis of the dynamics of fungal communities in soil via fungal-specific PCR of soil DNA followed by denaturing gradient gel electrophoresis. Journal of Microbiological Methods 43: 133-151

Vogt KA, Grier CC, Meier CE, Edmonds RL (1982 Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* ecosystems in western Washington. Ecology 63: 370-380

Wallander H, Nilsson LO, Hagerberg D, Bååth E (2001) Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. New Phytologist 151: 753-760

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 *in* M. A. Innis, D. H. Gelfand, J. J. Sninksky, and T. J. White, editors. PCR Protocols: a Guide to Methods and Applications. Academic Press, New York

Yano Y, Lajtha K, Sollins P, Caldwell BA (2005) Chemistry and dynamics of dissolved organic matter in a temperate coniferous forest on andic soils: Effects of litter quality. Ecosystems 8: 286-300

Zak JC (1992) Response of Soil Fungal Communities to Disturbance. Pages 403-425 *in* G. C. Carroll and D. T. Wicklow, editors. The Fungal Community: Its Organization and Role in the Ecosystem. Marcel Dekker, Inc., New York

APPENDICES

Appendix A. Root biomass and ectomycorrhizal colonization for the reciprocal transplant samples harvested in 2002.

Methods. Roots were removed from bulk soil by sieving. After bulk separation of roots from soil, roots were rinsed in deionized water. Roots were then placed on a 500-um sieve and rinsed again. Dead roots and other organic material were then removed. Ectomycorrhizal infection was visually quantified. Roots were then dried in paper sacks at 65°C for 3-days and weighed.

Table A1. Dry weight of roots and ectomycorrhizal infection from 2002 background samples.

Site	Treatment	Sample Code	Roots (g dry weight)	ECM[1]
CF	Background	CF27B	0.82	++
CF	Background	CF29B	1.63	+
CF	Background	CF31B	0.50	++
CM	Background	CM27B	0.54	-
CM	Background	CM29B	3.27	-
CM	Background	CM31B	0.60	-
LF	Background	LF27B	1.06	++
LF	Background	LF29B	2.16	+++
LF	Background	LF31B	2.09	+
LM	Background	LM27B	2.36	-
LM	Background	LM29B	6.81	-
LM	Background	LM31B	2.55	-

[1]None = -, low = +, moderate = ++, and high = +++.

Table A2. Dry weight of roots and ectomycorrhizal infection from 2002 closed remaining samples.

Site of origin	Treatment	Sample Code	Roots (g dry weight)	ECM[1]
CF	Closed remain	CF20	0.76	++
CF	Closed remain	CF22	0.88	-
CF	Closed remain	CF24	0.81	+++
CM	Closed remain	CM20	1.05	-
CM	Closed remain	CM22	0.89	-
CM	Closed remain	CM24	1.30	-
LF	Closed remain	LF20	1.73	+
LF	Closed remain	LF22	3.89	++
LF	Closed remain	LF24	2.04	+
LM	Closed remain	LM20	2.04	-
LM	Closed remain	LM22	2.85	-
LM	Closed remain	LM24	1.77	-

[1]None = -, low = +, moderate = ++, and high = +++.

Table A3. Dry weight of roots and ectomycorrhizal infection from 2002 open remaining samples.

Site of origin	Treatment	Sample Code	Roots (g dry weight)	ECM[1]
CF	Open remain	CF21	1.81	++
CF	Open remain	CF23	1.32	-
CF	Open remain	CF25	0.31	+
CM	Open remain	CM21	0.16	-
CM	Open remain	CM23	2.76	-
CM	Open remain	CM25	0.72	-
LF	Open remain	LF21	4.63	++
LF	Open remain	LF23	2.54	-
LF	Open remain	LF25	2.83	+
LM	Open remain	LM21	3.25	-
LM	Open remain	LM23	2.09	-
LM	Open remain	LM25	6.82	-

[1]None = -, low = +, moderate = ++, and high = +++.

Table A4. Dry weight of roots and ectomycorrhizal infection from 2002 closed transfer samples.

Site of origin	Site of incubation	Treatment	Sample Code	Roots (g dry weight)	ECM
CF	CM	Closed transfer	CM26	1.16	-
CF	CM	Closed transfer	CM28	1.94	-
CF	CM	Closed transfer	CM30	1.21	-
CM	CF	Closed transfer	CF26	0.42	+
CM	CF	Closed transfer	CF28	0.40	-
CM	CF	Closed transfer	CF30	0.35	+
LF	LM	Closed transfer	LM26	1.18	+
LF	LM	Closed transfer	LM28	1.05	-
LF	LM	Closed transfer	LM30	1.61	-
LM	LF	Closed transfer	LF26	0.17	-
LM	LF	Closed transfer	LF28	0.78	-
LM	LF	Closed transfer	LF30	0.56	-

[1]None = -, low = +, moderate = ++, and high = +++.

Table A5. Dry weight of roots and ectomycorrhizal infection from 2002 open transfer samples.

Site of origin	Site of incubation	Treatment	Sample Code	Roots (g dry weight)	ECM
CF	CM	Open transfer	CM27	2.06	-
CF	CM	Open transfer	CM29	2.62	+
CF	CM	Open transfer	CM31	2.02	-
CM	CF	Open transfer	CF27	missing	++
CM	CF	Open transfer	CF29	0.5	++
CM	CF	Open transfer	CF31	0.34	++
LF	LM	Open transfer	LM27	0.48	+
LF	LM	Open transfer	LM29	4.43	-
LF	LM	Open transfer	LM31	2.76	-
LM	LF	Open transfer	LF27	1.06	-
LM	LF	Open transfer	LF29	1.56	-
LM	LF	Open transfer	LF31	2.76	+

[1]None = -, low = +, moderate = ++, and high = +++.

Appendix B. List of plant species at Carpenter and Lookout, H. J. Andrews Experimental Forest

Family	Species	Site[1]
Aceraceae	*Acer circinatum* Pursh	CM, LM
Apiaceae	*Ligusticum grayi* Coult. & Rose	CM, LM
Apiaceae	*Perideridia gairdneri* (H. & A.) Math.	CM, LM
Asteraceae	*Achillea millefolium* L.	CM, LM
Asteraceae	*Anaphalis margaritacea* (L.) B. & H.	CM, LM
Asteraceae	*Aster ledophyllus* Gray	LM
Asteraceae	*Cirsium callilepis* (Greene) Jeps.	LM
Asteraceae	*Eriophyllum lanatum* (Pursh) Forbes	CM, LM
Asteraceae	*Rudbeckia occidentalis* Nutt.	CM
Asteraceae	*Solidago canadensis* L.	LM
Berberidaceae	*Achlys triphylla* (Smith) DC.	CF, LF
Caryophyllaceae	*Moehringia macrophyllum* (Hook.) Fenzl.	CT
Caryophyllaceae	*Silene douglasii* Hook.	LM
Ericaceae	*Vaccinium membranaceum* Dougl.	CM
Ericaceae	*Vaccinium parvifolium* Sm.	CF
Fabaceae	*Lupinus latifolius* Agardh	CM, LM
Fabaceae	*Lupinus polyphyllus* Lindl.	LM
Fabaceae	*Vicia americana* Muhl.	CM, LM
Grossulariaceae	*Ribes sanguineum* Pursh	CM
Grossulariaceae	*Ribes lacustre* (Pers.) Poir.	CF
Hydrophyllaceae	*Hydrophyllum occidentale* (Wats.) Gray	CT
Hydrophyllaceae	*Phacelia heterophylla* Pursh	LM
Liliaceae	*Erythronium grandiflorum* Pursh	CM
Liliaceae	*Calochortus subalpinus* Piper	CM, LM
Liliaceae	*Lilium columbiana* Hanson	CM, LM

[1]CM = Carpenter meadow, CT = Carpenter transition zone, CF = Carpenter forest, LM = Lookout meadow, LT = Lookout transition zone, LF = Lookout Forest

Appendix B (continued).

Family	Species	Site[1]
Liliaceae	*Maianthemum dilatatum* (Wood) Nels. & Macbr.	CF
Liliaceae	*Xerophyllum tenax* (Pursh) Nutt.	CF, LF
Liliaceae	*Zigadenus venenosus* Wats.	LM
Onagraceae	*Chamerion angustifolium* (L.) Holub	CM, LM
Onagraceae	*Gayophytum diffisum* T. & G.	LM
Pinaceae	*Abies amabilis* (Dougl.) Forbes	CF, LF
Pinaceae	*Abies grandis* (Dougl.) Forbes	CF, LF
Pinaceae	*Pinus monticola* Dougl.	CF, LF, LT
Pinaceae	*Pseudotsuga menziesii* (Mirbel) Franco.	CF, LF
Pinaceae	*Tsuga mertensiana* (Bong.) Carr.	CF, LF
Poaceae	*Agrostis variabilis* Rydb.	CM, LM
Poaceae	*Bromus carinatus* H. & A.	CM, LM
Poaceae	*Elymus glaucus* Buckl.	CM, LM
Poaceae	*Melica subulata* (Griseb.) Scribn.	CM, LM
Poaceae	*Poa pratensis* L.	CM, LM
Polemoniaceae	*Gilia capitata* Sims	LM
Polemoniaceae	*Phlox diffusa* Benth.	LM
Polemoniaceae	*Polemonium carneum* Gray	CM, LM
Polygonaceae	*Polygonum bistortoides* Pursh	LM
Polygonaceae	*Polygonum douglasii* Greene	LM
Polygonaceae	*Polygonum newberryi* Small	CM
Polygonaceae	*Rumex acetosella* L.	CM, LM
Polypodiaceae	*Pteridium aquilinum* (L.) Kuhn.	CM, LM
Portulacaceae	*Claytonia lanceolata* Pursh	CM, CT
Ranunculaceae	*Anemone lyallii* Britt.	CF

[1]CM = Carpenter meadow, CT = Carpenter transition zone, CF = Carpenter forest, LM = Lookout meadow, LT = Lookout transition zone, LF = Lookout Forest

Appendix B (continued).

Family	Species	Site[1]
Ranunculaceae	*Aquilegia formosa* Fisch.	CM, LM
Ranunculaceae	*Delphinium menziesii* DC.	LM
Ranunculaceae	*Delphinium nuttallianum* Pritz.	CM
Rosaceae	*Amelanchier alnifolia* Nutt.	CM
Rosaceae	*Fragaria vesca* L.	CM, LM
Rosaceae	*Fragaria virginiana* Duchesne	LM
Rosaceae	*Holodiscus discolor* (Pursh.) Maxim.	CT
Rosaceae	*Potentilla glandulosa* Lindl.	LM
Rosaceae	*Potentilla gracilis* Dougl.	CM, LM
Rosaceae	*Prunus emarginata* (Dougl.) Walp.	CM
Rosaceae	*Rubus parviflorus* Nutt.	CM, LM
Santalaceae	*Comandra umbellate* (L.) Nutt.	LM
Scrophulariaceae	*Castilleja hispida* Benth.	CM, LM
Scrophulariaceae	*Collinsia parviflora* Lindl.	CT
Scrophulariaceae	*Mimulus guttatus* DC.	LM
Scrophulariaceae	*Orthocarpus imbricatus* Torr.	CM, LM
Scrophulariaceae	*Penstemon newberryi* Gray	LM
Violaceae	*Viola glabella* Nutt.	CT, CF

[1]CM = Carpenter meadow, CT = Carpenter transition zone, CF = Carpenter forest, LM = Lookout meadow, LT = Lookout transition zone, LF = Lookout Forest

Appendix C. List of dominant vascular plant species at the DIRT Plots, H. J. Andrews Experimental Forest.

Family	Species
Aceraceae	*Acer circinatum* Pursh
Aceraceae	*Acer macrophyllum* Pursh
Aristolochiacae	*Asarum caudatum* Lindl.
Asteraceae	*Adenocaulon bicolor* Hook.
Berberidaceae	*Achlys triphylla* (Smith) DC.
Berberidaceae	*Berberis nervosa* Pursh.
Berberidaceae	*Vancouveria hexandra* (Hook.) Morr. & Dec.
Caprifoliaceae	*Linnaea borealis* L.
Cornaceae	*Cornus canadensis* L.
Cornaceae	*Cornus nuttallii* Aud.
Cupressaceae	*Thuja plicata* Donn.
Ericaceae	*Gaultheria shallon* Pursh
Ericaceae	*Rhododendron macrophyllum* G. Don
Ericaceae	*Vaccinium parvifolium* Sm.
Fumariaceae	*Dicentra formosa* (Andr.) Walp.
Liliaceae	*Clintonia uniflora* (Schult.) Kunth.
Liliaceae	*Trillium ovatum* Pursh
Orchidaceae	*Goodyera oblongifolia* Raf.
Oxalidaceae	*Oxalis oregana* Nutt.
Pinaceae	*Pseudotsuga menziesii* (Mirbel) Franco.
Pinaceae	*Tsuga heterophylla* (Raf.) Sarg.
Polypodiaceae	*Polystichum munitum* (Kaulf.)
Primulaceae	*Trientalis latifolia* Hook.
Ranunculaceae	*Anemone deltoidea* Hook.
Taxaceae	*Taxus brevifolia* Nutt.

Appendix D. List of dominant mosses and liverworts at the DIRT Plots, H. J. Andrews Experimental Forest.

Species	Growth form	Substrate	Abundance[1]
Antitrichia curtipendula	pleurocarp	litterfall from canopy	3
Atricum selwynii	acrocarp	on moist soil and litter	2
Dicranum fuscescens	acrocarp	decaying wood	3
Dicranum scoparium	acrocarp	decaying wood	2
Eurynchium oregana	large pleurocarp	soil, decaying wood	4
Hylacomium splendens	pleurocarp	soil	3
Isothecium myosuroides	thin pleurocarp	branches, decaying wood, conifer bark	4
Leucolepis menziesii	acrocarp	soil	3
Porella navicularis	leafy liverwort	decaying conifer logs	3
Rhizomnium glabrescens	acrocarp	soil	2
Rhytidiadelphus triquetrus	pleurocarp	soil	3
Scapania bolanderi	leafy liverwort	bark of decaying log	3

[1]Abundance codes: 1 = none to very low, 2 = low, 3 = moderate, 4 = high