AN ABSTRACT OF THE THESIS OF

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Title: Effects of Spatially Dispersed Green-Tree Retention on Ectomycorrhiza Diversity

Abstract approved:

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The Demonstration for Ecosystem Management Options (DEMO) study originated out of the changing management priorities associated with federal forest lands in the Pacific Northwest which included an objective to maintain mature and old-growth forest characteristics in managed stands.

The DEMO project examines the effects that different levels and patterns of green tree retention have on various aspects of biodiversity including the diversity of ectomycorrhizal fungi (EMF). Ectomycorrhizal fungi play an important functional role in forest ecosystems. Ectomycorrhizal fungi are essential for nutrient uptake by the host tree, they have been shown to play a critical role in forest regeneration success, and EMF provide an important food source (in the form of sporocarps) for many small mammals.

As part of the DEMO study, this thesis documents EMF diversity in the control unit (100% retention) and the spatially dispersed 40% basal area retention treatment (40%D). The measures of diversity studied included EMF constancy,

abundance, and species richness. Constancy and abundance measures were analyzed only for the common ectomycorrhizae (EM) types.

Morphological techniques for identification of the EMF were employed together with molecular techniques to achieve the finest level of identification possible. Characters used to assess morphology include mantle structures, emanating hyphae, rhizomorphs, root morphology, chemical reactions, and mantle color. Molecular techniques were used for identification purposes and for checking EM morphotype groups. Molecular techniques used included DNA extraction of the internal transcribed spacer region, polymerase chain reaction amplification, restriction fragment length polymorphism, and DNA sequencing.

Significant responses to the 40%D treatment were determined by testing both pre and post EMF diversity within the treatment and by comparing changes in diversity between the control and the 40%D treatment. Overall, within the 40%D treatment, the majority of the common EMF species did not decrease significantly.

A moderately significant difference was detected between the pre-treatment and post-treatment mean number of EM types per soil core (measure of species richness). This decrease in richness of about 50% in the post-treatment$_2$ samples seems to indicate a delayed response to the treatment since the post-treatment$_1$ samples were not different from the pre-treatment conditions. Since no dominant types were lost, the decline in richness occurred in the less common EM types.

This research demonstrates that the 40%D treatment can maintain a relatively high legacy of EMF. However, there is a loss of some rare EM types and

a reduction in abundance and constancy in some of the more common EM types. These results lend support for the consideration of 40% dispersed green-tree retention as a silvicultural option when management goals include the maintenance of EMF diversity. Timber harvest via implementation of ecosystem-based forest management practices will require the continuing study of complex below-ground systems for the development of potential silvicultural treatments during further iterations of adaptive management.

Effects of Spatially Dispersed Green-Tree Retention on Ectomycorrhiza Diversity

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CHAPTER 1—INTRODUCTION

The forest products industry has been a cornerstone of the Pacific Northwest (PNW) economy and social structure. Historically, clearcutting practices have been the dominant form of timber removal in the PNW. However, new understanding of the ecological importance of intact, old forests have changed societal attitudes towards clearcutting as a sustainable harvesting method. Due to increased understanding of forest ecosystems and a desire to preserve mature and old-growth characteristics in managed forests, alternatives to clearcut logging practices have gained popularity (Jensen and Bourgeron, 1994). The Demonstration for Ecosystem Management Options (DEMO) study originated out of the changing management priorities associated with federal forest lands in the Pacific Northwest (Aubrey et al., 1999), including an objective to maintain mature and old-growth forest characteristics in managed stands. Issues such as the listing of the northern spotted owl as threatened have intensified the need to alternatively manage federal forest lands for ecosystem integrity as well as timber. The Northwest Forest Plan was created to help deal with these issues. This plan specifies spatial patterns and minimum levels of green-tree retention on federal forest lands when timber is harvested (Franklin et al., 1999).

Although the Northwest Forest Plan was based on the best scientific knowledge available, many alternative harvest designs have not been scientifically

tested (Franklin et al., 1999). One alternative harvest design that is currently being studied by DEMO scientists is green-tree retention. Green-tree retention harvests retain some live trees in harvested units (Aubrey et al., 1999). The DEMO study is one of the first large-scale, long-term studies that rigorously test the effects of different levels of green-tree retention and spatial patterns on various ecological characteristics of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) dominated forests in the PNW.

The DEMO study was designed to provide scientifically sound information to aid the advancement of harvesting strategies that maintain or increase various ecological features found in mature and old growth forests (Aubrey et al., 1999). In addition to harvesting effects on wildlife, insect populations, hydrology, and a number of other ecosystem components, ectomycorrhizal fungi (EMF) are a subject of study in the DEMO experiment. In order to maintain biological diversity, it is important to understand the effects of different timber harvest regimes on both long-term and short-term EMF diversity. Since forest trees rely on fungal symbioses for survival (Trappe and Luoma, 1992) and EMF may be particularly vulnerable to disturbance events (Perry et al., 1989; Stockdale, 2000) occurrence of EMF species is of particular interest as an indicator of forest biological diversity.

Ectomycorrhizal fungi play an important role in the regeneration, survival, and resilience of temperate forest ecosystems (Amaranthus and Luoma, 1997). It has been argued that ecological stability is linked to the diversity of the ecosystem (Pimm, 1991). If ecological stability is linked to diversity, Perry et al. (1989) argue

that recovery of an ecosystem is dependent on the pre-disturbance stability of that ecosystem. In addition, Perry et al. (1989) contend that above- and below-ground diversity act to stabilize an ecosystem through periods of stress and other environmental disturbances. This theoretical relationship between ecological stability and biodiversity has not been adequately tested, but some evidence (Tilman and Dowing, 1994) and arguments (Perry et al., 1989; Pimm, 1991) point to the existence and importance in the maintenance of ecosystems.

When a forest is logged, ectomycorrhiza diversity and abundance decline due to the loss of host trees (Harvey et al., 1980; Stockdale, 2000). Ectomycorrhizal fungi are highly diverse, and studies indicate EMF may partition their resources (Sudhakara and Natarajan, 1997). Under this assumption, trees with high EMF diversity may have high ecological stability in the face of disturbance. Thus, it is important to document changes in EMF diversity and abundance due to timber harvest prescriptions.

Current societal demands on timber resources are increasing at the same time timber harvest on federal lands is decreasing due to a shift in management emphasis from resource extraction to ecosystem management (Jensen and Bourgeron, 1994). By preserving a high level of biodiversity at a landscape scale, it is possible that ecosystem stability and many elements of biodiversity (such as wildlife, plants, and fungi) can be maintained locally (Perry et al., 1989).

The DEMO study will bring science a step closer to documenting the interaction between EMF diversity and timber harvesting treatments. The

knowledge gained from this study has the potential to help forest managers balance the timber needs of society with the goal of maintaining a high level of biodiversity across the landscape. The DEMO study is located in southern Washington and western Oregon and is based on a randomized block experimental design with six treatments (13 hectares each) (Aubrey et al., 1999). However, for the purposes of this thesis, the following two treatments were studied:

1) Control (100% retention)

2) 40% basal area retention in a dispersed pattern (40%D).

This thesis will address the following research questions:

- What is the post-treatment EMF diversity of the 40%D retention?
- How does post-treatment EMF diversity differ from pre-treatment diversity in the 40%D retention?
- How does EMF diversity in the 40%D treatment compare to EMF diversity in the untreated control?

Three objectives were developed to answer those questions:

(1) document the ectomycorrhiza diversity in the 40%D treatment

(2) compare pre-treatment data to post-treatment data on the 40%D treatment

(3) compare the ectomycorrhiza diversity in the 40%D treatment to the untreated control

CHAPTER 2—LITERATURE REVIEW

ECTOMYCORRHIZAE

"Mycorrhizae" refers to symbioses between fungal hyphae and the roots of many vascular plants including gymnosperms and angiosperms. "Ectomycorrhizae" refers to a category of mycorrhizal symbiosis in which the fungus grows on the outside of feeder roots and between the cells of the vascular plant roots (Alexopolous et al., 1996). Ectomycorrhizae (EM) are particularly abundant in forested ecosystems and are often found on the roots of gymnosperms, especially *Pinaceae*. The mycorrhizal symbiosis gives trees greater efficiency in water and nutrient uptake, greater resistance to fungal pathogen infections, and increased drought resistance. In exchange, carbohydrates generated by the tree nourish the fungi (Smith and Read, 1997).

Ectomycorrhizal symbioses are formed by about 8000 plant species (Dahlberg, 2001), the best estimate of the number of ectomycorrhizal fungus (EMF) species being 6000 (Molina et al., 1992). Ectomycorrhizal plants include some of the most economically important and ecologically dominant trees world-wide, including the obligate EM families Pinaceae, Betulaceae, and Fagaceae (Smith and Read, 1997). The EMF symbiosis is particularly diverse and important with Douglas-fir (*Pseudostuga menziesii*) forests with an estimated 2000 different EMF species associated with Douglas-fir throughout its range (Trappe, 1977).

ECTOMYCORRHIZA DIVERSITY AND SILVICULTURAL TREATMENTS

Ectomycorrhiza diversity can provide many important ecosystem services. Perry et al. (1989) contend EM diversity can be important in stabilizing below ground mutualists after a catastrophic disturbance. In addition, Simard et al. (1997) showed that seedlings associated with a higher diversity of EMF were better able to adapt to environmental changes compared to their counterparts with reduced EMF diversity.

At the individual tree level, high EMF diversity and individual differences in EMF nutrient acquisition were shown to increase the capability for nutrient uptake and growth for individual trees (Baxter and Dighton, 2001). Sudhakara and Natarajan (1997) found that *Pinus patula* seedlings inoculated with two different species of EMF grew taller and put on more biomass then seedlings that were inoculated with only one species of EMF. Similarly, Baxter and Dighton (2001) found that *Betula populifolia* seedlings had a higher root to shoot ratio as the number of EMF types present on host roots increased from one to four species of EMF. In addition, they showed that EMF diversity explained a significant amount of variation in total phosphorous uptake and concentrations of nitrogen and phosphorus in plant shoots.

Ectomycorrhizae and clearcuts

There are two ways EMF naturally re-colonize clearcuts: (1) through mycelium that is connected to a host, and (2) through sexual or asexual propagules

that are not connected to a host, such as spores and sclerotia (Dahlberg and Stenström, 1991). After a clear-cut, the mycelium from the surrounding forest can provide an inoculation front, however live roots with mycorrhizae are limited in the distance they penetrate into a clear-cut. Distances into clearcuts that EMF have been found range from 4.6 meters (Harvey et al., 1980) to 5 to 6 meters (Parsons et al., 1994). Luoma, (unpublished data) found sporocarps of *Rhizopogon villosulus* 9 meters from a mature forest edge into a burned clearcut. Taylor and Bruns (1999) argue that EMF found in disturbed soils tend to enter the system via resistant propagules while mycelial expansion tends to dominate in the undisturbed ecosystems. Recolonization by resistant propagules include spores blown in by wind, spores brought in by animals, and spores in the root zone that survived the tree cutting process.

Active EM roots have been shown to persist for some time following a clear-cut. Ferrier and Alexander (1985) reported EM persistence from 9 months following a clear-cut, and Persson (1982) reported active EM root tips for up to 18 months following a clear-cut.

Hagerman et al. (1999) studied EMF diversity along a gradient from mature subalpine fir (*Abies lasiocarpa*)/ Engelmann spruce (*Picea engelmannii*) forest into clearcuts. This gradient ranged from 40 meters inside the forest to 165 meters into clearcuts. During the growing season immediately following harvest, they found no statistical difference in EMF diversity (as measured by the Shannon index) between the uncut forest and distances up to 165 meters into clear-cuts. However, during the

second growing season, a significant reduction in EMF diversity was found at distances of 16 meters or greater into clear-cuts. By the third growing season of the study, active EMF root tips were nearly absent at distances 2 meters or greater into the clearcuts. Hagerman et al. (1999) suggested that EMF readily survive on root tips for several months but very few survive to the third growing season in the absence of living trees. The presence of apparently active EM in clearcuts may be accounted for by root grafting or the limited saprotrophic capabilities of some species of EMF.

Mah et al. (2001) found EMF diversity was lower on naturally regenerated spruce seedlings located in clearcut areas as compared to those growing in mature spruce forests. Within the clearcut sites, the authors discovered that EM diversity was higher on seedlings that were planted on the site compared to seedlings that were naturally regenerated. In addition, a shift in abundance and frequency of EM resulted from the clearcut treatments when compared to the uncut controls.

Ectomycorrhizae and selective cuts

In the DEMO study, EMF diversity was assessed in the 15%D treatment. One to two years after the 15%D treatment was implemented, Stockdale (2000) found a 50% decrease in EM morphotype richness in areas furthest from remaining live trees. Conversely, there was no significant decrease in mean EM type richness within the dripline of the residual live trees in the treatment when compared to pre-treatment data. This finding suggests that retention trees have important ecological

functions as legacy structures and have the potential to serve as an EMF inoculum source for the regeneration of seedlings. This finding also suggests that maintenance of retention trees may improve success on hard-to-regenerate areas when EMF are limiting.

Kranabetter and Wylie (1998) studied EMF community structure across forest openings (50-75 meters in diameter) in mature and old-growth western hemlock (*Tsuga heterophylla*) dominated forests located in British Columbia. Average EMF richness decreased 27% between the forested interior and the forest edge, and was 40% lower in the openings (15 and 20 meters from the edge). The authors suggested that the "late-stage" EMF from the surrounding mature forests are not adapted to the gaps and will not colonize these gaps until mature forest redevelop.

Debellis et al. (2002) discovered that in a naturally regenerated, mixed yellow birch (*Betula alleghaniesis*) and sugar maple (*Acer saccharum*) forest, the birch associated EMF diversity (as measured by Simpson's and Shannon's diversity indices) showed no decrease in response to a selective cutting treatment. The selective cutting treatment consisted of a series of gaps. The gaps were sampled 3-12 years after treatment. The gap sizes range from 0-50 meters (small gaps) and from 50-250 meters (large gaps). The authors suggested that a combination of rapid regeneration of EM hosts, minimal soil disturbance, and relatively small gap sizes was responsible for the lack of detectable change found in the EMF community structure as measured by the selected diversity indices.

ECTOMYCORRHIZAE AND COMMUNITY STRUCTURE

In uncut forests, EMF communities are diverse on a local scale irrespective of the aboveground plant diversity (Goodman and Trofymow, 1998; Hagerman et al., 1999). The distribution of EM types is patchy, with a clumped spatial distribution rather than a random distribution (Horton and Bruns, 2001; Taylor, 2002). Community structure shows dominance by a few EMF species, while a large number are relatively rare (Gardes and Bruns, 1996b; Gehring et al., 1998).

Mature forests (approximately 90 years old) tend to be similar in aspects of EMF community structure (although not necessarily species composition) to old growth forests, greater than 90 years old. Goodman and Trofymow (1998) found that the communities in these two age classes did not differ with respect to species richness, equability, and diversity indices of EMF types.

Community structure also varies vertically in the soil profile. Byrd et al. (2000) found that a few common species dominated the upper soil profile layer from 0-5cm while the soil layers from 5 to 15cm contained many rare EMF species. In another study testing vertical distributions of EMF, Taylor and Bruns (1999) found 40% of the total EMF biomass occurred in the mostly organic top soil layer, 38% in the middle mineral soil layer, and only 22% in the lower layer.

A large part of the EMF community is likely generalist with regard to host plant associates. Horton and Bruns (1998) working in a mixed conifer forest found that most species of EMF were common to root systems of both Douglas-fir and

Bishop pine (*Pinus muricata*) and that only 10% of the total number of species were host specific. Horton et al. (in press) found that fungi specific to Douglas-fir colonized 14% of its EM biomass in a mixed Douglas-fir/Hemlock forest..

MORPHOTYPING

Morphotyping is commonly used to differentiate among EM root types. The characters used to assess morphology include mantle structures, emanating hyphae, rhizomorphs, root morphology, chemical reactions, and mantle color (Agerer, 1991; Ingleby et al., 1990; Goodman et al., 1996). Morphotyping EM root tips is advantageous because some characteristics such as color and shape can be used to efficiently sort EM root tips into groups (Horton and Bruns, 2001).

However, morphotyping has disadvantages. For instance, similar EM root tip types cannot be distinguished using characteristics such as color and shape alone. In addition, Gardes and Bruns (1996b) argue that morphological methods can leave up to 50% of EMF types unclassified. Although Gardes and Bruns (1996b) show that a high percentage of EMF can remain unmorphotyped, Jonsson et al. (2000) show that the alternative molecular identification techniques can leave up to 54% of the EM unidentified. In addition, it has been suggested that the skill of the observer has a dramatic influence on the outcome of morphotyping analysis (Gardes and Bruns, 1993). To obtain high percentages of species level identification both morphotyping and molecular techniques are required (Horton and Bruns, 2001).

MOLCULAR TECHNIQUES

Recent advances in molecular techniques have increased the ability to study belowground ecosystems more accurately. Restriction fragment length polymorphism (RFLP) techniques have been applied to sample EMF community structure for about 10 years (Gardes and Bruns, 1996a; Horton and Bruns, 2001) but within this short time span molecular techniques have become widely used in EMF community research. Molecular methods have been primarily used for identification purposes (matching unknown EM to identified sporocarp RFLP patterns) and for checking EMF morphotype groups both within and across geographical regions (Kårén et al., 1997; Horton and Bruns, 2001).

Most EMF community studies use restriction analysis within the internal transcribed spacer (ITS) region (Figure 1) located within the larger ribosomal RNA (Horton and Bruns, 2001). The ITS region is between 650-900 base pairs in length (in fungi) and it is located between the small subunit (SSU) of rRNA and the large subunit (LSU) of rRNA. Between the LSU and SSU lies a highly conserved 5.8s rRNA region which separates two non-coding spacer regions referred to as ITS-1 and ITS-2 (Horton and Bruns, 2001). The ITS-1 and ITS-2 regions are highly variable and allow for species-level differences to be detected using RFLP comparisons (Kårén et al., 1997). Unique RFLP patterns are made possible by indels, insertions, or deletions in nucleotides which result in sequence differences (Horton and Bruns, 2001).

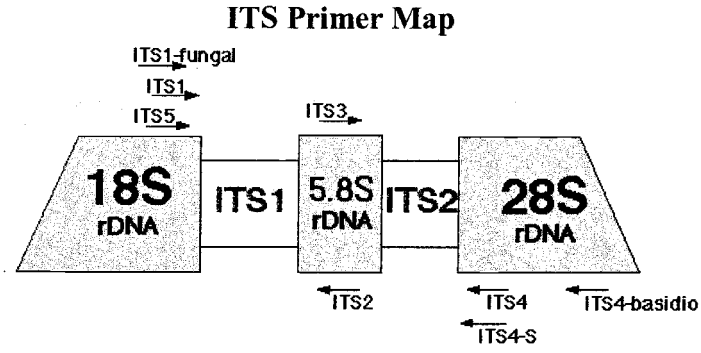

Figure 1: Internal Transcribed Spacer Region primer map-section of DNA routinely amplified in EMF diversity studies

Primers flanking the ITS region have been created allowing for polymerase chain reaction (PCR) amplification of the fungal DNA (Gardes and Bruns, 1996a). RFLP comparisons utilize the fungal DNA that has undergone PCR by digesting the DNA with restriction endonuclease (enzymes) and running that product out on a electrophoresis gel. The electrophoresis separates the DNA from the ITS region into fragments based on the molecular weight of each fragment. Within the ITS region, the location where the fragment is cut is dependent on the restriction enzyme that is being used. Each restriction enzyme cuts the DNA at different locations. Only two or three enzyme digests are needed to distinguish between most EMF species. This approach allows for the matching of EMF root tips from different locations (Horton and Bruns, 2001). RFLP matching has gained wide

popularity because it can quickly separate EMF into species, is relatively inexpensive, and is not difficult to learn (Horton and Bruns, 2001).

Relying on RFLP matching alone can be misleading for large-scale diversity studies. Some species cannot be differentiated or identified using ITS-RFLP tools. In a study looking at ITS variation in EMF collected throughout the boreal forests of Fennoscandia, Kårén et al. (1997) found that 16% of the EMF sampled exhibited intraspecific polymorphism. In addition, they also found that many EMF belonging to the genus *Cortinarius* were indistinguishable at the species level. Horton (2002) found intraspecific RFLP polymorphism in 14% of the 44 EMF species sampled. The six polymorphic species were distributed among the genera *Laccaria, Inocybe, Tricholoma, Rhizopogon,* and *Lactarius*.

Local diversity studies should have good correspondence between unique RFLP patterns and morphotype differences. In almost all local-scale cases, there will not be any RFLP matches between two different species (Horton and Bruns, 2001; Horton, 2002). However, as the scale and geographical area increases, the probability of intraspecific variation (due to population isolation and varying evolutionary rates) can result in multiple RFLP types for the same species. For example, *Cenococcum geophilum* is a very common EM type found in most studies at many geographical locations. Multiple studies have found a high level of genetic variability in this and other EMF species (Farmer and Sylvia, 1998; Horton, 2001; Jany et al., 2002; Douhan and Rizzo, 2005). Genetic inconsistencies such as this

can result in overestimating the true diversity of EMF when relying solely on RFLP analysis.

ITS-RFLP analysis works well for stand-level studies. However, as the number of samples and enzymes increase, the complexity of finding RFLP matches increases. In addition, minute differences of RFLP patterns due to variations in electrophoresis gel conditions can create problems when working with large sample sizes. As a result, scientists interested in EMF diversity over broader areas often do not rely on RFLP matching exclusively. Rather, a combination of morphotyping, RFLP matching, and sequencing should be employed in order to achieve the highest level of accuracy (Horton and Bruns, 2001).

Many studies follow RFLP analysis with direct gene sequences of the most common unknown EM types (Horton and Bruns, 2001; Horton, 2002). Sequencing allows for the potential identification of unknown EM types or it allows placement into a species group if the corresponding sequence can be matched using sequence databases such as Gen Bank (Horton and Bruns, 2001). Unfortunately, there are limited numbers of fungi in even the largest sequence databases, so EM types are often left unknown. As a general rule, sequence dissimilarities greater than 2% are considered different species.

SUMMARY

Ectomycorrhizal fungus diversity is an important biological attribute of Pacific Northwest conifer forests. Limited research has documented the response of

EMF diversity to different silvicultural treatments and in natural stands. There are no published reports on the response of EM diversity in situations similar to the relatively high green-tree retention level of the 40%D treatment in the DEMO study.

Reduction in EM tree host basal area may result in an overall decline in EMF diversity in thinned stands. As thinning treatments become more intense, the EMF diversity may decrease in proportion to the level of harvesting.

When a tree is cut, the EMF that depends on that tree eventually die due to the loss of their carbohydrate source. After a thinning treatment, an initial decrease in total EMF biomass is expected. As the retained trees grow and send roots into the unoccupied space, the EMF community should begin to recover. The EMF that remains on the live tree roots may come to occupy the niches that were created by the thinning. However, it is unknown how the different thinning intensities will affect this hypothesized recovery trajectory.

The cryptic nature of EM makes their study difficult. Discovery of how to utilize the PCR has allowed techniques like ITS-RFLP matching to be applied in fungal ecology. This technique is valuable in the process of identifying EMF. My study uses the traditional approach of morphotyping EM in combination with PCR/RFLP to obtain a high level of species resolution in assessing the response of a 40% basal area green-tree retention experiment.

CHAPTER 3—METHODS

STUDY SITES

Located in the Gifford Pinchot National forest in southern Washington, the Hamilton Butte block ranges in elevation from 975 to 1280 meters, has slopes of 40-53 degrees with a general east-southeast aspect, and the mature trees were 70-80 years old. The Butte block had no previous history of timber harvesting. The 40%D treatment unit contained a class 3, non-fish-bearing perennial stream.

The Dog Prairie block is located in the Umpqua National Forest in the south-central Cascades of Oregon. Elevation ranged 1460-1710 meters, slope 34-52 degrees with a aspect southwest, and the pre-harvest forest age was about 165 years. Dog Prairie was previously thinned in 1986.

The Watson Falls block was also located in the Umpqua National Forest elevation ranged 945-1310 meters, slope 4-7 degrees with a flat aspect, and forest age 110-130 years. Watson Falls was salvage logged between 1970-1978 (Aubrey et al., 1999).

STUDY DESIGN

The DEMO study consists of 6 blocks, each with 6 treatments. Three of these blocks (Butte, Watson Falls, and Dog Prairie) were used for the ectomycorrhiza diversity study. Within these blocks, the 40%D treatment was investigated as the subject of this thesis.

The DEMO study was implemented with a randomized block design. One treatment unit was represented in each block. Each treatment unit was 13 hectares of relatively homogenous forest with respect to species composition and forest structure (Aubrey et al., 1999). The treatments (Figure 2) were:

1) 100% basal area retention (100%)

2) 75% basal area retention of overstory trees in an aggregated pattern (75%A)

3) 40% basal area retention of overstory trees in a dispersed pattern (40%D)

4) 40% basal area retention of overstory trees in an aggregated pattern (40%A)

5) 15% basal area retention of overstory trees in a dispersed pattern (15%D)

6) 15% basal area retention of overstory trees in an aggregated pattern (15%A)

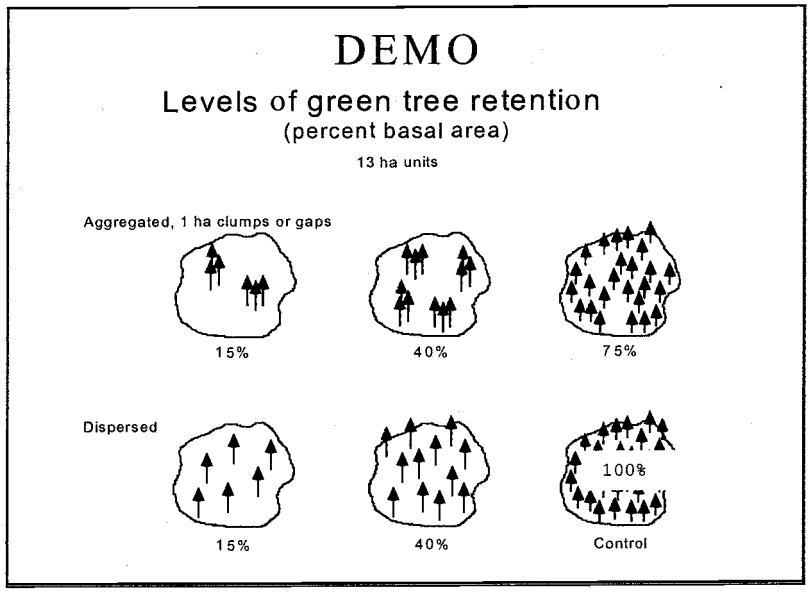

Figure 2. Schematic of the DEMO green-tree retention treatments depicting the levels and patterns of basal area retention.

Within-block variation was controlled to the extent possible by applying each treatment similarly with respect to harvest method, snag and log retention, and slash treatment (Aubrey et al., 1999) However, harvesting methods did differ among blocks due to logistical and topographical constraints. Butte and Dog Prairie were helicopter logged where as the Watson Falls block was logged with ground based equipment. The experimental treatments (tree harvest) for Butte were applied during the spring and summer of 1997. Dog Prairie and Watson Falls were harvested in the summer of 1998. The treatments were randomly assigned within the blocks so that causal inferences could be drawn. Only the 40%D and 100% retention treatments were compared in this study. Pre- and post-harvest structural

descriptions of the treatments are presented in table 1 (Unpublished data on file with the Forest Science Data Bank, Oregon State University).

Table 1: Stand structural descriptions of the 100%, 40%D treatments

Block	Trees/ha	Basal area (m2/ha)	Relative stand density	Stand density index
Butte				
100%	1119	58.2	11.48	1143
40%D, pre-[1]	1019	48.7	9.8	972
40%D, post-	520	28.6	5.6	555
Dog Prairie				
100%	295	89.6	11.36	1241
40%D, pre-	372	72.4	10.26	1095
40%D, post-	147	37.7	5.0	540
Watson Falls				
100%	310	43.7	6.71	704
40%D, pre-	389	47.7	7.59	790
40%D, post	152	26.9	3.9	415

[1] pre-= pre-treatment, post-= post-treatment

SAMPLING

EM were sampled using a 5.5 x 15 cm cylindrical soil corer. Soil cores for this project were collected from 1994 to 2002. Refer to Table 2 for time, location, and number of soil cores collected throughout the study.

Table 2: Time, location, and number of soil cores collected for the 40%D and control treatments from 1994-2002

		Pre-treatment										Post-treatment									
		1994	1995		1996		1997		1998		1999		2000		2001		2002				
Block	Treatment	F[1]	S[2]	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S
Watson	40%D	6	6									6	6			6	6				
Falls	100%	6	6									6	6								
Butte	40%D				6	6				6	6					6	6				
	100%				6	6				6	6										
Dog	40%D						6	6				6	6			6	6				
Prairie	100%						6	6				6	6								

[1] fall (f)
[2] spring (s)

Soil core samples through spring 2000 were obtained from each end of the permanent mushroom plots (Luoma et al., 2004) during one fall and one spring sample period for a total of 12, each, pre-treatment and post-treatment cores from each unit (Table 2). The permanent mushroom plots were 3 strip plots (2 x 50m) that were systematically placed to maintain a dispersed sampling pattern throughout each treatment (figure 3).

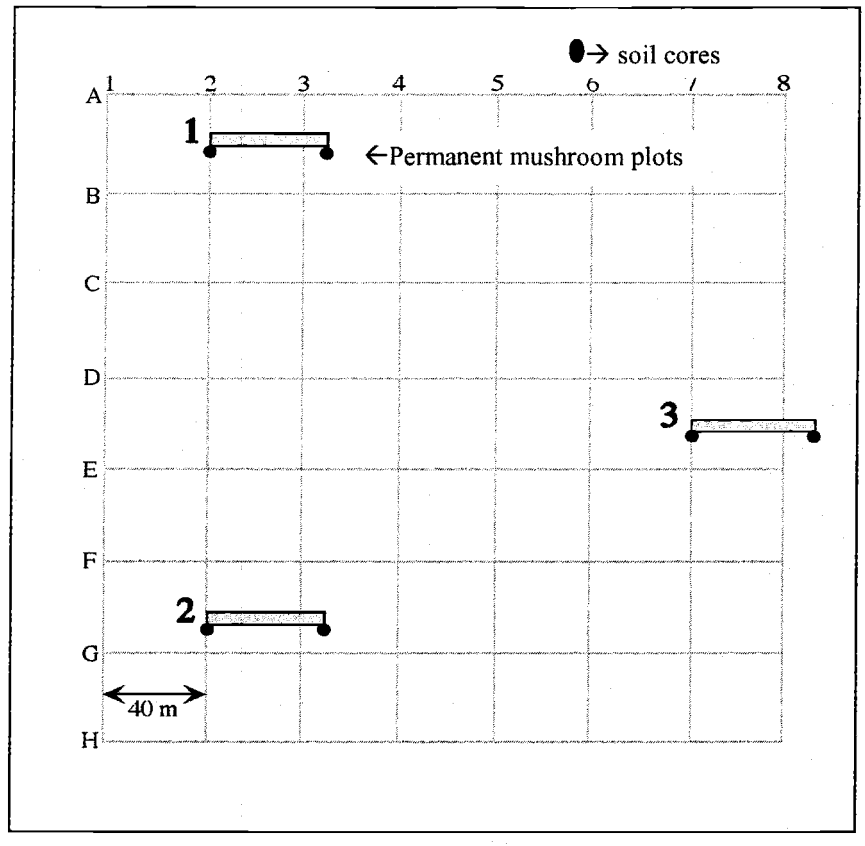

Figure 3. Soil core locations for samples obtained through spring 2000

In fall 2001 and spring 2002, six soil cores were collected each season, per 40%D treatment (Watson falls, Dog Prairie, Butte). Three soil cores each season were collected using the permanent mushroom plots. During the fall 2001 sampling, three soil cores were collected at the middle of the baseline of each permanent mushroom plot (Figure 4). The other three soil core sampling locations were systematically dispersed throughout each 40%D treatment unit. The hole left by each core was marked with a flagged pin to facilitate re-finding the sample point

the following spring. For the spring sample, soil cores were collected adjacent to the fall soil core collection sites. A total of 36 soil cores were collected for the 2001/2002 sample. The dispersal of half of the soil cores away from the permanent mushroom plots was done to spatially broaden the sample of the 40%D treatment.

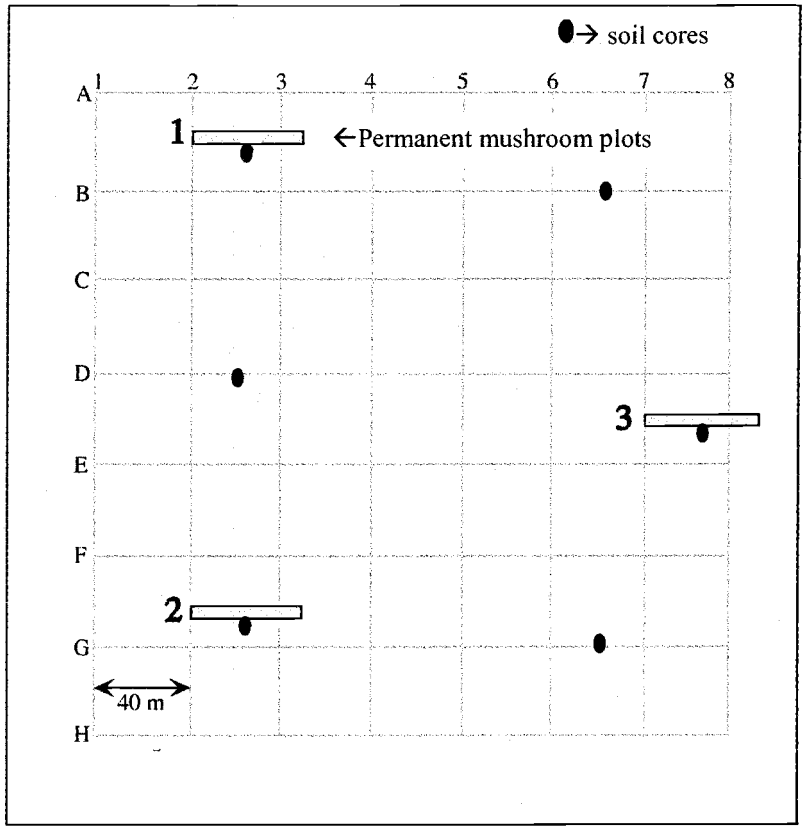

Figure 4. Sampling locations for soil cores collected in fall and spring 2001

QUANTIFYING EM DIVERSITY

The soil cores were stored at 5°C until they were processed. Processing occurred within 3-6 months. Processing time for an individual soil core ranged from a few hours up to 3 days depending on the complexity of the morphotypes. Soil cores were washed either by hand or with an elutriator. The remaining material was then screened with a 1mm sieve to further remove soil particles (sand, rocks, woody debris, etc.). Any clumped root tips were not intentionally broken up.

Cleaned soil cores were then spread evenly in a 25.4 cm^2 water filled tray, with a plastic grid insert that divided the space into 36 equally sized squares. Morphotyping and molecular methods were used to identify EMF and provide a basis for quantifying diversity estimates. Each square was assessed for presence or absence of EM using a stereomicroscope. The number of squares of occurrence for each EM morphotype was recorded and divided by the total number of squares (36). This value provided an estimate of within-core frequency for each fungal morphotype within each soil core (Eberhart et al., 1996).

MORPHOLOGICAL IDENTIFICATION OF EM

Ectomycorrhiza morphotyping was based on many characteristics including the anatomy of mantle scrapings, emanating hyphae, and rhizomorphs, root morphology, chemical reactions, mantle color, presence of clamp connections, and other characteristics detailed in Ingelby et al. (1990) and Goodman et al. (1996).

Morphotype characteristics were then compared to existing EM descriptions from Oregon and Washington DEMO sites in a relational database (Filemaker Pro. 5.5 v1) created and maintained by Joyce Eberhart. The comparisons resulted in either a morphological match or creation of a new description. Each EM type was assigned a morphotype identity. EM descriptions in the database were restricted to the EM found in the DEMO study locations.

MOLECULAR IDENTIFICATION OF EM

DNA extraction

From each soil core, two EM root tips of each morphotype group were each placed in separate 1.5ml Eppendorf tubes with 300µl CTAB and stored at 5°C until the DNA could be extracted. Before the 2001 sampling period, DNA was extracted using the CTAB extraction method without the β-mercaptoethanol (Gardes and Bruns, 1993). Samples that did not amplify using this method were re-extracted using the GENE Clean III kit from Q-Biogene as according to the manufacturer. During the 2001-2002 sampling period, DNA was routinely extracted using the GENE Clean III kit. Extracted DNA samples were stored in 50µl TE Buffer (1mM Tris-HCL, 0.1 M EDTA) at -20°F until they were used in a PCR reaction.

Polymerase Chain Reaction

The DNA was amplified using the polymerase chain reaction (PCR) (Mullis and Faloona, 1987). The internal transcribed spacer (ITS) region located between the nuclear small and nuclear large rDNA was amplified using primers ITS-1f and ITS-4 (White et al., 1990; Gardes et al., 1991). Initially DNA was diluted at a ratio of 1:100 with a "PCR Cocktail" (50µl ITS-1F primer, 50µl ITS-4 primer, *Taq*, 10x dNTP's, 10x PCR buffer, and sterile water). When amplification was not successful at that ratio, the dilution was decreased to 1:10 or bovine serum albumin (BSA) was added to the "PCR Cocktail" at a concentration of 1-2µl per 25µl reaction or a 1:10 dilution was used with BSA. PCR was carried out at a denaturing temperature of 94°C and an annealing temperature of 55°C over the course of 35 cycles. To check for successful amplification, each sample was run in an electrophoresis gel, using a 2.5% agarose gel for 30 minutes at 110 volts. PCR gels were stained with ethidium bromide and visualized using Gel Analyzer.

Restriction Fragment Length Polymorphism

Successful PCR amplification of the ITS was followed by applying RFLP to the product using the methods described in Horton and Bruns (2001). The PCR product was digested with the enzymes *Hinf*1 and *Dpn*II (Promega). Digested samples were run in a 1% agarose/ 2% NuSeive (GT Technologies) electrophoresis gel for 2 hours at 100 volts. ITS-RFLP gels were stained with ethidium bromide and visualized using Gel Analyzer.

ITS-RFLP band molecular weight was assessed using Scanalytics 4.0. Gels that were unscorable due to smears, faint and/or unrecognizable bands, potential contamination, etc. were thrown out and the samples were rerun. To maintain consistency, gels were scored using the following criteria:

1. Moving from the heaviest molecular weighted band to the lightest, the first scored band was the brightest band
2. The bands became progressively fainter moving from heavier molecular weight to lighter molecular weight. If a band was found to be brighter then the one above it, that band was considered contamination and was not scored.
3. Bands below 100 base pairs were not scored
4. Any questionable gels were re-run and those results were not used.

If using the scoring criteria outlined above resulted in the total ITS-RFLP molecular weight being unequaled to the molecular weight of the PCR product (from the PCR gels used to assess amplification) then the gel was rejected.

These molecular weight values were then compared to other ITS-RFLP gel values using a relational database created by Joyce Eberhart (using Filemaker Pro. 5.5v1). Potential matches were then rerun on the same ITS-RFLP gel for confirmation. Any questionable ITS-RFLP matches were re-digested using the enzymes *Hae III*, *RSA*, or *ALU* (Promega). ITS-RFLPs were used to compare

samples to one another, correct misidentification of morphotyping, and to identify EMF to species using known ITS-RFLP patterns from sporocarps.

SEQUENCING

To provide taxonomic information about the most common EM types, the ITS region was sequenced using the ITS-1f and ITS-4 primers. Samples were purified using QIAquick PCR purification kit and the PCR product was run on an agarose gel to quantify DNA. Samples were then sent to the Central Services Lab at Oregon State University, where the unknown EMF DNA was sequenced using an ABI 3100 capillary sequence machine. DNA sequences were edited using Sequence Editor and entered into the National Center for Biotechnology Information (NCBI) BLAST search program where unknown EMF DNA were matched with known fungal sequences located in the GenBank database (if corresponding sequences were available). Sequence dissimilarities >2% were considered different species (Horton and Bruns, 2001).

CHAPTER 4—STATISTICAL ANALYSIS

DIVERSITY

Ectomycorrhiza diversity was measured using three parameters—constancy, frequency (as a measure within-core abundance), and number of EM types per soil core.

Constancy

Constancy measured the general commonness of an EM type in its distribution throughout an experimental unit. For a particular EM type, constancy was calculated by dividing the number of soil cores in which the EM type was found (within an experiment unit) by the total number of soil cores taken from that experimental unit. Constancy was expressed as the percentage of the total number of soil cores taken in an experimental unit.

The constancy measure was used to determine which EM types would be used in the abundance analysis. For each treatment, EM types that had a mean constancy $\geq 20\%$ were compared for differences in mean abundance.

Abundance

Within-core frequency was used to measure EM type abundance. It was calculated for each soil core by summing the squares in which an EM type was found and dividing that number by 36 (the total number of squares in the

subsampling tray). That fraction was expressed as a percent (Eberhart et al., 1996). Mean within-core frequency was used to assess dominance in the EM community.

Number of EM Types per Soil Core

The mean number of EM types per soil core is a measure of local species richness. The number was calculated on an experimental unit basis by summing the number of EM types in each soil core and dividing that number by the total number of soil cores in the experimental unit.

ANALYSIS OF VARIENCE

Each of the above described measures of EM diversity and was used as a response variable for both the within-treatment and between-treatment comparisons of green-tree retention effects. When appropriate, this was followed by Fisher's protected least significant difference (PLSD) to determine whether treatment responses differed from one another. A repeated measures analysis was used on all of the within-treatment analysis to adjust for repeated sampling of the same experimental unit over time.

To meet the assumptions of normality and constant variance, data were square root or log transformed for within-treatment analysis. For the among treatment analysis, transformation was applied to non-normal data using the hyperbolic arcsine $[\ln(x+\sqrt{x^2+1})]$ (Luoma et al., 2004).

Because this type of data is highly variable, we increased the alpha level in order to increase our ability to detect a difference if the difference was there (power). We chose an alpha level of 0.1 as appropriate to discuss statistically significant differences prior to beginning the data analysis with the attendant risk of Type I error. In other words, there is a 1 out of 10 probability of a falsely concluding a treatment effect due to chance alone. All analyses were performed with Statview® 5 (SAS Institute, 1998).

Within-Treatment Analysis

For the control (100%) treatment, changes in the response variable are related to changes over time or spatial variation in the EMF community structure. Pre- and post-treatment soil core samples also provided a baseline sample of EMF diversity in mature Douglas-fir forests.

Soil cores from the 40%D treatment were assigned to 3 groups to test for treatment effects. These groups were (1) the pre-treatment soil cores, (2) the post-treatment soil cores collected between fall 1998 and spring 2000 (to be referred to as post-treatment$_1$ cores), and (3) the post-treatment soil cores collected in fall 2001 and after spring 2002 (to be referred to as the post-treatment$_2$ cores). For the pre-treatment soil cores and post$_1$ cores, both the morphotyping and the molecular work was performed by J. Eberhart. Post-treatment$_2$ soil cores were morphotyped by and the molecular work was performed by O. Kolaczkowski. All of the pre-treatment and 40%D treatment soil cores were collected, morphotyped, and molecularly

analyzed using the same methods. The statistical hypothesis tested was H_0: $\mu_{pre\text{-}treatment}=\mu_{post\text{-}treatment1}=\mu_{post\text{-}treatment2}$.

Between-Treatment Analysis

Analysis of varience was used to test between-treatment differences comparing the mean change from the pre-treatment to post-treatment condition. Changes in constancy, abundance, and mean number of EM types per soil core were tested for between-treatment differences. The statistical hypothesis tested was H_0: $\mu_A=\mu_B$ where μ_A was the mean change in the control (100% basal area retention) treatment and μ_B was the mean change in the 40%D treatment. Because there were few morphotyping differences between the two data sets they were pooled for the between-treatment analysis.

CHAPTER 5—RESULTS

WITHIN TREATMENT DIVERSITY

A total of 199 EM types were found and described in the DEMO study during the period of investigation reported here. Some of the initial EM groupings that resulted from the morphotyping were merged or separated based on the results of the RFLP's. However, the majority of the EM retained their initial morphotyping groupings, particularly the most common types. Due to the many rare EM collected, RFLP's patterns were not compared for all rare types. As a result, the uncommon EM may be more prone to observer bias, but that is balanced by each rare type's distinctiveness.

Control-100% Treatment

Constancy—Values of common EM types in the 100% treatment changed little between pre- and post-treatment samples (Table 3). The only statistically significant change between the pre- and post-treatment constancy was for an unknown *Piloderma* sp., which decreased 17% ($p=0.09$).

Table 3: Mean Constancy[1] (%) of EM types in pre- and post-treatment samples from the Control (100% basal area retention) treatment, n=3

Mycorrhiza type	Pre-treatment	Post-treatment	p-value[2]
Cenococcum geophilum	97 (2.78)[3]	100 (0)	0.42
Lactarius rubrilacteus	42 (24.06)	47 (21.7)	0.41
Truncocollumella citrina	28 (13.89)	25 (12.73)	0.42
Suillus lakeii	22 (14.7)	33 (19.25)	0.25
Ruhizopogon villosulus	44 (13.89)	56 (20.03)	0.26
Inocybe group	36 (10.02)	19 (5.56)	0.43
Rhizopogon vinicolor[4]	50 (22.05)	50 (25)	0.43
Piloderma sp.	53 (14.7)	36 (10.02)	**0.09**
Russula xerampalina	31 (7.35)	25 (8.33)	0.58
Amphinema byssoides	19 (5.56)	42 (16.67)	0.18
Cortinarius group	31 (2.77)	19 (7.35)	0.20
Gautiera sp.	28 (14.7)	19 (10.02)	0.97
Phialophora finlandia	8 (4.81)	28 (14.7)	0.25
Genea harkenssii	3 (2.78)	3 (2.78)	0.42

[1] defined as the mean percent of soil cores with that EM type present
[2] Tested by Fisher's Protected Least Significant Differences ($p<0.1$) using transformed values
[3] Standard error in parentheses—standard errors based on untransformed MSE from ANOVAs.
[4] Includes *R. vesiculosus*

Abundance—For the control treatment, the *Cortinarius* group EM type had the single statistically significant difference between pre- and post-treatment (Table 4). Its post-treatment within-core frequency dropped to 4% from a pre-treatment abundance of 14% ($p=0.06$). The EM with the highest within-core frequency was

Cenococcum geophilum, which had a mean abundance value of 77.5% within soil cores. Following *C. geophilum* was *Lactarius rubrilacteus* with a mean abundance of 20% in pre-treatment cores and 28% in post-treatment cores.

Table 4: Abundance[1] (%) of EM types in pre- and post-treatment samples from the Control (100%) treatment (n=3).

Mycorrhiza type	Pre-treatment	Post-treatment	p-value[2]
Cenococcum geophilum	78 (15.89)	77 (16.21)	0.29
Lactarius rubrilacteus	20 (16.01)	28 (16.04)	0.30
Truncocollumella citrina	4 (2.17)	6 (3.34)	0.61
Suillus lakeii	9 (8.08)	13 (10.33)	0.31
Rhizopogon villosulus	17 (6.69)	18 (8.77)	0.65
Inocybe group	8 (1.52)	5 (3.74)	0.39
Rhizopogon vinicolor[4]	8 (3.05)	7 (3.54)	0.41
Piloderma sp.	15 (7.12)	16 (3.43)	0.70
Russula xerampalina	10 (1.11)	15 (4.0)	0.42
Amphinema byssoides	10 (4.98)	13 (1.0)	0.43
Cortinarius group	14 (3.94)	4 (0.41)	**0.06**
Gautiera sp.	19 (11.51)	13 (6.56)	0.97
Phialophora finlandia	3 (2.43)	7 (5.23)	0.69
Genea harkenssii	1 (1.16)	6 (5.86)	0.42

[1] Calculated as the mean percent of subsamples (36) occupied by that type within each soil core.
[2] Tested by Fisher's Protected Least Significant Differences ($p<0.1$) using transformed values
[3] Standard error in parentheses based on untransformed MSE from ANOVAs
[4] Includes *R. vesiculosus*

The mean number of EM types per soil core (as a measure of species richness) for the control (100%) treatment was 9, both pre- and post-treatment ($p=0.96$).

40%D Treatment

In the statistical analysis the post-treatment soil cores from the 40%D treatment were separated into two groups; post-treatment$_1$ and post-treatment$_2$. This was done because the two groups were morphotyped by different researchers at different times. The subscript 1 in post-treatment$_1$ represents the first post-treatment sampling time period during which Joyce Eberhart analyzed the soil cores. The subscript 2 in post-treatment$_2$ represents the second post-treatment sampling period during which I collected and analyzed the soil cores following Eberhart methods.

Constancy—Mean *C. geophilum* constancy was lower in post-treatment$_2$ soil cores, however, the difference was not statistically significant ($p=0.15$) (Table 5). Mean *Truncocollumella citrina* ($p=0.06$) and *Rhizopogon vinicolor* ($p=0.03$) constancies were lower in the post-treatment$_2$ soil cores as compared to pre-treatment and post-treatment$_1$ cores. *Piloderma* sp. constancy was significantly lower in both post-treatment sample groups ($p=0.05$).

Table 5: Mean Constancy[1] (%) of EM types in pre- and post-treatment samples from the 40%D treatment (n=3).

Mycorrhiza Type	Pre-treatment	Post-treatment$_1$	Post-treatment$_2$	p-value[2]
Cenococcum geophilum	97[a]	100[a]	64[a]	0.15
	(2.78)[3]	(0)	(18.22)	
Lactarius rubrilacteus	19[a]	19[a]	17[a]	0.46
	(7.35)	(7.35)	(8.33)	
Truncocollumella citrina	50[a]	47[a]	22[b]	**0.08**
	(16.67)	(19.44)	(12.11)	
Suillus lakeii	28[a]	19[a]	8[a]	0.39
	(20.03)	(10.02)	(8.33)	
Rhizopogon villosulus	69[a]	67[a]	n/a[4]	0.93
	(18.22)	(25)		
Inocybe group	33[a]	42[a]	11[a]	0.22
	(17.35)	(14.43)	(2.78)	
Rhizopogon vinicolor[5]	44[a]	64[a]	8[b]	**0.04**
	(14.7)	(23.73)	(8.33)	
Piloderma sp.	58[a]	28[b]	28[b]	**0.07**
	(8.33)	(14.7)	(2.78)	
Russula xerampalina	25[a]	25[a]	47[a]	0.41
	(12.73)	(14.43)	(18.22)	
Amphinema byssoides	11[a]	17[a]	6[a]	0.50
	(11.11)	(9.62)	(2.78)	
Cortinarius group	53[a]	39[a]	42[a]	0.65
	(16.9)	(15.47)	(16.67)	
Gautiera sp.	19[a]	11[a]	3[a]	0.73
	(10.02)	(5.56)	(2.78)	
Phialophora finlandia	39[a]	42[a]	6[a]	0.22
	(20.03)	(20.97)	(5.56)	
Genea harkenssii	8[a]	14[a]	6[a]	0.44
	(8.33)	(13.89)	(5.56)	

[1] Defined as the percent of soil cores with that EM type present
[2] Tested by Fisher's Protected Least Significant Differences ($p<0.1$) using transformed values
[3] Standard error in parentheses based on untransformed MSE from ANOVAs.
[4] n/a= not assessed
[5] Includes *R. vesiculosus*

Abundance—*Cenococcum geophilum* in the post-treatment$_2$ samples was significantly less abundant than in the pre-treatment samples or the post-treatment$_1$ samples ($p=0.05$) (Table 6). *Truncocollumella citrina* abundance was significantly lower in post-treatment$_2$ soil cores than in the pre-treatment soil cores or the post-treatment$_1$ soil cores ($p=0.05$).

Table 6: Abundance[1] (%) of EM types for the 40%D treatment (n=3).

Mycorrhiza Type	Pre-treatment	Post-treatment$_1$	Post-treatment$_2$	p-value[2]
Cenococcum geophilum	84[a] (6.3)[3]	92[a] (4.61)	29[b] (17.48)	**0.09**
Lactarius rubrilacteus	11[a] (3.0)	4[a] (1.6)	7[a] (4.68)	0.40
Truncocollumella citrina	23[a] (11.76)	20[a] (10.41)	3[b] (2.24)	**0.05**
Suillus lakeii	9[a] (7.67)	1[a] (.95)	2[a] (2.47)	0.40
Rhizopogon villosulus	32[a] (15.0)	20[a] (10.87)	n/a[4]	0.64
Inocybe group	20[a] (12.47)	8[a] (3.16)	2[a] (1.01)	0.26
Rhizopogon vinicolor[5]	7[a] (2.86)	9[a] (4.13)	2[a] (2.23)	0.23
Piloderma sp.	25[a] (9.32)	10[a] (6.69)	6[a] (0.97)	0.15
Russula xerampalina	13[a] (6.65)	11[a] (8.64)	21[a] (11.38)	0.29
Amphinema byssoides	8[a] (8.03)	6[a] (3.28)	4[a] (2.21)	0.55
Cortinarius group	28[a] (12.38)	12[a] (5.99)	20[a] (12.36)	0.33
Gautiera sp.	15[a] (7.72)	7[a] (3.41)	2[a] (2.4)	0.74
Phialophora finlandia	19[a] (9.99)	13[a] (8.13)	1[a] (1.24)	0.12
Genea harkenssii	4[a] (3.86)	3[a] (3.4)	1[a] (1.31)	0.44

[1] Calculated as the mean percent of subsamples (36) occupied by that type within each soil core.
[2] P-values from Fisher's Protected Least Significant Differences test using transformed data
[3] Standard error in parentheses based on untransformed MSE from ANOVAs
[4] n/a= not assessed
[5] Includes *R. vesiculosus*

Mean Number of EM Types per Soil Core—Post-treatment$_1$ EM type richness did not differ from the pre-treatment soil cores (Table 7). Average EM type richness

was lower in post-treatment$_2$ soil cores by 5.3 types as compared to the pre-treatment soil cores ($p=0.10$).

Table 7: The mean number of EM types per soil core for the 40%D treatment

Pre-treatment soil cores	Post-treatment soil cores$_1$	Post-treatment soil cores$_2$
10.3[a]	9.12[a]	5.0[b]
(0.4)[1]	(0.05)	(1.7)

[1]Standard error in parentheses—standard errors based on square root transformed MSE from ANOVAs. Values that do not share a superscript letter are significantly different by Fisher's Protected Least Significant for Means ($p=0.10$).

BETWEEN-TREATMENT COMPARISONS

Constancy—The control and 40%D treatments showed no statistically significant different changes in mean constancy (Table 8).

Table 8: Change in mean constancy[1] from the pre- to post-treatment condition as compared between the control and 40%D treatment (n=3).

Mycorrhiza Type	Control (100%)	40%D	p-value[2]
Cenococcum geophilum	3 (2.78)[2]	-7 (11.37)	0.56
Lactarius rubrilacteus	11 (5.56)	27 (27.9)	0.58
Truncocollumella citrina	-6 (5.60)	-30 (18.06)	0.18
Suillus lakeii	22 (11.11)	-11 (13.68)	0.23
Rhizopogon villosulus	22 (14.69)	n/a[5] (14.5)	n/a
Inocybe group	-33 (28.90)	-15 (11.87)	0.43
Rhizopogon vinicolor[4]	0 (16.67)	-1 (3.68)	0.94
Piloderma sp.	-33 (16.7)	-34 (5.01)	0.95
Russula xerampalina	-11 (5.56)	-11 (14.7)	1.0
Amphinema byssoides	44 (22.22)	-1 (6.05)	0.19
Cortinarius group	-22 (11.11)	-18 (16.02)	0.58
Gautiera sp.	-17 (41.94)	-15 (9.72)	0.96
Phialophora finlandia	39 (24.21)	-25 (14.4)	0.24
Genea harkenssii	0 (0)	1 (1.39)	0.42

[1] Absolute % change in number of soil cores with an EM type present
[2] Tested by Fisher's Protected Least Significant Differences ($p<0.1$) using transformed values
[3] Standard error in parentheses based on untransformed MSE from ANOVAs.
[4] Includes *R. vesiculosus*
[5] not assessed

Within-core Frequency—The control and 40%D treatments showed no statistically significant different changes in abundance (Table 9).

Table 9: Change in abundance from the pre- to post-treatment conditions as compared among the control and 40%D treatments

Mycorrhiza Type	Control (100%)	40%D	p-value[1]
Cenococcum geophilum	-1[2] (0.63)[3]	-23 (12.91)	0.21
Lactarius rubrilacteus	8 (5.89)	-6 (1.04)	0.13
Truncocollumella citrina	2 (2.27)	-11 (5.68)	0.13
Suillus lakeii	4 (2.47)	-7 (5.98)	0.28
Rhizopogon villosulus	1 (2.6)	n/a[5]	n/a
Inocybe group	-3 (3.95)	-15 (11.78)	0.29
Rhizopogon vinicolor[4]	1 (1.01)	-2 (0.32)	0.49
Piloderma sp.	-1 (9.18)	-16 (6.36)	0.22
Russula xerampalina	4 (3.04)	3 (9.5)	0.92
Amphinema byssoides	3 (4.14)	-3 (6.02)	0.42
Cortinarius group	-11 (4.26)	-13 (13.57)	0.92
Gautiera sp.	-7 (14.66)	15 (7.15)	0.74
Phialophora finlandia	3 (6.2)	-12 (5.84)	0.25
Genea harkenssii	5 (4.71)	-2 (1.15)	0.42

[1] Tested by Fisher's Protected Least Significant Differences ($p<0.1$) using transformed values
[2] Absolute % change, see methods for details of calculations
[3] Standard error in parentheses based on untransformed MSE from ANOVAs.
[4] Includes *R. vesiculosus*
[5] not assessed

Change in Richness—Average EM type richness held steady in the uncut control and decreased slightly in the 40%D treatment, but the difference in the change in

mean number of types per soil core was not statistically significant (p=0.28) (Figure 5).

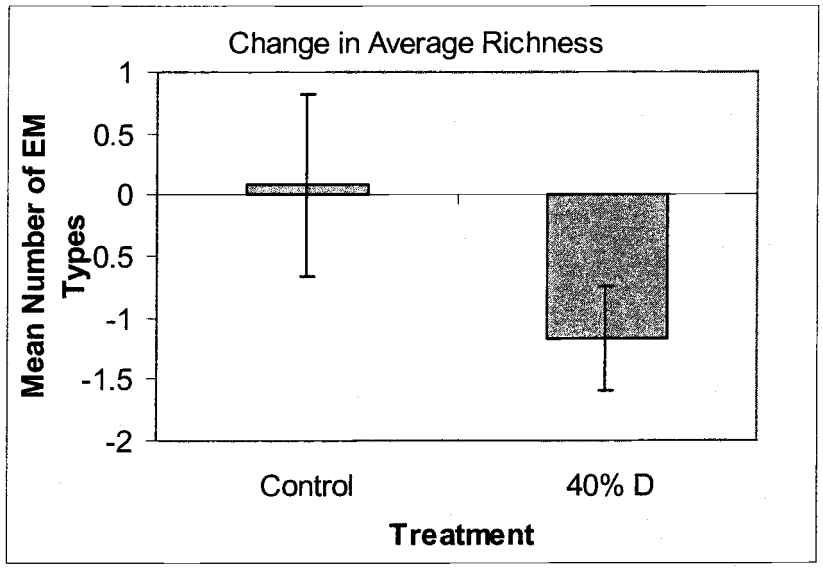

Figure 5. Change in mean number of EM types per soil core (p=0.28)

CHAPTER 6—DISCUSSION

WITHIN TREATMENT RESPONSES

Control—100% Retention

The control treatment accounts for effects of weather and other natural influences on the abundance of EM types. If an EM type changes significantly in the post-treatment control sample (tree harvest in this case), such change can be attributed to effects other then the removal of the trees. Further it is assumed that non-treatment influences may apply equally to treated units. Therefore, changes in treated units must be significantly greater than changes in the control in order to be considered a treatment effect. For both the abundance and constancy measurements, a majority of the EM types did not differ between pre- and post-treatment samples. Only an unknown *Piloderma* species had a significant change in its constancy (Table 3).

A decrease of 17% in the constancy of the *Piloderma* species in the control may, by extension, be expected in the 40%D treatment as well. Only a change significantly different from 17% can be attributed to the effects of the experimental treatment rather than external factors.

Abundance, as used here (within-core frequency) is a measure of the dominance of a certain EM type in the community. In the control, none of the common EM type frequencies showed significant changes (Table 4). Therefore,

any significant change within a treatment that is also significantly different from the control may be considered a treatment effect.

The mean number of EM types was used as an index of species richness at the community level. The mean number of EM types takes into account all of the EMF found, including the rare and uncommon species. The mean number of EM types did not change significantly from the pre- to post-treatment controls, indicating that the overall diversity did not change greatly from year-to-year due to nominal influences.

40% Basal Area Retention (40% D)

Cenococcum geophilum constancy was 33% lower in the post-treatment$_2$ soil samples as compared to the pre-treatment condition (Table 5). This dramatic drop was not statistically significant, however, due to variance associated with its high constancy in the Hamilton Butte block. The Hamilton Butte block had a constancy of 100% while the Watson Falls block and the Dog Prairie block had a constancy of 50% and 42%. As noted in the methods, the Butte block had a class 3 non-fish-bearing perennial stream that may be associated with environmental conditions that ameliorated treatment effects on EM types. Therefore, although no statistical significance was detected, it may be premature to conclude that the treatment had no effects on the presence of *C. geophilum.*

In addition, *C. geophilum* abundance significantly declined by 55% from the pre- to post-treatment$_2$ (Table 6). Although *C. geophilum* showed the largest

decrease in both constancy and within-core frequency, this EM type remains the most dominant and most common EM type, post-treatment, in the 40%D treatment. Two primary interpretations of the decreased abundance of *C. geophilum* in post-treatment$_2$ samples are possible. One is that the observed difference is attributable to between-observer differences. The other is detection of an actual treatment effect. If a treatment effect is assumed, then this delayed change in the EM communities is important because *C. geophilum* is a dominant member of EM community in many ecosystems (Horton and Bruns, 2001; Dahlberg et al., 1997; Kårén et al., 1998). *C. geophilum* seems resistant to decay which may contribute to a perceived time in its apparent response to the treatment.

A similar consideration may be taken into account with regard to the moderately significant difference between the pre-treatment and post-treatment mean number of EM types per soil core (Table 7). The decrease in richness of about 50% in the post-treatment$_2$ samples seems to indicate a delayed response to the treatment since the post-treatment$_1$ samples were not different from the pre-treatment conditions. Since no dominant types were lost (Table 5) the decline in richness occurred in the less common EM types.

Delayed changes in EMF community composition up to a few years following a harvesting treatment, due to the ability of EM to persist, have been noted by other researchers (Ferrier and Alexander, 1985; Persson, 1982). Ferrier and Alexander (1985) reported EM persistence for nine months following a clear-cut, and Persson (1982) reported active EM root tips for up to 18 months following

a clear-cut. Persistence of EM following clearcutting for nine to eighteen months post-harvest, suggests it is reasonable to expect EM to persist longer when more trees (hosts) are left on a site, as found in the 40%D treatment results presented here.

Due to the inherent complexity of ecological systems, there maybe other factors influencing the response of the EM to the treatments. Although, it is argued that there may be a decrease in the diversity of EM 3-4 years from tree harvest, it is possible that other ecological processes are affecting the presence of the EM in addition to or instead of a slow decline due to loss of host photosynthate.

Long-term ecological studies are naturally difficult and prone to different researchers finding somewhat different results from the same data set (Horton and Bruns, 2001). The pre-treatment and post-treatment$_1$ soil cores were processed and analyzed by a different researcher than the post-treatment$_2$ soil cores. Therefore, another possible explanation for the decline in EM in post-treatment$_2$ samples is researcher bias. However, potential bias was addressed by ensuring that both researchers used the same criteria for EM morphotyping. In addition, the same morphotype database was used and the protocols for the molecular techniques were followed.

Overall, the results were similar over time and between researchers. Therefore some general conclusions can be drawn. The 40%D treatment was successful in maintaining a legacy of EM on site. However, in response to the 40%D treatment, rare EM types were less abundant in the samples or became

undetectable while some common types declined, but remained present in the system.

Statistically significant EM abundance increases were not seen in response to the 40%D treatment. However, *Russula xerampalina,* was higher in post-treatment$_2$ in both constancy and within-core frequency. If this represents an undetected real increase in its dominance on roots, loss of competitors or increased limiting resources may provide mechanisms for higher abundance. Alternatively, this EM type may be better adapted to younger forests, and the 40%D treatment imparted younger forest characteristics thereby favoring this EM type (Flemming et al., 1984).

At the stand level, overall EMF diversity may have decreased in the 40%D treatment, but a viable population of EMF remained three to four years post harvest. In terms of harvesting practices, retaining 40% basal area allowed enough EMF to survive so that regeneration of the forest soil ecosystem can be facilitated by the presence of legacy EMF. Perry et al. (1989) postulated that diversity in the EMF community stabilized the plant-soil system during environmental fluctuation and assisted in the recovery of the aboveground ecosystem. However, it is unknown what level of EM legacy is necessary in this type of silvicultural treatment to maintain a stable ecosystem in case of further disturbance (Perry et al., 1989).

TEST FOR TREATMENT EFFECTS

These results were used in conjunction with the within-treatment results to determine significant treatment effects. Although a significant change from pre- to post-treatment may be detected, for a change to be attributed to treatment effects, the analysis used here required two conditions be met. One was that there be a significant pre-treatment to post-treatment within-treatment change and the other was that the change be significantly different from the change in the control.

The mean constancy in the control data for the *Piloderma* sp. (Table 3) showed a significant decrease from pre- to post-treatment indicating that there existed a non-treatment related factor causing this decline. Out of the 14 common EM types, the *Piloderma* sp. was the only one that showed a significant difference. It should be noted that because there is a 1 out of 10 chance of a false positive (Type I error) it could be that this significant decrease is due to chance alone. This also holds true for the *Cortinarius* group within treatment abundance measure (Table 4).

A trend of decreasing species richness was indicated in the 40%D treatment by the difference in the mean number of EM types (Table 7). The control showed little change in post-treatment samples, while the 40%D treatment initially showed no response to the treatment in the post-treatment$_1$ samples, but then decreased by about 50% in the post-treatment$_2$ soil cores.

Another treatment in the DEMO study is a 15%D retention cut. Stockdale (2000) summarized EM constancy for the 15%D treatment for 10 of the most

common EM types—all of which are included as common types present in the 40%D and the control. Looking only at data collected greater then 3m beyond retention tree driplines, all of the 15%D common EM types decreased compared to the control except for the *Genea harkenssii* which slightly increased. The dripline was a conceptual device and used as a surrogate for root density. The results show a strong response in EM constancy to the higher level of disturbance in the 15%D treatment.

A direct comparison between the 40%D post-treatment$_2$ and the 15%D treatment will not be made here. However, Stockdale (2000) showed a sharp decline in mean number of EM types comparing soil cores from inside tree crowns to open areas greater then 3m outside the dripline. In addition, Stockdale documented a 1/3 reduction in the amount of fine root tips outside the dripline as compared to inside the dripline. Although these results are striking, one must also consider that active EM root tips can survive without a supply of photosynthate for up to three years (Hagerman et al., 1999). This makes it likely that residual EM from the pre-treatment forest persisted in the 15%D treatment in the short-term. It is possible that EM root tip density in the 15%D treatment could have been on a downward trajectory as the residual EM died due to lack of photosynthate. Regardless of the effect of residual EM root tips in the 15%D treatment, the striking results in areas away from retention trees demonstrates the importance of legacy trees in the maintenance of EM root tips following disturbance.

The 40%D treatment showed some statistically significant declines in individual EM type diversity, but the large declines found in the 15%D treatments (Stockdale, 2000) were not present. As a management option, it seems unambiguous that the maintenance of 40% of the basal area of the forest can create conditions closer to those found in an undisturbed forest. The higher EMF diversity found in the 40%D could contribute to ecosystem health and stability as the forest recovers from disturbance (Amaranthus and Perry, 1987; Perry et al., 1989).

Using the isolated trees of the 15%D treatment, it was found that at distances greater then 7m from green tree (open areas) diversity of EMF was greatly reduced. However, change in EMF diversity in response to distance from trees was not addressed in this study due to high tree densities and greater crown coverage. The 50% canopy cover of the 40%D provided much higher levels of shading than experienced in the 15%D treatment. The goal of the dispersed sampling used in the 40%D treatment was to capture the unit level response rather than to investigate variations in relation to individual trees.

As the amount of basal area harvested increases, EM diversity (at a community level) decreases (Harvey et al., 1980; Hagerman et al., 1999; Mah et al., 2001). Because the soil cores in this study were taken between 2-4 years post-treatment, within the range of time that roots of harvested trees may still be viable (Ferrier and Alexander, 1985; Persson, 1982), the mean number of EM types may drop further in response to the treatment before the forest system begins to recover and the mean number of EM types increase again.

Each individual EMF seems to respond to the treatments differently. Some, such as *C. geophilum*, decrease in dominance, commonness, or both. Without knowing the individual EMF species and how they function in the ecosystem, it is impossible to draw any mechanistic conclusions from these results. It is unknown whether the observed decreases are due to loss of living tree roots that serve as host habitat, an increase in temperatures associated with forest thinning, loss of nutrients from a site, or a combination of these and other factors.

Although mechanistic conclusions cannot be drawn, Stockdale 2000 showed a significant 45% decrease in the mean number of EM types per soil core in the 15%D treatment outside the dripline of retained trees. The 40%D treatment in this study showed no change in the mean number of EM types (Figure 3). The 40%D treatment maintained more individual EMF species compared to the 15%D treatment. Although it is difficult to assess the impacts of the treatments on the individual species level, the mean number of EM types present did decrease in response to decreasing basal area.

Small sample size in this study may contribute the inability to find significant differences in the majority of the diversity indices tested here. The inability to detect significant differences in some of the EM that exhibited large changes in diversity may be a result of the low power associated with a small sample size. It would be premature to strongly conclude that there the 40%D treatment had no effect on some of these EMF due to the limited sampling and inability to sample over a longer time period.

Luoma et al. (2004) in another DEMO study, compared effects of the different DEMO treatments (including the 40%D treatment) on sporocarp production—both hypogenous and epigeous. Those results lent support for tree retention in a spatially dispersed treatment mixed with some areas of aggregated tree retention. The potential effects of mixed aggregated and dispersed patterns in the same treatment is a worthy question for future study. This mix of spatial patterns could provide for maintenance of EMF throughout the treatment with hotspots of high diversity in the aggregated areas as a function of the reduction of edge effects.

EM are a fundamental building block of forest ecosystems in the Pacific Northwest, loss of EM diversity at the landscape level is a concern in the face of widescale disturbances. As diversity is lost, less severe disturbances are able to damage forest ecosystem (Perry et al., 1989). Current knowledge does not allow assessment of where the 40%D treatment lies on a continuum of risk to diversity loss. The answer to this question may help determine how much diversity is required to maintain stability in all forested systems, especially in the face of hypothesized global climate change scenarios.

CHAPTER 7—CONCLUSION

Overall, within-treatment differences for the majority of the common EMF were not significant when comparing pre-treatment to the post-treatment conditions for all of the diversity measurements. Although the majority of the within-treatment EMF did not respond significantly to the treatment, some of the EMF that did decline seemed to have a delayed response to the treatment indicating that it may be imprudent to end EMF diversity studies after only a few years. The between-treatment differences were similar in that the majority of the EMF did not show a significant response to the treatment.

A moderately significant trend of decreasing species richness was noted in the 40%D treatment by the difference in the mean number of EM types from the pre- and post-treatment$_1$ soil cores to the post-treatment$_2$ soil cores (Table 7). Since the majority of the common types did not show a significant reduction, the decrease in diversity in the post-treatment$_2$ soil cores must be coming from the rare EM types. It is important to note that the soil cores in this comparison were taken within the range time frame in which harvested tree roots are still viable, indicating that a further drop in diversity may become detectable in future years.

This research demonstrates that the 40%D treatment can maintain a relatively high legacy of EM. However, there is a loss of some rare EM types and a reduction in some of the more common EM types. In terms of harvesting practices, retaining 40% basal area allowed enough EMF to survive so that regeneration of

the forest soil ecosystem can be facilitated by the presence of the EMF. However, it is unknown what level of EM legacy is necessary in this type of silvicultural treatment to maintain stability.

Each individual EMF seems to respond to the treatments differently. Some, like *C. geophilum*, decrease in dominance, commonness, or both. Without knowing the individual EMF species and how they function in the ecosystem, it is impossible to draw any mechanistic conclusions from these results.

These results lend support for the consideration of 40% dispersed green-tree retention as a silvicultural option when management goals include the maintenance of EMF diversity. This level of retention clearly maintains a high level of ectomycorrhizal fungus diversity. The maintenance of a diverse EMF community can provide important ecosystem services such as stabilizing the below ground ecosystem in the face of possible disturbances (Perry et al.,1989), increasing the rate at which new seedlings put on biomass and height (Sudhakara and Natarajan, 1997), and increasing the efficiency of nutrient uptake by trees (Baxter and Dighton, 2001). Timber harvest via implementation of ecosystem-based forest management practices will require the continuing study of complex below-ground systems for the development of potential silvicultural treatments during further iterations of adaptive management.

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