The Influence of Forest Floor Moss Cover on Ectomycorrhizal Abundance in the Central-Western Oregon Cascade Mountains

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Abstract:

Mycorrhizal fungal associations are pervasive in land plants; however, mosses are uniquely non-mycorrhizal. The central-western Oregon Cascades (CWOC) has an overstory dominated by ectomycorrhizal gymnosperms while mosses copiously carpet the forest floor. Both ectomycorrhizal fungi (EMF) and mosses can heavily influence ecosystem dynamics where they dominate, especially through the regulation and cycling of nutrients and water. A manipulative experiment was performed in which the moss layer was removed from half of otherwise naturally moss-covered plots and the abundance of infected ectomycorrhizal root tips (EMT) was monitored over a one year period. It was found that the removal of forest floor moss mats significantly decreased the abundance of EMT in the soil beneath, whereas plots not subject to manipulation showed a significant increase in EMT one year after manipulation. Soil phosphatase activity significantly increased in both harvested and non-harvested plots in Year 1; harvested plots showed a negative correlation between soil phosphatase activity and EMT, while non-harvested plots showed a positive correlation. Neither biomass nor the dominant moss species, *Eurhynchium oreganum* and *Hylocomium splendens*, had a significant differential effect on EMT reduction in the harvested plots one year later. This study confirms that forest floor moss cover in the CWOC provides suitable microclimate for the proliferation of ectomycorrhizal root tips, and its removal causes a significant reduction in the abundance of EMT one year later. These results have important implications for ecosystem function and land use in the Pacific Northwest. More research is needed to identify the specific avenues responsible for decreased EMT abundance associated with moss mat removal.

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Glossary of Terms

1) Arbuscule
    a) The exchange structure produced by arbuscular mycorrhizal fungi in which nutrients and water are given to the plant host in return for photosynthate. It is dendroid in form and penetrates the cell walls of the root cortex, but not the plasma membrane.
2) Arbutoid Mycorrhiza(ae)
    a) A certain type of mycorrhizal association formed in members of the plant order Ericales
3) Aseptate Hypha
    a) A hypha lacking septa, which are thin divisions between hyphal cells.
4) Autogenic
    a) Produced from within or self-generating
5) Biotroph
    a) An organism that is dependent upon another living organism for all or some of its nutrient requirements.
6) Bryophyta
    a) The division consisting solely of mosses in the orders Bryales, Sphagnales, Andreaeales, Tetraphidales, Polytrichales, and Buxbaumiales.
7) Bryophyte
    a) Any member of the divisions Marchantiophyta (liverworts), Anthocerotophyta (hornworts), or Bryophyta (mosses).
8) Ectohydric Mosses

a) Mosses that receive their water and nutrient supplies primarily from the atmosphere.

9) Embryophyte
   a) Land plant.

10) Feather-Moss
   a) Pleurocarpous, ectohydric mosses with erect stems that have a feather-like, or frond-like, appearance. These mosses tend to carpet the forest floor in temperate-coniferous and boreal forests, while they are patchily distributed in mixed temperate forests.

11) Hartig-Net
   a) The intercellular, hyphal network within the root formed by an ectomycorrhizal fungus.

12) Hypha(ae)
   a) One of the filament-like threads that make up the body of a fungus.

13) Hyphal Coil
   a) An intracellular nutrient/photosynthate exchange organ of arbuscular mycorrhizal fungi.

14) Lamina
   a) A moss leaf.

15) Mantle
   a) Ectomycorrhizal hyphae that form a sheath on the surface of root tips in ectomycorrhizal plant species.

16) Moribund

a) Dying or at the point of death; no longer growing.

17) Moss

a) Any member of the division Bryophyta.

18) Mycelium

a) The filamentous vegetative portion of a fungus. It is composed of hyphal aggregations.

19) Mycorrhiza(ae)

a) Literally a "fungus-root," but for the purposes here mycorrhizae are considered dual organs of absorption formed when symbiotic fungi inhabit the healthy tissues of most terrestrial plants (Trappe, 1996).

20) Phosphatase

a) Any of a group of enzymes that act as a catalyst in the hydrolysis of organic phosphates.

21) Pleurocarpous Moss

a) Generally, those mosses that grow horizontally across a substrate.

22) Propagule

a) A structure with the capacity to give rise to a new organism.

23) Protonema

a) The filamentous gametophyte stage of mosses.

24) Terricolous

a) Living on/in the ground or soil.

25) Tracheophyte (Vascular Plant)

a) Any plant that contains lignified vascular tissue for transport; a vascular plant.

26) True Moss

a) Any member of the order Bryales within the division Bryophyta.

Preface

My decision to go to SUNY ESF was pretty easy, well, as far as life-changing choices are concerned. At my first college fair in high school, SUNY ESF was set up at a very approachable booth, leading me to spend nearly the entire college fair talking with the ESF representative. That following summer I was employed at Camp Unirondack in the Adirondack Mountains. It was the best time of my life and I knew that ESF's connection with the Adirondacks would put me in a good place for future academic study. Little did I know that my entire career path would take a sudden turn during my first semester at ESF.

The following fall I began my ESF career in the Sadler Hall Learning Community as an Environmental Science (E.S.) student, hoping to change the world some day. Although the latter part of that thought process remains intact, the former changed when I took Dr. Kimmerer's Botany course during my first semester. Dr. Kimmerer was so passionate about plants, presenting and relating them in a way I had never known before. By the middle of the semester I had changed majors and set out on the long path towards an EFB degree with a concentration in plant biology.

During my second semester I decided to continue taking courses with Dr. Kimmerer and enrolled in the Ecology of Mosses. At the end of the semester, Dr. Kimmerer announced an opportunity to apply to the Undergraduate Mentoring in Environmental Biology (UMEB) program. It consisted of two years of undergraduate research funding in environmental biology, a sizable stipend, and an independent project developed and carried out with the help of a mentor or advisor. Being an ambitious freshman I decided to enroll in UMEB. Following that decision I was

asked to join the Honors Program. I considered the feasibility of conducting two separate independent research projects, when it dawned on me that I could combine the two into a more comprehensive work. I ran the idea by Dr. Bennett and made the decision to do a joint Honors/UMEB research project.

Let me just say this right at the start: as far as the Honors thesis combined with a UMEB project is concerned, to say that it has been the most work I have ever done in my entire life would still be an understatement. Nothing could have possibly prepared me for the research plan I would eventually adopt, except both of my advisors struggling to reduce all of my crazy ideas into a feasible project. They offered insightful questions such as "is that really possible?; can you manage that all alone?; have you considered joining a graduate student's project to make it a bit easier?" The questions were aimed to help me consolidate my ideas into a rational project, but my ambition had no rational substrate to attach itself. I felt like I could do anything, no matter how long or how difficult. I simply had too many ideas. As Dr. Kimmerer mentioned in one of our project brainstorming sessions, "It's like you're a kid again, anything in the world that you have a question about can likely be explored." I guess I took her a bit too seriously.

The first objective was to complete a literature review on 20 scientific papers in the fields we were interested in. I had no other basis, at this point, than my love for botany and my recently developed bryophyte-centric view of the discipline. In talking with Dr. Kimmerer I became enthralled with the feather mosses and epiphytic mosses of the Pacific Northwest, mosses on a scale I had never imagined, carpeting the forest floor with lush mats and dangling from tree limbs. This was nothing like

the landscape of a Northeastern forest. Ergo, I researched everything and anything I could about bryophytes, from their nutrient and water relations to the over-harvesting that was being conducted in northern temperate ecosystems, including the Pacific Northwest. Paper after paper, I was literally downloading bryophyte information into my brain, but nothing really satisfied my research interests.

Simultaneously with the literature review for UMEB I was enrolled in Dr. Tom Horton's Mycorrhizal Ecology course. I had taken General Ecology with him the previous semester, and, like the Kimmerer story, his sheer excitement about mycorrhizae convinced me to continue with his teachings. Honestly, before General Ecology I had never even heard about mycorrhizae before, let alone knew where the new concept would take me. During Mycorrhizal Ecology, the entire field of plant ecology really began to make sense. It all seemed to fall into place once I understood what was going on below ground. However, the most interesting I made during my two years at ESF was soon to come; mosses were not known to be mycorrhizal. What? An entire phylum that did not exhibit mycorrhization of any tissue, it just did not make sense to me. After all I had learned about land plants and mycorrhizae, why were the true mosses, Bryophyta, not mycorrhizal? This was the question that drove me to read the subsequent papers for thesis development.

I met with Dr. Kimmerer to discuss reasons why mosses do not have a mycorrhizal association. During that time, one of her fellow bryologist colleagues, Dr. Janice Glime, was writing a comprehensive work on bryophyte ecology. Dr. Kimmerer allowed me to photocopy an unpublished section entitled, "Mosses and Ecosystem Roles." Within this paper the non-mycorrhizal status of mosses was

confirmed, however the most interesting idea I had discovered was revealed. Dr. Glime presented research concerning the indirect effects that mosses may have on the mycorrhizal community; another way to put it is that mycorrhizae may have evolved a way to benefit their symbiosis without the direct mycorrhization of mosses. One paper in particular sparked my interest, however when I looked at the citation it was in French.

I filed for an interlibrary loan for the original document. Within a week it had arrived and I immediately spent about three hours on an English-French translation website trying to decipher exactly what it said. The paper, "Influence d'un tapis de mousses sur la mycorrhization de *Pinus silvestris*" by Kilbertus and Mangenot, was published in Oecology Plantarum in 1972. The title of the paper translates to, "The Influence of a Moss Carpet on the Mycorrhization of *Pinus silvestris."* The researchers conducted a laboratory experiment to test the effect of moss cover on soil ectomycorrhizal abundance. They grew *P. silvestris* in pot culture and observed, one year later, that the ratio of ectomycorrhizae to root dry weight was significantly higher under moss than under bare soil. This was an important finding because it indicates that there is something beneath a moss mat that is beneficial to mycorrhizae, thus causing them to be more abundant. This study formed the foundation for my UMEB and Honors Thesis research.

I followed up by contacting Gerard Kilbertus to ask him about his work years ago. The crux of my e-mail involved asking him to explain what he did, because the only perfect translation I was able to decipher was that of the abstract. His response included a copy of the French paper and a response saying, "It is me the Gerard

Kilbertus! I thank you for the interest in this work. Please find herein the publication you desired. If there are problems, send me your postal address and I will mail it to you." I guess he did not fully understand what I was asking, however I pushed through and translated the important parts myself, to gain a proper understanding of the entirety of the work. I had a good running model from which to design my research topic.

I could not stop thinking about what it was that I wanted to do with this new information. Did I want to sample hundreds of mosses in an attempt to find one that was mycorrhizal? Or did I want to verify the results of Kilbertus and Mangenot's work in the lab? I began reading papers about the ecology and dynamics of both mosses and mycorrhizal fungi, to try to elucidate a reason why moss cover would influence mycorrhizal abundance. I looked into the nutrient dynamics of each component, their ecophysiology, and even their life histories and phylogenies. Eventually, I realized that I wanted to take this question of abundance into the field, and quantify it in a more precise manner. Biomass can be a difficult measurement when precision is concerned, so I decided to look at physical abundance of live ectomycorrhizal root tips in soil cores from naturally moss-covered areas compared to areas where the moss mat was experimentally removed. In doing so I set out with an even more ambitious goal, to determine a complete ecological framework for an indirect association between mosses and mycorrhizal fungi, thus proposing a possibly new ecological understanding of temperate forest dynamics where mosses are a dominant part of the ecosystem.

Finally I had solidified a topic and, although seemingly too long for me to complete, I busted out over the course of the next three years and completed it with utter satisfaction, and surprise. Because of the UMEB component, my funding was significantly increased, allowing me to travel to the Central Oregon Cascade Mountains during two consecutive summers to perform the necessary field work. I'll tell you, what you put down on paper, in terms of field methods, never actually works when you get to a site you have never been to before, as I have learned first hand and my advisors continually warned me about. And believe me–I had never been out of the northeast prior to my research.

Speaking of doing more than you should, in developing my project it seemed that the only feasible way for me to complete the field study was to assume two summers of field work instead of the one required by both programs. The reason for this was in ectomycorrhizal response time. Approximately 90% of ectomycorrhizal root tips turn over in nine months. That meant that I would need to allow at least nine months to elapse after removing the moss layer from half of my plots (discussed in detail in the methods section) to allow for the ectomycorrhizae to respond to the moss harvest, if they would at all. I proposed this to Dr. Kimmerer and luckily was allowed to continue my research for a year longer than students tend to have. Thus, my junior and senior years were poised for a completely different arrangement, with laboratory research slotted to take up a significant amount of time.

From the time I got to the H.J. Andrews Long Term Ecological Research Station (LTER) in Blue River, Oregon, my project was in a perpetually dynamic state. I can not remember how many e-mails, 180° turns, and frustrating moments I had in

setting up my field sites. I was out for days, weeks even, and realized only now that a lot of that data I collected is not all that pertinent to the overall research question. I just thought it was at the time; but hell, if I did it I might as well include it! Regardless, while I was in Oregon during both summers, I learned more than I could have imagined about field research. I know now that things do not have to be perfect. An example of this was marking the boundaries for my five, $40m^2$ sites. I spent an entire morning (4 hours), during my first year, trying to measure and mark an exact 40x40 m site, only to get to site two and realize that it was not physically possible on that type of terrain. So I just formulated a general visual boundary of all of my sites and established 16 random plots within those bounds, which then took a total of only six hours total. It is things like that which a novice field researcher, working by himself, does not realize at first. Who knows, maybe I am not as sharp as I think. That is the other answer I guess.

Overall, I think the experience of developing and implementing the Honors Thesis and UMEB project was the overwhelming highlight of my college career. It has undoubtedly prepared me for graduate school and has enabled my scientific writing skills to remarkably improve. I am humbled by the time and effort all of my professors commit to research in the name of biology. I hope that one day I will fill their shoes, or at least be happy with the shoes that I am filling, wherever I end up.

Advice to Future Honors Students

What you will learn while carrying out your research is unparalleled by any other undergraduate experience you may have, aside from those that are outside of academia (like your social life, which I suggest not letting fall by the wayside). The honors program is a once in a lifetime opportunity that will augur great rewards in the future, whether they are in the job market or post graduate study; the experience is simply unmatched. If you respect the program and give it your all, graduate school will seem tenfold easier, at least I think it will. You basically develop a project just like you would in graduate school. If you are eligible I certainly would not pass up this opportunity. It is well worth it and I highly recommend doing an Honors Thesis.

The best advice I can offer any prospective honors student is to not bite off more than you can chew, at risk of sounding incredibly cliché. I know from experience (hundreds of hours in the lab, unnecessarily late nights, endless meetings, nearly undoable field work, loss of social life at times, etc…) that when a professor (Dr. Horton and Dr. Kimmerer in my case) warns you not to do so much, they probably know better than you do, seeing as they have been living research for their entire careers. That is the only negative aspect of the Honors Program. Know your limits and always plan on everything taking twice to three times as long as you predict it will. Otherwise, it is worth more than anything else you will do at ESF. Here is a list of key advice tidbits that I offer you in your quest toward honors thesis completion and graduation.

1. Nothing ever goes as planned, especially field work.

2. Be able to be spontaneous and on top of your game when things you thought would work out don't. Be adaptable and willing to change.
3. If you are dead set on a project idea, don't get too excited until you run it by your advisors and they give you a reality check.
4. Set numerous deadlines for yourself, and lay it all out in a timeline. It really helps to have a visual of your entire project coming to fruition.
5. Don't be afraid to ask for help or admit you need help. Your advisors are always there for you no matter how busy they might be; you just have to get them at the right time.
6. Don't bite off more that you can chew; keep your project ideas to something you won't have trouble finishing.
7. You can find help in odd places; just by talking with your friends about your project can really shed light on a recurring issue or idea you just can't get a hold on, even a discussion late on a Friday night (they might think you're weird, but really, who cares?).
8. Do not procrastinate about any part of the project. I know this is easier said than done (I really procrastinated), but if you write things up as you go along… your methods, for instance… it won't be utterly difficult to recall what you did two years ago.
9. Research something that you are really interested in. Don't settle for any old project. This is a once in a lifetime opportunity that will hopefully stay with you for a long time.
10. Good Luck!!!!!

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Introduction

1. Overview of Mycorrhizae

Mycorrhizal associations, in the strict sense, are considered to be between a fungus and vascular plant. This interpretation is consistent with the derivation of the term "mycorrhiza" from the Greek words 'mykes' (fungus) and 'rhiza' (root). To encompass pre-root associations present in many cryptogamic plants, Trappe (1996) defines mycorrhizae as "dual organs of absorption formed when symbiotic fungi inhabit healthy tissues of most terrestrial plants."

Of the numerous mycorrhizal categories that have been defined, the arbuscular mycorrhizae (AM) and ectomycorrhizae (EM) will be covered herein. AM fungi, the most ancient type of mycorrhizal fungi (Wang & Qiu, 2006), are obligate biotrophs of the phylum *Glomeromycota* (Schüßler et al., 2001) that form intracellular nutrient and carbon exchange organs within plant cells known as arbuscules/hyphal coils, as well as storage structures (vesicles) that can also function as asexual propagules. Aseptate hyphae from these organisms breach plant cell walls yet remain separated from the cell cytoplasm by the cell membrane (Peterson & Massicotte, 2004). Typically the cells infected are epidermal and cortical tracheophyte root cells; however the term "plant cells" will be used to account for bryophytic associations.

Ectomycorrhizae are derived fungal associations with specific woody trees and shrubs, although a few herbaceous plants also employ this habit (Cairney, 2000); however it must be noted that this association occurs only with tracheophytes. The extant family *Pinaceae* is known to be almost entirely EM (Cairney, 2000), 95% in an

estimate by Newman and Reddell (1987). EM fungi are known to facilitate photobiont access to both mineralized forms of nitrogen and phosphorus as well as provide plant access to recalcitrant organic elements through extensive extraradical mycelial breakdown and absorption. As opposed to AM fungi, EM fungi do not produce any intracellular structures in plants, aside from arbutoid morphologies in ericaceous plants, rather they function intercellularly with the formation of a Hartig-net (hyphae between root cells that function as the fungal/plant exchange site), and a mantle (interwoven hyphae covering root tips that function in storage and protection). The basic EM morphology present in many gymnosperms, especially the Pinaceae, involves presence of the Hartig-net between several layers of root cortical cells (Brundrett, 2004).

Today, approximately 80% of tracheophytes are known to form AM symbioses while liverworts and hepatics are also known to produce AM-like symbioses (Schüßler, 2000). Including bryophytes, over 90% of all embryophytes form mycorrhizal associations (Cairney, 2000; Wang & Qiu, 2006). A study cited by Giovannetti and Sbrana (1998) conducted on a Croatian island identified 75% of the embryophytes as having AM symbioses, with 18% exhibiting other mycorrhizal forms and only 7% being non-mycorrhizal. This supports the claim that very few land plants are non-mycorrhizal and such plants could be considered “outliers” within the kingdom Plantae. It has been found that non-mycorrhizal habits occur in plants associated with disturbed sites due to lack of nutrient competition, as well as hydric habitats due to greater aqueous nutrient mobility/water availability (Cairney, 2000 and references therein); interestingly these are sites which mosses tend to inhabit.

2. Overview of Mosses (Bryophyta)

Mosses, although seemingly minute and typically overlooked, function as important components of ecosystems worldwide. As Janice Glime began her book, Bryophyte Ecology (2006), "It is time that the scale be refined to examine the role of bryophytes in ecosystem processes… while the scale is small the role can be crucial." This crucial role can perhaps be attributed to the non-mycorrhizal status of mosses (noted later), and their unique physiology/ecology as compared with the more familiar vascular plants.

Uniquely, only mosses, with the exception of the liverworts, have vegetative structures (leaves, thallus, stem, and rhizoids) composed entirely of haploid gametophytic tissue with a dependent diploid sporophyte. The vegetative tissues of all other plants consist of the diploid sporophyte, with severely reduced gametophytes involved in reproduction. As Glime (2006) points out, the scale at which mosses are examined needs to be reduced from that of tracheophytes. Mosses: (1) lack lignin for support; (2) are poikilohydric whereby their moisture level is mediated by the external environment; (3) require an aqueous environment for fertilization limiting dispersal; (4) have gametophytic structures, protonema and lamina, that are typically a single cell layer thick and lack a true cuticle; (5) have astomatal lamina causing gas exchange to be regulated through diffusion across exposed acuticular leaf surfaces rather than by potassium pumps in guard-cells; and (6) vegetative reproduction is prolific among mosses leading to dense mats, on various substrates, of a single species and genotype.

The propensity of moss lamina to be only a single cell thick provides ample surface area for nutrient absorption, rather than by roots in the soil. The nutrient absorption strategy of mosses, specifically pleurocarpous mosses, is also unique and stems from their poikilohydric habit. Mosses have an incredible affinity for cations via passive psysio-chemical uptake by highly negative charges on their cell walls. This phenomenon is known as Cation Exchange Capacity (CEC), and increases the availability of cations to mosses (Büscher et al., 1990). Surrounding the outside of moss lamina are carbohydrates known as polygalacturonic acids (Kimmerer, 2004). These carbohydrates provide the negative charge that binds cations dissolved in precipitation, fog, surface water, throughfall, stem flow, substrate and dry deposition. The bound cations can be stored on cation exchange sites until actively or passively accepted into cells whereby they are utilized, stored, or in acrocarpous mosses transported around the gametophyte via non-lignified leptoids.

Both the specific nutrient dynamics and ecological position of mosses (interceptors of nutrients before they enter the soil), as well as desiccation tolerance mechanisms, will be discussed in detail in a subsequent section. What then do mycorrhizae and mosses have to do with each other, seeing as these two components are the focus of the current research, and why is this association unique? The thesis will begin to address this issue by comparing embryophyte and mycorrhizal fungal phylogenies, superimposing one on top of the other.

3. Inter-Kingdom Coevolution: What about the Mosses?

The evolution of embryophytes marked the beginning of autotrophic land domination, which subsequently allowed for the radiation of all other organisms into terrestrial niches. This new, hostile, and physiologically challenging environment offered very little in terms of evolutionary ease. Water and nutrients became harder to access, the protection of the hydrosphere was lost, all of which required stronger absorptive, anchorage, and supportive structures to be developed. All of these drastic physical changes suggested the need for embryophytes to find help in other organisms, both for their survival and eventual dominance of the terrestrial surface.

But where could this help be obtained? An inter-kingdom coevolution seems a likely compromise, where each component assists the other to cope with stressful environmental conditions. Perhaps, as Pirozynski and Malloch (1975) have hypothesized, "embryophytes are the product of an ancient and continuing symbiosis (mycorrhizae) of a semi-aquatic green alga and an aquatic fungus, an oomycete." Wang & Qiu (2006) further this claim stating that, "all available evidence seems to point to an origin of mycorrhizas at the beginning of land plant evolution." As the earliest land plants, the bryophytes should associate with mycorrhizal fungi for these claims to be substantiated.

Bryophyte Phylogeny:

It is generally accepted that embryophytes are a monophyletic group (Bateman et al., 1998) evolving from a freshwater alga of the charophyte lineage (Charales) during the Ordovician period, approximately 480 million years ago (mya) (Kawai and Otsuka, 2004; Nishiyama et al., 2004; Chapman & Waters, 2002;

Nickrent et al., 2000; Qiu and Palmer, 1999; Bateman et al., 1998). The earliest record of a land plant, a fossilized spore from 475 million years ago in the mid-Ordovician, was found by Wellman et al. (2003) and was identified as having liverwort affinities. Considering all embryophyte taxa, the literature clearly shows that descendants of the three extant bryophyte divisions (Marchantiophyta/liverworts, Anthocerotophyta/hornworts, and Bryophyta/mosses) were the earliest plants to colonize land (Shaw & Renzaglia, 2004; Kugita et al., 2003; Nishiyama & Kato, 1999).

Currently, more data is emerging to support the basal position of liverworts in embryophyte evolution (Wang & Qiu, 2006; Groth-Malonek et al., 2005; Kawai & Otsuka, 2004; Wellman et al., 2003; Karol et al., 2001; Pruchner et al., 2001; Nickrent et al., 2000; Qiu et al., 1998) and these data are becoming increasingly more accurate and comparable. Hepatics are emerging as basal to the sister clade of all other bryophytes (Groth-Malonek et al., 2005) and will be accepted here. Recently, after reviewing over 650 papers dating from 1987 to present, Wang and Qiu (2006) stated that, "liverworts are clearly favored as the earliest divergent lineage of extant land plants according to emerging molecular evidence."

With this consideration of liverwort placement, where does the evidence place mosses and hornworts? As Shaw and Renzaglia (2004) point out, it has been recently postulated that hornworts, rather than mosses, are the closest living relative of tracheophytes, thus deviating from past studies positing the opposite (Kenrick, 2000 and references therein). This former hypothesis has commanded much support, however recent studies point to a joint sister group of mosses/hornworts to

tracheophytes (Groth-Malonek et al., 2005; Kawai & Otsuka, 2004). The phylogenetic tree for the Groth-Malonek study can be found as Appendix 1 with the proposed path of mycorrhizal evolution highlighted. These relationships among embryophytes support the non-mycorrhizal habit exhibited by mosses, a phenomenon to be evidenced later. As the following text intends to show, mosses evolved "differently" from their embryophyte and tracheophyte counterparts in that the omnipresent land plant reliance on, and association with, AM fungi was not kept, or "lost" in this group, ultimately allowing for a more competitive strategy with vascular plants, extant in ecosystems across the globe.

How does evidence from fungal phylogenies compare to illustrate an inter-kingdom coevolution between embryophytes and mycorrhizal fungi? Fungal evolution occurred during the fungus-animal split around 900 mya (Blackwell, 2000). The main terrestrial fungi diverged from aquatic chitrids approximately 550 mya (Berbee & Taylor, 1993). Lutzoni et al. (2004) attempted to assemble the fungal tree of life and determined a paraphyletic origin of the Chytridiomycota, the sister group to all other fungi, as well as a paraphyletic Zygomycota which evolved out of the chytrids. From the Zygomycota came the monophyletic Glomeromycota and ultimately the Basidiomycota and Ascomycota, the most derived phyla. The Glomeromycota, interestingly, was not considered a separate fungal phylum until 2001 (Schüßler et al., 2001) and currently contains the AM fungi with more that 150 described species.

The phylogeny of the Glomeromycota will solely be considered here because EM associations were not present at the time of terrestrial plant radiation; rather, EM

evolved later in response to numerous factors including tracheophyte secondary growth, established organic soils, and temperature/fluctuating environments (Malloch et al., 1980). EM will be addressed in a later section, with direct importance to the current study.

The oldest recorded AM fungal hyphae and spores were discovered by Redecker et al. (2000) in a dolomite rock formation in Wisconsin and dated at 460 million years old, corresponding closely in time with fossilized liverwort-like spores from 475 mya (Wellman et al., 2000). Other fossil evidence includes mixed AM colonies of *Gigasporineae* and *Glominae* in the cortex of *Antarcticycas* roots in Antarctica (Phipps & Taylor, 1996) as well as Rhynie chert colonization of *Aglaophyton* from 400 mya (Taylor et al., 1995), both of which lend support to early presence of AM associations. The claim by Taylor et al. (1995) was further supported by definite arbuscules identified in *Aglaophyton major*, an early Devonian plant lacking tracheids with secondary wall thickenings (Remy et al., 1994). These two latter papers indisputably determined an AM symbiosis with plants earlier than 400 mya, thus indicating an earlier evolution of the AM condition.

Molecular evidence confirms the fossil dates on the origin of the Glomeromycota. Simon et al. (1993) determined the origin of "AM-like" fungi to approximately 400 mya. Redecker et al. (2000) identified glomalean divergence to have occurred long before the time proposed by Simon et al. (1993) and place it along side the radiation of land plants 475 mya. Another analysis concluded that AM emergence occurred 490 mya (Berbee and Taylor, 1993), while Schüßler (2002) re-determined that *Geosiphon pyriformis* (Kutz.) v. Wettstein, the only known

cyanobacterial endosymbiotic associate, is in the Glomerales, thus indicating that the proposition of Pirozynski and Malloch (1975) is entirely probable and that AM-like associations existed even before land plants evolved. Wang and Qiu (2006) allude to the fact that mycorrhizal evolution may even predate embryophyte evolution if the "fungal association in the extinct charophyte *Palaeonitella*, as reported in Taylor et al. (1992), is mycorrhizal." These latter cases strongly support the inter-kingdom co-evolution of land plants with AM fungi.

In a comprehensive review on the evolution of mycorrhizal systems, Cairney (2000) provided further time scale data for AM evolution. He makes the statement that, "Arbuscular mycorrhizas evolved concurrently with the first colonization of land by plants some 450-500 million years ago and persist in most extant plant taxa." The dates presented above for both AM and embryophyte evolution fit together perfectly. Although one may not yet state definitively, as Cairney audaciously did, that plants radiated onto the land in tandem with AM fungi, the suggestions for such a coevolution permeate every facet of phylogenetic literature (Wang and Qiu, 2006; Heckman et al., 2001; Schüßler et al., 2001; Wilkinson, 2001; Read et al., 2000; Redecker et al., 2000; Blackwell, 2000; Cairney, 2000; Selosse & Tacon, 1998; Remy et al., 1994; Simon et al., 1993; Berbee & Taylor, 1993; Taylor, 1990; Pirozynski & Malloch, 1975).

The phylogenies of both embryophytes and glomalean fungi coincidentally parallel each other. The necessity for inter-kingdom cooperation to colonize land seems probable as the hardships faced by the first land plants included: (1) the fact that neither plant nor fungus was individually equipped to exploit a terrestrial habitat,

plants lack heterotrophic efficiency in nutrient extraction and water absorption while fungi lack autotrophic photosynthetic superiority (Pirozynski & Malloch, 1975); (2) the geometrical inadequacy of the underground axis in autotrophs at attaining nutrients in non-aqueous mediums required thinner absorbent structures such as fungal hyphae (Read et al., 2000); (3) the threat of pathogenic attacks on plants if not protected by a symbiotic partner, or buffer (Read et al., 2000); and (4) the direct exposure of typical habitats to intense solar radiation due to the absence of shading objects, leading to increased transpiration and thus the need for faster water acquisition methods provided by the mycobiont (Blackwell, 2000).

Pruchner et al. (2001) has developed an interesting inter-kingdom coevolutionary hypothesis. They looked at the intron sequences of mitochondrial genes and found that mosses share three group II introns with anthophytes, yet none with the complex thalloid liverwort *Marchantia polymorpha*. They deduced this relationship to signify differential intron gains from fungal sources in liverworts and other embryophytes. Qui et al., (1998) also notes that liverworts are the only land plants lacking particular introns at certain points of the mitochondrial genome, perhaps granting them basal embryophyte status, as previously discussed. This may mean that the early plant associates, glomalean fungi, may have contributed introns differently to different diverging groups and thus associated differently. Although horizontal gene transfer has never been reported in eukaryotes, it seems that research in the field is leading to confirmation of this phenomenon, or as Peter Gogarten has hypothesized, a “new paradigm for biology” (Gogarten, 2000). The Pruchner et al. (2001) study validates the theory that mosses are the sister clade to tracheophytes and

also supports the idea that the driving force to this sisterhood was either the mycorrhizal (tracheophyte) or non-mycorrhizal (moss) association chosen during evolutionary divergence and niche differentiation.

The data clearly support the theory of inter-kingdom coevolution between plants and fungi. Everything seemingly fits into place with mycorrhizal fungi aiding in bryophytic dominance of the initial paleoecosystem. This habit seemingly was passed on through evolution to the vascular plants from their direct ancestors, either hornworts or a sister group of hornworts and mosses. Mosses must not have assumed the mycorrhizal status, whereas higher plants did. If this non-mycorrhizal status is sound, there should not be extant any mycorrhizal moss taxa, whereas the liverworts and hornworts should exhibit mycorrhizal/mycorrhizal-like associations.

4. <u>Hepatic/Hornwort Mycorrhizal Associations</u>:

The idea that liverworts and hornworts evolved employing an arbuscular mycorrhizal relationship needs to be evidenced in extant taxa to be legitimized. Read et al. (2000) explains that hepatics and hornworts often form symbioses with fungi. This provides further reason to accept the idea that mycorrhizae were present at the very beginning of embryophytic land colonization. Added support for this type of symbiosis, to be dubbed mycorrhizal, stems from the fact that the same fungi form AM associations in vascular plants. Some may choose to call this association "mycorrhizoid" or "mycothalloid" but herein it will be considered mycorrhizal.

Russell and Bulman (2005) have documented a "specialized symbiosis" between the liverwort *Marchantia foliacea* and an AM fungus in the genus Glomus. Every thallus examined was colonized by aseptate fungal hyphae in the parenchymatous tissue, while hyphae in cells of the upper thallus were extensively coiled and surrounded by active arbuscules. This provides unequivocal evidence of AM fungal colonization in the basal most land plant group, the thalloid liverworts (Marchantiidae). Therefore it is wholly possible that fungi evolved concurrently with land plants.

Hornwort associations with AM fungi are also prominent in the literature. Boullard (1988) reported on AM associations in a number of hepatic families, described therein as mycothallic associations. One important paper documenting an AM-like symbiosis between *Glomus claroideum* and *Anthoceros punctatus* was that of Schüßler (2000). After 20 days, branched hyphae were apparent in the thallus; after 45 days arbuscules and vesicles were clear; after 60 days a transfer was made of the liverwort to a low nutrient agar substrate where the hyphae spread and formed new spores five weeks later; after four months over 1000 spores were formed in each Petri dish. This was the first time that a Glomalean fungus was found to associate with a hornwort under laboratory conditions, yet had previously been noted in the field (Stahl, 1949). This implies that as a sister group to the tracheophytes, hornworts could have been mycorrhizal before vascular evolution and thus transferred this habit to the remainder of succeeding embryophytes.

These data clearly show that liverworts, the most basal extant phylum, can form AM associations. Therefore the habit could have evolved during the radiation

of land plants or could even have even been present in the progenitors of land plants (Taylor et al., 1992). The data also confirm the ability of hornworts, a sister group to vascular plants, to form AM symbioses with all of the necessary distinguishing features of that association. This highlights the possibility for hornworts to have been more closely related to tracheophytes through the ability to form mycorrhizal associations.

5. "True" Moss/Arbuscular Mycorrhizal Associations:

The literature abounds with claimed "associations" between mosses (Bryophyta) and mycorrhizal fungi. For the most part these connections have not involved literal mycorrhization, and the unequivocal demonstration of AM fungal structures within moss tissue, namely arbuscules, have never been identified. It has been proposed by Read et al. (2000), in an evaluation of symbiotic fungal associations in "lower" land plants that only mosses and *Equisetum* appear to lack any sort of mycorrhizal or mycorrhizal-like structures. Selosse (2005) also states that mosses have no symbiotic fungi. Wang and Qiu (2006) further state that, "The continuous phylogenetic distribution of mycorrhizas throughout land plants, with the sole major exception of the mosses, tends to suggest that these plant-fungus interactions began when land plants originated." These aforementioned propositions support the theory presented with caution by C. Jeffery in that, "mosses may have arisen independently of fungi and presumably led to the diversification within this group" (Pirozynski & Malloch, 1975). Read et al. (2000) reported that it is of physiological interest that mosses appear to resist colonization by mycorrhizal fungi

so effectively. Some reasons for this include: (1) the poikilohydric status of mosses that usurps the need for mycorrhizal water acquisition from the soil by absorbing water directly from the air and rain while also possessing the ability to survive severe desiccation; (2) the high cation-exchange capacity of mosses that allows them to successfully bind and sequester nutrients in the quantities needed without the outside help of mycorrhizae; (3) and the fact that many mosses do not need protection from fungal and bacterial pathogens because they seem to have evolved successful anti-bacterial and anti-fungal phenolic compounds, especially in Sphagnum.

Although some papers have evidenced a mycorrhizal status in some mosses (Mago et al., 1992; Rabatin, 1980), Read et al. (2000) explains that, "careful scrutiny of the data has indicated that the fungi are confined to dead or moribund host cells and are thus almost certainly saprophytic or parasitic." A very recent study, however, has threatened to disprove the non-mycorrhizal status of mosses. Zhang and Guo (2007) contend that arbuscular mycorrhizal structures and fungi were found to be associated with 24 moss species belonging to 16 families in China and suggest that AM fungal structures commonly occur in most mosses. It seems curious that never before has anything of this nature been documented, let alone to this degree. The methods and conclusions are debatable and the authors ultimately concede that, "we cannot certify that the mosses formed a mutualistic symbiosis with AM fungi in the present study." More work is needed in this area as well as careful scrutiny of this data, therefore, the contention herein is that mosses are non-mycorrhizal, and uniquely so.

6. Ectomycorrhizal Development and Coevolution: Shaping the *Pinaceae*

With the conclusion of the AM symbioses, ectomycorrhizae will be the only mycorrhizal morphology to be discussed in detail hereafter. It must be noted that the pervasive AM symbiosis is thought, by the current author, to be the reason plants radiated onto and persist in terrestrial habitats. As evolution progressed, however, a new type of mycorrhizal association evolved under new selection pressures to further improve plant dominance in increasingly temperate/marginal environments; thus the ectomycorrhizal association was born.

Being the most frequent and widespread mycorrhizal type in the forests and woodlands of cool-temperate and boreal zones (Alexander, 2006), ectomycorrhizae appear particularly adapted to nutrient/water acquisition in these zones; especially where periodic nutrient fluxes to the mycorrhizosphere occur (Halling, 2001). EM communities are typified by low photobiont diversity with high mycobiont diversity, the antithesis of AM communities (Mallock et al., 1980). Regions harboring EM forests, in some cases, have an understory dominated by mosses, such as boreal forests and temperate coniferous forests. It seems curious how the EM association evolved in such a way.

The great range of taxonomic clades containing ectomycorrhizal fungi has led many to hypothesize that the habit arose independently numerous times (LePage et al. 1997); at a minimum in the largely ectomycorrhizal family Pinaceae, of which 95% are ectomycorrhizal (Newman & Reddell, 1987) and at least twelve times in various angiosperm lineages (Bruns & Shefferson, 2004; Mallock et at. 1980). Bruns and Shefferson (2004) note that the intriguing nature of this evolution indicates that the

complex morphology of EM fungi (introduction: part 1) must have been "invented" on multiple separate occasions, and lost on others. Concurrently, the diverse plant lineages would have had to assume this association independently. Hibbett et al. (2000) considers this gain/loss of ectomycorrhizal habit an indicator that mycorrhizae are unstable, evolutionarily dynamic associations. Regardless, EM fungal phyla, including the Zygomycota (*Endogone*) as well as diverse ascomycete and basidiomycete lineages (Bruns & Shefferson, 2004), indicate this dynamism must have temporally beneficial aspects to persist in extant taxa. The immense benefit EM fungi provide their photobiont will be discussed later, specifically in temperate coniferous forests of the Pacific Northwest.

The first unequivocal evidence of fossil ectomycorrhizae, from British Columbia, dates to 50 mya (LePage et al., 1997). A Hartig net extending to the endodermis, typical dichotomous branching of root tips, coralloid root clusters, a pseudoparenchymatous mantle, as well as simple-septate, contiguous extramatrical hyphae were observed in fossil roots of a presumed *Pinus* species. The morphological similarity of this mycorrhizal form to the extant genus *Rhizopogon* (LePage et al., 1997) can shed light on coevolution between the *Pinaceae* and this homobasidiomycete (mushrooms, puffballs, and allies) genus, suggesting a likely origin of the ectomycorrhizal symbiosis prior to 50 mya; the plant taxa currently employing the habit, namely the *Pinaceae*, evolved long before the Eocene. This assumption was substantiated by Berbee and Taylor (1993) in which their molecular clock of fungal evolution placed homobasidiomycete origin around 220 mya. The authors maintain that many of the same mushroom-forming basidiomycetes also form

ectomycorrhizal associations, predicting that EM fossils should date back to that time. Halling (2001) stated that ectomycorrhizal fungi likely diversified simultaneously in the Jurassic (206-144 mya) at about the time when EM gymnosperms were becoming established. With these evolutionary dates considered, how do they coincide with extant plant taxa represented in the temperate coniferous forests of the Pacific Northwest?

As the *Pinaceae* dominate the central-western Oregon Cascades (CWOC), focus will be placed on its evolution and the mycorrhizal symbioses within it. It was noted that fossilized material resembling existing *Pinaceae* dates from 200 mya, the late Triassic (Hibbett et al., 1997). This corresponds to the resupinate basidiocarp origin of about 220 mya as noted in Cairney (2000) and proposed by Berbee and Taylor (1993). Interestingly, a third event, the breakup of Pangea and major continental creation/shift occurred at about the same time (Triassic) as the evolution of ectomycorrhizal fungi and the extant *Pinaceae* (Bortolotti & Principi, 2005). Genetically, it seems that even EM associations in the *Pinaceae* are related with ancestral AM conditions; seedling stages of the typically EM species *Pseudotsuga menziesii* (Cázares & Smith, 2004; Cairney, 2000) and *Tsuga heterophylla* (Cázares & Smith, 2004) have been observed to show AM infection. This provides further reason to believe that EM coevolution with the *Pinaceae* subsequently diversified and dispersed them among worldwide habitats. However, in EM associations, host specificity has evolved with different fungal symbionts (Wang & Qiu, 2006) and can thus explain specific adaptations to particular environments.

What then is the adaptive advantage conferred to the *Pinaceae* by coevolving with EM fungi, an association that subsequently enabled them to dominate the landscape? Prior to the emergence of the *Pinaceae* the habitats they currently occupy were either uncolonized, had not emerged yet (through volcanism, continental drift, and uplift), or were colonized by poor competitors not designed for proliferation under such conditions, thus their eventual extinction or range shift. From the Miocene to the beginning of the Holocene, 10,000 years ago, there was a significant diversification and increase in the number of *Pinaceae* species along with an increase in geographic range (LePage, 2003), which, as the author explains, is linked to habitat creation due to mountain-building events and subsequent global cooling.

The Cascade Mountains, part of the Western Cordillera and mostly volcanic in origin with basalt/andesite composition, began to form during the middle Miocene (LePage, 2003). This created novel, harsher environments in the Pacific Northwest subject to colder temperatures and countless environmental stresses, especially at the highest elevations. As LePage (2003) quotes Read (1984), "the mycorrhizal strategy employed by plants broadly corresponds to the environment in which they occur." This makes functional sense; tropical environments that are dominated by AM associations have high turnover rates and warm temperatures, whereas temperate/high-altitude environments dominated by EM associations are colder, more seasonal, and have biomass accumulation greater than decomposition (LePage, 2003). As Smith and Read (1997) explain, soil nitrogen and phosphorus are less extractable to plants in these soils, thus the need for EM fungi to aid in mineral acquisition.

Considering the two tree species of greatest importance in the forests of the CWOC at middle elevations, *P. menziesii* (Douglas-fir) and *T. heterophylla* (western hemlock) in the *Pinaceae*, it seems that EM fungi may have significantly contributed to the structure of this association. LePage (2003) and references therein, eloquently highlight the evolution and biogeography of the *Pinaceae* and discusses reasons for the current composition of ecosystems dominated by species in this family. Notably, all but one species is endemic to the northern hemisphere; the others occur throughout the boreal, montane, and subalpine of North America in greatest abundance, as well as the Pacific Northwest evergreen coniferous forests (Waring & Franklin, 1979).

It was during the Late Cretaceous and Early Tertiary that modern genera of the *Pinaceae*, aside from the basal clades, first appeared. An evident conclusion by LePage (2003) from examining conifer phylogenies was that the *Pinaceae* is distinguished from the other families. Wang et al. (2000) determined that the most basal clade to all other genera in the family is *Cedrus*, in turn giving rise to the *Larix-Pseudotsuga* clade, which is sister to the *Pinus* and *Picea-Cathaya* clades in the more derived taxa, as well as the *Tsuga-Nothotsuga* clade in more basal taxa. These relationships are illustrated in Appendix 1. The earliest known fossils of *Tsuga* in North America are from the Eocene and include seeds, cone scales, and seed cones (LePage, 2003). *Pseudotsuga* fossil presence in North America dates to the Eocene as well (Hermann, 1985); interestingly, the modern *Pseudotsuga* needles, cones, and seeds are almost indistinguishable from their ancestors highlighting temporal similarities. *P. menziesii* and *T. heterophylla* have become closely associated since their respective genera emerged during the Eocene and is likely coincidental with the

evolution of the EM symbiosis in each. It becomes curious then as to how the associations present in the CWOC developed and, the extent to which they depend on each component part.

With ectomycorrhizal coevolution occurring many times between woody plants and saprotrophic fungi, during or before the Cretaceous, it seems likely that this association was a compromise with the changing climate to proliferate both plant and fungus. It has even been suggested that mycorrhizal migration may have been the rate-determining step in some plant migrations, especially in modern times (Wilkinson, 1998). As Cairney (2000) concludes about mycorrhizal evolution, "ongoing parallel evolution of the partners in response to environmental change on both widespread and more local scales may most readily explain extant patterns of mycorrhizal diversity and specificity." This association has undoubtedly helped build the structure of many extant forest communities, especially in temperate zones, that are familiar today.

7. The Central-Western Oregon Cascades: Why do Forest Floor Feather-Mosses Matter and What is their Function in the Ecosystem?

Ecosystem biologists are realizing that bryophytes may play an integral role in nutrient cycling, water retention, and water availability in ecosystems (Glime, 2006), serving as effective traps for water and nutrients (Turetsky, 2003). Mosses likewise have great influence in areas where they are most abundant. The ecological roles of mosses have not been explored in depth and further study is needed to fully appreciate their complex interactions with ecosystem structure and function. New

pathways of energy and nutrient flow may become apparent when mosses are studied and scrutinized in the ways other aspects of forested ecosystems already have been.

The CWOC, especially in the region studied, is known to support a uniquely robust moss community due to its generally moist climate and dominance by coniferous trees. Binkley and Graham (1981) documented that mosses account for 20% of the biomass (1075 kg/ha) and 95% of the photosynthetic tissue in an Oregon Cascade forest understory at the H.J. Andrews Long Term Ecological Research Center (HJA). *Eurhynchium oreganum* and *H. splendens* were found to comprise 99% of the biomass of the terricolous moss layer, 92% and 7% respectively. From this study the authors concluded that, "…moss biomass can represent an important portion of total production and nutrient cycling and should be considered in studies of ecosystem function." This is a clear indication of the substantial role forest floor mosses fill as an ecosystem component in the CWOC; but in what specific ways may mosses influence ecosystem processes?

The research sites described hereafter have a forest floor dominated by *E. oreganum*, *H. splendens* (Binkley and Graham, 1981), and *Rhytidiadelphus spp.* (Rambo and Muir, 1998), which are categorized as feather-mosses. It should be noted that species-specific differences in ecological roles do exist among feather-mosses (Bates, 1994), but overall the water and nutrient acquisition/retention strategies of pleurocarpous feather-mosses are presumed to be similar and may contribute to their potentially significant role as regulators of soil microclimate and overall dynamics.

The extent to which feather-moss mats cover the forest floor of the CWOC is known to heavily influence soil microclimate. To put it into perspective, the moss mat can be visualized as a literal boundary between atmosphere and soil through which anything entering the soil must pass (e.g. a filter). There are many influences that forest floor moss cover (FFMC) may have on soil microclimate; however, their effect on the major functions of EM fungi, water and nutrient acquisition for host plant(s), will be considered herein.

Mosses have high water holding capacities (WHC), therefore the effects on soil moisture must be considered. On one hand, FFMC can initially intercept all forms of precipitation, thus depriving the soil of moisture, while on the other they can increase soil water retention by creating a buffer that reduces evaporative losses (Glime, 2000). Schofield (1985) contended that when there is dew or rainfall of short duration, the bryophytes can absorb all of it, depriving the roots of any (noted by Glime, 2000). Feather-mosses were found to insulate the mineral soil beneath them in the discontinuous permafrost zone of interior Alaska (Bonan, 1991), thus decreasing evaporation, stabilizing soil temperature fluxes, slowing biological activity, and influencing rates of percolation. Glime (2000) also noted that a study by van Tooren et al. (1985) found soil moisture in a patchy chalk grassland was 2-4% higher beneath bryophytes, a percentage that would likely increase in a system with less patchy cover.

Feather-mosses, virtually all of which are ectohydric (Glime 2006), have a large amount of smaller capillary spaces formed by overlapping leaves and shoots which trap water, or, to put it more simply, they act as the “sponges” of the forest. As

in sponges, direct contact between moss and water must be established for absorption; once in contact, capillary action can transport that water to other areas of the moss, however, lack of roots prohibits water uptake from deep in the soil. Significant moisture sources available to mosses include rain, dew, stemflow, runoff, snowmelt, and throughfall, all of which contact moss before soil. It seems as if the prevalence of mosses may influence water availability in the mycorrhizosphere, and regulate water available to tracheophytes.

Furthermore, mosses have an innate propensity to sequester nutrients/ions that enter the system, thus making them unavailable in the soil for some time. The acuticular, unistratose lamina (leaves) of ectohydric feather-mosses immediately absorbs the moisture they are exposed to, able to reach complete hydration in a matter of seconds/minutes (Glime, 2006); that very moisture contains dissolved nutrients. Mosses can also acquire some nutrients from the substrate that contacts the gametophyte (Glime, 2006; Økland et al., 1999; Binkley and Graham, 1981; Bates and Farmer, 1990). Whether it is from non-root inhabited apical soil zones or atmospheric deposition, including dry deposition, mosses must be quick to absorb these essential elements, as well as sequester them to facilitate their competition with tracheophytes in the system. If mosses are accessing nutrients before they become available to tracheophytes, how proficient are they in holding onto those nutrients and what effects does this have on the ecosystem?

Nutrient sequestration by mosses is accomplished in many ways, foremost it seems by the high cation-exchange capacity (CEC) of their cell walls, which is a non-metabolic and selective absorption of cations (Koedam & Büscher, 1983). It results

from cell wall sites that have large concentrations of polygalacturonic acid (Kimmerer, 2004; Büscher et al., 1990). A protruding carboxyl group ($COOH^+$) freely exchanges its H^+ for other cations (Glime, 2006). The bound cations remain at exchange sites, sequestered, until they are absorbed into moss tissue or are less frequently released to the soil.

Glime (2006) compared data, from two previous studies, on the CEC of bryophyte gametophores and tracheophyte root cell walls. The moss with the most inefficient CEC was almost five times more effective at binding cations than the tracheophyte with the most efficient CEC; the most efficient moss was 31.6 times more effective. In a nutrient absorption study, Koedam and Büscher (1983) confirmed that mosses have selectively preferential cation-exchange sites, favoring divalent (Ca, Mg) over monovalent cations (K, S) when offered in similar amounts.

Moss tissue also acts as a strong chelating agent (Turetsky, 2003 – reference therein) and may thus sequester essential metals, making them unavailable in the soil. For a long time, mosses have been considered a viable estimate for atmospheric trace element deposition because of their high nutrient holding capacities (Berg et al., 1995). This is especially true for metal deposition (Berg & Steinnes, 1997). The metal complex formed in chelation is stable and not readily dissolved or released from chelation sites, ultimately stored on/in moss tissue.

Other ways that mosses obtain nutrients, thus denying them to the soil, abound. These include: (1) nocturnal distillation, where mosses can “steal” nutrient rich soil water typically reserved for tracheophytes and condense it on active photosynthetic tissues where the nutrients can be used for new growth or stored

(Carleton & Dunham, 2003); (2) nutrient translocation, via external capillary action or inter/intracellular transport, from senescent/moribund tissues litter to new growth (Skre & Oechel, 1979); and (3) mineralization-immobilization on moss tissues (Weber & Van Cleve, 1984). With all of these unique nutrient sequestration abilities, what is the efficiency at which forest floor moss cover may deny the tracheophytes of nutrients?

The atmosphere is not the sole source of nutrients to the soil. Tracheophytes can therefore receive their nutrition through mineral weathering, decomposition of organic matter already present in the soil, nitrogen-fixing microbes, and various other sources. However, it seems as though FFMC has the potential to limit nutrients outside the immediate system from reaching the soil. This can be seen in countless studies that document the exceptional ability of mosses to sequester nutrients, and the ectomycorrhizal fungal component of the system may be consequentially affected.

As Hart and Parent (1974) stated, chemicals are delivered in a dissolved form with precipitation or adhere to particulate matter in the air only to be deposited as dry fallout between storms. These chemicals may become even more concentrated due to canopy capture. They measured concentrations of sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), and nitrate (NO_3^-) under *P. menziesii* throughfall in Utah as compared with precipitation in the open. It was found that concentrations were 3-16 times greater beneath the *P. menziesii*, indicating that plant canopies enrich the chemical composition of precipitation falling beneath. Weetman and Timmer (1967 and references therein) noted that the cation nutrient concentrations in solutions washed from tree canopies was easily absorbed by living

moss segments of *H. splendens*. If atmospheric deposition/precipitation is passing through terricolous mosses before reaching the rest of the system, what significance does this have? Whether in a canopy-free area or beneath a dense mix of tree branches, forest floor mosses are still the foremost recipients of this input in the CWOC. So where do the nutrients end up?

Oechel and Van Cleve (1984) found that for the combined input of N, P, Ca, Mg, and K, in the throughfall and litterfall of an Alaskan black spruce forest, bryophyte accumulation always exceeded deposition, except in the case of Ca; thus the surface ion exchange capacities for all mosses studied showed the potential for element retention to be substantially greater than the total flux to the forest floor. This indicates that in a system with a healthily robust feather-moss mat, the soil was being deprived of all atmospheric nutrient deposition, while at the same time having nutrients taken from it by the mosses. In this system at least, forest floor moss cover was significantly inhibiting nutrient availability for tracheophytes.

In an experiment by Bates (1989), that assessed the uptake and retention of P and K by the feather-moss *Pseudoscleropodium purum* in wet deposition, it was found that only 6.3% of the P and 12.1% of the K added to the system over 74 days was retrieved in the moss throughfall. Although levels in the living moss tissue did not account for the missing nutrients, it was hypothesized that the elements bound in litter and by microorganisms may eventually return to living moss tissue by mass flow nutrient translocation over time, and thus be unavailable in the soil. Interestingly, this heavy P and K sequestration occurred in a moss that is known to have a low capacity to utilize nutrients received in wet deposition. The capacity of

other mosses that can better sequester nutrients in wet deposition, therefore, can limit nutrient entry into tracheophyte-accessible soil layers even further.

Bates (1990) determined that *P. purum* had the ability to retain "luxury" amounts of orthophosphate in its tissues. As a limiting nutrient to plant growth, P scarcity would likely result in EM recruitment by plants. This study indicates that feather-mosses have the ability to differentially bind and sequester limiting nutrients for future use, thus limiting the trace amounts of phosphorus that enter the system from the atmosphere even further, perhaps never even reaching the soil under certain circumstances.

Another study by Bates (1994) attempted to wash cations out of *P. purum* with the application of distilled water mists, applied 8 times/day, over the course of 10 weeks. It was found that *P. purum* effectively conserved K, Ca, and Mg during this period; however N and P were not conserved as well. This indicates that this feather-moss is able to significantly retain metal cations even when tissue flushing is attempted, giving further weight to the efficient CEC forest floor feather-mosses may have.

In terrestrial systems, the most limiting element to plant production is nitrogen (N). It is a component in chlorophyll, plant genetic material (DNA/RNA), amino acids which build proteins, enzymes such as RUBISCO, hormones, and numerous secondary metabolites such as alkaloids. Forsum et al. (2006) identified the important nitrogen sources to mosses in boreal forest throughfall were ammonium (NH_4^+), nitrate (NO_3^-), and amino acid N. Weber and Van Cleve (1983) found that 30-100% of the nitrogen isotope N^{15} applied to boreal feather-mosses, including *H.*

splendens, was immobilized, retained in the moss mat, and slowly released to the soil horizons below. They concluded that the feather-moss layer acted as a filtering agent and severely limited N export to the soil for vascular plant uptake. Thus, feather-mosses, including the *H. splendens* present in the current study, acted as a nutrient sink for the ecosystem.

Eckstein and Karlsson (1999) evaluated the pattern of N^{15} movement in *H. splendens*. They found that current year segments were a strong sink for nitrogen; while one-year-old segments increased their N^{15} pool (i.e. sequestered more). The segments older than two years lost 50% of the N^{15} initially taken up; however all of this lost N^{15} was recovered from the current growth and one-year-old segments. They note that the redistribution of N^{15} to new growth in *H. splendens* helps to reduce N losses from moss to soil, and may lead to increased residence time of N in ecosystems. This provides solid evidence for efficient nutrient retention and flux control by forest floor feather-mosses, particularly in terms of N.

Considering substrate nutrient uptake and sequestration, Bates and Farmer (1990) showed that the feather-moss *Pleurozium schreberi* exhibited bi-directional movement of inorganic Ca ions between moss and soil through intercellular transport, indicating that mosses could acquire Ca from the soil to be used in growing apical portions, thus taken up and sequestered from the system. Økland et al. (1999) showed similar significant nutrient uptake by *H. splendens* from water that had been in contact with the soil. Binkley and Graham (1981) noted that *E. oreganum* and *H. splendens* had only 75% of their nitrogen content accounted for by rainfall. All of

these findings support the claim that mosses acquire nutrients from the substrate they contact, like a sponge, and similarly sequester those nutrients.

An interesting finding by Weetman and Timmer (1967) indicated that, in an upland black spruce forest, the nutrient concentration in the green feather-moss segments tended to increase with decreasing light intensity. This means that as light becomes a limiting factor to growth, such as is the case in a forest with heavy canopy cover, moss nutrient storage tends to increase. Perhaps this may be an attempt to decrease the probability of nutrients limiting future growth. Therefore, it seems pertinent to assess canopy cover when moss nutrient dynamics are an essential part of the scientific question.

It seems as though data support the fact that forest floor mosses act as reservoirs/sinks for nutrients entering forest systems, strategically binding them among their tissues and preventing their quick release to the soil. Further evidence of this phenomenon will be presented later, when effects of mosses on ectomycorrhizal fungi are considered. However, even though mosses seem exceptionally proficient at nutrient sequestration, is there any leaching of bound nutrients from mosses, and if so what is the significance?

Along with the ability of mosses to store nutrients comes the nutrient leakage inherently associated with their physiology. Moss poikilohydry leads to severe desiccation during times of insufficient rainfall. During a desiccation event cellular membranes lose integrity and leach certain ions/nutrients upon rehydration (Bewley 1979); most of the leakage occurs in a large pulse during the first two minutes of rewetting (Gupta 1976) and increases with the length of the rain event (Turetsky

2003), where leakage quantity depends on the mosses ability to repair photosynthetic and protein synthesizing machinery (Bates 1992). Although leaked nutrients can be reabsorbed by moss tissue and bound again after rehydration (Gupta 1976) some may enter the soil beneath. Dissolved organic carbon (DOC), nitrogen, phosphorus, potassium, other essential elements, DNA, RNA, amino acids, phospholipids and proteins may be pulsed from mosses upon rehydration (Turetsky 2003). In the Pacific Northwest, spring and fall months bring the most snow melt/rain (Figure 2) and may be correlated with seasonal nutrient leakage from mosses. Therefore, the nutrients retained in moss tissue may be seasonally pulsed to the soil in predictable snow-melt/rain events, or trapped and released in small quantities throughout the year during small, intermittent precipitation events.

Desiccation is the process of drying up entirely whereas the antithesis is rehydration (Proctor, 2000). There are three ways by which organisms deal with desiccation, which include evasion, avoidance, and tolerance (Glime, 1993). Tracheophytes can be considered drought avoiders (homeohydric) by internally regulating cellular moisture. Bryophytes typically exhibit tolerance, defined as the ability to survive and maintain their activity despite water stress, while simultaneously trapping moisture in their gametophytic undulations (Glime, 1993). There is an inherent need, however, to protect against cellular damage caused by constant drying and rewetting from the poikilohydric habit. If moss rehydration mechanisms were not more efficient than those of the tracheophytes, cells would die, thus killing the individual; severe competitive disadvantages would therefore exist, which are not present naturally; the result would be mosses being out-competed by

tracheophytes. Rather, to compete with tracheophytes, mosses have evolved an effective desiccation tolerance strategy, one that involves repair, differential reabsorption of leached elements (initial reabsorption being more efficient), and rapid photosynthetic recovery (Proctor, 2000). It must be noted, however, that the speed of desiccation events are important to the degree of cellular damage incurred. Typically, most cellular damage is found in bryophytes that are rapidly-dried rather than those that are slowly-dried (Gupta, 1976; Proctor, 2000). This is logical because a cell requires time to protect itself from desiccation damage.

When a cell becomes desiccated numerous events occur, including protoplasmic shrinking, leaving a gas filled cell lumen; photosynthetic arrest whereby chlorophyll becomes bound to a protective protein; decrease in respiration; and cessation of protein synthesis (Procter, 2000). Moss cells remain in this state until the next precipitation event or water transport by less-efficient means occurs. In order to maximize desiccation tolerance, a moss must recover and physiologically respond rapidly. These rehydration responses include: (1) limiting carbon loss during desiccation and initial rehydration; (2) maximizing carbon synthesized at low water contents – photosynthesizing at low water levels; (3) speedily repairing cellular damage incurred during rehydration; (4) limiting nutrient loss upon rewetting; (5) limiting physiological deterioration during rewetting; (6) withstanding multiple dry-wet-dry periods; and (7) controlling the rate at which drying occurs via growth form, morphology, and anatomy (Kimmerer, 2004; Proctor, 2000).

Now that all of these points are understood, it is appropriate to take a closer look at each of the important physiological processes that occur during desiccation

and rehydration on a cellular level, to see how mosses recover from an essential "dry death" and the possible ecosystem implications of this habit. Specifically, the effects of desiccation on protein synthesis, cellular respiration/photosynthesis, and cell membrane stability and support will be addressed.

Desiccation tolerant mosses are affected by dehydration on a regular basis; the capacity to synthesize proteins decreases as water is lost, however, it is easily regained when rewetted. This is a trait not common in tracheophytes, which reach a permanent wilting point and subsequently die. The stability of polysomes (ribosome aggregations actively translating mRNA into polypeptides) is very important to the re-initiation of protein synthesis and varies with the speed at which a desiccation event occurs. In rapidly dried mosses approximately half of the polysomes present in the non-desiccated control were retained, whereas none were retained in the slowly dried moss (Bewley, 1979). It is hypothesized therein that the principal cause of polysome loss during desiccation is the runoff of ribosomes from mRNA in coordination with failure to reform the initiation complex. Thus, during a slow-drying episode runoff is allotted more time to occur and leaches all polysomes off mRNA complexes; however, in fast-dried mosses critical water loss finishes before runoff can be detrimental, leaving the polysomes within the cell (Bewley, 1979). Interestingly, it is still debated why mosses with no remaining polysomes resume protein synthesis faster than one with in-tact polysomes, especially if the slow-dried moss has to recombine separated mRNA with ribosomes. This resumption of protein synthesis after being in a completely dried and contorted state is unique to

bryophytes. It is likely a major contributor to their proliferation alongside tracheophytes in many environments.

Respiration is the process by which cells breakdown organic compounds in the mitochondria to make ATP, a usable energy source. Photosynthesis is the opposite process in which CO_2 is removed from the atmosphere and synthesized into organic compounds in the chloroplast. Photosynthesis, in particular, requires the presence of water; the photolysis of water in the light reaction could not occur without it. To maximize production of photosynthate while being poikilohydric, mosses tend to exhibit a spike in photosynthetic rate at "less-than-saturated" levels (Proctor, 2000). This ensures excess photosynthate build up prior to desiccation, thus balancing rehydration stress. Significant photosynthate has also been found to leach out of moss cells upon rehydration due to the permeability in cell membranes prior to repair (Proctor, 2000). Respiration bursts upon rehydration are necessary to generate enough energy to synthesize and reabsorb leached entities. Upon rehydration, mosses exhibit a spike in oxygen consumption for approximately 24 hours, known as "resaturation respiration" (Bewley, 1979); rapidly dried mosses nearly double their consumption while slowly dried mosses only moderately increase consumption.

Significant structural changes in plant cells also occur with desiccation. The protoplasm, predominantly composed of water, completely shrinks and clumps together inside the cell membrane. The phospholipid bilayer, when not in the presence of water, loses its hydrophobic/hydrophilic arrangement and becomes slightly dissociated from itself, leaving holes through which substances can leach out. The high CEC of moss cell walls, along with other processes, function to trap much

of the leached cations and retain them for future reincorporation into the cell. But is this reincorporation significant or do mosses still lose a good proportion of their sequestered elements upon rehydration?

Bates (1997) studied desiccation effects on nutrient leakage of two ecologically contrasted mosses. It was observed that when N, P, and K were applied, the quantities of nutrients reabsorbed by the mosses followed this order: initial rehydration stages, end of rehydration episode, and middle of the hydration period. This indicates that with an increased rehydration period the moss under investigation reabsorbed fewer nutrients due to a longer mid-rehydration time.

Although Gupta (1979) recorded significantly large leaching rates for four ecologically contrasted mosses, the major flaw of this laboratory study was noted and seemingly accepted. Moss specimens were subjected to laboratory submergence in a great excess of water which would never occur in nature. Gupta states that "although this yields a picture of the maximum potential rate of loss… rainfall would normally be absorbed instantaneously by dry shoots, and the very high WHC of most bryophyte wefts or cushions would ensure that excess water would not be available to act as a leaching medium for a considerable period of time." For 90%, 72%, 58%, and 10% of labeled solutes to be washed from mosses (of differing desiccation tolerances), as was seen in this study, torrential and prolonged periods of rain would have to occur following exceedingly long drought periods. This phenomenon does occur in the CWOC; however, not with the periodicity that would have substantial impacts on moss leachates.

A very recent paper by Startsev and Lieffers (2006) tested the capacity of feather-mosses to release N to water and reabsorb it within 64 hours. The mosses were submersed in distilled water. The fully hydrated pre-treatment mosses showed no nutrient leakage, while the dehydrated pre-treatment mosses lost only 8% of their total N within two hours of rehydration; however, over the course of 16 hours, two thirds of the leached N was recovered. Startsev and Lieffers interpreted these data to suggest that "the strong ability of mosses to quickly re-absorb released N from surrounding solutions suggests that leakage of N from dried mosses after rewetting, as a source of N to the ecosystem, is not as large as suggested by previous literature." This is of exceptional importance and validates the idea that forest floor feather-mosses act as a boundary layer between soil and atmosphere, exerting a heavy influence on nutrient fluxes in ecosystems where they abound.

Let us finally touch on moss decomposition by saprobic/mycorrhizal fungi as a source of nutrient addition to the soil. Mosses do not produce lignin, which has poor organic matter quality; therefore it is assumed that moss litter quality would be higher and decay more rapidly, being quickly recycled to the system. In reality, moss organic matter is very slow to decompose, being found to decompose slower than tracheophyte litter in many systems, including a Scots pine forest (Liu et al., 2000). Oechel and Van Cleve (1986 and references therein) contend that moss litter decomposes at 1-10% of the rate of tracheophyte litter. As Turetsky (2003) suggests, this could be due to large phenolic and nonpolar compound concentrations in mosses.

Weetman and Timmer (1967) estimate a time span of four to eight years for the nutrients stored in *H. splendens* to be released to the system by the decomposition

of old moss segments, estimated by comparing the proportion of nutrient weight in one year's moss growth as compared with that of dead moss. This is assuming of course, that the mosses will not reabsorb and translocate any of those released nutrients back into live tissues via mass transport.

Feather-mosses appear to play very significant roles in ecosystems where they are in high abundance, particularly in moisture and nutrient dynamics; forest floor feather-mosses in the CWOC likewise have a similar affect. Eckstein (2000) even states that, "large feather-mosses of the forest floor may act as autogenic ecosystem engineers." This means that, "mosses may directly or indirectly modulate the availability of resources to other species by causing physical state changes in biotic or abiotic materials (Jones et al., 1994). This situation may affect a significant pathway not yet elucidated in any ecosystem, that from moss to mycorrhizal fungus to mycorrhizal plant. How, then, would ectomycorrhizal fungi react to feather-mosses carpeting the forest floor of CWOC forests?

8. <u>The Role of Ectomycorrhizae in Central-Western Oregon Cascade Processes and their Function in the Ecosystem</u>:

It has already been discussed that: (1) mosses are, from the preponderance of evidence in the literature, non-mycorrhizal; (2) clades closely related to the mosses form mycorrhizal associations with AM fungi; (3) ectomycorrhizal fungi evolved later in evolutionary history than AM fungi and tend to associate with plants in marginal habitats; (4) the forests of the CWOC are dominated by members of the *Pinaceae*, of which approximately 95% are ectomycorrhizal; and (5) feather-mosses

carpet the forest floor in the CWOC and act as highly efficient filters for nutrients and water that enter the system from areas external to it. It is also known that the climate of the CWOC results in severe summer drought and abundant winter rain. With all of these points considered, what is the function of EMF in ecosystem processes and how may this relate to FFMC at the sites studied? The following sections will attempt to determine how FFMC may influence EMF dynamics in the soil beneath, whereas this section will specifically focus on EM fungal nutrient and water dynamics.

Ectomycorrhizal fungi perform innumerable ecosystem functions worldwide, as well as in the CWOC. The most abundant trees at sites in the present study (table 2) are known EM species (Wang & Qiu, 2006; Newman & Reddell, 1987), thus reflecting EMF importance in the CWOC. It is only now, with advances in molecular techniques, that these ecosystem functions are being elucidated and understood. A diagram of mycorrhizal associations in northern hemisphere systems is presented as Appendix 2. As Horton and Bruns (2001) contend, we are at the forefront of a revolution in ectomycorrhizal ecology, a revolution that has discerned a great deal in regards to the ecosystem dynamics of EMF.

Nutrient and Water Relations of Ectomycorrhizal Fungi:

Ectomycorrhizal fungi predominantly serve as nutrient absorption organs for their host plants, in which inorganic and organic forms of N, P, and trace elements are absorbed and translocated in return for plant photosynthate. Nitrogen and phosphorus are certainly the two most important nutrients which EMF obtain for their hosts (Read & Moreno, 2003). Soil water acquisition is a second major function of EMF,

especially when soil moisture is low. A diagram and table on the role of mycorrhizae in nutrient acquisition is presented as Appendix 3. For the purposes here, these two primary functions will be considered; ectomycorrhizal fungi can be regarded, herein, as extensions of host root systems that assist in the survival of their associate(s) and thus are "…overwhelmingly the most important absorbing organs of ectomycorrhizal trees," (Harley 1978).

The physical attributes of EMF that confer advantages to the host(s) include: (1) the greater surface-to-volume ratio fungal hyphae exceed that of roots; (2) the reduced carbon cost of producing a hypha with similar absorptive capabilities as a root; (3) the ability to explore and exploit the soil more effectively; (4) carbonic acid leechates; and (5) that EMF bind nutrients more effectively than non-mycorrhizal roots via fungal specific enzymatic reactions, including high cation exchange capacities. The specific acquisition of nutrients and water will now be discussed.

Most studies of EM fungal nutrient acquisition have focused on N and P, however EMF have been found to play important roles in the uptake of most essential nutrients, particularly K and cations, for use by the fungus and associated photobiont(s). Other studies have found that the employment of EM fungi by plants returns a greater energy return on investment (EROI) than a non-mycorrhizal habit. It also seems as if EM nutrient sources are much more diverse than those available for fine plant roots, which include: (1) atmospheric deposition and substrate absorption of simple mineral ions including ammonium, nitrate, phosphate, and cations; (2) organic intermediates including amino acids, DNA, and simple sugars; (3) possibly structural and nutritional polymers including lignin, cellulose, and protein; (4) possibly natural

substrates including litter, necromass, and woody debris, and finally (5) recalcitrant mineral mobilization from bedrock and soil rocks. Much of the ability of EM fungi to absorb nutrients comes from surface enzyme production. Studies supporting the aforesaid claims will now be evidenced, and the effects of FFMC will be considered.

Overwhelming evidence in the literature suggests that EM fungi are more efficient at attaining nutrients than non-mycorrhizal roots. Phosphorus is undoubtedly the most studied nutrient in this regard. Kramer and Wilbur (1949) found that when P^{32} was applied to excised roots of *P. taeda* and *P. resinosa*, EM portions of the roots accumulated much larger quantities of P than non-mycorrhizal portions, and they accumulated those quantities at faster rates. A similar study by Harley and McCready (1950) looked at *Fagus* roots and determined the EM tips had 5 times the rate of P absorption as did non-mycorrhizal roots. Bowen (1973) determined that the uptake of N, P, and K by ectomycorrhizal *Pinus strobus,* compared to non-mycorrhizal *P. strobus,* were 1.8, 3.2, and 2.1 times more efficient, respectively. It was also found that *P. pinaster* had twofold higher uptake rates for NO_3^- in vitro (Plassard et al., 1994). Other studies have constructed efficiency models to assess the validity of the former results.

Yanai et al. (1995) presented a quantitative comparison of nutrient acquisition efficiency by fine roots and mycorrhizal fungi of trees. They used a biophysical model of the soil-root system, defining the efficiency of nutrient acquisition as "…the amount of carbon expended per unit of nutrient taken up, averaged over the lifetime of the root." They determined that if the C cost per unit mass and P uptake kinetics for roots and ectomycorrhizal fungi were considered to be equal, then the efficiency

of hyphae in nutrient acquisition is orders of magnitude greater than non-mycorrhizal roots (Appendix 4). This indicates that in soils of limited nutrient availability, EMF would be more prolific to balance the cost of nutrient acquisition for the host plant(s). It would not be advantageous for the trees in nutrient-limited systems to invest so heavily in roots when EM fungi perform a far more efficient job.

Allen et al. (2003) constructed a model to describe the increasing effects of the complex mycorrhizal community on plant productivity. It was found that EMF accessing inorganic material increased P uptake by one unit, water uptake by 50%, and drew one unit of carbon from the host in return, when compared to a plant root. Ectomycorrhizal fungi accessing organic material increased the N and micronutrient supply to the plant by one unit each, took up 80% more water, and drew one unit of carbon in return. This model, although hypothetical, evidences the greater efficiency of EMF nutrient/water acquisition in return for host C investment.

Tuomi et al. (2001) used another cost-benefit model to assess the benefits of ectomycorrhizal associations to host plants. Their phytocentric model assumed that colonization percentage evolved towards an optimum which maximizes plant growth or fitness. They noted that although the cost-efficient EM habit will confer benefits to mycorrhizal over non-mycorrhizal plants, in terms of carbon cost per unit of acquired mineral nutrient, the EM association may even evolve under less-beneficial circumstances, provided that photosynthesis and/or growth are nutrient-limited. They note that EM plants can be superior even in conditions where non-mycorrhizal short roots are more cost efficient than mycorrhizal ones. This indicates the high value of mineral nutrients acquired for carbon assimilation by EMF; the plant would give up

additional carbon to its EM fungal associate(s) to access more nutrients and increase potential assimilation.

It has already been shown that feather-mosses have a high capacity to sequester cations. Ectomycorrhizal fungi have also been shown to have a high CEC, far greater than that of plant roots. Marschner et al. (1998) found the CEC for two EM fungal isolates, 2000-3000 μmol g^{-1} and 800-1200 μmol g^{-1}, to significantly exceed the CEC for EM tree roots, 60-700 μmol g^{-1}. They concluded that the high CEC of the fungal mycelium can be explained by the high surface area per unit weight, and that EMF could thereby substantially enhance nutrient uptake of trees. This suggests that if cation addition to soils is already limited by mosses, as the case may be in the CWOC, ectomycorrhizal fungi would be better at accessing these rare/pulsed nutrients than uncolonized roots, conferring a preferential advantage of mycorrhization beneath FFMC.

The extraradical mycelium of EMF can also significantly regulate soil water absorption for plants in a forest system. Ectomycorrhizal mycelial strands provide a pathway for transport of physiologically significant amounts of water (Brownlee et al., 1983). Ectomycorrhizal tree seedlings have been shown to have a higher resistance to drought than non-mycorrhizal seedlings (Bowen, 1973). Wu et al. (1999) found that the enhancement of $^{15}NO_3^-$ uptake caused by mycorrhizal formation was more pronounced during water stress, indicating that EMF aid in plant avoidance of water stress. During times of soil drought, however, it has also been shown that nocturnal water translocation from plant to ectomycorrhizal fungi, contrary to what one may think, occurs to keep the hyphae functioning as nutrient absorption organs

(Querejeta et al., 2003). This latter study showed that the capacity of EMF to absorb nutrients is vital for host function, so much so that water is actually given to the fungus by the tree in times of prolonged drought to maintain proper function of both symbionts. These data suggest that EM fungi can access scarce soil moisture and increase drought tolerance of the host plant(s), as well as aid in plant nutrient uptake during periods of drought when soil nutrients are less mobile.

It seems, however, that with the higher nutrient/water absorption rates of EMF comes: (1) higher mycorrhizal respiration rates (Harley, 1978 and references therein); (2) greater plant transpiration rates (Allen et al., 2003); and (3) more photosynthate allocation to the fungus (Rygiewicz & Anderson, 1994), which can even result in a greater fungal biomass than that of the hosts own root system (Fogel & Hunt, 1982). The mycorrhizal enhancement of photosynthetic machinery, however, leads to an increased carbon gain of 10%-40% for the host plant (Allen et al., 2003); this is associated with increased stomatal aperture for increased CO_2 intake. Therefore, Harley (1978) states that, "It is my belief that the rapid absorptive properties of the fungus and the cumulative properties of the fungal sheath are the basis of this selective advantage."

In a comprehensive review of mycorrhizal nutrient uptake, Allen et al. (2003) determined that mycorrhizal roots obtain phosphorus, nitrogen, zinc, copper, nickel, sulfur, magnesium, boron, iron, calcium and potassium from the soil more efficiently than non-mycorrhizal roots, especially at low fertility levels. Hatch (1937), as cited in Harley (1978), found that only under conditions of low nutrient availability did mycorrhizal infection of roots significantly increase nutrient absorption. In soils with

high nutrient availability, mycorrhizal infection was even found to be suppressed, although this suppression is not necessarily universal in natural systems where EMF may still proliferate in the presence of sufficient nutrients (Tuomi et al., 2001). The increased abundance of EMF in nutrient-poor soils leads one to consider alternate pathways of nutrient absorption that deviate from those known for plant roots. How and where could the limited, bound, and scarce nutrients be accessed such that EM plants may support a healthy mycorrhizal community?

It has been suggested that EMF may access N in forms unusable to plants and convert it, within the fungal tissue, to readily absorbable plant forms, subsequently transferring it to the fungal/plant interface for the latter components absorption (Finlay et al., 1988, 1989). The former two studies showed that when ^{15}N-labelled ammonium chloride and sodium nitrate were added to the fungal mycelium, a decrease in enrichment levels throughout the mycelial transport pathway suggested the rapid conversion of ^{15}N inorganics into amino acids within the mycelium, amino acids that were subsequently recovered in plant tissue. The conversion of NH_4^+ to glutamine (NH_4^+ + glutamate) must be accomplished within fungal tissue; this is the result of high concentrations of NH_4^+ being toxic to mycorrhizal fungi. The conversion of inorganic nitrogenous compounds into amino acids indicates that EM fungi alter N forms to make them easier to use by host plants.

Lindahl et al. (1999) show that ectomycorrhizal fungi will access P pools within the mycelia of saprotrophic fungi when their mycelia share a similar microsite. A clear morphological confrontation response between the two fungal types was exhibited in a microcosm. The ectomycorrhizal fungus formed dense patches of

hyphae near the saprotroph, attaining 25% of the ^{32}P present in the saprotophs mycelium; this P was ultimately transferred to the plant host, *P. silvestris*. This indicates exploitation of a saprotrophic fungus by an EM fungus, and is proposed by the authors to perhaps be a "short cut" in nutrient cycling in forest systems. It must be noted, however, that the opposite was also found. Cairney and Meharg (2002) note that a contrasting study referenced within indicates that the vigor of an EM fungus was reduced when it contacted a saprotroph. Carbon allocation from host to EMF was similarly reduced.

Further, it has been shown that EM fungi are able to directly utilize organic nitrogen sources (Finlay et al., 1992). Although large differences occurred in regards to EM species ability to utilize protein as a nitrogen source, proteins were found to be used by certain EM fungi and can therefore constitute a source of nitrogen in forest systems. This may be especially important in moss dominated systems where novel nitrogen input seems to be limited.

The ability of EM fungi to obtain nutrients directly from minerals, such as bedrock and soil particulates, through chemical weathering is being regarded as more important than previously thought. Ectomycorrhizal fungi have the ability to produce extra-hyphal enzymes, including oxalate, citrate, and malate (Malajczuk, 1982; Landeweert et al., 2001; Allen et al., 2003) to mobilize recalcitrant nutrients. Landeweert et al. (2001), and references therein, note that EMF species can solubilize calcium phosphates deposited on agar and mobilize K^+, NH_4^+, and Ca^{2+} trapped inside mineral interlayer spaces. In a positive feedback loop, the depletion of cations on the growing medium causes an increase in mineral weathering to find novel

nutrient sources. A diagram of the mineral nutrient mobilization by EMF is presented as Appendix 5.

In temperate forest soils, a large proportion of the P pool is bound in organic compounds such as nucleic acids and phospholipids (Allen et al., 2003). Ectomycorrhizal fungi have the ability to access this P through abundant phosphatases produced externally on their hyphae (Landeweert et al., 2001; Alvarez et al., 2004). EM fungi are able to mineralize these organic P reservoirs and convert them into plant accessible nutrients (Smith & Read, 1997). It is generally assumed, then, that an increase in EM abundance will similarly exhibit an increase in phosphatase activity; if more fungal mass is produced a nutrient must be limiting and thus more phosphatase should be produced to access that nutrient.

An in vitro study assessed the production of phosphatase by mycorrhizal fungi (Dighton, 1983). For birch, it was found that a significant negative correlation existed between phosphatase production and extractable PO_4^- in the rooting zone, however there was no correlation between phosphatase production and PO_4^- with pine. This would indicate that, for birch, as P availability increases phosphatase activity would decrease due to the non-limiting status of P. It was also found that phosphatase production, per gram of mycorrhizal fungus, was greater than that of the tested decomposer fungi. Thus, it was concluded that, "…sheathing mycorrhizas have the capacity to solubilize P from inorganic and organic complexes… the P released is not always incorporated into fungal biomass but may be supplied to the plant host." The inorganic phosphate, in forms such as aluminum and iron phosphate, is not even available for plant uptake; rather the fungus must enzymatically liberate

the bound phosphate ions. The potential action of EMF as decomposers to access P in natural systems may in fact circumvent the need for decomposition and mineralization by saprotrophic organisms. It must also be noted, that when phosphatase activities of EMF are considered, different isolates have been shown to produce strikingly different activities (Kieliszewska-Rokicka, 1992); however, for the purposes of this study, because isolates were not differentiated in the field, general phosphatase activities are assumed to react similarly within sample plots.

In contrast to non-mycorrhizal roots, ectomycorrhizal fungi appear to cost less carbon to produce, have a greater surface-to-volume ratio, can explore the soil more effectively, have a greater ability to bind nutrients and water, and can access nutrient forms not available to plants. This would be advantageous in systems where nutrients are limited, have a predictable release regime, or are intermittently pulsed to the soil, such as is the case in the CWOC where FFMC regulates soil nutrients and water to some extent.

Long Distance Transport by Ectomycorrhizal Fungi:

Rhizomorphs of EM fungi are known to travel long distances and may also transport nutrients and sugars equally as far. Anastomosis (hyphal fusion) between genetically similar individuals of the same species has been noted (Brasier 1992; Giovannetti et al 1999). Brasier (1992) likewise claimed that in the higher fungi, those known to form EMF associations (basidiomycetes), adjacent hyphae of the same species have a strong propensity to fuse; however it is noted that "…support for altruism has waned…and fungal thalli have been shown to conform to… the selfish

gene" hypothesis. Allen et al. (2003) provided insight into the integration of mycorrhizal diversity and function across landscapes. They presented data on metacommunities, defined as "populations of communities, each open to others through varying degrees of connectivity," which can ultimately lead to translocation through those connections. It therefore seems likely that their conclusion holds true. "Plant productivity and the stoichiometry of nutrient availability within individual patches (single plant and associate or multiple plants connected by a single fungal species) would affect the state of adjacent patches through… the flow of nutrients between patches… as a function of fungal facilitation of connectivity between patches."

The "humongous fungus," a single genet of *Armillaria bulbosa*, is known to occupy a minimum of 15 hectares and weigh in excess of 10,000 kg (Smith et al 1992). Although habitually a facultative tree-root pathogen, the size of this fungus emphasizes the possibility for similar growth and interconnectedness in ectomycorrhizal fungi via anastomosis. The bidirectional transfer of carbon, nitrogen, and phosphorus between plant species via interconnected mycelia has also been shown (Tiwari et al., 2004; Simard et al., 1997 and references therein). Interconnected mycelial transport can lead to the acquisition of nutrients by EMF from areas that are not nutrient limited; these nutrients could even be translocated to nutrient deficient areas, resulting in mycorrhizal patch dynamics that are nearly impossible to define, let alone comprehend. It must be noted, however, that there is much dissention concerning the gravity of hyphal interconnectedness and elemental/water translocation (Pawlowska & Taylor, 2004; Horton, unpublished),

therefore much more research is needed to solidify the extent of common mycorrhizal networks in ecosystem function.

Ectomycorrhizae in *Pseudotsuga menziesii* Forests of the Region:

Fogel and Hunt (1982) assessed the contribution of mycorrhizae to nutrient cycling in a *P. menziesii* ecosystem in the Oregon Coast Range. In this young second-growth stand it was found that mycorrhizal fungi accounted for 6% of total tree biomass, where foliage was only 4%. In fact, mycorrhizal standing crop was 2-4 times greater than that of fine roots, explained by the prolific branching habit of many mycorrhizal fungi. If extraradical mycelial networks were included, the mycorrhizal biomass estimates would likely increase significantly. Support of the stands mycorrhizal root system required an average of 73% of the NPP over 2 years; the fine roots and mycorrhizae accounted for the bulk of the total stand throughput (50-58%) and uptake of organic matter (51-55%). This study indicates that the belowground ecosystem, including EMF, plays a major role in nutrient cycling in the CWOC, as well as carbon allocation by host plants.

A subsequent study by Hunt and Fogel (1983) assessed fungal hyphal dynamics in the same stand as above. Soil mycelial mass was greatest during the fall and spring, while it was significantly lower in the summer. This likely corresponded with the wet seasons (Figures 2 & 4) in the region. The single EM fungus *Cenococcum geophilum* contributed up to 66% of the monthly hyphal volume, which is noted by the authors to be an underestimate. They conclude that soil hyphae in this *P. menziesii* stand, including mycorrhizal extraradical mycelia, turnover yearly and

thus "…constitute a rapidly cycling pool of nutrients and may contribute to ecosystem stability by immobilizing nutrients and thus reducing leaching from the root zone."

Concluding Remarks:

It appears that the employment of ectomycorrhizal fungi by plants in the CWOC is the perfect answer for the nutrient dynamics of the temperate coniferous forest system. Numerous nutrient inputs to the system studied seem to be regulated by FFMC to a great extent. Evergreen conifers have reduced leaf-litter that falls on mosses; all forms of wet and dry deposition initially filter through mosses, which have an innate propensity to sequester nutrients contained therein. Forest floor mosses have been shown to intermittently leach small quantities of inorganic and organic nutrients, as well as undergo pulsed release during extremely wet conditions. With the thorough review of the nutrient and water dynamics of ectomycorrhizal fungi, it seems as if their proliferation beneath FFMC would benefit the associated tracheophyte community. Ectomycorrhizal fungi are more efficient at trapping scarce and pulsed nutrients than bare roots, while simultaneously being able to exploit recalcitrant nutrient sources in the soil, nutrient sources which bare roots cannot access. The only question to ask at this point, aside from the experimentally tested influence of FFMC on EM abundance, is to what extent have moss-ectomycorrhizal relationships been observed in previous studies. Do empirical data validate the premise of the current study?

9. <u>The Interactions of Ectomycorrhizal Fungi and Mosses: Extent and Relevance</u>:

The influence that FFMC has been shown to have on EM systems varies. The unique non-mycorrhizal status of mosses has already been discussed; however their indirect associations with EMF are extremely important to ecosystem dynamics. The following is a comprehensive review of such associations involving saprotrophism and increased associated abundance, which support the theory that mosses and mycorrhizae play important roles in the nutrient regime of temperate ecosystems, thus warranting further study.

Kilbertus and Manġenot (1972) conducted a laboratory experiment to test the effect of moss cover on soil ectomycorrhizal abundance. They grew *Pinus silvestris* in pot culture and observed, one year later, that the ratio of mycorrhizae to root dry weight was significantly higher under moss cover than under bare soil. This finding led to the development and implementation of the current thesis. It was conducted in a laboratory microcosm and indicated that there could be many factors beneath a moss mat promoting EM proliferation; however the experiment was confined to the laboratory and the mechanism by which moss cover increased EM abundance was not elucidated. The only valid explanation focused on the ability of moss cover to create a favorable microclimate for ectomycorrhizal proliferation.

Chapin et al. (1987) suggests that ectomycorrhizae may be an avenue by which phosphorus moves out of the moss mat to underlying spruce roots and subsequently to apical portions of the vascular plant. They applied ^{32}P to the feather-mosses (*P. schreberi* and *H. splendens*) in an Alaskan black spruce forest to test the

role of bryophytes in a boreal forest nutrient regime. In one in situ test, the effect of physically severing all root/hyphal connections to areas external to the plot resulted in a significantly lower amount of ^{32}P lost from the plot and increased phosphorus retention by the bryophytes. This indicates that mosses export phosphorus from their tissues and that limiting exportation pathways results in greater P retention by the mosses. Chapin et al. (1987) also found that by limiting the action of mycorrhizal fungi beneath a feather-moss mat, with the application of a fungicide, the transport of phosphorus from the experimental plot was reduced. This, in coordination with the severing result, indicates that fungi provide a pathway by which phosphorus can be transferred out of feather-mosses to other parts of the forest system, presumably supporting both fungal and vascular plant nutrient requirements. It should also be considered that P retention in mosses, with the application of a fungicide, limits the ability of EM fungi to "steal" those nutrients sequestered in the moss, thus making them unavailable to their host(s).

Weetman and Timmer (1967) found fine black spruce roots to be most prolific in the region of feather moss decomposition, tending to grow upwards among masses of yellow, black, and white ectomycorrhizal hyphal strands. They noted that it seemed probable that mosses, quite apart from competing with the trees may actually be one of their main sources of nutrients. Upon further analysis, it seems as though fine tip proliferation creates more sites for EM fungi to colonize and access the scarce nutrients needed for spruce growth, thereby reducing energy expenditure of the tree that would be necessary for deep soil exploration and fine root production. Why not increase the absorptive surface area and efficiency of roots, via EM fungi, and

proliferate around mosses, such that during short-lived nutrient pulses and intermittent releases of dilute ions, the available nutrients are obtained for use by other system components and are thus, not washed through the soil?

Bates and Farmer (1990) tested the sources and effects of Ca on mineral content and growth of the calcifuge feather-moss *P. schreberi*. They applied two separate treatments of Ca to the moss mats; calcium chloride applied as a top down rain, and $CaCO_3$ as powder to the soil beneath a cut/peeled back mat. A greenhouse experiment was also performed to compare the effects of Ca concentration and the pH of simulated wet deposition on moss growth. From these experiments they suggested that nutrients released from mosses could be used/exploited by other organisms in the substratum or remain in the forest litter. Also, they indicated that inorganic ions could move upwards from the soil through the moss litter and senescent tissues, ultimately ending up in the growing apices of *P. schreberi*. This study recognizes the bi-directional movement of inorganic ions between moss and soil through intercellular transport, thus shedding light on other organismal associations that might benefit from the nutrient translocation, perhaps ectomycorrhizal fungi and their host plants.

Wells and Boddy (1995) observed the movement of ^{32}P (orthophosphate) through saprotrophic basidiomycete mycelial cord systems to the apical regions of *Hypnum cupressiforme* shoots. Radioactive ^{32}P was applied to *Fagus sylvatica* wood block inocula and allowed to distribute for five days. Upon harvest of identified radioactive areas, it was found that *Phanerochaete velutina* was attached to *H. cupressiforme* at their live/moribund bases. This was the first study directly

observing that terricolous mosses can rapidly gain phosphorus from fungal mycelium in the substratum. This indicates that a phosphorus exchange site may exist between saprotrophic fungi and moss senescent/moribund tissues; perhaps the differential ion binding ability of moss and mycorrhizal fungus favors movement from fungus to moss under certain circumstances.

Carleton and Read (1991) performed an in vitro experiment on nutrient transfer of ^{32}P and ^{14}C between *P. schreberi*, an ectomycorrhizal fungus (*Suillus bovinus)*, and the conifer *Pinus contorta* (lodgepole pine). It was detected that mycelial connections facilitated the transfer of phosphorus from formerly labeled moss shoots to the roots and shoots of pine seedlings. No labeled phosphorus was detected in the peat substrate thus indicating no leaching; the translocation of phosphorus from moss to tree was specifically mediated by connected EM fungal mycelium, and thus was very efficient. Labeled carbon was observed to act similarly. It must be noted that the *P. schreberi*/*S. bovinus* association was saprotrophic, as the *P. schreberi* shoot was dead and buried in the soil. The experiment also photo-documented the approach and eventual colonization of the *P. schreberi* shoot by *S. bovinus*. Carleton and Read (1991) also observed that the mycelial fans reached all parts of the moss shoot and documented a structure that was "superficially" comparable to the mantle produced by ectomycorrhizal fungi on portions of the *P. schreberi* shoot, which in reality was likely encapsulation by saprotrophic hyphae. This indicates that ectomycorrhizal fungi can colonize *P. schreberi* saprotrophically and therefore may be a nutrient connector between bryophytes and vascular plants,

accessing the sequestered nutrients in moss tissue that would otherwise remain immobilized.

Zackrisson et al. (1997) conducted an experiment on the interference mechanisms of the feather-moss *P. schreberi,* the ericacaeous shrub *Empetrum hermaphroditum,* and ericoid mycorrhizal fungi on the establishment and growth of Scots pine seedlings. It was found that the three interference species do in fact inhibit Scots pine seedling performance. This result led to the hypothesis that a three-part interacting system of the biotic components feather-mosses, ericoid fungi, and ericaceous dwarf shrubs may both block tree regeneration and immobilize nutrients. This is important because it shows that ericoid fungi are indirectly associated with mosses, even though it may be in a way that harms one vascular plant at the expense of another. Ectomycorrhizal fungi have been seen to act similarly, in many respects, to ericoid mycorrhizal fungi (Read & Moreno, 2003).

Zobel et al. (1999) conducted an assessment of small-scale plant community dynamics in an experimentally polluted and fungicide-treated birch-pine forest. It was found that the experimentally polluted sites showed a decrease in bryophyte cover and an increase in ericaceous shrub cover. This may be due, in part, to mycorrhizal fungi buffering the pollutant load the shrubs were exposed to, indicating that both mosses and mycorrhizae together sequester a large portion of the polluting metal cations. The fungicide treated sites, on the other hand, resulted in increased bryophyte cover and production. This finding suggests that ectomycorrhizal fungi of birch and pine may be efficiently binding/stealing nutrients and organic leachates from FFMC before they are able to reabsorb them. This would mean that EMF

proliferation beneath FFMC would be advantageous and benefit both tracheophyte and fungus.

In a final study, Oechel and Van Cleve (1986) contend that mosses may control ecosystem function and can have major effects on vascular plant productivity and nutrient cycling. In the Taiga ecosystem studied, they hypothesized that mosses may inhibit the growth of vascular plants by accessing nutrients first and sequestering them for long periods of time. They found that mosses accessed threefold more nitrogen, phosphorus and magnesium than was accessed by black spruce. This would ultimately lead to the eventual removal of the vascular component from the system, however they still persist. Perhaps to compete with mosses, vascular plants recruit a third component, ectomycorrhizal fungi, in larger quantities beneath moss cover to better absorb the leached nutrients, electrolytes, and photosynthates during moss rehydration, better absorb the scarce nutrients due to moss mat sequestration, or saprobically colonize and grab nutrients from the senescent/moribund moss tissue before leaching. This would account for the lack of a documented mycorrhizal association with a member of the bryales; mosses are already equipped to capture and retain nutrients, however the vascular plants need inter-kingdom assistance.

10. <u>Tying it All Together</u>:

It seems as if the plausible mechanisms to explain the effects of FFMC on EM abundance are many. Further exploration, including soil properties and rainwater chemistry of the CWOC, will be performed in future reports; however, the aforementioned evidence is certainly compelling and makes one think about the complexities of an association such as this. From the information presented it seems

probable that: (1) mosses could have evolved separately from other embryophytes to never assume the mycorrhizal habit of sister clades; (2) forest floor feather-mosses may function as key ecosystem regulators of nutrient and water regimes, especially in the CWOC; (3) ectomycorrhizal fungi are more efficient absorptive organs of plants, as opposed to bare roots, and therefore may be more abundant beneath FFMC than bare soil; and (4) the removal of FFMC may significantly influence EM abundance in the soil beneath the site of removal.

11. <u>Objectives and Hypotheses</u>:

This study applied existing laboratory findings on the interactions of mosses and ectomycorrhizae to a field situation, where mosses play a significant role in ecosystem regulation, especially the key finding by Kilbertus and Manġenot (1972). Due to the undergraduate nature of the field research, exploration into the specific mechanisms that may effect EM abundance associated with the removal of moss cover were, for the most part, only theoretically assessed from previously published data. The following was hypothesized:

1) H_0: The removal of forest floor feather-moss cover in the central-western-Oregon Cascades will have no influence on the abundance of ectomycorrhizal root tips beneath.
1) H_a: The removal of forest floor feather-moss cover in the central-western-Oregon Cascades will significantly reduce the abundance of ectomycorrhizal root tips beneath.

2) H_0: Dominant moss species, *Eurhynchium oreganum* or *Hylocomium splendens*, will not have a differential effect on the abundance of ectomycorrhizal root tips.

2) H_a: Dominant moss species, *Eurhynchium oreganum* or *Hylocomium splendens*, will have a differential effect on ectomycorrhizal abundance prior to and following harvest.

3) H_0: Moss mat biomass will not have an effect on the abundance of ectomycorrhizal root tips.

3) H_a: Moss mat biomass will be positively correlated with ectomycorrhizal abundance prior to harvest; after harvest those plots with the greatest initial biomass will show a significantly greater loss of ectomycorrhizal abundance.

4) H_0: The activity of soil phosphatase will show no correlation to the abundance of ectomycorrhizal root tips.

4) H_a: The activity of soil phosphatase will be positively correlated with the abundance of ectomycorrhizal root tips.

Methods and Materials

Site Description ~

1. Regional Context:

The current study was conducted in the United States Pacific Northwest bio-region, specifically the central-western Cascade Mountains of Oregon. All research areas were located within the McKenzie River Ranger District of the Willamette National Forest, within and nearby both the H.J. Andrews Experimental Forest (HJA) in Blue River, Oregon, and the Cougar Reservoir, located 10 km south-west of the HJA (Figure 1, Appendix 6). The HJA is the Lookout Creek Watershed.

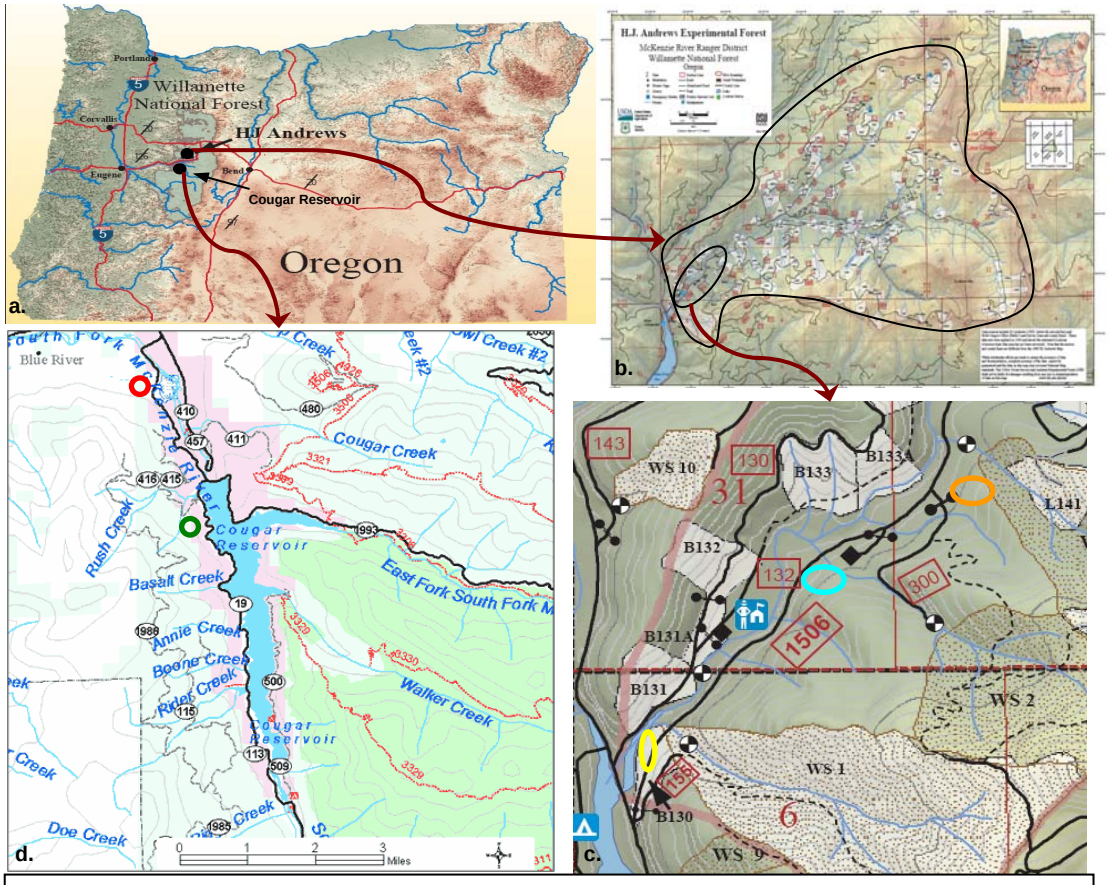

Figure 1: Locations of the five study sites within the greater context of Oregon State: a. State of Oregon, b. HJ Andrews LTER with sites 1-3 delineated, c. Sites 1-3 at the HJ Andrews LTER, d. Sites 4-5 at Cougar Reservoir.

Site 1 Site 2 Site 3 Site 4 Site 5

Blue River, Oregon, located at N44°9′15″ and W122°20′21″, is a component of the Pacific Northwest-North Pacific Ocean Bioclimactic region where conditions are cool and wet; the Pacific lies approximately 193 km to the west (Zobel et al., 1976). Both the HJA and the Cougar Reservoir lie 5 km north and south of Blue River, respectively. A regional maritime climate causes wet, mild winters and dry, warm summers with three moist seasons (fall, winter, spring), and one dry season (summer) from June through August (Dyrness et al., 1974). The dry months only receive 5% of the average annual rainfall (Figure 2) (McKee et al., 1996). The mean monthly temperature ranges from 1°C in January to 18°C in July and August (Figure 3); elevation changes can alter these numbers (McKee et al., 1996). Because of high summer temperatures and lack of precipitation, the potential evapotranspiration exceeds available water supplies by approximately 84 mm (Figure 4); the potential evapotranspiration for the HJA is 538 mm (Rothacher et al., 1967). This leads to a soil water deficit during the dry season (McKee et al., 1996), as well as susceptibility to fire.

These climactic conditions favor the development of massive, long-lived conifers. Precipitation averages 2,202 mm yearly at elevations encompassing the study sites (McKee et al., 1996), with the wettest season occurring during the coldest months (Figures 2 & 3). However, snowpack is functionally non-existent below 762 m (McKee et al., 1996), in which all the study sites fall. This wet, cold winter, in conjunction with the extremely dry summer, causes a severe decrease in photosynthetic output of *P. menziesii* during the "growing season," as well as during the coldest months (Figure 5). The photosynthetic peak for this

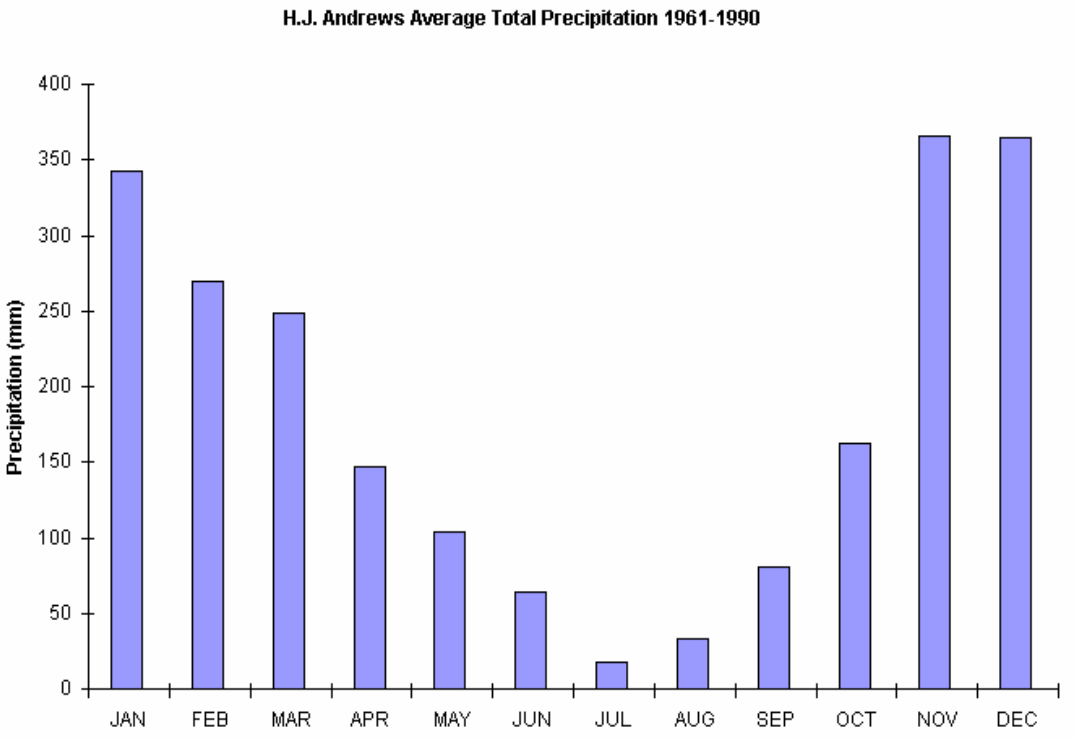

Figure 2: Graph of precipitation averages at the HJA over 30 years. Taken from McKee et al., 1996

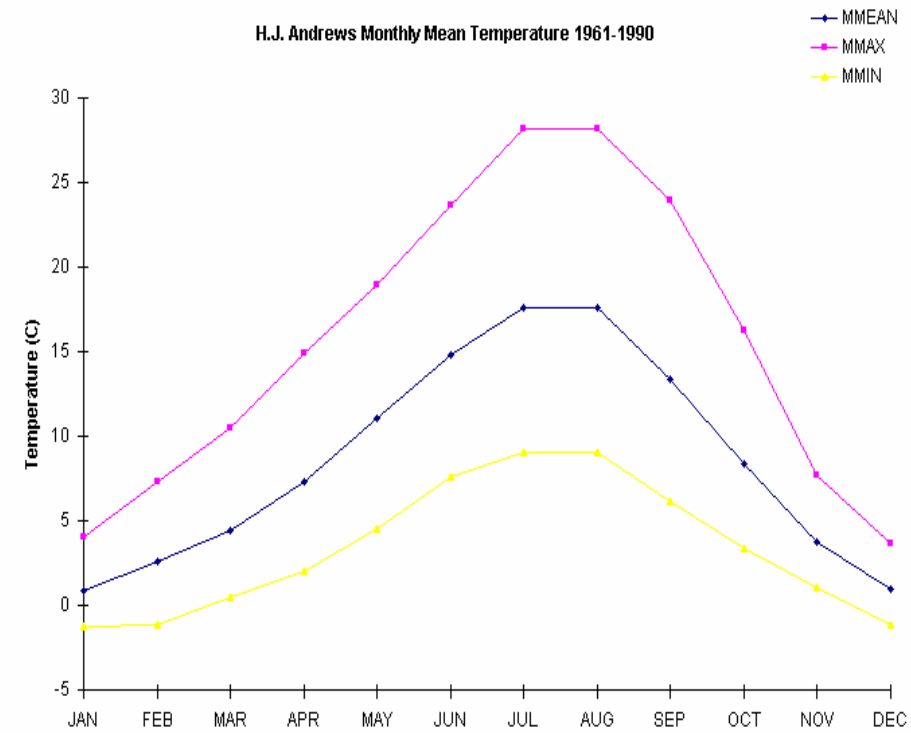

Figure 3: Graph of temperature averages at the HJA over 30 years. Taken from McKee et al., 1996

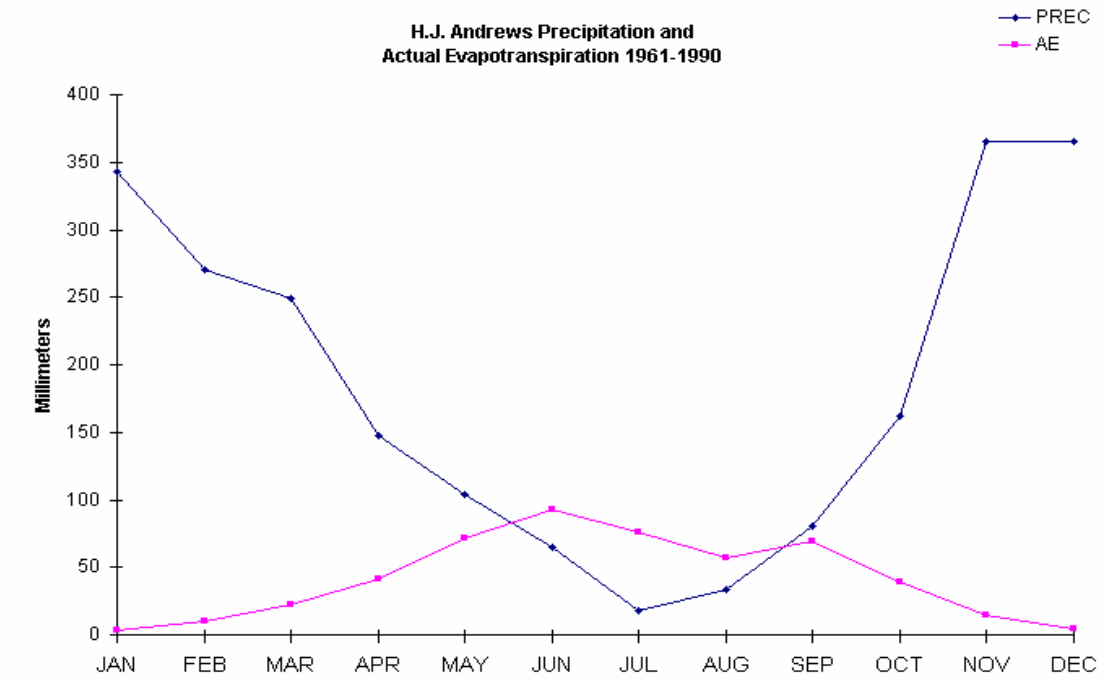

Figure 4: Graph of precipitation and actual evapotranspiration averages at the HJA over 30 years. Taken from McKee et al., 1996

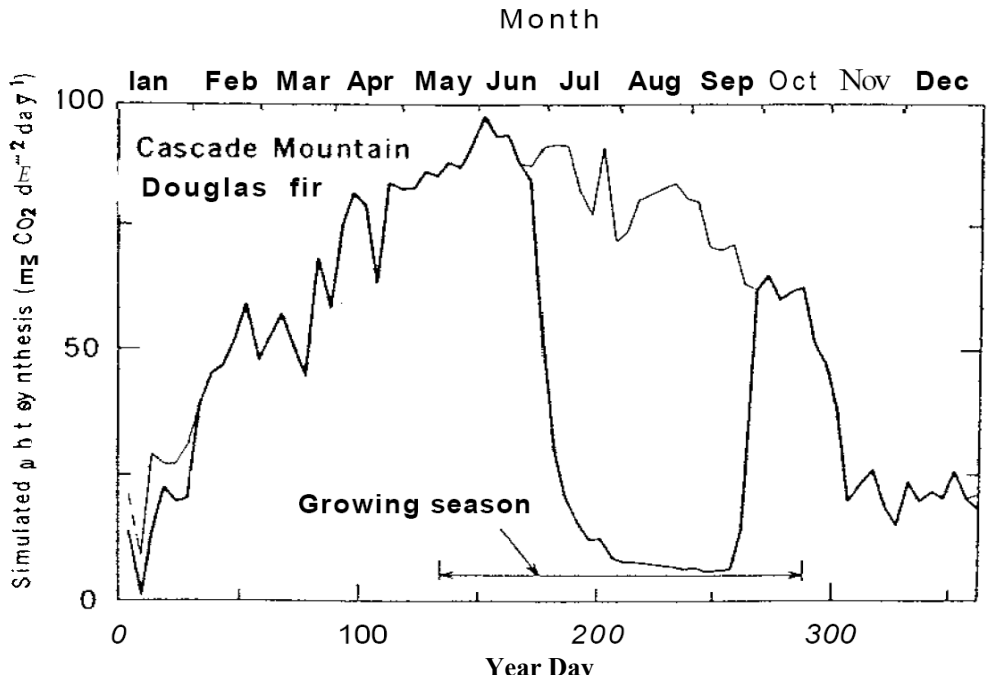

Figure 5: Graph of photosynthetic capacity of *P. menziesii* throughout the year, growing in the western Cascade Mountains of Oregon. The lighter line shows potential photosynthesis without constraints due to moisture stress, frost, or low soil temperature; the thick line incorporates these constraints. A high proportion of photosynthesis occurs outside the "growing season." Taken from Waring and Franklin, 1979.

dominant species is immediately before the dry season, with a smaller peak immediately following the dry season (Figure 5). Other species will likely react similarly.

The HJA is biologically diverse, typical of a rich north temperate ecosystem. There are over 500 documented tracheophyte species within the HJA alone, with over 100 more in the surrounding region. A typical 0.5 ha plot in an upland site contains between 35-40 vascular plant species, while riparian zones can contain close to 80 (Zobel et al., 1976). The five sites in this study had more than 28 tracheophyte species each, excluding epiphytes (Table 1).

The forests in this region are representative of mature Pacific Northwest *P. menziesii* dominated conifer forests at the elevations studied. The general forest structure for the study sites are as follows: (1) canopy trees include dominance by *P. menziesii*, as well as co-dominance by *T. heterophylla* and *Thuja plicata* (western red cedar); (2) understory trees include *Acer cirinatum* (vine maple) and *Acer macrophyllum* (bigleaf maple); (3) understory shrubs include *Mahonia nervosa* (dull-Oregon grape), *Gaultheria shallon* (salal), *Vaccinium parvifolium* (red huckleberry) and *Rhodendron macrophyllum* (pacific rhododendron); (4) understory herbs include *Oxalis oregana* (redwood sorrel), *Viola sempervirens* (wood violet), *Ribes spp.* (currants/gooseberries), and *Rubus spp.* (raspberries); (5) simple vascular plants include *Polystichum munitum* (sword fern) and *Pteridum aquilinum* (bracken fern); and (6) mosses include *Hylocomium splendens, Eurhynchium oreganum, Rhytidiadelphus triquetrus* and *Leucolepis acanthoneuron* (Table 1). The bedrock

supporting these plants is entirely Tertiary volcanic rock, comprised mostly of andesite and dacite (Zobel et al., 1976).

2. Individual Site Descriptions:

Within the HJA/Cougar Reservoir region, five experimental sites were established. Each individual site was located within 10 km, due south/south-west of the HJA headquarters, N44°2' and W122°2' and ranged in elevation from 428-536.9 m. If the 450-year-old age class proposed by Dyrness et al. (1974) is sound, such that *P. menziesii* dominates at an average diameter at breast height (dbh) over 120 cm. Most sites were estimated to be around 400 years old. The only glaring exception was Site 1, known to be approximately 50 years old. Site 4 also had *P. menziesii* with a lower average dbh, likely due to the presence of a large gap.

The five 40 m^2 sites (Figure 1, Appendix 3) were chosen by Kari O'Connell (site director at the HJA) and Susan Fritz (Botanist for the McKenzie River Ranger District). The sites corresponded, as reasonably as possible, to the following criteria: (1) the sites should be dominated by *P. menziesii*; (2) the dominant moss species consist primarily of *H. splendens* and *E. oreganum*; (3) the site areas be restricted to approximately 40m^2; (4) the entire site be carpeted with a robust feather-moss mat, or there be at least 20 areas, 1.5m^2 each, that have nearly 100% moss cover; and (5) the sites have relatively consistent abiotic characteristics including moisture, terrain, canopy cover, elevation and aspect. There were a few deviations from the criteria due to lack of suitable sites which will be subsequently noted.

At each site, initial measurements were made of general forest structure and composition, which will be considered here rather than in the results, because these data were part of a preliminary site classification prior to the actual experiment. A complete survey for presence of tracheophytes and terricolous mosses was undertaken using 45 and 20 minute timed meanders, respectively, with the most prevalent plants visually estimated by percent cover. Any novel species found on individual plots were added to the total species count for each site (Table 1). The importance values of tree species were assessed using a point-centered-quarter method (Mitchell, 2001). Canopy cover was measured at eight random plots using a spherical densiometer (Model-A; Robert E. Lemmon, Forest Densiometers). Soil moisture was taken at seven points in each plot using a TDR 100 Digital Moisture Probe®. All preliminary data were recorded during late June to early July of 2005. Additional data was recorded for individual plots at each site and will be reported later.

Site 1**:** (Figure 6)

This rocky, dry, west-southwest facing site was once dominated by old-growth *P. menziesii* in the 1960's. It was clear-cut 40+ years ago and replanted as a *P. menziesii* monoculture with *Pinus ponderosa* (ponderosa pine) along the north-northwest cliff bordering Lookout Creek (LC). Few snags and deadfall were present on the site due to clearing at the time of clearcutting. It runs west-southwest between Forest Service Road (FSR) 1506 and LC, recessed 20 m from each. The soil quality transitioned from richer and less rocky near the road to rockier near the LC cliff. The

average elevation was 428 m, with the center of the site located at UTM 10T-559122, 4895122.

The most common plants were *P. menziesii, V. parvifolium, G. shallon, M. nervosa, Rubus spp., Whipplea modesta* (whipple vine), *P. munitum,* and *E. oreganum*. *Pseudotsuga menziesii* was the overwhelmingly dominant tree with an importance value of 284.35/300 (table 2). The average dbh of all *P. menziesii* was 25.74 cm. The canopy cover averaged 91.81%, while the soil had an overall moisture content of 10.06%. These data are summarized in Table 3.

Site 2: (Figure 6)

This site has a west-northwest aspect, very dense understory, heavily shaded moist forest floor, and is very hilly with heavy deadfall. The soil has a rich, fine-grained organic layer without many large rocks/soil aggregates. The lowland area bordering the stream has noticeably moister soil. It is an old-growth *P. menziesii/T. heterophylla* codominant stand, the former with the greatest IV (table 2), which borders Watershed 2 and lies within Reference Stand 7 of the HJA. It is located off of FSR 1506. The average elevation was 483 m, with the center of the site located at UTM 10T-559824, 4895946.

The most common plants were *P. menziesii, T. heterophylla, T. plicata, V. parvifolium, G. shallon, M. nervosa, O. oregana, P. munitum, Blechnum spicant* (deer fern), *E. oreganum, H. splendens,* and *R. triquetrus/loreus*. The average dbh of *P. menziesii* was 124.22 cm. The canopy cover averaged 94.35%, while the soil had an overall moisture content of 18.31%. These data are summarized in Table 3.

Site 3: (Figure 6)

This site has a west-northwest aspect, with obvious snags and deadfall. The understory shrubs are less dense than in Site 2, making it easier to walk. The soil was dark, rich, and lacked large rocks. This site is an old-growth *P. menziesii/T. heterophylla* codominant stand, the former with the greatest IV (Table 2), which is set back from the intersections of FSR 1506 and RSR 300. The average elevation was 500 m, with the center of the site located at UTM 10T-560286, 4896091.

The most common plants were *P. menziesii, T. heterophylla, V. parvifolium, G. shallon, M. nervosa, R. macrophyllum, O. oregana, Linnaea borealis* (twinflower), *P. munitum, Blechnum spicant* (deer fern), *E. oreganum, H. splendens,* and *R. triquetrus*. The average dbh of *P. menziesii* was 122.81 cm. The canopy cover averaged 94.44%, while the soil had an overall moisture content of 15.5%. These data are summarized in Table 3.

Site 4: (Figure 6)

This site has an east-northeast aspect, with rich soil that is rocky in places. The border of the site is old-growth *P. menziesii*; however a large gap, created approximately 40 years ago, has led to the establishment of *T. heterophylla* and *T. plicata* in greater abundance. Thus, *T. heterophylla* is a strong codominant. This is the only site where *T. heterophylla* has the highest IV (table 2).

Site 4 is not located on HJA property; rather it lies across the McKenzie River Valley (rt. 126) and up the West Cascades Scenic Byway/South Fork Road. Off of

this road, on the way to Cougar Reservoir, a right turn on FSR 19 will lead to Site 4 (appendix 3). It is flat with an average elevation of 418 m, the lowest elevation of all sample sites. The center of the site is located at UTM 10T-559067, 4888440.

The most common plants include *T. heterophylla, P. menziesii, T. plicata, A. cirinatum, R. macrophyllum, G. shallon, M. nervosa, L. borealis, Clintonia uniflora* (queens cup), *P. munitum, H. splendens,* and *R. triquetrus/loreus.* The average dbh of *P. menziesii* was 85.92 cm. The canopy cover averaged 93.14%, while the soil had an overall moisture content of 12.25%. These data are summarized in Table 3.

Site 5 – (Figure 6)

This site has a generally north-facing ravine flanked by two hills facing east and northwest. The site has rich soil and is heavily covered by deadfall and decayed wood which provides preferential habitat for *H. splendens*. Sizable rocks were present, but sparse. It is an old-growth site that has three codominant species, *P. menziesii, T. heterophylla,* and *T. plicata*. This codominance is likely due to gap prevalence. The site itself is located past the Cougar Reservoir dam off South Fork Road on the first FSR road to the right. Site 5 has the steepest slope and the highest average elevation, 536 m. The site center is located at UTM 10T-560211, 4885703.

The most common plants include *P. menziesii, T. heterophylla, T. plicata, R. macrophyllum, G. shallon, M. nervosa, Trientalis borealis ssp. latifolia* (starflower), *L. borealis, Coptus laciniata* (goldthread), *P. munitum, P. aquilinum, H. splendens, E. oreganum, R. triquetrus/loreus*. *P. menziesii* dbh averaged 113.51 cm, while the soil had an overall moisture content of 21.1%, the moistest site (Table 3).

Figure 6: Photos of Site 1 (a/b), Site 2 (c/d), Site 3 (e/f) Site 4 (g/h), and Site 5 (i/j)

Table 1: Species Presence on Each Site

	Site 1	Site 2	Site 3	Site 4	Site 5
TREES					
Douglas Fir (Pseudotsuga *menziesii)*	X	X	X	X	X
Western Hemlock (Tsuga *heterophylla)*	X	X	X	X	X
Western Red Cedar (Thuja *plicata)*	X	X	X	X	X
Vine Maple (Acer *cirinatum)*	X	X	X	X	X
Bigleaf Maple (Acer *macrophyllum)*	X			X	X
W. Flowering Dogwood (Cornus *nuttallii)*	X	X	X	X	X
Pacific Yew (Taxus *brevifolia)*	X	X	X	X	
Chinquapin (Castanopsis *chrysophylla)*	X	X		X	X
Ponderosa Pine (Pinus *ponderosa)*	X				
Black Hawthorn (Crataegus *douglasii)*	X	X		X	
SHRUBS					
Salal (Gaultheria *shallon)*	X	X	X	X	X
Dull Oregon Grape (Mahonia *nervosa)*	X	X	X	X	X
Red Huckleberry (Vaccinium *parvifolium)*	X	X	X	X	X
Thimbleberry (Rubus *parviflorus)*	X				
Oceanspray (Holodiscus *discolor)*	X				
Common Snowberry (Symphoricarpos *albus)*	X				
Pacific Rhododendron (Rhododendron *macrophyllum)*		X	X	X	X
Trailing Snowberry (Symphoricarpos *mollis)*	X				
Cascara (Rhamnus *purshiana)*	X				
HERBACIOUS PLANTS					
Inside-out Flower (Vancouveria *hexandra)*	X	X	X	X	
Prince's-Pine (Chimaphila *umbellata)*	X		X		X
Menzies' Pipsissewa (Chimaphila *menziesii)*	X	X	X		X
Starflower (Trientalis *borealis ssp. latifolia)*	X		X	X	
Bunchberry (Cornus *canadensis)*			X		
Redwood Sorrel (Oxalis *oregana)*		X	X		X
Vanilla Leaf (Achiys *triphylla)*	X	X	X	X	X
Twinflower (Linnaea *borealis)*	X	X	X	X	X
Western Tea Berry (Gaultheria *ovatifolia)*					
Beargrass (Xerophyllum *tenax)*	X			X	
Rattlesnake-Plantain (Goodyera *oblongifolia)*	X		X	X	X
Violet (Viola *sempervens)*	X	X	X	X	X
White Veined Wintergreen (Pyrola *picta)*			X		
False Solomons Seal (Smilacina *racemosa)*	X	X			
Star-Flowered F. Solomons Seal (Smilacina *stellata)*		X			X
Rasberries *(Rubus spp.)*	X	X	X	X	X
Gooseberries and Currants (Ribes *spp.)*					
Anemone (Anemone *spp.)*	X	X		X	X
Pathfinder (Adenocaulon *bicolor)*	X	X	X	X	
Foamflower (Tiarella *trifoliata)*		X	X	X	
White Flowered Hawkweed (Hieracium *albiflorum)*	X				X
Sweet-Scented Bedstraw (Galium *triflorum)*	X	X	X		X
Purple Trillium (Trillium *chloropetalum?)*		X	X	X	X
White/Pink Trillium (Trillium *ovatum)*		X	X	X	X
Wild Strawberry (Fragaria *vesca)*	X				
Whipple Vine (Whipplea *modesta)*	X				X
Queens Cup (Clintonia *uniflora)*		X		X	
Rosy Twistedstalk (Streptopus *roseus)*		X		X	
False Lilly of the Valley (Mainthemum *dilatatum)*					
Wild Ginger (Asarum *caudatum)*	X			X	
Indian Pipe (Monotropa *uniflora)*		X		X	
Goldthread (Coptus *laciniata)*					X
Simple Vascular Plants					
Sword Fern (Polystichum *munitum)*	X	X	X	X	X
Bracken Fern (Pteridum *aquilinum)*	X	X		X	X
Deer Fern (Blechnum *spicant)*		X			
Maidenhair Fern (Adiantum *pedatum)*					
Mosses					
Hylocomium splendens	X	X	X	X	X
Rhytidiadelphus triquetrus	X	X	X	X	X
Rhytidiadelphus loreus		X		X	X
Eurhynchium oreganum	X	X	X	X	X
Leucolepis acanthoneuron	X	X			X
Rhizomnium glabrescens	X	X			X
Dicranum scoparum					
Polytrichum juniperum	X				
Polytrichum commune					
Tortula ruralis					
Unknown *Dicranum sp.*	X	X	X		X
Unknown *Brachythecium sp.*	X				X
Unknown *Mnium sp.*		X			
Unknown wispy hanging epiphyte	X	X	X	X	X

Table 2: Importance Values (IV) of Trees at Each Site as Determined by the Point-Centered Quarter Method

	** P. menziesii*	** T. heterophylla*	*T. plicata*	*T. brevifolia*	*A. cirinatum*	*C. nuttallii*	*A. macrophyllum*	** P. ponderosa*
Site 1	284.35	7.36	0	0	0	0	7.36	8.29
Site 2	132.22	90.33	44.18	17.51	11.81	3.94	0	0
Site 3	146.25	120.00	11.52	22.23	0	0	0	0
Site 4	104.08	135.73	35.97	10.76	0	13.45	0	0
Site 5	113.51	94.23	92.26	0	0	0	0	0

* Indicates association with EM fungi

Table 3: Site Characteristics Assessed Prior to Experiment

	Average Elevation (m)	Aspect	Average Canopy Cover (%)	Average Soil Moisture (%)	Doug-Fir IV (out of 300)	Average Doug-Fir DBH (cm)	Central GPS Position
Site 1	428	W-SW	91.81	10.06	284.35	25.74	UTM 10T-559122, 4895122
Site 2	483.01	W-NW	94.35	18.31	132.22	124.22	UTM 10T-559824, 4895946
Site 3	500.23	W-NW	94.44	15.5	146.25	122.81	UTM 10T-560286, 4896091
Site 4	418.79	E-NE	93.14	12.25	104.08	85.92	UTM 10T-559067, 4888440
Site 5	536.9	N	86.9	21.1	113.52	115.24	UTM 10T-560211, 4885703

3. Manipulative Study – Design, Data Collection, and Preliminary Analysis:

The current study involved a manipulative field experiment (complete forest floor moss mat removal) to explore the influence forest-floor moss cover has on EM abundance on tracheophyte roots in the soil beneath. It was performed in Oregon at the HJA over the course of two summers, between June 20th – July 20th 2005 and between June 20th – July 4th 2006, as well as subsequent laboratory analyses in Syracuse, N.Y. Each summer will be considered separately as different methods were used.

Summer 2005 (Year 0):

At each individual study site 16 plots, measuring 1.5 m^2, were established (20 at site five) at randomly selected points if they did not meet the exclusion criteria, which is described later. Site 1 contained plots 1-16, Site 2 contained plots 17-32, site 3 contained plots 3 3-48, Site 4 contained plots 49-64, and Site 5 contained plots 65-84. Plot locations were determined by gridding out each $40m^2$ site into $2m^2$ segments. Each segment was assigned a sequential number. The Minitab® random number generator was used to isolate the desired number of plots per site. At each point ($2m^2$ segment) a $1.5m^2$ square frame was randomly thrown onto the ground (four sticks tied together). Plots were excluded and picked again if: (1) the plot was located on deadfall or an impermeable substrate, such as rock; (2) the plot contained a large tree or shrub; (3) the plot was not predominantly covered by *E. oreganum* or *H. splendens*; (4) the plot was located beneath a dense or shrubby canopy that would block or redirect rainfall; (5) the plot did not have close to 100% moss cover; (6) the plot was within two meters of a tree bole >10 cm dbh; or (7) the plot contained a rare or endangered plant.

Prior to experimental manipulation, from June 20th – July 11th, each plot was analyzed for: (1) soil moisture, using a TDR 100 Digital Moisture Probe®; (2) tracheophyte species presence and percent cover, estimated with an open hand at hip height constituting 4% of the plot; (3) moss species presence and percent cover; (4) percent of plot covered by a feather-moss mat; (5) average depth of moss mat; (6) biomass of moss mat; (7) soil phosphatase activity; (8) photo documentation of each

plot pre/post harvest; and (9) one 15 cm soil core, using a 15 cm AMS Split Core Soil Sampler® with a diameter of 5.08 cm and total volume of 304 cm^3.

The soil moisture probe was inserted at seven random points throughout each plot to a depth of 12 cm; average plot moisture content was calculated from these readings. Vascular plant species presence was estimated by identifying all species that were rooted in the plot, while percent cover was estimated with an open hand at hip height constituting 4% of the plot. Percent cover was only estimated to a height of 2 m, therefore most canopy covering the plot area was not counted.

Moss species presence was determined by identifying all terricolous moss species, while percent cover was estimated as the number of cm each species occupied out of 300 cm, determined by randomly throwing down three meter sticks inside the plot. Mosses touching the marked side of the meter stick were counted; if two species overlapped they were both counted. Any gaps without moss were not counted and the total moss cover on each plot was assessed using the 'hand = 4%' method described above.

The total weight of each harvested moss mat was determined by: (1) finding the dry weight of a random 10 cm^2 mat segment that was removed from the mat in the field, had its height measured at three random points, and was air dried for two weeks; (2) the weights were multiplied by the average of 13 random height measurements, 10 from the field mat and three from the 10 cm^2 segment; (3) this number was ultimately divided by the average height of the three points from the 10 cm^2 segment; (4) these corrected weights for the 10 cm^2 segment were multiplied by 225 to obtain the weight of the entire harvested moss mat.

Determining the total weight of each non-harvested moss mat was limited because a 10 cm^2 segment could not be removed; therefore a regression between harvested mat height and mass was created. Dominant moss species, *E. oreganum* and *H. splendens* were subsequently separated to achieve a more accurate regression; however certain plots had heavier influences from *Leucolepis acanthoneuron* and *Rhytidiadelphus triquetrus/loreus.* The former species was grouped with *E. oreganum* while the latter two with *H. splendens* due to similarity of growth forms, thus a more accurate height vs. weight correlation. Each plot was designated either *E. oreganum* or *H. splendens* dominated if their relative abundance, along with that of the similar species previously stated, exceeded 50% of the plot. The height measurements of the non-harvested mats were applied to each dominant species logarithmic regression equation to estimate the biomass of non-harvested mats.

Each soil core was split into two vertical sections in the field, the 0-7.5 cm and 7.5-15 cm soil layers. These layers were stored in Ziplock® bags and immediately transported to the HJA after each site was entirely cored and stored in a 4°C refrigerator. All sites were cored within two days on July 12th-13th. Each core had its roots separated, by vertical section, using four nested Dynamic Aqua Supply® brass sieves (2 mm, 1,000 μm, 500 μm, and 250 μm); no soil core was stored in the refrigerator for longer than three days. Initially the soil was sieved without the addition of water. Approximately 5 g of soil that passed through the sieves from the 0-7.5 cm soil sections was returned to the refrigerator in Ziplock® bags. This soil was kept field fresh and transported from the HJA to Bruce Caldwell at Oregon State University in Corvallis, Oregon, for analysis of soil phosphatase activity. Once the

soil for phosphatase analysis was separated, water was used to wash the remaining soil samples through the sieves. All discernable roots were removed and stored in 50 mL Corning® centrifuge tubes filled with 95% ethanol. Remaining soil was similarly packaged. The roots and soil were immediately stored at 4°C and subsequently shipped overnight air to the College of Environmental Science and Forestry in Syracuse, N.Y., where they were similarly stored at 4°C until EM analysis.

To evaluate soil phosphatase activity in each plot, a 1 g soil to 10 mL water slurry was made. One mL was incubated with one mL of 50 mM p-nitrophenylphosphate at 30°C for one hour. The reaction was terminated with 0.5 mL of 0.5 M $CaCl_2$ and 2 mL of 0.5 M NaOH. The reaction products were centrifuged and supernatant absorbance (410 nm) was measured in a spectrophotometer. Standard curves were prepared from p-nitrophenol and results were calculated as umol p-nitrophenol released per gram of soil dry weight per hour. A more detailed description of this procedure can be found in Caldwell et al. (1999).

For each study site, half of the plots were randomly selected using the Minitab® random number generator. Those sites were subjected to an extreme manipulation, in which the entire feather-moss mat was removed. The other half were left as controlled, completely undisturbed. Moss mat removal was imposed on July 18th. The harvested moss was transported to the HJA and left for future use by Suzan Fritz in McKenzie River restoration projects. Moss that could not be used in this manner was randomly scattered around the study sites, areas that would not influence the study plots, in hopes of proliferation via spore dispersal and/or vegetative propagation. The sites remained undisturbed, following manipulation, for

an entire year in order for ectomycorrhizae to respond to manipulation and turnover. The extent of plot manipulation can be seen in Figure 7.

Figure 7: Photo of a plot not subjected to harvest (a) and one that had the entire moss mat removed (b). These plots were both within site # 4.

Summer 2006 (Year 1):

The experimental sites were revisited almost exactly one year later, June 20th to July 4th, 2006. Soil cores were taken from the center of each plot to allow the largest buffer from external influence as possible (0.75 m). Soil cores and phosphatase analyses were processed as they were in year 0 (2005); however plots were cored 12 days earlier than they were in year 0, on July 1st, due to circumstances beyond the experimenters control.

Ectomycorrhizal Analysis (SUNY ESF - Syracuse, N.Y.):

Each year, there were a total of 168 EM samples to be evaluated for abundance of live, colonized root tips, two from each plot signifying the two vertical soil sections, 0-7.5 cm and 7.5-15 cm. Each sample took about 50 minutes to analyze. A method for EM root tip quantification was determined from the recommendations outlined in Grand and Harvey (1982), the methods outlined in Brundrett et al. (1996), and personal correspondence with Dr. Tom Horton, SUNY ESF.

Each respective vertical layer from each plot was analyzed individually for EM abundance, the total number of live EM root tips per sample. All of the 50 mL Corning® centrifuge tubes for a sample were emptied into a shallow water bath to dilute the ethanol. Clumps of soil and roots were placed on a 7x7 cm plastic Petri dish, with a 1 cm^2, grid and picked apart under a dissecting microscope for live EM root tips. Live EM tips were considered: (1) plump without any external shriveling; (2) having a visible mantle; (3) showing a white/healthy root stele when broken open;

and (4) able to recover to original form after being squeezed by a pair of forceps; in better cases the tips would emit a white exudate upon squeezing. Initial samples were subjected to cross-section analysis for presence of a healthy mantle and Hartig-net. Once comfortable with live EM tip identification, this latter procedure was discarded. A photographic comparison of root tips counted as alive and infected (Figure 8), and those that were not counted (Figure 9), is presented.

Any discernable root tips that showed healthy EM fungus infection were counted. This included short, stubby root tips. Figure 10 provides an example of the number of root tips counted on an individual sample, where the numbers of live EM tips in the pictured samples are denoted. The total numbers of live EM tips for each vertical layer of each plot during each year were recorded and were the most important data of this experiment.

Figure 8: Sample of root tips considered live, healthy, and infected in the EM abundance analysis.

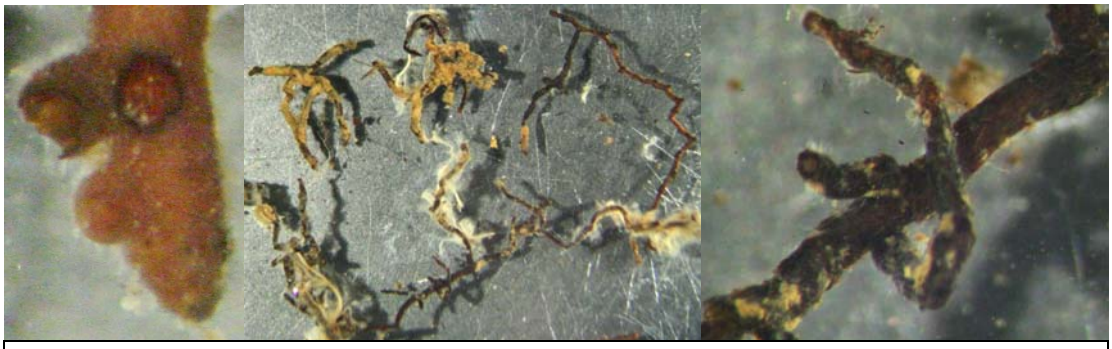

Figure 9: Root tips considered dead, therefore not included in EM abundance count. a) shows dead stele on two tips, however the other two had live steles and were counted; b) shows some infected and some uninfected roots, the infected roots were too shriveled and were likely not active at harvest; c) shriveled, dead root tips.

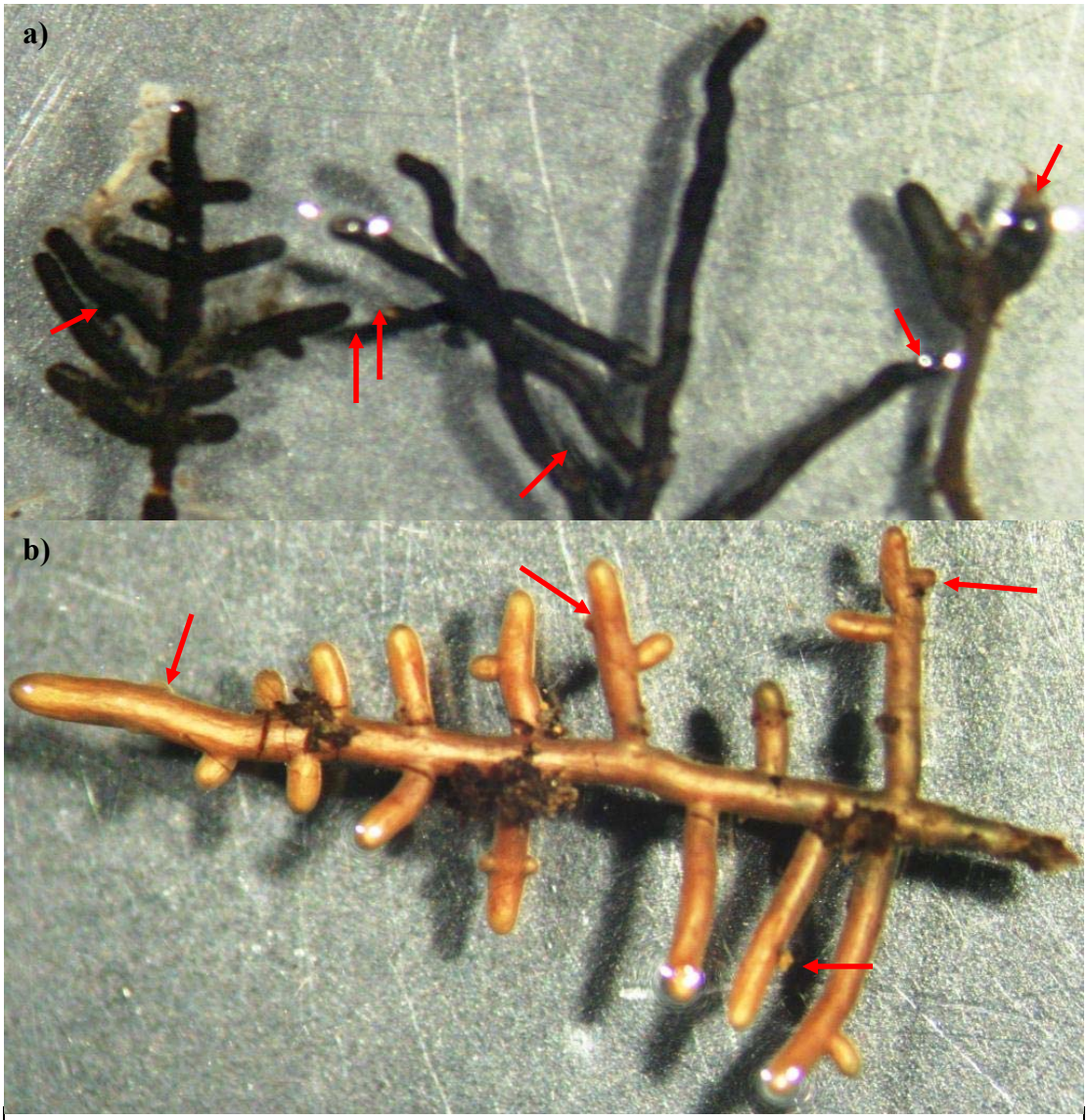

Figure 10: Count of live EM tips on different samples. a) 17 live EM tips; b) 21 live EM tips Exceptions due to lack of visibility or broken tips are noted with red arrows in each image.

4. Data Analyses:

Preliminary analyses, after site/plot characterization data were collected in Year 0 before any EM comparison could be made, involved the identification of trends between the different sites, individual plots on a site, and all plots together. Regression analyses were used to find correlations between the depth and biomass of the moss mats on each plot in an effort to extrapolate weights of the unharvested moss mats. Regressions were also used to find relationships between the two dominant moss species (*E. oreganum* and *H. splendens*), as well as moss mat biomass, on EMT reduction in the harvested plots.

PC-ORD® version 5.0 was used to conduct a nonmetric multidimensional scaling (NMS) ordination to identify site and plot relatedness for all biotic and abiotic data collected in Year 0. The primary matrix consisted of embryophyte percent cover on each plot, while the secondary matrix included the other biotic/abiotic variables (moss relative cover, soil moisture, canopy cover, soil phosphatase activity, moss mat depth, moss mat biomass, and relative cover of moss species).

Once EM tip data and phosphatase activities from Year 1 were collected, comparisons of the change in these two components following moss mat manipulations were made. Anderson Darling tests for normality revealed a non-normal data distribution in every case. Subsequently, Mann-Whitney Nonparametric tests were used to compare all EM root tip counts and phosphatase activities.

A second NMS ordination was constructed in which only harvested plots were included. Data from Year 1 was included in the secondary matrix such that the

influence of changes in EMT and soil phosphatase activities one year after manipulation could be assessed.

Finally, sites were considered separately. Mann-Whitney tests were used to determine whether within each site, the removal of forest floor moss cover had a similar effect as it did when all sites were considered together.

Results

Although the abundance of live ectomycorrhizal root tips (EMT) varied between treatments prior to manipulation (P = 0.02; Figure 11), it was found that, in the central-western Oregon Cascade Mountains, the overall removal of forest floor moss mats significantly decreased the abundance of EMT in the soil beneath (P = 0.0015; Figure 12). Interestingly, those plots that were not manipulated showed a significant increase in EMT from year 0 to year 1 (P = 0.0039, Figure 13), while the entire forest as a whole supported a statistically similar number of EMT during each sampled year (P = 0.7260, Figure 14). Therefore, I reject the null hypothesis (1); the removal of FFMC significantly reduced EMT one year after harvest.

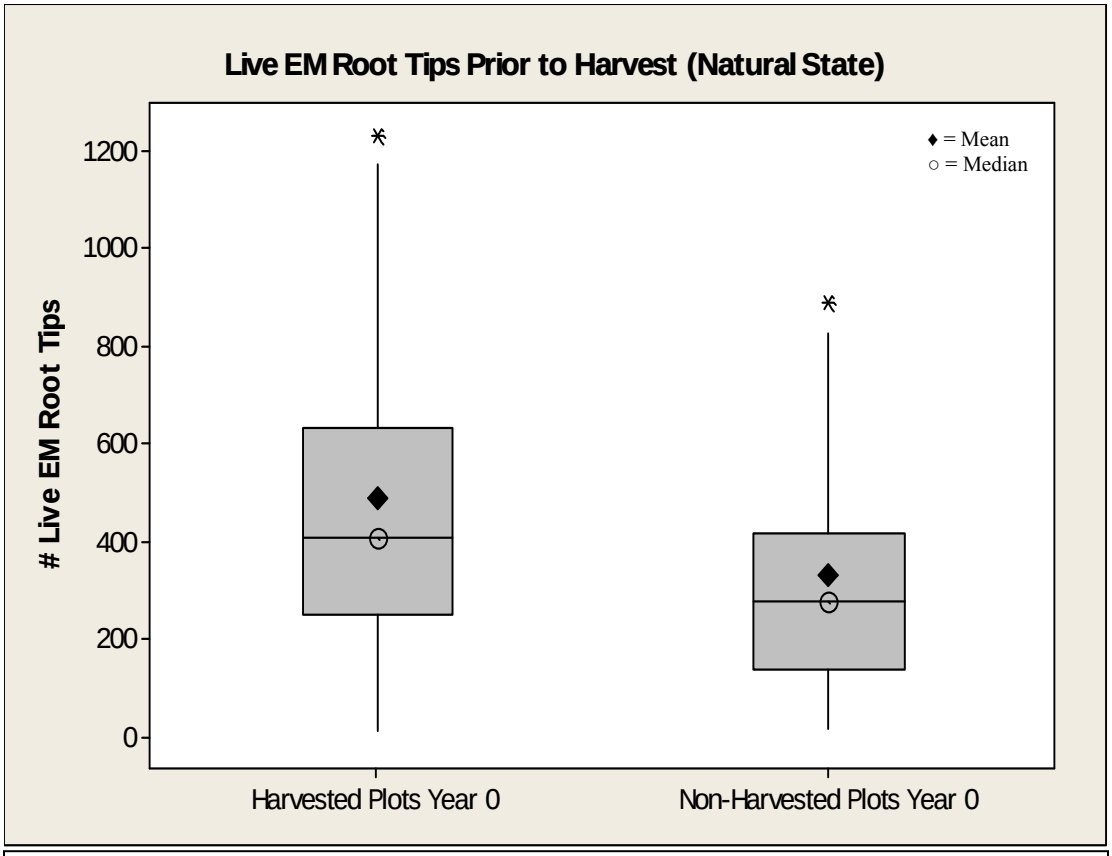

Fig. 11: Boxplot of live EM tips prior to moss removal; plots are separated based on future manipulation. There were natural variations between the number of EMT between treatments prior to manipulation (P = 0.02).

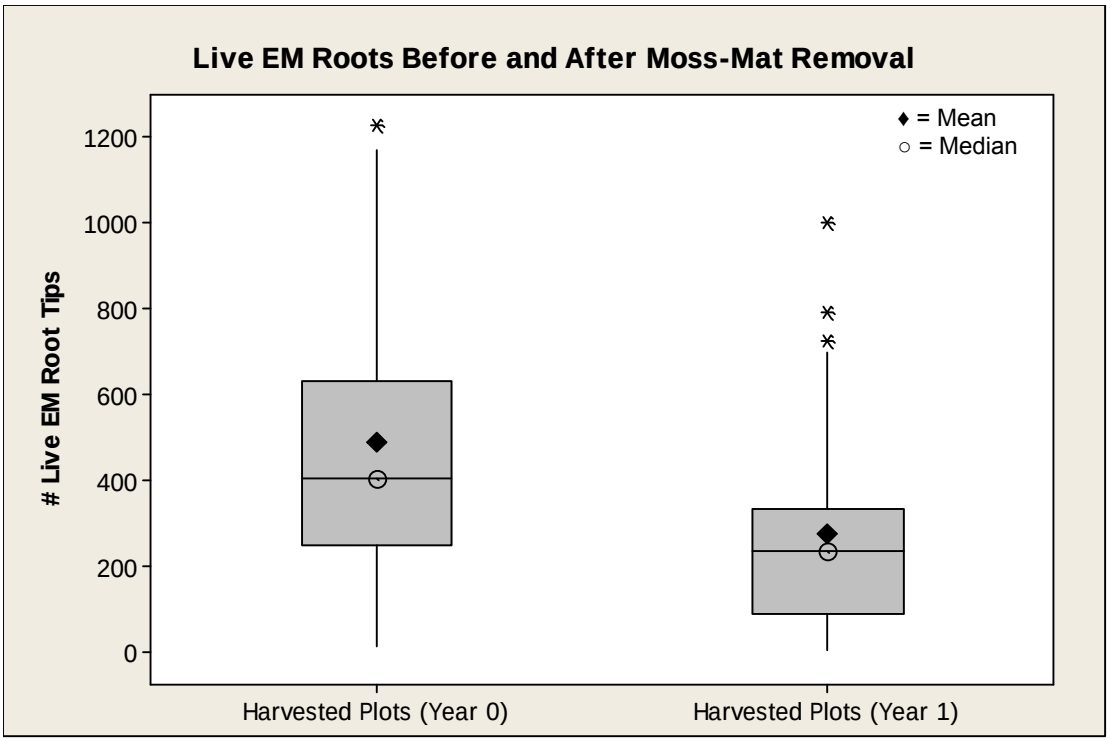

Fig. 12: Boxplot of live EM root tips in harvested plots before and after manipulation. There was a significant decrease in live EM roots after the moss mat was removed from the respective plots (P = 0.0015)

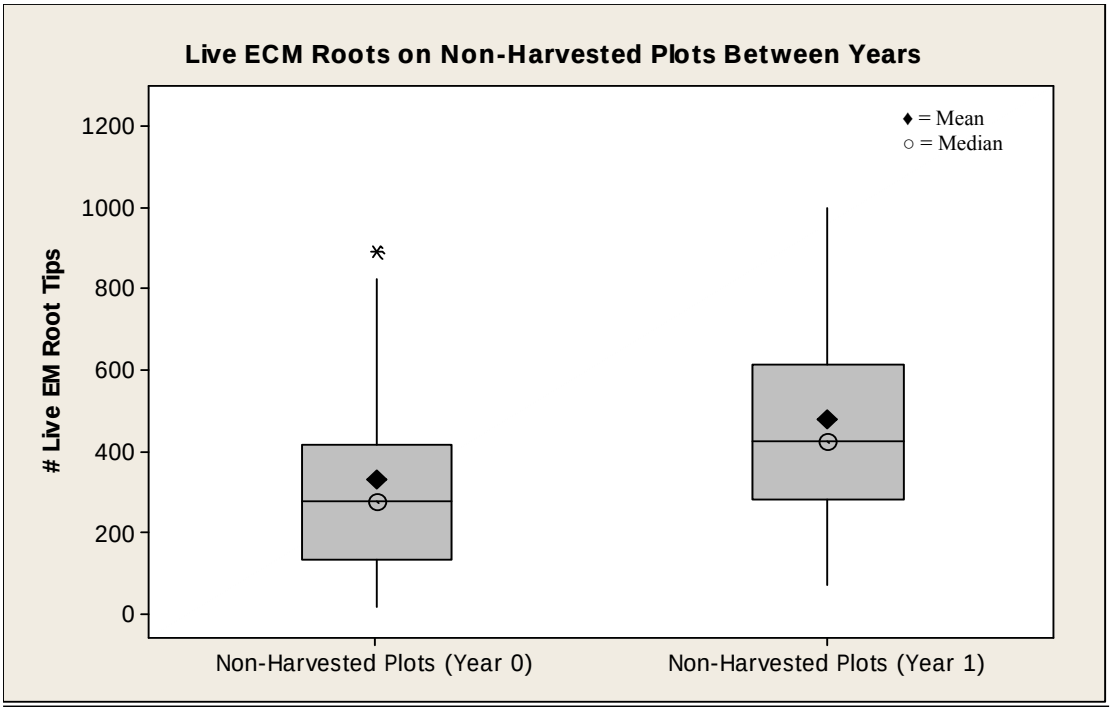

Fig. 13: Boxplot of EMT in non-harvested plots before and after manipulation. There was a significant increase in EMT between years with no moss mat removal (P = 0.0039). This indicates that in a natural state, year 1 provided better soil conditions for the proliferation of ectomycorrhizal fungi

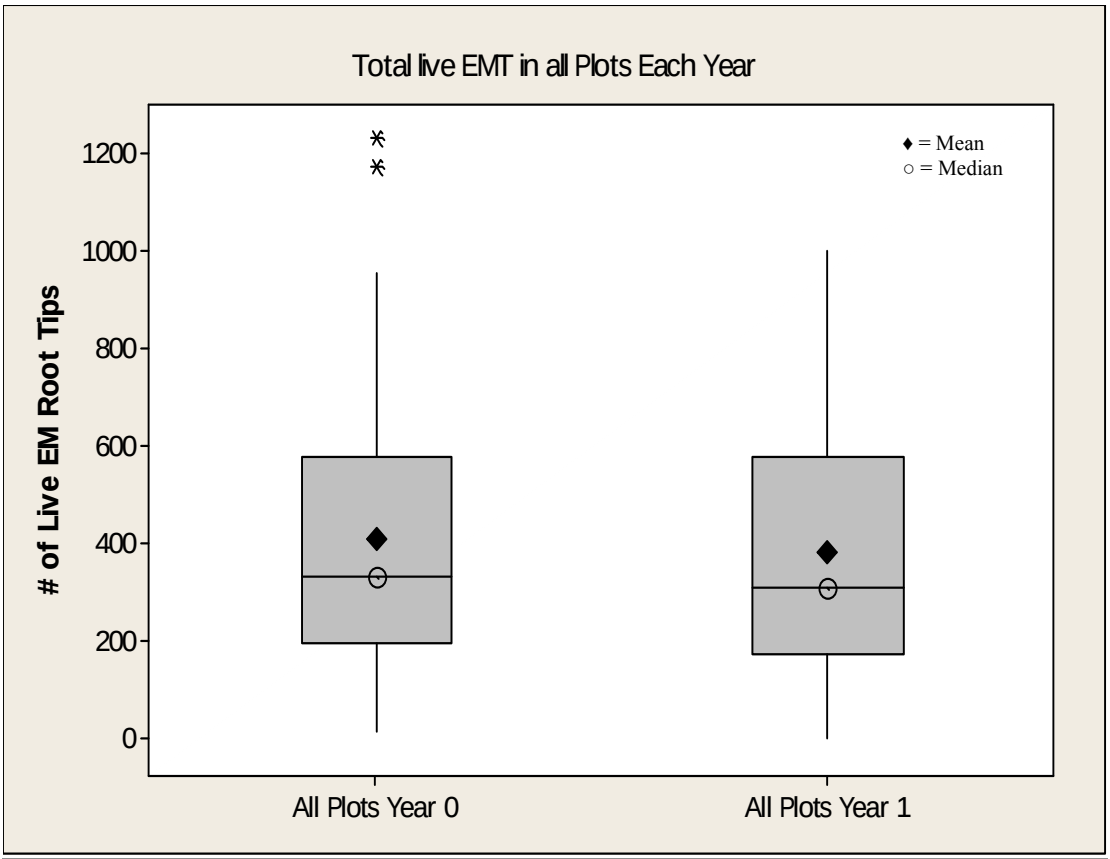

Fig. 14: Boxplot of total live EM tips from each year. There were no significant differences between the medians of each treatment (P = 0.72)

An NMS ordination of individual sample plots, for all data collected during year 0, indicates that: (1) the sampled plots were not homogeneous and represented environmental gradients both within and among sites; (2) Site 1 was the only site where its component plots grouped together in ordination space; (3) Site 2 contained the most plots considered as outliers in ordination space; and (4) the most important trends (vectors) influencing plot position in ordination space were *E. oreganum* % cover, *H. splendens* % cover, and moss mat depth with their respective R^2 values along axes one and two being -0.560, -0.712; 0.591, 0.776; and 0.447, 0.443 (Figure 15).

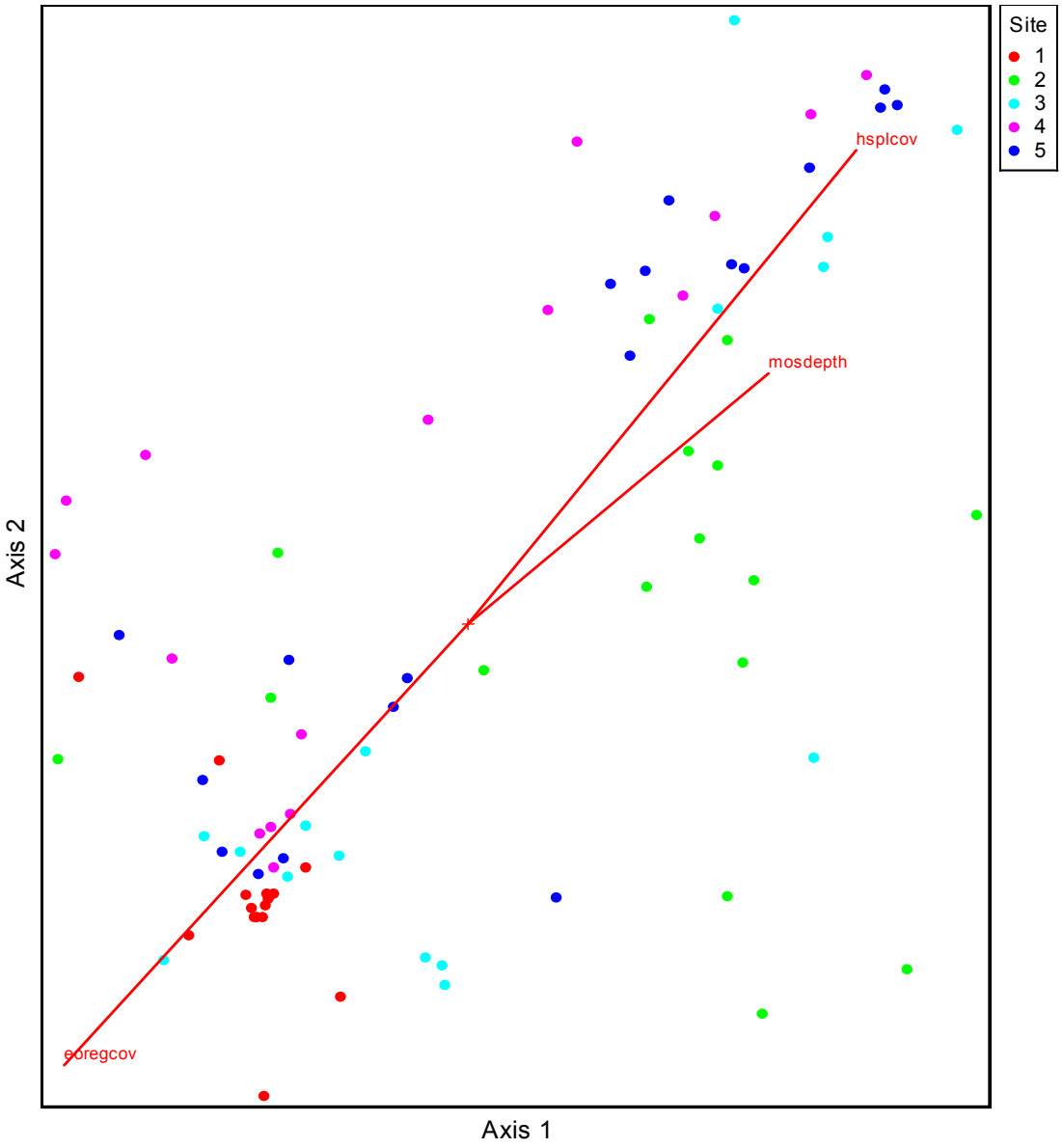

Figure 15: NMS ordination of all plots, harvested and non-harvested, from year 0. Primary matrix = percent cover of all embryophytes on each plot; Secondary matrix = relative cover of moss species, all biotic and abiotic variables collected during year 0. The three strongest vectors, from left to right, are *E. oreganum* % cover, moss mat depth, and *H. splendens* % cover.

Subsequently, the harvested plots were ordinated by themselves; another NMS ordination evaluated influences on plot relatedness that may not have been apparent with all plots considered together. It was found that the outlying plots in this comparison had a stronger domination by *R. triquetrus* while plots dominated by *E.*

oreganum and *H. splendens* tend to have defined groupings. The harvested plots were not homogeneous and represent environmental gradients both within and among sites. The four strongest vectors were *E. oreganum* % cover, *R. triquetrus* % cover, moss mat depth, and *H. splendens* % cover with their respective R^2 values along axes one and two being -0.749, -0.668; -0.095, -0.29; 0.484, 0.397; and 0.761, 0.735 respectively (Figure 16).

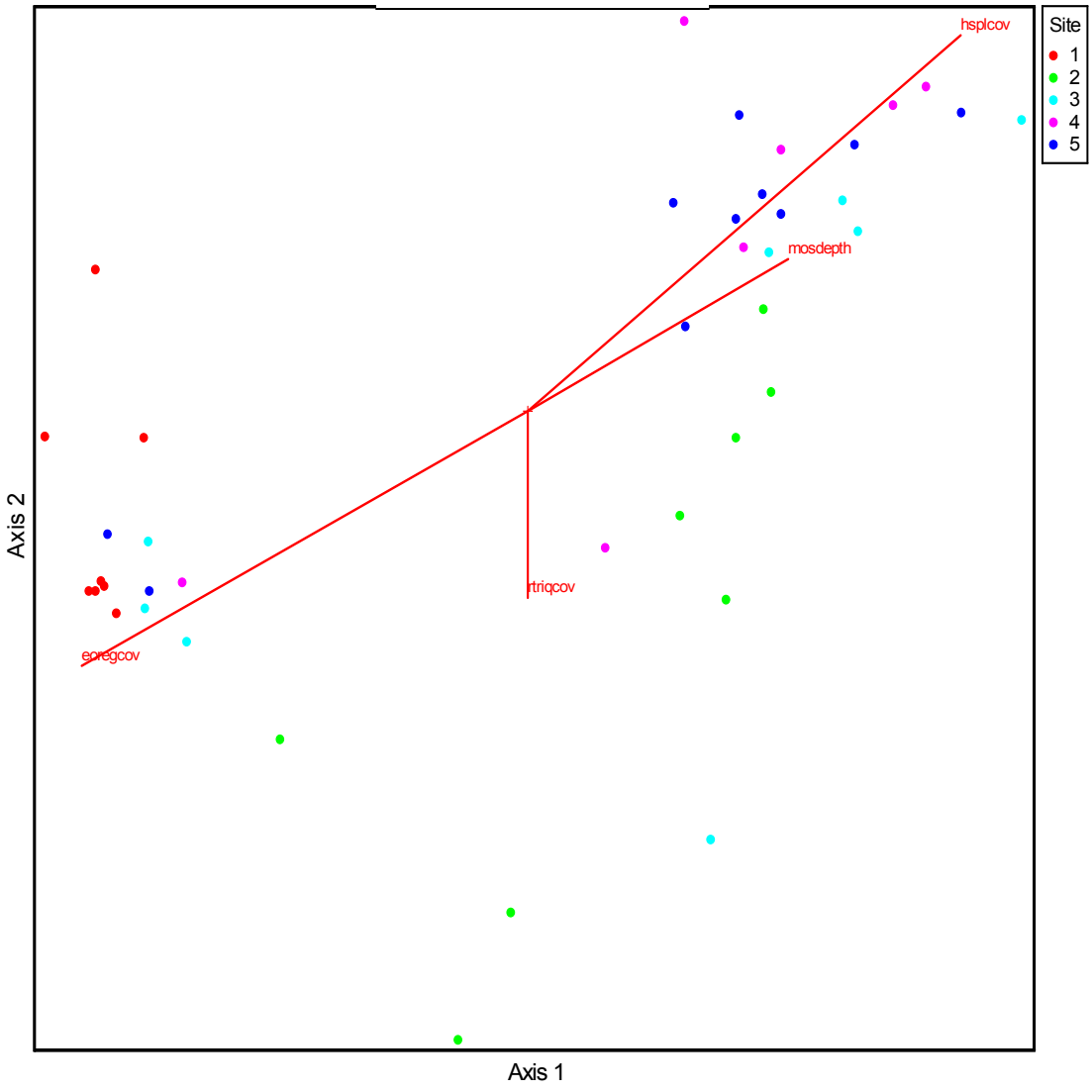

Figure 16: NMS ordination of all harvested plots with data from year 1 considered (change in EMT and change in soil phosphatase activities). The four strongest vectors, from left to right, are *E. oreganum* % cover, *R. triquetrus* % cover, moss mat depth, and *H. splendens* % cover.

The ordination representing EMT reduction following harvest showed a possible differential reduction in EMT between the two dominant moss species (Figure 17). Although there was not a significant correlation along either axis (R^2 = 0.018 and 0.074) when all variables were considered together, further investigation was carried out. A Mann-Whitney test found that, for the harvested plots, the dominant moss species did not have a differential affect on EMT reduction from year 0 to year 1 (P = 0.76); root tip reduction was 210.8 ± 261 (mean ± 1 s.d.) for *E. oreganum* and 213.3 ± 247.1 (mean ± 1 s.d.) for *H. splendens*. Histograms are included as Figures 18 & 19 respectively. Therefore, I fail to reject null hypothesis two; the dominant moss species did not have a differential effect on EMT abundance one year after harvest.

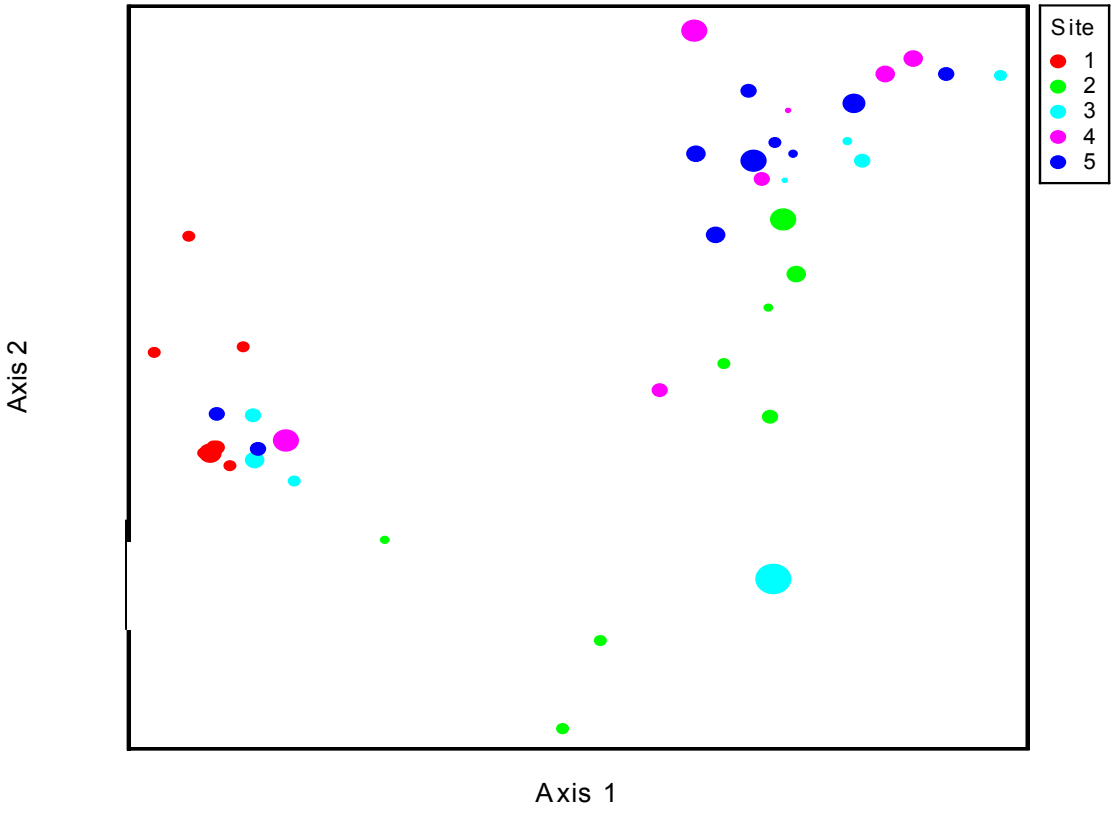

Figure 17: NMS ordination of harvested plots, considering the influence of EMT change on plot position in ordination space. Circle size represents EMT reduction from Year 0 to Year 1.

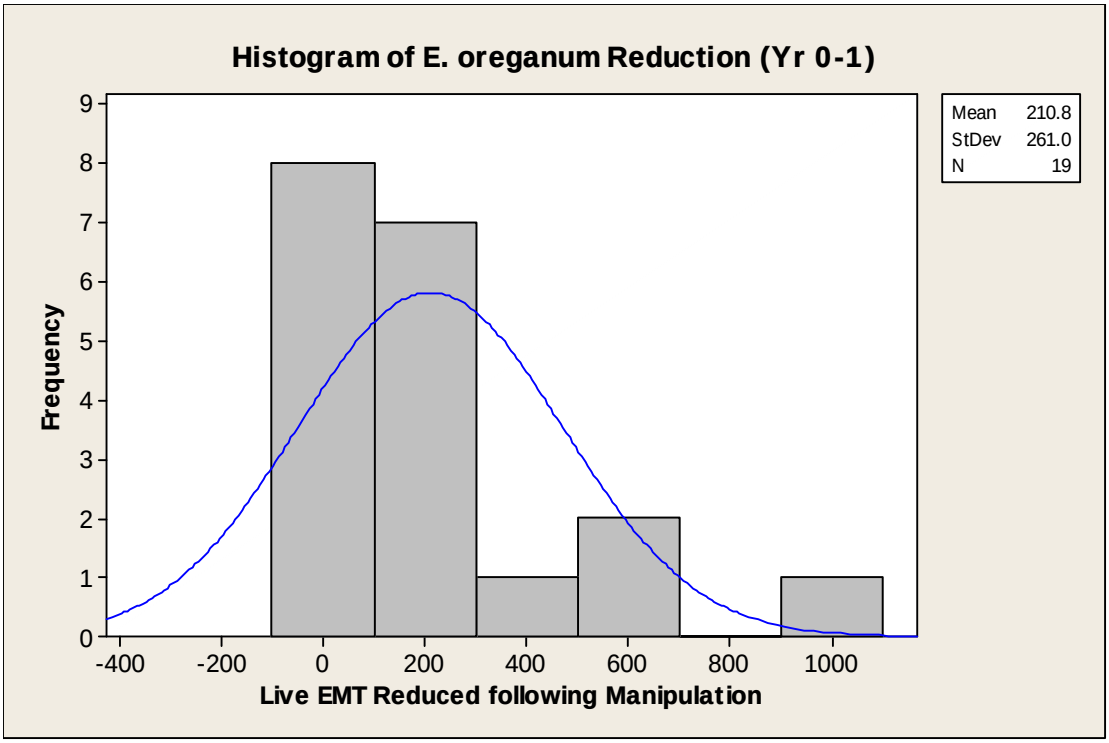

Figure 18: Histogram of the number of EMT reduced from year 0 in harvested plots dominated by *E. oreganum* following manipulation.

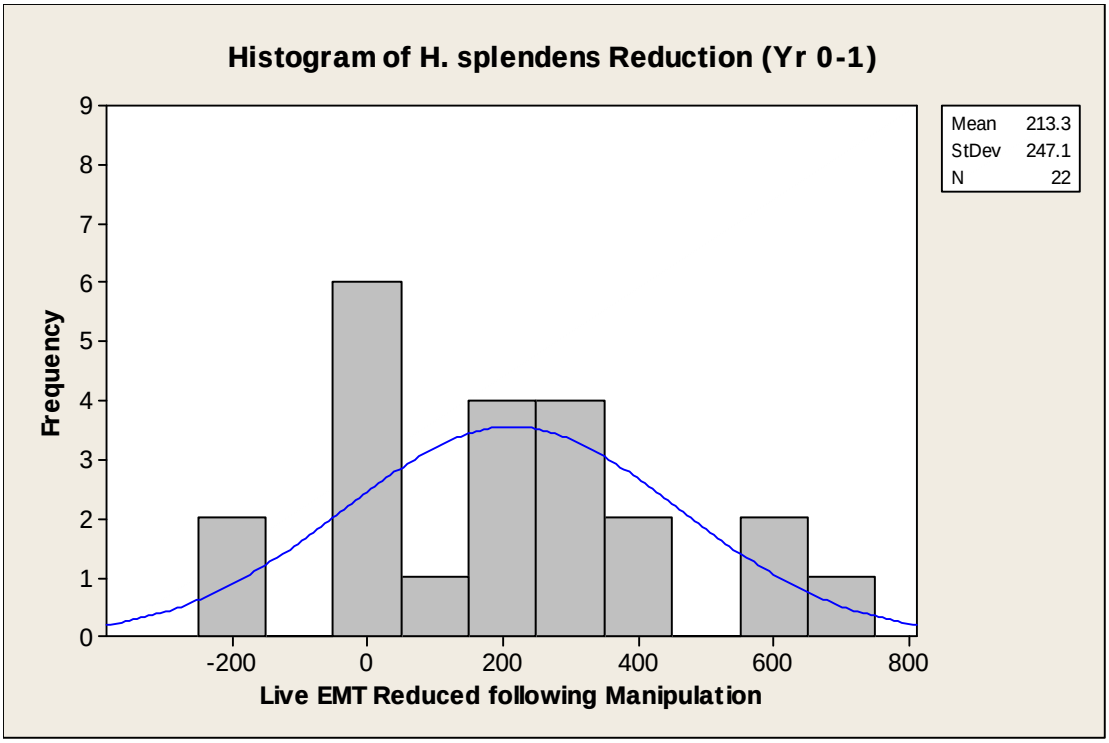

Figure 19: Histogram of the number of EMT reduced from year 0 in harvested plots dominated by *H. splendens* following manipulation.

The effects that moss mat biomass had on EMT were also assessed. It was found that when all harvested plots were treated together, moss mat height did not serve a significant predictor of moss mat biomass ($R^2 = 0.1954$, Figure 20); however when the two dominant moss species, *E. oreganum* and *H. splendens*, were separated and logarithmic regressions were performed individually, moss mat height proved to be a reasonable predictor of biomass ($R^2 = 0.4721$ and $R^2 = 0.5669$ respectively, figure 21). Regardless, there were no correlations between the biomass of a plots moss mat and the effect on EMT, even when dominant moss species were separated (Figure 22). Therefore, I fail to reject null hypothesis three; moss mat biomass has no effect on the abundance of EMT following moss mat harvest.

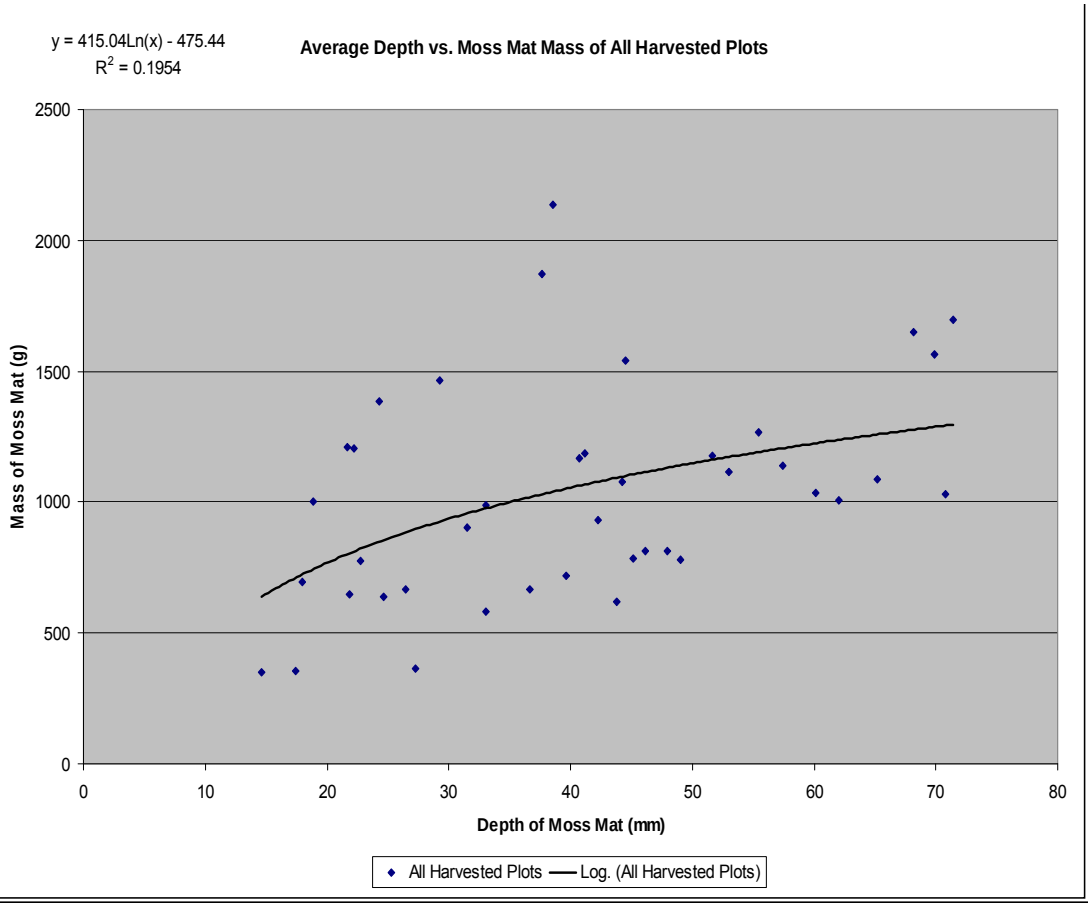

Figure 20: Logarithmic regression to see if moss mat biomass can be predicted using moss mat depth.

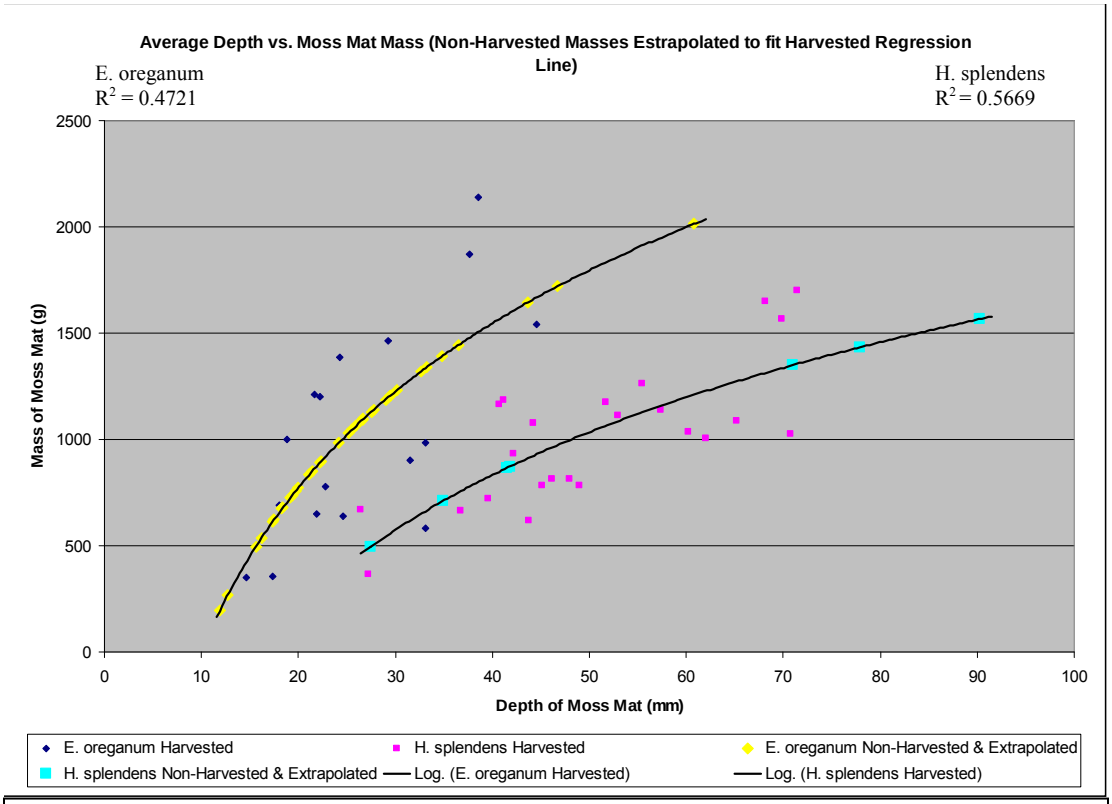

Figure 21: Logarithmic regressions of moss mat mass vs. height with dominant moss species separated and non-harvested plots extrapolated.

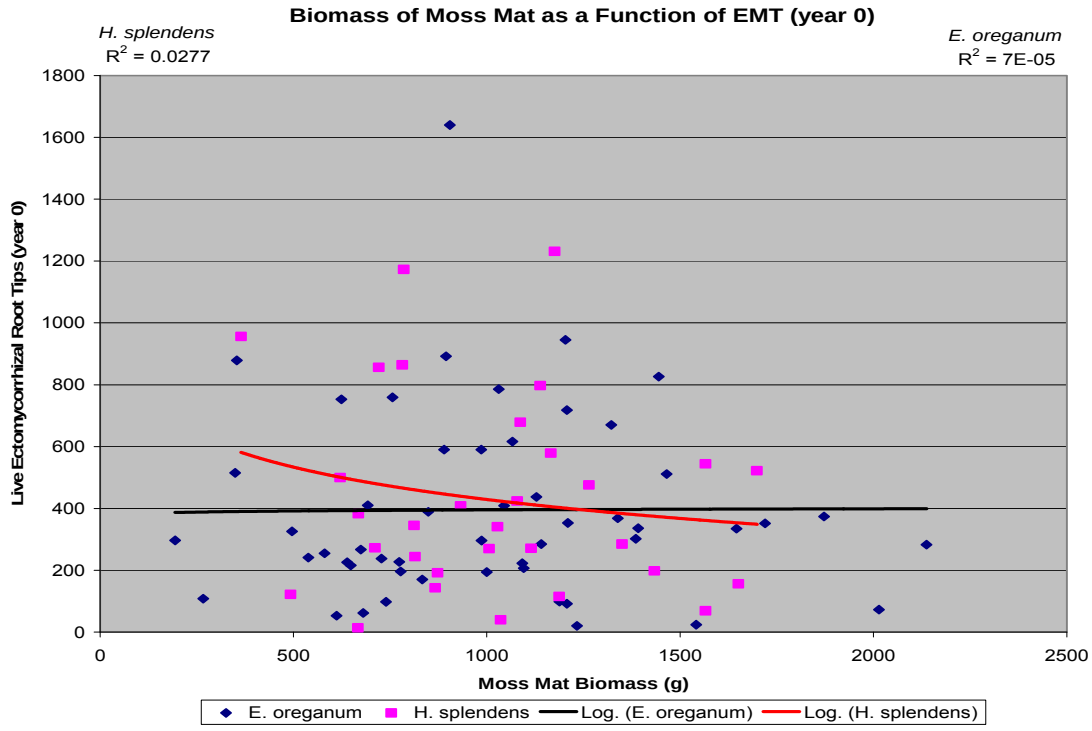

Figure 22: Logarithmic regression of moss biomass and EMT in year 0. There is no correlation between the two variables even with dominant moss species separated

Soil phosphatase activity in each plot did not mirror the trends exhibited by the EMT following moss mat harvest. Mann-Whitney tests determined that soil phosphatase activity was statistically similar in harvested and non-harvested plots prior to manipulation ($P = 0.0795$) and following manipulation ($P = 0.4150$); however both the harvested and non-harvested plots showed a significant increase in soil phosphatase activity in year 1 ($P = 0.0003$ and $P < 0.0001$ respectively). A summary of medians is presented in Figure 23. In every combination, including dominant mosses and moss mat biomass, there were significant increases in soil phosphatase activity in year 1 as compared to year 0. There was a negative correlation between soil phosphatase activity and EMT in the harvested plots, whereas there was a positive correlation in the non-harvested plots. Therefore, I reject the fourth null hypothesis; correlations do exist between soil phosphatase and EMT.

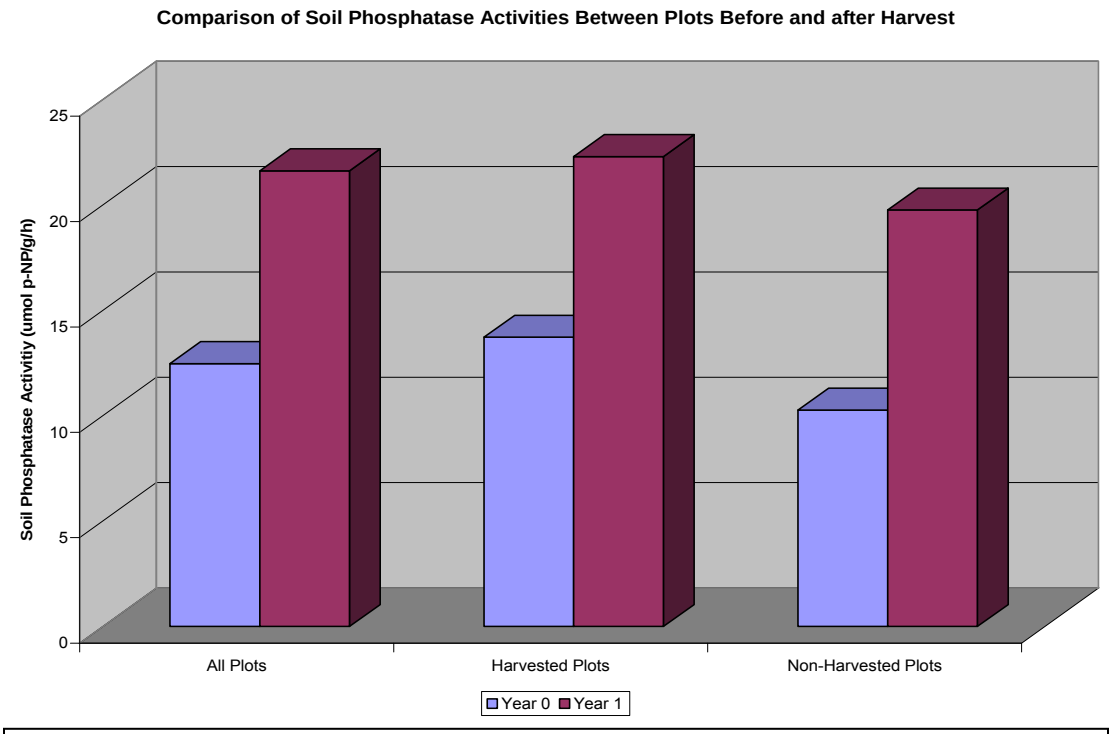

Figure 23: Median soil phosphatase activities for sample plots. Results from Mann-Whitney tests are presented above, however only medians are presented in the graph.

There are myriad comparisons to be performed between plots on the site level, as well as ecologically similar plots across all sites. These analyses may elucidate influences on EMT that were not determined herein, looking at all plots between all sites; however the general trend seen throughout all sites is consistent within each individual site. The removal of forest floor moss cover significantly reduced the number of EMT one year after manipulation; however the P-values did not reflect this trend for each site because the sample size was too small. Mann-Whitney values for these comparisons were: Site 1 (P = 0.0742); site 2 (P = 0.1409); site 3 (P = 0.372); site 4 (P = 0.0553); and site 5 (P = 0.033). A graph comparing medians is presented to evidence the obvious reduction in EMT on harvested plots within sites (Figure 24).

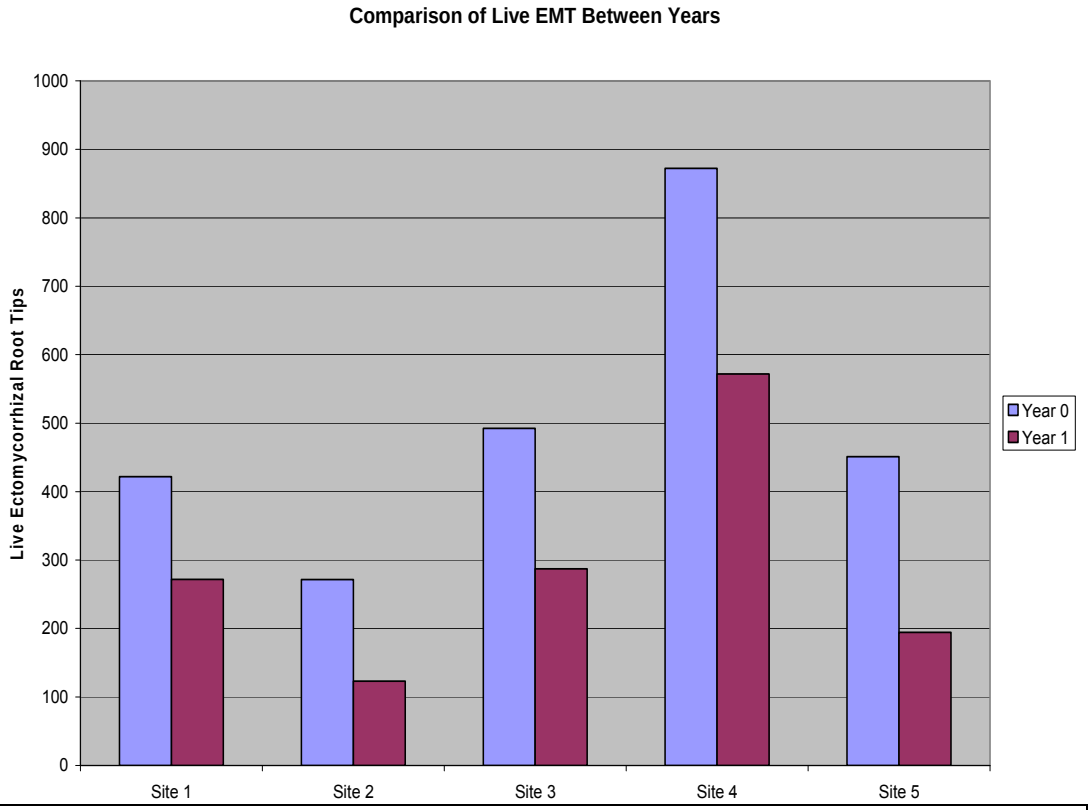

Figure 24: Live ectomycorrhizal root tips before and after manipulation, separated by sites. Bars represent median values for all harvested plots within the site, where P-values for Mann-Whitney tests are supplied in the above paragraph. Only Plot 5 has statistically less root tips following manipulation due to the small sample size within sites.

Further information was also collected to elucidate any cryptic patterns between treatments. Precipitation data was collected from the national weather service station at Leaburg in Lane County, Oregon, approximately 30 miles east of the HJA. Daily precipitation indicated that the year between initial coring (Year 0) and final coring (Year 1), from July 13th 2005 – July 1st 2006, had significantly greater precipitation than the year preceding the experiment, July 14th 2004 – July 1st 2005 (Figure 25; P = 0.012). Precipitation in the month prior to coring was twice as much for Year 1 than Year 0 (Figure 26). There was a large rain event in the month preceding year 1 coring (Figure 26).

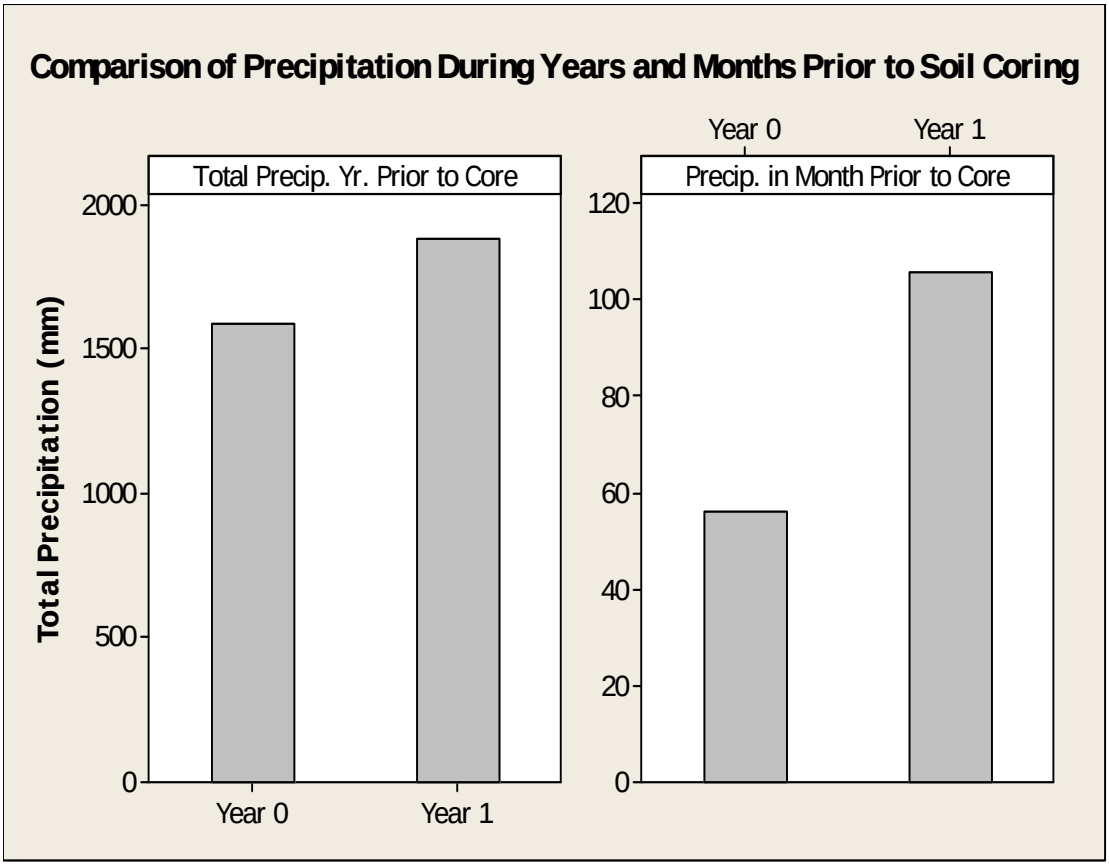

Figure 25: Comparison of the total precipitation in the study region for the entire year preceding ectomycorrhizal coring, as well as the month prior to coring. In both cases the rainfall prior to coring was less for Year 0 than Year 1 (P = 0.012).

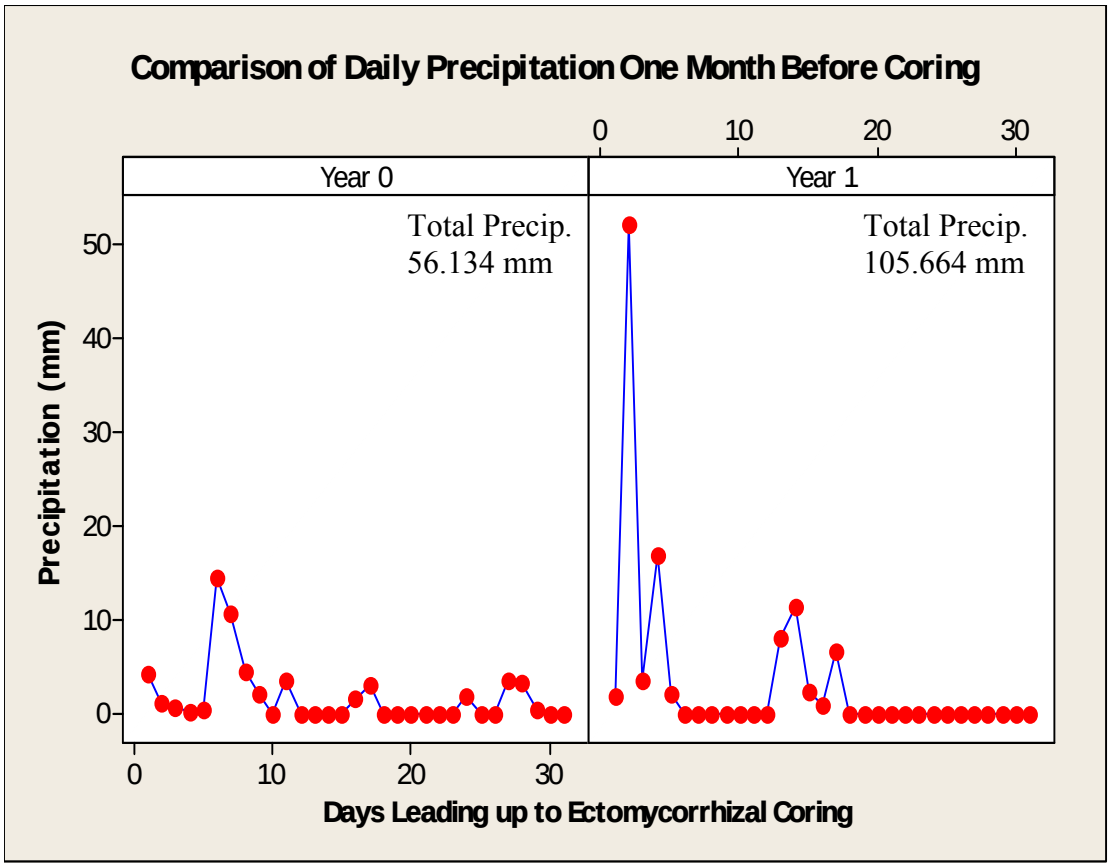

Figure 26: Precipitation for the month prior to ectomycorrhizal coring for both years. Year 1 had a much more intense rain event 30 days before coring than year 0, while experiencing twice the rainfall as year 0.

Discussion

Considering the vast yet incomplete data available on the ecosystem roles of both mosses and EMF in the literature, as well as the influences each may have on the other and the forest system as a whole, the research conducted here provides a valuable contribution to forest ecology and temperate ecosystem science. Further analyses of the data collected in this experiment may reveal additional relationships than those previously presented. Subsequent research will likely identify specific relationships to explain the results seen here; however, the following discussion attempts to analyze these results in the context of the literature review previously presented.

1. Abundance of Live Ectomycorrhizal Root Tips:

The primary finding (Figure 12) of this study, that the removal of FFMC significantly decreased the abundance of EMT one year later, is important. Before the moss mats were removed in Year 0 it was found that in a natural state, the harvested and non-harvested treatments exhibited differences in the number of EMT per soil core (Figure 11), with the non-harvested plots containing considerably less EMT than the harvested plots. Also, the non-harvested plots showed a significant increase in EMT one year after the initial measurement (Figure 13). There are myriad explanations for these trends, although empirical support is lacking.

Microscale Variability, EMT Turnover, and the Range of Moss Influence:

The increased abundance of EMT in the non-harvested plots following manipulation (Figure 13) may be attributable to more favorable environmental conditions for EM infection in Year 1. The total number of EMT for all of the plots between years (Figure 14) indicates, perhaps, that the forest system as a whole was able to keep EMT constant, perhaps a Clementsian "self-regulation". This idea ties into common mycorrhizal networks (CMNs) that may be functioning throughout the forest. As Selosse and Duplessis (2006) have indicated an individual fungal mycelium can associate with two or more plants. The extent of hyphal connections is questionable (Pawlowska & Taylor, 2004; Horton, personal correspondence) and seems restricted to one genet of a single fungus; however, the bidirectional transfer of carbon, nitrogen, and phosphorus between plant species via interconnected mycelia has been shown (Tiwari et al., 2004; Simard et al., 1997 and references therein). It may be possible that a *P. menziesii* rooted beneath both harvested and non-harvested plots would exhibit differential EM colonization by concentrating EM tips beneath mosses in an effort to acquire scarce materials, whereas the lack of moss cover decreases ectomycorrhizal presence because the bare roots can better handle absorption without prolific EM associations. If nutrients and water are not being trapped by mosses in areas with bare soil, trees may not necessarily need to associate with ectomycorrhizal fungi with such abundance; tree roots may be perfectly capable of dealing with water and nutrient acquisition independent of ectomycorrhizal fungi.

The number of EMT were statistically similar between treatments prior to harvest (Figure 11), which may be attributable to the fact that "the spatio-temporal

variation of EMF on root tips is… very high" (Stendell et al., 1999). As Taylor (2002) points out, our accurate assessment of EMF is impeded by skewed abundance distribution patterns, which can occur due to: (1) proliferation in and around nutrient patches; (2) the ecological and biological behavior of individual EM fungal species; (3) sampling effort; (4) time of sampling; and/or (5) soil chemistry (Horton & Bruns, 2001). Therefore, patterns of EMF abundance between plots may be statistically different when they should not be, and vice versa. When harvest was imposed, however, the fact that EMT abundance of harvested plots significantly decreased from Year 0 (Figure 2), while the opposite was seen for non-harvested plots (Figure 3), indicates that the substantial plot sample size was able to account for the majority of EMF variation.

Although coring for EMT did not occur on the same date each year, the 11 day difference almost certainly did not affect EMT abundance between years because of annual variation in seasonal progression. Seasonality was generally consistent and it seems as if ectomycorrhizal root tip turnover does not typically react on such a small scale. Ectomycorrhizal root tips have been found to turnover yearly in an Oregon *P. menziesii* stand (Hunt and Fogel, 1983). This Hunt and Fogel (1983) finding legitimized the allotment of one year between harvest and subsequent coring for EMT to react to manipulations. However, as Horton (personal correspondence) has mentioned, the cause of fine-scale root tip patchiness is still under question and could even be due to temporal variation at a small scale, an idea that must be considered.

Soil cores were taken from the top 15 cm of soil because: (1) the majority of roots and ectomycorrhizal root tips are present there (Godbold et al., 2003); (2) the zone of feather-moss influence is likely restricted to the upper soil layers, below which only a small amount of nutrients and water pass; and (3) depths below 15 cm would likely be regulated by factors other than FFMC. The efficiency of EMF to bind and absorb nutrients and water (Kramer & Wilbur, 1949; Harley & McCready, 1950; Bowen, 1973; Brownlee et al., 1983; Plassard et al., 1994) leached from mosses, may lead to a funnel-effect in the soil, causing the effects of feather-moss to decrease with increased depth. This identifies the rationale behind splitting each soil core into 7.5 cm sections; the greatest affect of FFMC on EMT abundance is presumed to be in the uppermost layers of soil. Differential effects of FFMC on EMT within respective soil layers were not included here because a strong overall relationship was found when considering the 15 cm soil core as a whole. The unreported data indicate, however, that EMT were significantly reduced in both respective soil profile layers, 0-7.5 cm and 7.5-15 cm. It may be interesting to include differences in future publications. For now, let us turn to the two overarching influences FFMC has on the soil microclimate and explore why such a decrease in EMT were observed following harvest.

Moisture and Temperature:

The removal of forest floor feather-moss mats influences soil moisture, temperature, and nutrient content (Glime, 2006 and references therein). Foremost, the moisture regime of the central-western Oregon Cascades is characterized by wet

winters and extremely dry summers; in some places less than 10 percent of the total precipitation falls during the summer (Waring & Franklin, 1979). At the HJA average annual precipitation varies with elevation; the sites studied receive approximately 228-254 cm annually. It seems possible that the low amounts of moisture received during the summer would initially be caught and retained by forest floor mosses (Glime, 2000; Schofield, 1985) with only a small percentage entering the soil beneath to become available for tracheophytes. This could play a major role in the significant reduction of EMT observed in the harvested plots.

Without moss cover, scarce rainwater can directly enter the soil without impediment and rapidly percolate to the root zone. This excess water may lead to reduced need for EMF; when it rains the water is readily available to roots in the first 15 cm of soil, perhaps circumventing the need for excess EMT. This could be further tested by separating EMT data of the harvested plots by depth to see if EMT are more abundant in the lower 7.5 cm during Year 1; as the water gets absorbed by the first 7.5 cm it becomes scarcer, perhaps requiring greater EMF abundance to absorb it in the lower soil layers.

The non-harvested plots, on the other hand, may have been deprived of rainwater by the efficient forest floor feather-moss barrier. Soil insulation by mosses (Bonan, 1991) may not have decreased evaporative losses significantly enough to keep the soil moist (van Tooren et al., 1985), especially during the dry days preceding harvest in Year 1 (Figure 26). The moisture gain from the heavy rain event that occurred 30 days prior to coring (Figure 26) may have simply led to excessive ectomycorrhizal growth to absorb as much as possible. It has been shown that EMF

aid in drought tolerance (Bowen, 1973) and enhance nutrient absorption during drought (Querejeta et al., 2003), which would expectedly increase their abundance beneath non-harvested plots in this situation, the precise pattern that was observed.

Nutrients and Ions:

Forest floor feather-mosses have previously been shown to act as sinks for atmospheric nutrients (Weber & Van Cleve, 1984), thus preventing rapid leaching to lower soil levels. Their ability to function as a reservoir, sequestering valuable nutrients in their tissues for long periods of time, may be a principal mechanism that explains their ability to influence ectomycorrhizal abundance. In the CWOC, mosses are in direct competition with tracheophytes for nutrients and efficiently act to capture and retain those nutrients, making them unavailable to the rest of the system.

Feather-mosses: (1) take up threefold more N, P, and Mg than the spruce trees in an Alaskan forest (Oechel and Van Cleve, 1986); (2) translocate nutrients from old to new tissue during growth (Skre & Oechel, 1979); (3) quickly immobilize N in tissues, retain it for long periods of time, and slowly release it to the soil (Weber & Van Cleve, 1983); (4) most leached nutrients are quickly and efficiently reabsorbed (Gupta, 1976); (5) have high CEC and sequester cations on cell-wall sites or intracellularly (Koedam & Büscher, 1983); and (6) do not associate with mycorrhizal fungi (Read et al., 2000; Selosse, 2005; Wang & Qui, 2006). Ectomycorrhizae: (1) are the most important absorbing organs of their host plants (Harley, 1978); (2) are more efficient at capturing and binding nutrients than non-mycorrhizal roots (Bowen, 1973; Plassard et al., 1994; Yanai et al., 1995); (3) have a greater surface area of thin

mycelium with a higher CEC than plant roots (Marschner et al., 1998); (4) are especially beneficial in nutrient absorption at low fertility levels (Allen et al., 2003), with infection even being found to be suppressed at high nutrient levels (Hatch, 1937); (5) can access nutrient forms that are unavailable to plants, such as rock minerals (Landeweert et al., 2001), mineral pools from saprotrophic fungal mycelia, (Lindahl et al., 1999), and diverse organic phosphates by producing abundant phosphatase crystals externally on mycelia (Alvarez et al., 2004), and (6) can transport absorbed materials long distances via rhizomorphs.

The combination of the physiological traits of both ectomycorrhizal fungi and mosses seemingly places them in "ecological harmony" with one another. Forest floor feather-mosses have evolved to exploit a unique spatial/physiological niche, being the first access, bind, sequester, and slowly release nutrients in atmospheric deposition. These releases can occur in very small quantities, such as during the dry summer when the fully desiccated mosses will sponge up every drop of moisture available, or in large and significant pulses when substantial rain events break long periods of drought, like during the onset of rainy seasons (Figures 2 & 4).

Dissolved organic carbon (DOC), nitrogen, phosphorus, potassium, other essential elements, DNA, RNA, amino acids, phospholipids and proteins may be pulsed from mosses upon rehydration (Turetsky 2003). These are exactly the substances that EM fungi are so efficient at attaining (Read & Moreno, 2003). The production of extraradical mycelial networks (Fogel and Hunt, 1982), which include dense mycelial mats (Aguilera et al., 1993), and efficient enzymes/nutrient exchange sites makes EMF a strategic partner for tracheophytes in situations such as this. The

scarce nutrients being released to the root zone beneath moss cover would likely only get utilized if EMF were actively exploring the soil.

Startsev and Lieffers (2006) performed a study on N leakage in feather-mosses only to find that no desiccation or handling treatment caused them to lose more than 3% of the N. They contend that feather-moss leakage of N to the system is not as large as previously thought, and they even go on to state that this small amount of residual N released to the system is likely to be the only source of that nutrient for plant roots. It therefore may be necessary for EMT to be in greater abundance beneath FFMC because N flow could be limited between mosses and trees in a forest, yet further work is certainly needed in this area. EM fungi must associate with roots, efficiently reach the minute nutrients, and absorb them before the moss is able to re-assimilate. Considering nutrients, it seems as though the finding that EMF proliferate beneath FFMC and are significantly reduced when it is experimentally removed may one day find an explanation in one of these possible mechanisms. Thus, null hypothesis one was rejected; there was a significant decrease in EMT with the removal of FFMC.

2. <u>Dominant Moss Species</u>:

It should be noted that species-specific differences in ecological roles do exist among feather-mosses (Bates, 1994). In Bates' (1994) study it was found that the mosses *Brachythecium rutabulum* and *Pseudoscleropodium purum* reacted differently to nutrient pulses of nitrogen and phosphorus, with the latter moss had a greater net uptake of N and P while also conserving them more efficiently under nutrient-limiting

conditions. As Glime (2006) has noted, the water holding capacity, CEC, growth form, and ecological habit of different moss species varies. Some may have increased external gametophytic spaces to retain excess moisture and further limit it to the forest soil beneath, whereas some may grow in a denser cushion form as opposed to a sparser tall turf. All of these species specific ecophysiological variations warrant the exploration of a differential influence on EMT abundance for the dominant moss species. Because there were two main dominants, *E. oreganum* and *H. splendens,* this was briefly undertaken (figures 18 and 19, ordinations). Although no differential influences were observed for dominant moss species as a whole, further data manipulations may elucidate more silent effects that went undetected, especially if EMT reductions in each soil layer are analyzed independently. This will be performed for future publications.

3. <u>Moss Mat Biomass</u>:

In central Alaska, as feather-moss abundance increases (biomass) soil temperatures and nutrient levels tend to decrease whereas soil moisture increases; therefore, moss production and biomass appear to be inversely correlated with tree productivity (Oechel & Van Cleve, 1986). Mosses can influence decomposition rates by reducing soil temperature and increasing soil moisture, thus reducing the efficiency of aerobic respiration as well as nutrient uptake by higher plants. An increase in biomass will simply add more tissue to a feather-moss mat. Those tissues will also enhance nutrient sequestration and water absorption. As Binkley and Graham (1981) have stated, “moss biomass can represent an important portion of

total production and nutrient cycling and should be considered in studies of ecosystem function."

As a result of this previously reported data it was thought that EMT would be increasingly more prolific as moss mat biomass increased. The ectomycorrhizal fungi may be needed for enhanced nutrient and water absorption if the increased moss mat biomass was sequestering more nutrients and blocking water from reaching the soil. This was not the case in the current study (Figure 22); however, future data manipulations will be undertaken to examine the less pronounced influences. More research is needed on specific microclimatic differences that variations in moss mat biomass may cause.

4. <u>Soil Phosphatase Activity</u>:

Ectomycorrhizal fungi can attain P by using phosphatase to mineralize organic phosphate. Acid phosphatase activity is typically an indicator of the physiological activity of mycorrhizal fungi (Genet et al., 2000). Haussling and Marscher (1989) found a positive correlation between phosphatase activity and length of fungal hyphae associated with EM mantles. Also, Kieliszewska-Rokicka (1992) found that the absorption of phosphate by pine seedlings was closely associated with the formation of mycorrhizal short roots, thus phosphatase production. Therefore, it was hypothesized that the activity of this enzyme in the soil would be positively correlated with the abundance of EMT; the higher the abundance of EMT the higher the activity of phosphatase in the soil. This assumption, however, did not prove to be the case for the harvested plots. Soil phosphatase activity was negatively correlated with

ectomycorrhizal abundance. Regardless of manipulation, the phosphatase activities during Year 1 were significantly greater than those during Year 0 (Figure 23). This result may reflect the fact that soil hyphae were not incorporated in EM quantification. If EM hyphae proliferated more in Year 1 than Year 0, the overall increase in phosphatase activity may find an explanation (Figure 23).

Kieliszewska-Rokicka (1992) determined that for *Paxillus involutus*, increased nitrogen levels resulted also increased the total acid phosphatase activity. An explanation for increased phosphatase activities in the harvested plots is the possibility of increased nitrogen availability in the soil from rainwater deposition. No longer is nitrogen being efficiently sequestered by mosses, rather it is entering the soil directly. Although the abundance of EM decreased in Year 1, phosphatase activities of the reduced fungi may have increased with the input of N on a regular basis in precipitation. The increase in soil phosphatase activities of the non-harvested plots can be attributed to the significant EMT increase in from Year 0 to Year 1. Therefore, null hypothesis four was rejected; soil phosphatase activity was negatively correlated with EMT abundance in the harvested plots, whereas it was positively correlated with EMT abundance in the control plots, perhaps because of the abovementioned reasons.

5. Moss to Ectomycorrhizal Fungus to Ectomycorrhizal Plant: A Three-Way Relationship:

In a recently published review of the fifth international conference of mycorrhizal, Selosse and Duplessis (2006) conclude with a section entitled "Mycorrhizal networks: linking plants and shaping communities." In this section they discuss the fact that even beyond linkages of trees by ectomycorrhizal fungi, mycorrhizas may "integrate into even larger networks of interactions." Although no such moss-EM fungus-EM plant relationship has been proposed, this idea highlights the possibilities for a complex inter-kingdom and inter-phylum interaction between the components, one that is proposed here concerning nutrients and water at the very least. The multitrophic interactive network that Selosse and Duplessis (2006) begin to place mycorrhizal fungi into, in the current case, can be reduced to superficially describe the substantial interactive networks that may be present at the "producer" level between mycorrhizal plants and non-mycorrhizal mosses, via mycorrhizal fungi.

Other data indicate a three way partnership may exist between the aforementioned components. Weetman and Timmer (1967), note that mosses may not be "competing" with trees for nutrients, but may serve as a nutrient source by means of the ectomycorrhizal fungal intermediate. All of the associations relevant here are discussed in depth in Section 9 of the introduction, therefore reiteration will not be undertaken.

The idea of ectomycorrhizal fungi acting as efficient acquisition mechanisms for their associated plants in moss dominated systems certainly requires further study; however, it holds some merit in the current literature and is further supported by this

study. The specific reason for EMT being more prolific beneath FFMC in the CWOC must be elucidated, but the possible reasons for the data observed here require the heaviest consideration when new research in the field is undertaken. Therefore, for this complex topic of a significant ecosystem-level association between forest floor feather-mosses, ectomycorrhizal fungi, and associated ectomycorrhizal plant species I leave the reader with this: From the data of the current study, as well as previous research, it seems likely that nutrients and water sequestered by mosses would require ectomycorrhizal plants to increase the abundance of ectomycorrhizal exchange sites (root tips) to better access the scarce nutrients and water released during ephemeral pulses or in regular, less-concentrated discharge. Thus, this three-way relationship may prove to be increasingly important to forest ecosystem science as it is further understood.

6. <u>Future Directions with These Data</u>:

The data collected for this experiment can be taken far beyond the current level of analysis. However, my statistical limitations and time constraints have limited analyses to the overall/general topics. In the future, possibly for paper publication, I would like to more fully perform various other data analyses, some of which will be discussed here.

Vegetation characterization of sites and plots were made in an attempt to ecologically distinguish certain areas from others and possibly isolate influences, other than moss mat removal, that may contribute to abundances of EMT. In plant ecology, it is known that the presence of certain species can properly identify

components of a system when the means to take detailed and time-consuming measurements are unavailable. Therefore individual plots will be assessed and grouped into species-identified environmental groups and assessed for EMT abundance and phosphatase activity in those smaller groupings.

Because high light intensity increases soil temperatures and thus evaporative losses, it would be interesting to look at plots located in canopy gaps to see whether or not harvest of the moss mat has less of an affect on EMT reduction because the soil is drier, thus trees would need more EM to acquire that limiting resource. Also, it seems as though rain events at the HJ Andrews correlate with decreased photosynthetic capacity of *P. menziesii* (spring and fall), which may not be able to fully use the increased moisture and nutrient leachates. It would be interesting to look at look at moisture and temperature data, as well as data on *P. menziesii* photosynthetic rates to try to draw conclusions about the capacity of the dominant mosses to sequester nutrients during wet seasons and the direr summer because it seems as if *P. menziesii* invests more heavily in EM fungi during wet seasons, as evidenced by sporocarp production.

7. <u>Limitations to the Current Study</u>:

This study only assessed areas that were naturally covered with a robust moss mat. Control plots of forest areas that were naturally devoid of forest floor moss cover were not included. It would be interesting to see if areas that have developed without FFMC would have a lower abundance of EMT than those areas covered with

moss. It would help confirm the validity of the observed decrease in EMT in the harvested plots.

Boundaries were placed on plot size in this study, with a maximum being 1.5 m^2. This was assumed to create a sufficient buffer for harvested plots, such that the soil core taken in Year 1 would have a minimum of 0.75 m separating it from FFMC. Mycorrhizal fungi are known to react on a microscale, typically mm to cm, and thus this distance was accepted. It is possible that FFMC from 0.75 m away could have affected EMT in the harvested plots; therefore, future studies should create a larger buffer zone.

In an effort to compare differential effects of the dominant moss species on EMT abundance before and after harvest, it became clear that statistical analyses would not be as strong because of the unequal sample sizes. Before manipulating the plots, equal numbers of *E. oreganum* and *H. splendens* plots should have been picked for harvest. Perhaps there was a differential effect; however, the skewed sample size reduced statistical strength. In the current study there were more *E. oreganum* dominated plots that were non-harvested and more *H. splendens* plots that were harvested.

Other limitations to the study included: (1) the changes in abundance of soil hyphae were not assessed; (2) the species identities of EMT were not identified; (3) the nutrient content of soils before and after manipulation were not assessed; (4) the corresponding greenhouse experiment was never assessed due to time limitations; and (5) sampling for EMT was not conducted during peak ectomycorrhizal fungal production in the fall (fruiting season) because as an undergraduate student classes

were underway by that time. Regardless, the study has a statistically strong result, one that will hopefully be further researched in the future to elucidate the complexities of such a relationship.

8: <u>Future Research</u>:

This study establishes the need for further experiments to be conducted in the future to parse out the possible causes of the observed decrease in EM root tips with the removal of forest floor moss mats. Many are offered here, however anything that could further piece this novel finding together would greatly benefit ecosystem ecology.

Considering nutrient passage through the CWOC, one might manipulate a field system such that the chemistry of precipitation during different seasons and event intensities could be simultaneously compared with the chemistry of the water after it passes through mosses (at different levels and durations of desiccation), and again after it passes through soil with only roots and soil with EM roots. A fungicide could be applied to achieve the 'only root' treatment. This design could be further complicated by attempting it with different moss species, different soil types, different EM tree species, or even different EM fungi (if even possible). This data could be compared to similar data from manipulated (harvested) and naturally moss-devoid areas. It seems much more feasible to do in vitro; however a field experiment would be more telling of natural systems.

There are numerous further studies that could be developed to further the results presented in this paper. The fields of bryology and mycorrhizal ecology are advancing every day; much less is known about these topics that one may think. Any information would help further ecosystem science and provide more pieces to the biological puzzle.

9: Conclusion:

It appears that the removal of forest floor feather-moss cover, predominantly *E. oreganum* and *H. splendens*, in the CWOC results in the significant reduction of EMT in the uppermost 15 cm of soil, one year after harvest. The unique, non-mycorrhizal status of mosses enables them to assume different modes of water and nutrient acquisition. Forest floor feather-mosses function as a filter between atmosphere and soil, regulating and limiting nutrients and water that reach the root zone. Ectomycorrhizal fungi, adapted to scarce water and nutrient acquisition, associate with tracheophyte fine roots to secure a carbon source in exchange for their increased absorptive efficiency. Perhaps it is moss mat removal that alters the soil microclimate and causes EM reduction following harvest; future work is needed in this regard to empirically isolate mechanisms for these observed patterns.

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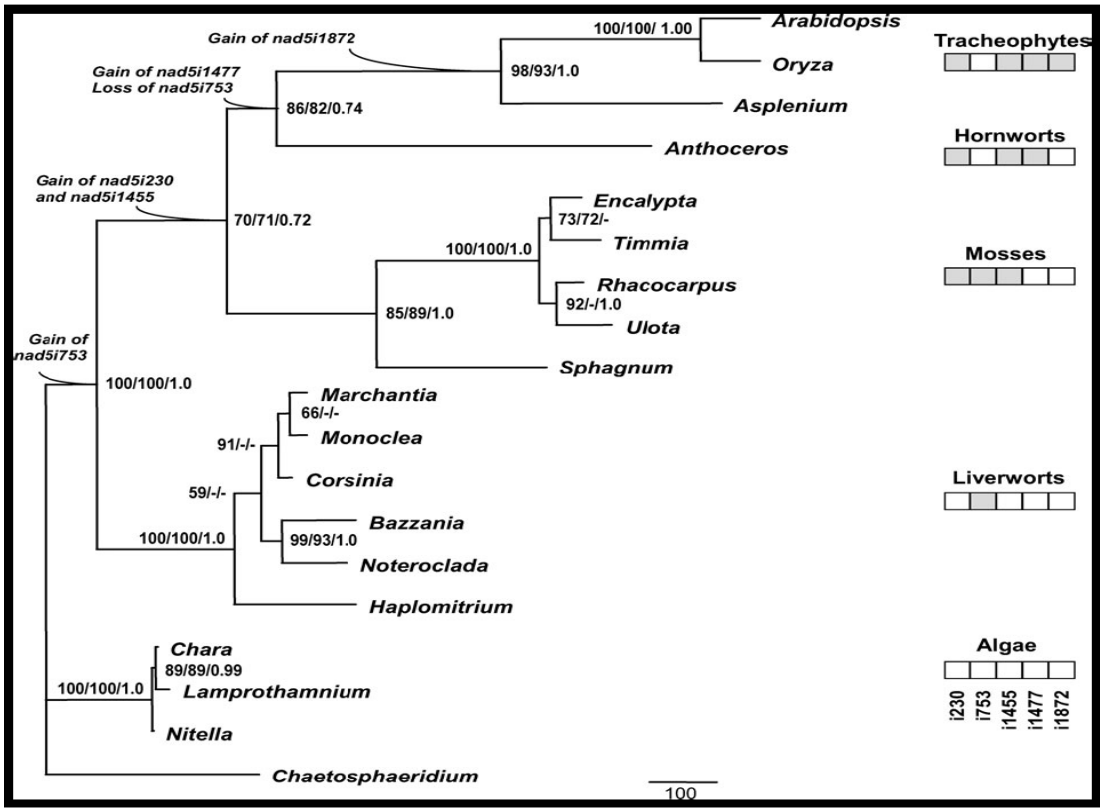
Appendices

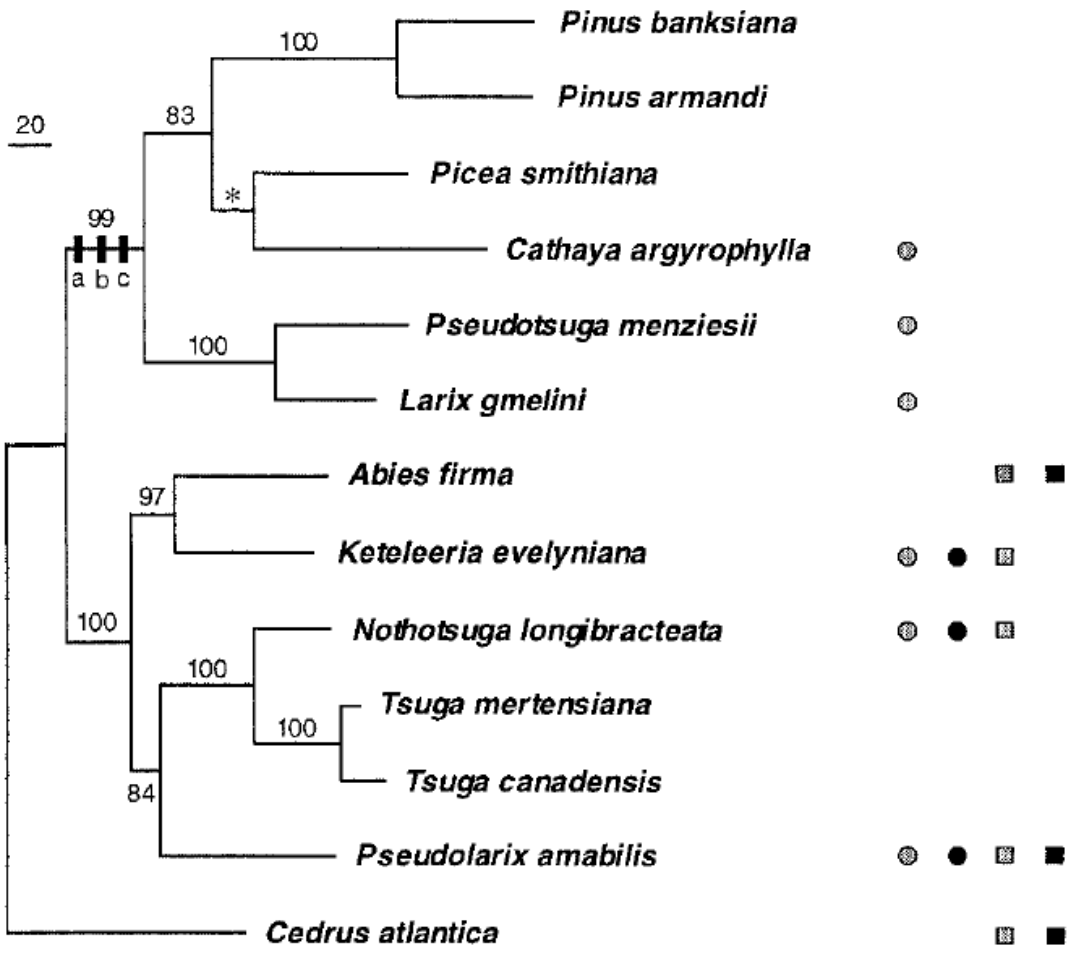
APPENDIX 1: Phylogenetic Trees

1. **Embryophyte phylogenetic tree as proposed by Groth-Malonek et al. (2005)**



- Liverworts are the most basal clade (mycorrhizal) with mosses branching next (non-mycorrhizal). Hornworts (mycorrhizal) are most related to the tracheophytes. Therefore mycorrhizal occurrence with land plants is proposed to have de-evolved or never have evolved at all in mosses.

2. ***Pinaceae*** **phylogenetic tree as proposed by Wang et al. (2000)**



- Phylogeny based on three gene sequences of every genus in the extant *Pinaceae*. *Cedrus* is the basal most clade. It gives rise to the *Larix-Pseudotsuga* clade, which is sister to the *Pinus* and *Picea-Cathaya* clades in the more derived taxa, as well as the *Tsuga-Nothotsuga* clade in more basal taxa.

Appendix 2: Role of Mycorrhizal Relationships throughout Global Ecosystems

1. Taken from Read and Moreno (2003)

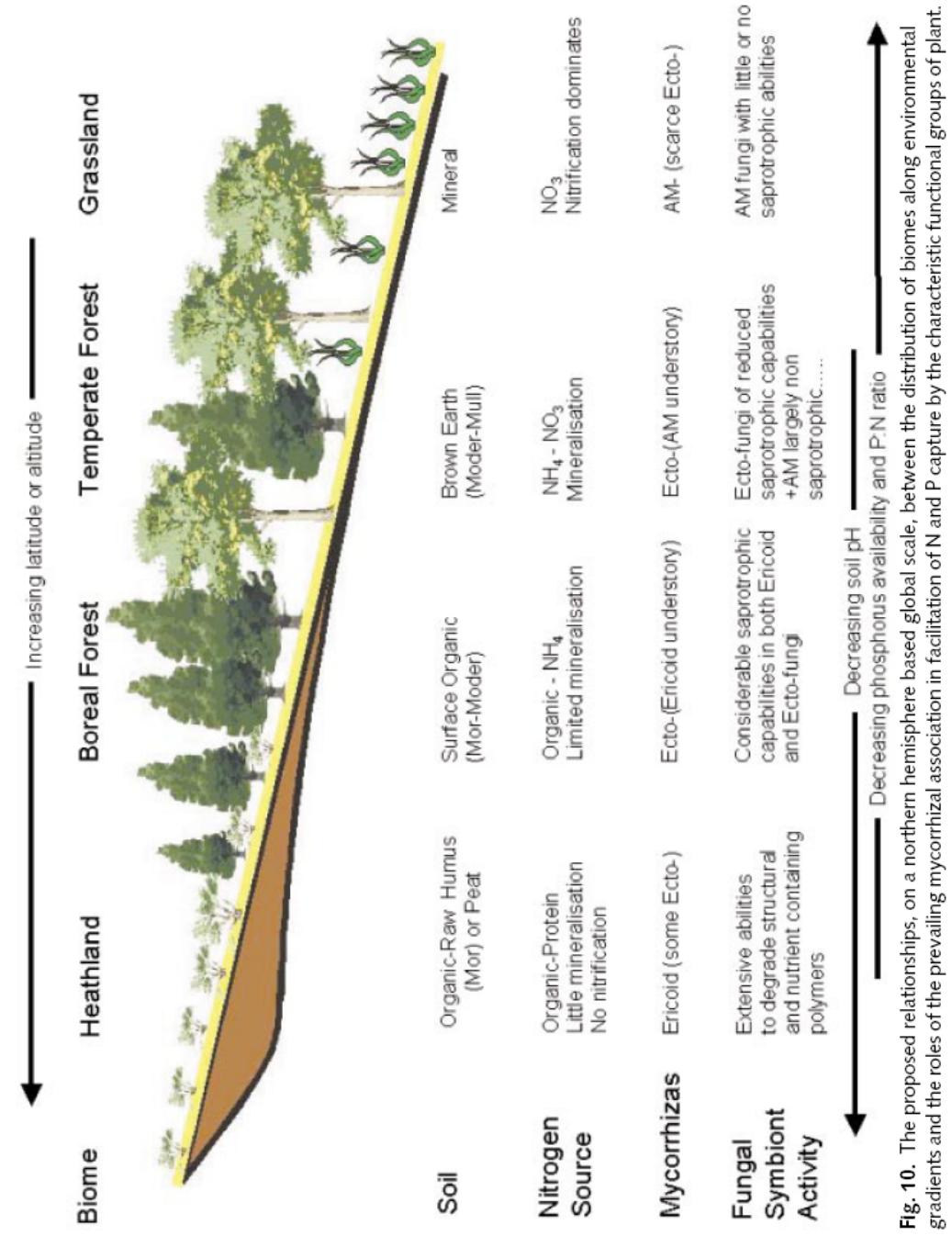


Fig. 10. The proposed relationships, on a northern hemisphere based global scale, between the distribution of biomes along environmental gradients and the roles of the prevailing mycorrhizal association in facilitation of N and P capture by the characteristic functional groups of plant.

Appendix 3: Role of Mycorrhizal Fungi in Nutrient Acquisition

1. Taken from Read and Moreno (2003)

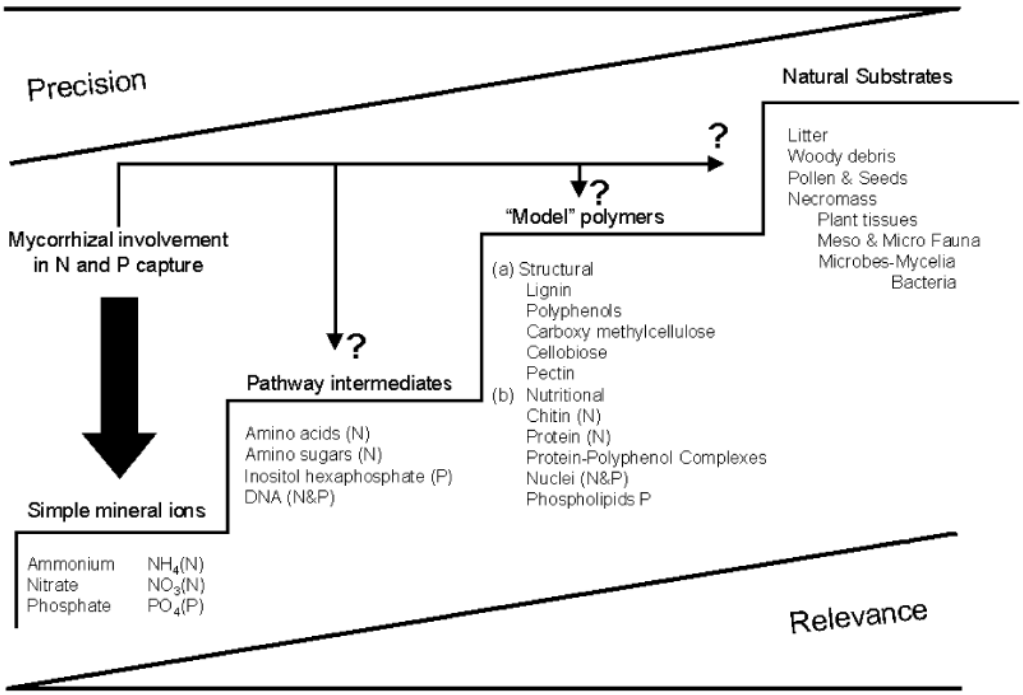


Fig. 1 Until recently almost all studies of the role of mycorrhizas in plant nutrition examined simple mineral ions as the sources of the key elements nitrogen (N) and phosphorus (P) (thick arrow lower left). While the processes of uptake of such elements can be observed in experimental systems with great precision, their primary repositories in most ecosystems are the organic residues of the soil microflora and fauna and of the plants themselves. It has been the convention to assume that N and P locked into organic macromolecules of these kinds was accessible only to specialist decomposers. However, new research using model polymers and natural organic substrates (thin arrows centre and top right) increasingly suggests that some classes of mycorrhizal fungi can mobilise these elements from the primary sources or their intermediates. By breaking into the mineralisation pathway and providing their autotrophic partners with access to the nutrients before they are re-immobilised in the microflora, these fungi would enable considerable increases in the efficiency with which plants are able to acquire and recycle N and P. The chemistry of primary nutrient sources in soils is often poorly characterised and as a consequence there may initially be a loss of precision in the new research, but it is argued that the advantages gained in terms of ecological relevance (bottom right) outweigh the disadvantages. Ultimately, identification of those compounds and processes which play key roles in the trophic cascades occurring in the 'black box' should provide the impetus necessary to ensure their chemical evaluation. This will enable the scientifically desirable balance between precision and relevance to be restored. Mycorrhizal fungi are increasingly seen to have the potential to be the drivers of nutrient mobilisation processes in some ecosystems.

Table 2 Extra cellular enzymes, known to be produced by selected ectomycorrhizal fungi, which would be expected to provide some abilities to degrade structural components of plant litter thereby contributing to decomposition processes and to 'unmasking' of nutrients. Italics indicate observations based upon gene presence rather than enzyme expression. For older literature see Leake & Read (1997)

Process	Substrate	Enzymes	Reference
Cuticle Degradation	Cutin, Lipid, Waxes	Fatty Acid Esterase	Hutchison (1990b), Caldwell *et al.* (1991)
Plant Cell Wall degradation	Pectin	Polygalacturonase	Hutchison (1990a)
	Cellulose	Cellulase	Maijala *et al.* (1991), Colpaert & van Laere (1996)
	Cellobiose	Cellobiohydrolase	Burke & Cairney (1998)
	Hemicellulose	Xylanase	Cao & Crawford (1993), Terashita *et al.* (1995), Cairney & Burke (1996b)
Oxidation of Phenolic	Monophenols	Tyrosinase	Hutchison (1990b)
Acids and Tannins	Polyphenols	Polyphenol oxidase	Bending & Read (1997), Colpaert & van Laere (1996), Günther *et al.* (1998)
		Peroxidase	Bending & Read (1997), Cairney & Burke (1994), Griffiths & Caldwell (1992)
		Laccase	Hutchison (1990b), Kanunfre & Zancan (1998)
Hydrolysis of Lignin	*Lignin*	*Manganese peroxidase*	*Chambers et al. (1999), Chen et al. (2001)*
		Lignin Peroxidase	*Chen et al. (2001)*

Italics indicate results based upon indirect methods of observation.

Appendix 4: Efficiency of Roots and Mycorrhizal Fungi in Nutrient Absorption

1. Taken from Yanai et al. (1995)

Uptake, Cost, and Efficiency of Roots and Hyphae

Fertility (umol P/liter)	Roots	Hyphae	Roots and Hyphae
	Uptake (umol P/day)		
190	6.7	323	165
100	6.3	302	154
50	5.4	264	134
	Cost (g C/day)		
all	0.045	0.075	0.06
	Efficiency (umol P/g C)		
190	150	4310	2760
100	140	4010	2570
50	122	3520	2250

* The combined effect of roots and hyphae assumes equal amounts of each. Hyphae were assumed to turn over monthly, roots annually. Taken from Yanai et al. (1995)

Appendix 5: Ectomycorrhizal Fungi as Mineral Nutrient Mobilizers

1. Taken from Landeweert et al. (2001)

Box 3. Nutrient mobilization by ectomycorrhizal fungi

As well as the quantitative effects on plant nutrient uptake (increase in uptake surface and exploited soil volume), the ectomycorrhizal fungus influences the uptake of plant nutrients in two qualitative ways:

(Fig. Ia) Via enzyme production, the ectomycorrhizal fungus can utilize organic nitrogen (N) and phosphorous (P) forms, which would otherwise remain largely unavailable to roots. Nutrient mobilization from amino acids, peptides, proteins, amino sugars, chitin and nucleic acids has been shown[a], together with transfer of N and P into the host plant[a,b]. Direct hyphal absorption of amino acids and simple peptides can also occur[a].

(Fig. Ib) The ectomycorrhizal fungus can mobilize P, potassium (K), calcium (Ca) and magnesium (Mg) from solid mineral substrates through organic acid excretion[c,d,e]. In addition, tunnels in weatherable minerals enable ectomycorrhizal hyphae to reach the interior of the minerals and access P from apatite inclusions. Essential nutrients become available to the host plant via the ectomycorrhizal mycelium[c]. Analogous to their organic nutrient mobilizing capabilities, the abilities of different ectomycorrhizal fungi to mobilize inorganic nutrients might be species specific[f].

References

a Chalot, M. and Brun, A. (1998) Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. *FEMS Microbiol. Rev.* 22, 21–44

b Antibus, R.K. *et al.* (1997) Root surface phosphatase activities and uptake of ^{32}P-labelled inositol phosphate in field-collected gray birch and red maple roots. *Mycorrhiza* 7, 39–46

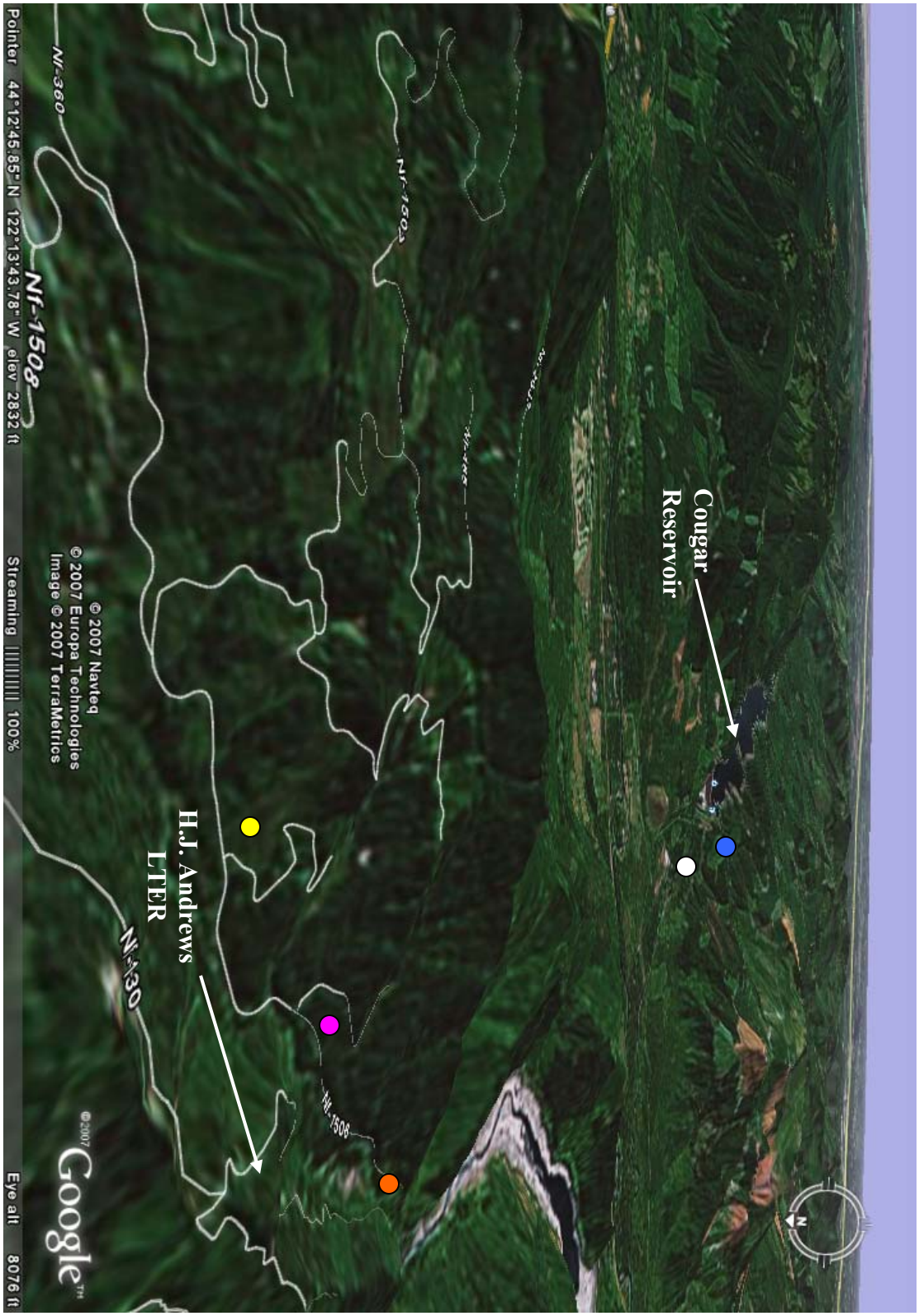
c Wallander, H. (2000) Uptake of P from apatite by *Pinus sylvestris* seedlings colonized by different ectomycorrhizal fungi. *Plant Soil* 218, 249–256

d Wallander, H. and Wickman, T. (1999) Biotite and microcline as potassium sources in ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings. *Mycorrhiza* 9, 25–32

e Wallander, H. (2000) Use of strontium isotopes and foliar K content to estimate weathering of biotite induced by pine seedlings colonised by ectomycorrhizal fungi from two different soils. *Plant Soil* 222, 215–229

f Lapeyrie, F. *et al.* (1991) Phosphate-solubilizing activity of ectomycorrhizal fungi *in vitro*. *Can. J. Bot.* 69, 342–346

<u>Appendix 6</u>: Image of Sample Area: The HJ Andrews/Cougar Reservoir Region



Site 1 Site 2 Site 3 Site 4 Site 5