CONTRASTING MYCORRHIZAL GROUPS THROUGH THE SOIL PROFILE

by

Franklin Diggs

A thesis
submitted in partial fulfillment
of the requirements for the
Masters of Science Degree
State University of New York
College of Environmental Science and Forestry
Syracuse, New York
January 2014

Approved: Department of Forest and Natural Resources Management

_____	_____
Ruth Yanai, Major Professor	Donald Leopold, Chair Examining Committee
_____	_____
David Newman, Department Chair Forest and Natural Resources Management	S. Scott Shannon, Dean The Graduate School

Acknowledgements

This work would not have been possible without the support of the Yanai and Horton labs. I would especially like to thank Dr. Yanai for her constant and enduring support through the years it has taken me to produce this. Her constant corrections, guidance, and advice have steered me better than any course I choose for myself. I would like to thank Dr. Horton for his innumerable mycorrhizal lessons and an endless supply of coffee; Jeremy Hayward, my unofficial mycorrhizal advisor; and Craig R. See for remembering what is important in life and being kind enough to remind me. A special thanks must go out to all the members that have ever worked on the Shoestring crew; the summers will be quite different now.

Table of Contents

List of figures iv
Abstract v
Chapter 1: The Relationship of Mycorrhiza to Soil Depth: A Historic Perspective 1
Chapter 2: Mycorrhizal groups decline similarly with depth: a case study from a northern-hardwood ecosystem 16
Abstract 16
Introduction 17
Temperate forests of New Hampshire: 18
Objectives 19
Methods 20
Site description 20
Sampling methods 22
Sample treatment 23
Statistical methods 24
Results 25
Discussion 27
Acknowledgements 29
Figures 31
Literature Cited 34
Resume 39

List of figures

Figure 2. 1. Root colonization by three major root endophytes. The upper most plot shows colonization by ectomycorrhizas, the middle plot shows AM colonization and the final plot shows dark septate endophyte colonization. 31

Figure 2. 2. Root length density in each of the stands by depth increment. 32

Figure 2. 3. Proportion of arbuscular mycorrhizal hyphal length in which (A) vesicles or (B) hyphal coils were encountered 33

Abstract

F. M. Diggs. Contrasting Mycorrhizal groups through the soil profile. 39 pages, 0 tables, 3 figures, 2014.

Mycorrhiza, the association between plants and fungi, can be separated into groups based on morphology. The distribution of this association is well understood in terms of the global distribution of plant communities, but in terms of depth, there are fewer investigations. The history and development of current mycorrhizal theories regarding depth are explored in Chapter 1. These assumptions are tested through observational data gathered within Chapter 2. Three broad groups were investigated here, the ectomycorrhizas, the arbuscular mycorrhizas, and the dark-septate endophytes. These three groups broadly associate with the plants in our region of investigation. I had thought that plant colonization by any of these groups would respond to depth, as the soil environment changes by, instead this investigation finds that depth is unsatisfying in explaining much of mycorrhizal distribution.

Key Words: Dark-septate endophytes, MELNHE, spodosols, vertical stratification.

F. M. Diggs
Candidate for the degree of Master of Science, January 2014
Ruth D. Yanai, Ph.D.
Department of Forest and Natural Resource Management
State University of New York College of Environmental Science and Forestry,
Syracuse, New York

Ruth D. Yanai, PhD _____

Chapter 1: The Relationship of Mycorrhiza to Soil Depth: A Historic Perspective

In the 1890's, A. B. Frank, asserted that the fungi he was observing on plant roots on the trees in Prussia were not parasites as his colleagues believed, but were instead a kind of symbiosis, a term he coined. This was not the first observation of mycorrhizas, another term which he coined, but Frank was able to correctly describe their symbiosis and role within a plant community. It has been over a hundred years since this discovery. Within that time many parts of this symbiosis have been described, and mycorrhizal investigations have become their own field of study. Much of this investigation has been on the level of physiology of a single plant, or even on a single root. However, the role of many environmental factors on the development and distribution of these mutualisms remains unknown and previous studies provide conflicting information concerning their role in the formation of these plant-fungal communities. I will attempt to briefly summarize the course of mycorrhizal research from Frank to the present with a focus on the idea that depth may be a controlling variable in the distribution of mycorrhizas. Within this narrative, I hope to show how mycorrhizal research priorities have shifted, grown more broad, and how, in the present, the methodologies allow us to address new questions.

The turn of the century, from the 19th to the 20th, was marked by radical change. This maybe true concerning any historical transition, but the change from the 19th to the 20th century seems stark through the lens of history. It is a period filled with contention and strife. For some context, in France, the Dreyfus was to

leverage anti-Semitism over Europe for the next half century, gold was discovered in Alaska, Jack the Ripper haunted the streets of London, and, in what would become Germany, Otto von Bismarck, the man who unified the nation, was dismissed by Wilhelm II. The scientific world was being rapidly advanced, Darwin had published not a century past, Mendeleev's periodic table seemed to be correct and his table could incorporate even the newly discovered "noble gases," and, in a personal favorite escapade in the history of science, Roentgen had discovered "Roentgen rays," now known as "X-rays."

It was during this time that an administer in Prussia appointed A. B. Frank to discover what made truffles grow, how to grow them, and where to find them (Frank 1885; Trappe 2004). Frank failed in growing truffles, but he no doubt found and enjoyed a few. What he did achieve was finding that truffles grew in conjunction with certain trees; he posited that they were grown in "symbiotismis" (which would now be symbiosis) in what he called a "mycorrhiza." In the same paper, he described a greater abundance of (ecto)mycorrhizas in the shallow soil, with colonization declining through the soil profile.

Frank's bold paper was a lot for the biological community to digest. Darwin had said of cooperation that it was his theory's most difficult conundrum, yet these mutualists seemed to be cooperating across species. Mycorrhizal research for the next several decades would test his claim, that these associations where mutualistic in nature, and test whether these symbioses were a real phenomenon or a theoretical construct. Questions as to the abundance, communities, and distribution were impossible to address before a basic understanding of the

physiology of the mutualism was explored. The question of mycorrhizal distribution across the soil depth profile was also set aside during this time, leaving Frank's paper as the sole description of depth distribution before the twentieth century, and it is one of a meager handful of such works created by end of the twentieth century's first half (Frank 1885).

The question of depth is important. Soils are formed over geological time by the breakdown of parent material and the incorporation of organic matter. The processes that form soil are both chemical and physical, but water plays a key role. As water percolates through the soil it picks up soluble material to then deposit the material in lower horizons, or to leach the material out of the system. In areas without organisms that turn the soil, this can result in extreme differences in the chemical and physical behavior of the soil. This makes Spodosols model soils in which to look for effects of depth.

Modern scholarship now recognizes at least six groups of mycorrhizas, but Frank dealt with only with those that were associated with Ascomycota and Basidiomycota. While he did recognize that those forming structures inside cell walls were different than those that did not, he had only ericatious and orchid roots as endomycorrhizal (Frank 1885; Koide and Mosse 2004; Trappe 2004). This is in contrast to the literature sinse his death, which refer to those mycorrhizas that form with glomeromycota as endomycorrhizas.

It is now known that most plants do not form this association, most plants form arbuscular mycorrhiza, and the story of mycorrhizal distribution in depth would be incomplete without a discussion of their history. I must here admit some

bias, as my work is concerned with only the arbuscular and ectomycorrhizal groups, with some attention given to the enigmatic dark-septate endophytes. Because of this focus this review will concentrate on these mycorrhizas to the exclusion of the others. This may not be as great a gap as it first seems, as many of the other mycorrhizas are formed in association with rare plants such as orchids, or with plants that parasitize the mycorrhizal networks (mycoheterotrophy) or are formed in ecosystems that are not in the scope of the current work. All mycorrhizas are morphologically defined, and these symbioses may not be functionally different from their ectomycorrhizal counterparts despite their strange forms and structures.

Nägeli first described the arbuscular mycorrhizas in 1842. His observations are mainly descriptive. It was not until the turn of that century that arbuscular-mycorrhizal features began to be fully described. By 1925, the arbuscule, the vesicle, and the appresorium of arbuscular mycorrhizas had been described (Koide and Mosse 2004).

Research during these early investigations into the mycorrhizal condition were both enhanced by and suffered from the influence of plant pathologists. For example, the Bordeaux mixture was developed to treat downy mildew in 1885(Money 2006), and Koch's postulates grew in prominence (Koch 1978). They were:

1: The microorganism must be found in abundance in all of the organisms manifesting symptoms of the disease, but should be absent in healthy organisms.

2: The microorganism must be isolated from an infected organism and grown in pure culture.

3: The cultured microorganism should cause the disease when introduced into a healthy organism

4: The microorganism must be able to be reisolated from the diseased hosts and shown to be identical to the organism first isolated from a diseased organism in (1).

These techniques began to lead to the identification of disease-causing pathogens. Meanwhile, plant diseases continued to ravage the world with little treatment. For example, the potato blights hit England and Ireland in the mid and late 1800's, coffee rusts hit Sri Lanka in the 1870's, and the downy mildew epidemic hit Europe. This shifted the focus of academic research on fungi from questions of basic ecology to the utilitarian questions raised by crisis after crisis.

The investigative techniques used for plant pathology are not always appropriate for mycorrhizas. So, while during this time the appresorium was discovered, Koch's postulates also led to a half-century long struggle to get arbuscular mycorrhizas to grow in pure culture, according to his second postulate. Fungi did not have a good reputation, they were decomposers and diseases, and few scientists were willing to investigate the kingdom. Instead, the botanists of the time preferred to spend their time working with the plants that were their speciality. Even those scientists who chose to work on the fungi were limited, as the microscopic and subterranean or internal nature of the organisms made (and continues to make) investigations of the fungi in natural systems difficult. Until 1970, when Phillips and Hayman (1970) developed arbuscular staining with KOH and Tryphan blue, investigations were laborious and no techniques were standardized. The confusion of early mycorrhizal investigations may be glimpsed in

Lohman's 1927 conjecture that arbuscular mycorrhizas might just be ectomycorrhizas on different plants, and that these fungi might be nitrogen fixers (Koide and Mosse 2004; Lohman ML).

The 1970s saw the techniques of tissues staining applied to mycorrhizal questions. This allowed questions of arbuscular mycorrhizal distribution, as techniques became standardized and consistant (Phillips and Hayman 1970; Koide and Mosse 2004). Though molecular techniques have allowed for new tools in mycorrhizal investigations over the last two decades, Koide and Mosse consider this period, the 1970's, as the beginning of modern mycorrhizology. They quote Harley (1969) that the study of arbuscular mycorrhiza had emerged as a "reputable pursuit" (Harley 1969; Koide and Mosse 2004). Harley's publication was the first textbook attempting to codify mycorrhizal research to that point.

It was during this period, from the 70's through the 90's, that radiolabeling began to be used within mycorrhizal studies, and the larger scale ecological questions started to be addressed. If mycorrhiza were mutualists, what were they doing? During the 60's, and especially 70's, the arbuscular mycorrhiza became linked to the phosphorous cycle (Gerdemann 1968). Prior to that time, it was assumed that most plants were nitrogen limited and that the arbuscular mycorrhiza would have something to do with the nitrogen cycle (Koide and Mosse 2004). However, the early 1970's tied arbuscular mycorrhiza to the phosphorous cycle, showing that plants in phosphorus-limited environments benefited from colonization, and that higher amounts of phosphorus were present in infected

plants (Mosse 1973). About the same time the ectomycorrhizal condition began to be linked to the nitrogen cycle (Olson 1963; Catalfomo and Trappe 1970).

The global distribution of mycorrhizas started to become clearer during this time. Went and Stark (1968) described arbuscular mycorrhizas as being "primarily in the Amazon rain forest," whereas by Mosse's (1973) review, it was "easier to think of families without arbuscular mycorrhizas than to list those with it". This coupling of the ectomycorrhizas to nitrogen and arbuscular mycorrhiza to phosphorous that begin to emerge in the 1970's was one of the first major functional distinctions between these two groups, and this idea has only gained momentum as time and experimentation have given it credibility. This may, to some extent be a bias based on experimental approaches, as nitrogen methodologies are thought to be more difficult than those for phosphorus, and the work of Dr. Read with nitrogen led many of his successors in ectomycorrhizal studies to focus on this nutrient, while the arbuscular mycorrhizal researchers were beginning to focus on phosphorous.

I do not know the backgrounds of the scientists working with mycorrhizas during the 1950's and 1960's, but in retrospect, it seems that mycorrhizology was a realm dominated by botanists, with a very select few fungal enthusiasts among the ranks. Indeed, you can read mycorrhizal history, if you want, as a struggle between mycocentric and phytocentric scientists. Around the 1970s then, the pendulum began to swing away from a photobiont-dominated point of view. Prior to this, mycorrhizas had been largely interpreted as extensions of the root system, with Katznelson et al. (1962) treating, at least linguistically, mycorrhizas as roots.

However, in 1970 Trappe pressed for fungal identification (Catalfomo and Trappe 1970). Since it was methodologically impossible to trace hyphae from fruit bodies back to root tip, though some published methodologies claimed to do so, a method to identify ectomycorrhizas morphologically began. It continues to this day, as Agerer (1987-2002) publishes morphological description of mycorrhizas, advocating a naming system that includes both plant and fungal partners. To an emerging scientist, born after 1980 and not encountering mycorrhizas until the twenty-first century, this approach seems antiquated. Tom Bruns, in 1990, and again in 1993 led a team in publishing protocols for generating fungal specific primers, and then sequences for fungal specific primers themselves (White et al. 1990; Gardes and Bruns 1993). Indeed, these primers and this technique were so useful to the field that the 1990 paper has received more than 10,000 citations, (Affairs et al. 2002). It must be said that if you can identify fungi morphologically, it is probably better than identifying them genetically, if the morphological distinctions hold at the genetic level, because morphological identification is cheaper. The ease of genetic techniques, however, makes Agerer's morphological approach look like a horse-and-buggy next to your automobile; it gets you there, it just takes more time and a lot more work, and can still be wrong.

Between the 1970's and 1990's, ectomycorrhizal researchers turned outward, moving away from the function of the plant root towards the function of the plant community. However, in order to ask the really large-scale questions, a better idea of global mycorrhizal distribution and diversity was needed. It was during this time that some of the first descriptions from China and Australia started

to appear in the English literature, as well as more thorough reporting from the Amazon (R. Singer and de Ja 1979; Malajczuk et al. 1982). Without a doubt, the most notable publication during this period was "Mycorrhizal Symbiosis" (Harley and Smith 1983). This book has become "the bible" for mycorrhizologist, though Harley seemed to view it as a continuation of his previous summary. Though Harley has left us, Smith and Read continue to publish periodically updates to this textbook providing over 10,000 citations (Harley 1969; Harley and Smith 1983; Smith and Read 2008).

This period can also be remarked upon for the increased dialog between these two groups, the ectomycorrhizal and arbuscular mycorrhizal camps. To a large extent, these two camps had become separated by their different settings and motivations. Arbuscular mycorrhizologist spent a good part of the middle of the century on applications in crop settings (Koide and Mosse 2004), whereas ectomycorrhizal researchers tended to work in temperate and boreal forests, with some exceptions, like Trappe, who frequented rainforests in his quests for truffles. To this day a given researcher's interests tend to fall into one mycorrhizal group or another, much like a plant root tends show fidelity to its mycorrhiza.

From the late 1970's through the early 1980's ectomycorrhizal and arbuscular mycorrhizal researchers were beginning to review questions of depth distribution. Arbuscular mycorrhizas were shown to decrease remarkably in agricultural settings below 40 cm depth (Jakobsen and Erik Nielsen 1983; Levy et al. 1983). Arbuscular mycorrhizal fungi were also found to colonize more strongly in low water conditions, and their growth, as well as root growth, was found to be

inhibited by plowpans (Levy et al. 1983). One of the more interesting attempts during the period of the 1970's through the 90's to show relationships between these two groups came from the idea that maybe AM are succeeded by EcM (Lapeyrie and Chilvers 1985), though so many late-successional species are AM that the idea of a successional gradient within mycorrhizas was immediately contested. Succession is just one area where the role of plant hosts and the role of their fungal partners are so intertwined as make any conclusions on such a time scale dubious. To what extent is succession a switch from shade-intolerant to shade tolerant species? To what extent is it a switch from plants that are colonized by fungi from spores to fungal colonization by mycelia? Do late successional plants establish because of a greater mycorrhizal resource network not required by early successional species? Successional models could be accurate, and in many seem to be, but the interdependence of these communities makes it difficult to create a comprehensive model.

Contemporary mycorrhizal research has access to new tools and can explore new questions. Most notably, the advent of molecular genetic technologies allows for the identification of fungi from otherwise unidentifiable hyphae. The emphasis in mycorrhizology now is more and more taxonomic. In the ectomycorrhizal world, Agerer (2000) suggested that ectomycorrhizal forms might relate to their function in a ecosystem, and put forth "exploration types." This served to refocus those mycorrhizologists still interested in microscopy and has spawned numerous studies as to whether these exploration types matter in natural settings. Molecular tools have allowed researchers to identify fungi from root tips alone, getting rid of all of

the difficult to distinguish morphological identifiers and replacing them with laboratory procedures. For those interested in mycorrhizal behavior through the depth profile, these techniques have allowed new questions to be asked. Contemporary with the research carried on in during the course of this thesis, Taylor conducted a survey of Alaskan fungi, capturing what he believes is an accurate prediction as to their abundance (Taylor et al. 2013). He found that fungi do segregate based on soil horizon, and that this segregation is happens along phylogenetic lines, that is, two species of the same genus may be found on top of each other, one in the lower horizon, and one in the upper, with little overlap.

Prior to my investigation, the only investigation that I found that looked at the distribution of ectomycorrhizas and arbuscular mycorrhizas across the depth profile and within the same ecosystem was Neville et al.'s 2002 study of *Populus tremuloides.* This species is of special interest because the trees are clonal and hectares of forest can be genetically identically. Also, while it prefers to host EcM fungi, it can and does host AM fungi. Neville believed that by focusing on a clonal species growing in monoculture, he would be able to detect vertical stratification by mycorrhizal group, as the area he chose to study was carefully selected to diminish the effects of the confounding factors inherent in a more diverse plant community. As such, these settings would allow AM to colonize roots more thoroughly in the mineral horizon, a hypothesis which his evidence supports after some data transformation and non-parametric tests. The new tools and methods have allowed for a reinvigoration of old questions, including the question of soil horizons as an influencing factor on mycorrhizal behavior.

Until this point, I have delved into the history of mycorrhizas, attempting to show that investigations into the relationship to the soil-depth profile were, in the beginning, impossible, and more recently, a low priority. It is important to address, even if only briefly, why soil depth matters

EcM fungi and AM fungi are thought to specialize in different soil environments (Treseder and Cross 2006). EcM fungi are usually found in forests in which there is considerable accumulation of organic litter (Allen 1993; George et al. 1995). The EcM condition is thought to have evolved from saprotrophic fungi that once broke down this organic litter for their entire sustenance, and though evolution has now linked them to trees, they still dominate in those environments where they presumably evolved, environments with heavy organic accumulation (Wang and Qiu 2006; Smith and Read 2008; Tedersoo et al. 2009). This evolutionary history has left the fungi with a suite of enzyme-relics that seem to be absent from both plants and AM fungi, enzymes that specialize in the acquisition of mineral nutrients from organic debris (Dighton 1991; Allen 1993, Martin et al. 1998). Ectomycorrhizas often dominate temperate forests, areas in which nitrogen is traditionally thought of as limiting to plant growth, and so the role of ectomycorrhizas in the acquisition of nitrogen has become of particular concern to those studying ectomycorrhizal symbiosis (Martin et al. 1998).

The biosynthesis of many plant compounds results in a pool of nitrogen in recalcitrant organic forms that are accessible mainly to saprotrophic and ectomycorrhizal fungi within these ecosystems (Martin et al. 1998). Bacteria may also be able to break down many of these compounds, but in forest systems with low pH and high organic matter concentrations fungal decomposition, both from mycorrhiza and from saprotrophs,

tend dominate over bacterial decomposition (Lindahl et al. 2007). In many of these ecosystems, primary productivity is high, but with little to no nitrogen fixation. Therefore, in these systems, below ground competition focuses on nitrogen (Lindahl et al. 2007). EcM, unlike saprotrophic fungi, can seek nitrogen in the environment to the exclusion of carbon. Whereas saprotrophic fungi must seek after sources rich in both carbon and nitrogen, EcM have a bank of photosynthate that allows them to seek this mineral to the exclusion of carbon, which is supplied via the plant. While both saprotrophic fungi and EcM fungi will seek the same organic sources of nitrogen, EcM can mine nitrogen not only from fresh organic debris fallen to the forest floor, but also from those parts of the soil where the C:N ratio would make it inefficient for saprotrophic fungi.

The glomeromycotan fungi that form AM symbiosis are common in environments dominated by herbaceous plants and environments that have exposed mineral soil (Treseder and Cross 2006; Wang and Qiu 2006). They are far more common than EcM fungi, which associate almost exclusively with woody roots, and AM are common in soil environments with a high pH, where nitrogen is not limiting, and in low latitudes. However, as the tropics become better cataloged, this final generalization has been called into question. Certainly tropical plants such as those in the family Dipterocarpaceae, or Pisonia, show fidelity to the EcM condition (Redhead 1982; Wang and Qiu 2006). It is even possible that the Diptocarps were among the first plants to adopt the EcM condition (Bacon and White 2000; Berbee and Taylor 2007; Tedersoo et al. 2009).

It is certain that the majority of terrestrial plants form arbuscular mycorrhizal symbiosis. This seems to be the more primordial form, and evidence shows that even

trees that are typically thought of as exclusively EcM, such as pine, can still contain AM fungi or can be induced to the AM condition (Cázares and Trappe 1993). Such observations support the idea that AM is the ancestral mycorrhizal condition (Smith and Read 2008). All of these lines of evidence have created a model in which EcM are thought of as nitrogen specialists, and AM as phosphorous specialists, EcM as the association for the high latitudes, and AM as the association for the low latitudes.

Soils and plant communities develop over time, not just space, and as such, there has been some attempt to describe general and specific patterns of mycorrhizal associations within a community over time. In general, non-mycorrhizal plants colonize novel environments more quickly than their mycorrhizal counterparts (Lapeyrie and Chilvers 1985). This is presumably because non-mycorrhizal plants are not limited to colonizing environments at the same time as their fungal associates, or colonizing areas that have an established mycorrhizal network. In addition, many of these plants have adaptations, such as windblown seeds and basal rosette growth forms, that reflect a history of colonizing environments that may not have other plants or fungi (Smith and Read 2008). If we accept that mycorrhiza derive most of their nutrition from organic sources that they are able to exploit using extracorporeal enzymes, those environments where soil is absent should resist immediate mycorrhizal establishment. This is reflected in the proliferation of non-mycorrhizal plants in extreme latitudes, as well the lack of mycorrhizas in bryophytes, save for a few liverworts (Koide and Mosse 2004; Wang and Qiu 2006; Brundrett 2009). Just as the model for mycorrhizal distribution across the globe is an over-simplification, the model for mycorrhizal succession is also crude.

However, it still provides a useful framework for thinking about the development of mycorrhizal communities over time.

From a fungal perspective, almost all plant communities are mycorrhizal communities. Differences in the functioning between these two mycorrhizal groups rarely result in sharp ecotones between the two fungal communities. Rather, in many places, such as in the northern hardwood forest, the AM and EcM systems coexist. While mycorrhizal communities do seem to gradually shift from one system to another as one moves across the landscape of the globe, at the local scale, mycorrhizal distribution is seemingly random or unpredictable. The local distribution of plant roots and fungi may, in fact, be sorting or segregating by processes that we have not yet examined.

With the ideas gathered from biogeography, evolutionary theory, and succession, better hypotheses can be generated concerning the mycorrhizal relationship with the soil depth profile. There is nevertheless a paucity of information concerning the interactions and behaviors of both mycorrhizal groups across soil depth. These mycorrhizal systems coexist and compete with each other, and our understanding of the processes that regulate this coexistence is poor. With a fungal diversity between 6-18 times greater than the plant community (Taylor et al. 2013), it is obvious that different strategies must have evolved to prevent niche exclusion. Segregation by soil horizon may be one way to limit competitive interactions between these groups. Though roots and mycorrhiza may be present at all levels of throughout the soil profile, those fungi better adapted to mineral environments should favor mineral horizons in soils with sharp vertical stratification.

Chapter 2: Mycorrhizal groups decline similarly with depth: a case study from a northern-hardwood ecosystem

Abstract

Ecosystems with both ectomycorrhizal and arbuscular mycorrhizal symbioses may show differences in colonization by fungi with soil depth. Theory suggests that there could be vertical partitioning if arbuscular mycorrhizal fungi have strong preference for mineral soils and ectomycorrhizas for organic soils. Beech, maple, and birch are often found growing near each other in the northeastern United States, yet each plant shows a high degree of fidelity to one mycorrhizal group or another. While these plants are often competing above ground in terms of basal area or crown dominance. It remains unclear whether the below ground component of these plants, the mycorrhizas, exhibit vertical partitioning.

I sampled roots from two stands of northern hardwoods at two depth intervals: 0-10 & 30-50 cm. I found that both groups colonized roots more thoroughly in the shallow soil compared to the deep (Ectomycorrhizas, $p<0.001$: Arbuscular mycorrhizas $p<0.001$). The shallow soils also had greater abundance of arbuscular mycorrhizal structures (vesicles $p=0.14$, hyphal coils $p=0.10$). The ratio of colonization by EcM and AM fungi did not differ between shallow and deep horizons ($p= 0.45$), or between mineral and organic horizons ($p=0.29$). However EcM and DSE showed preference for organic soil (EcM: $p=.05$, DSE: $p=0.04$). The ratio of colonization did not change by organic or mineral horizons ($p=0.90$). Our results indicate that these groups do not strongly segregate by depth or soil horizon.

Introduction

The coexistence of arbuscular mycorrhizas (AM) and ectomycorrhizas (EcM) networks is a poorly understood phenomenon (Allen 1993; Neville et al. 2002; Pringle and Bever 2002). While observations of mycorrhizal biogeography have revealed many patterns within each of the major mycorrhizal groups, as well as patterns between these groups across the landscape of the globe, few studies have attempted to analyze patterns in places where these mycorrhizal types co-dominate (Neville et al. 2002). However, the observations of mycorrhizal spatial patterns, as well as analysis of mycorrhizal enzymes, have created a picture of multiple mycorrhizal groups performing similar yet different functions within the environment (Martin et al. 1998; Treseder and Cross 2006). This paradigm could be summarized as follows: AM fungi specialize in the acquisition of phosphorous in environments where it is limited, while EcM acquires nitrogen in environments where nitrogen is more limited. AM fungi are thought to specialize in mineral soil environments, whereas EcM seem to favor environments with a high amount of organic accumulation (Treseder and Cross 2006; Tedersoo et al. 2009). This paradigm leaves many questions for the intermediate, temperate zones where both groups of mycorrhizas compete and coexist. This paradigm does, however, provide a possible mechanism for coexistence, that of vertical segregation, with AM dominating lower, mineral soil, and EcM dominating in the shallow organic horizons(Neville et al. 2002). I tested whether there was a segregation by soil depth between these two groups using the proportion of fine roots colonized and then adjusted by root length for each group within two mid-aged stands of northern hardwoods.

Both EcM and AM colonization have been shown to differ by depth (Dickie et al. 2002; Kuyper and Landeweert 2002; Baier et al. 2006a; Shukla et al. 2013). However, these studies have often been conducted in systems completely dominated by one of these groups, and few have worked on systems in which these groups co-dominate. In addition, while depth has been examined, the average depth that studies explore is 13 $\pm$ 3 cm, and few observations go as deep as 50 cm (Pickles and Pither 2014).

To investigate the way in which these two networks may interact or respond to environmental factors, I sampled roots from two stands within a northern hardwood ecosystem, at sites within the White Mountain National Forest. In this study, I took roots from the field and measured the length of the roots as well as the proportion of the roots that were colonized by the different groups of mycorrhizas. I used these measurements, as well as combination and transformation of these measurements to try establishing whether or not soil depth (as a proxy for soil environmental factors) could be a variable by which these groups were separation. I also tested organic and mineral components in the soil as factors by which mycorrhizas could vary. Further, I tested whether the measures of root colonization were appropriate or easily adaptable to the study of mycorrhizas in complex, natural systems.

Temperate forests of New Hampshire:

Northern hardwood forests occupy an area of interest for the mycorrhizologist because they are positioned at an intermediate space in terms of the latitudinal gradient, where EcM and ericoid mycorrhizas dominate nearer the poles, and AM seem to dominate nearer the equator. The northern hardwood forests are dominated by American beech (*Fagus grandifolia*), yellow birch (*Betula alleghaniesis*), and sugar maple (*Acer*

saccharum) (Kricher et al. 1998). If we consider the three typical northern hardwood tree species just mentioned, we find sugar maple has strong fidelity to AM, while yellow birch and American beech predominately are EcM (Wang and Qiu 2006). Younger stands of northern hardwoods contain early successional species, notably pin cherry (*Prunus pensyvanica*), which is strongly AM, whereas older forests sometimes often contain shade tolerant or late successional species, such as Eastern hemlock, that are strong EcM hosts. This pattern does not necessarily mean that the fungal community is driving succession, but we do observe the pattern that many early successional species are AM and many later successional species are EcM. Since this observed pattern does fit a model whereby the fungal community is driving or is at least connected to succession, we intend to incorporate succession into our experimental design. In order to prevent successional patterns from deluding the pattern that may exist in depth, we chose to sample from stands that are within the middle of this possible successional gradient. . We identified sites for our study that avoid the extremes of both the potential latitudinal gradient and the potential successional gradient in order to capture the complexity that both gradients may impart.

Objectives

The differences in habitat preference for the two groups of mycorrhizas found in northern hardwood forests could lead to observable differences in rooting behavior of plant species and the colonization of roots by mycorrhizal fungi across the soil profile. To test whether there was such an observable difference in root colonization by these groups, AM and EcM, two stands of northern hardwood forests were sampled for roots in the north-eastern United States.

In addition to examining whether mycorrhizal types segregate based on soil-depth categories, we chose to examine some of the specialized microscopic structures arbuscular mycorrhizas create and how they vary with depth. Arbuscular mycorrhizas use a number of specialized structures to store, transfer, and use nutrients (Mosse 1973; Smith and Read 2008). The presence or absence of mycorrhizal features may be an indication of mycorrhizal activity. These structures should provide a better clue as to how mycorrhizal function changes with depth than the degree of colonization. As the largest yearly input to the forest system comes from litterfall, we expect these structures to be greatest in the shallow soils. The greater presence of functional structures in nearer the forest floor is predicted regardless of patterns of vertical segregation within mycorrhizal group.

In addition to looking at mycorrhizal structures, the ratio of AM/EcM colonization was examined. This is because, if mycorrhizal activity is greater in the shallow soil across all mycorrhizal types, the signal for vertical segregation may be missed, however, the ratio made between the colonized roots may be a more robust measure for detecting a vertical segregation signal, if total colonization declines similarly, this ratio could still change. This ratio is expected to vary across the depth profile, with proportion to which AM colonized a root being in greater in the mineral soils.

Methods

Site description

This study was conducted in Bartlett Experimental Forest in the White Mountains of New Hampshire. The soils at the site are mixed to poorly sorted

Haplorthods developed in glacial drift derived mainly from granite and gneiss (Brissette 2015). Mean monthly temperatures average -8°C in January and 21°C in July, with an average annual precipitation of 1300 cm (Brissette 2015).

Bartlett stands C5 and C7 were chosen from among the sites being used in the Multiple Element Limitation in Northern Hardwood Ecosystems experiment. White and yellow birch dominate C5, with the two species accounting for more than two thirds the standing biomass; red and sugar maples make the second largest contribution. C5 is about 35 years in age, young enough that a few pin cherries are still standing in the plots, though more pin cherries sit on the ground fallen and decaying. Both sites are located roughly at 44° N 71° ‘ W; more detail is provided in a map of the experimental plots within Bartlett Forest found in the appendix. The stand C7 is older, roughly 110 years since its last cutting. It is co-dominated by beech and maple. If we use the basal area of trees greater than 10 cm and classify all trees into either AM or EcM host categories based on Wang and Qiu (2006), then C5 would be 17% AM and 83% EcM, and C7 would be 38% AM and 62% EcM.

There are differences in the rooting behaviors of the plants in these stands, for example maples often proliferate small low-order roots in high-nutrient microenvironments, and beech is able to graft roots and sprout clones from roots (Kessler 1966; Williamson 1975; Eshel and Beeckman 2013). Stands in Bartlett and nearby Hubbard Brook Experimental Forest have been tested for differences in the rooting behavior of these trees. Though fine roots were not found to segregate by species in the first 25 cm of soil, the average rooting depth of birch was found to be more shallow than that of American beech, which in turn was more shallow than

sugar maple (Yanai et al. 2008). While these averages were not statistically difference, the trend among the averages is that of the most strongly EM species having a shallower average rooting depth and the most strongly AM species having the deepest average rooting depth.

Sampling methods

Each of the stands is characterized by four 0.25-ha plots. Each plot is divided into nine 10 m x 10 m subplots surrounded by a 10 m buffer, an area in which no samples were collected. Within each stand, two types of cores were collected pertaining to two different depths. Shallow and deep samples were collected from each of the corner and the center sub-plots, for a total of five samples per plot or 20 samples per stand.

Shallow cores were collected on September 22 and October 10, 2010, using a polyvinyl chloride core with a diameter of 2 inches, hammered into the soil to a depth of 10 cm, after removing the Oi (litter layer). These cores were split into mineral and organic components, resulting in 37 organic and 34 mineral samples, as in 3 cases an organic layer was not present and in 5 cases the organic layer was deeper than 10 cm. The average depth of the organic layer (Oe and Oa horizons) in the shallow cores was 3.2 ± 0.5 cm.

The deep cores were taken using a gas-powered rotary corer with a 10-cm diameter diamond-tipped cylindrical drill bit (Levine et al. 2012) in July 2010. Intact root branches were separated from the 30-50 cm depth increment and frozen until they could be processed for use in this study. There were a total of 32 deep soil cores, as 2 were missing from C5 and 3 from C7. The depth of the power cores was

measured from the top of the mineral soil, as is conventional; this does not correspond to the depths reported for the shallow cores, where zero corresponds to the surface of the Oe horizon. We refer to the shallow cores as 0-10 and the deep cores as 30-50 even though the depths differ by the thickness of the Oea (3 cm).

Sample treatment

Roots from each sample were washed free of soil material over 2 mm and 0.2 mm mesh sieves. Roots < 1 mm in diameter and > 3 cm in length were preserved in ethanol until analysis.

Under a dissecting microscope (50x), roots were sorted into ectomycorrhizal and nonectomycorrhizal categories based on root morphology. When a root tip was questionably ectomycorrhizal, a cross section of the root tip was examined under 400-1000x magnification in tryphan blue or Chlorozal black E for the presence a Hartig net and mantle. The lengths of ectomycorrhizal and nonectomycorrhizal roots in each sample were measured using a grid intersection method (Brundett et al. 1996).

Plant tissues were opaque, and needed to be cleared before the AM roots could be quantified for the extent of colonization. Roots were first exposed to 10% by volume potassium hydroxide solution for a 20-minute autoclave cycle at 22 ATM, 120 °C. Roots were then exposed to 3% hydrogen peroxide for 10 minutes. Hydrogen peroxide interferes with the stain, so the roots were subjected to an acid wash in 1% nitric acid. Chlorazol black E was used selected to stain the fungi on account of its long history of use and its supposed selectivity in staining chitin over keratin or cellulose (Elewski 1996; Cannon 2011). The roots were then subjected to a 15 minute autoclave cycle, 22 atm, 120 °C in a mixture of 0.3% Chlorazol black E and equal parts water, lactic acid, and glycerol

by volume. Finally, the roots were transferred to a 50% glycerol 50% water solution for distaining for a period of 12 hours to several days (Brundett et al. 1996).

For each sample, 25 root segments (2 cm in length) were selected by floating the roots in a petri dish and picking one segment from each of the 25 sections of the dish (Brundett et al. 1996; Biermann and Linderman 2006). There were 12 samples that had fewer than 25 segments of AM roots. These had a range from 4-23 segments. The root sections were then placed parallel on glass slides and examined at 400x magnification and presence or absence of the following fungal structures was noted at each transect: coenocytic hyphae, septate hypha, clamped hypha, microsclerotia, vesicles, arbuscules, hyphal coils, and other structures (McGonigle et al. 1990; Brundett et al. 1996). Sugar maple, one of the more prevalent AM hosts in our sites, produces arum-type arbuscules, which may have been scored as hyphal coils as dendritic hyphae as these structures can be difficult to distinguish after staining.

To quantify colonization of the roots by EcM fungi, ectomycorrhizal roots were examined under the dissecting microscope. The number of root tips that were colonized by EcM fungi was counted and compared to the total number of tips.

The length of colonized roots was estimated as the percent colonization multiplied by the root length in the sample. The length per unit volume was calculated by taking the quotient of this and the volume of core.

Statistical methods

For each group of fungi (AM, EcM, and DSE), we compared colonization of roots by depth (shallow versus deep) and by horizon (organic versus mineral). We also analyzed the ratio of EcM to AM colonization and the of AM features (vesicles,

arbuscules, and hyphal coils) as dependent variables. In each of these analyses of variance, either depth (deep or shallow) or horizon (organic or mineral) was treated as a categorical variable with two levels, with stands incorporated into the model as a blocking factor.

Power analysis was conducting using PWR package in R (Champely 2012) on the tests where AM colonization was compared to EcM colonization by depth.

It is generally considered inappropriate to conduct hypothesis tests using percent data without transformation (DeVore and Berk 2007). Currently, the trend is to use Arcsine or Logit transformations for conducting hypothesis testing on proportional or percent data (Warton and Hui 2010). However, neither transformation strongly changed the results of the test, and since both arcsine and logit transformations alter observations at near extremes (conventionally (>0 and <0.05), and (>0.95 and <1.0) where more than 10% of our data lie, our statistics are presented from untransformed data.

Results

Root length density declined significantly with depth. The length of EcM roots was 12.2 cm/cm3 in the top 10 cm (including the Oea horizon) and 5.1 cm/cm3 30-50 cm deep in the mineral soil ($p = 0.05$). The length of AM roots was 9.1 cm/cm3 in shallow samples and 4.2 cm/cm3 in the deep samples ($p = 0.01$) (Figure 2.2).

The proportion of EcM root tips colonized declined from an average of 64% in the top 10 cm to 34 in the 30-50 cm depth ($p < 0.001$). Similarly, the proportion of root length with AM features declined from 66% in the top 10 cm to 34% at 30-50 cm depth ($p < 0.001$). The pattern of decline was similar between EcM and AM colonization: the ratio of the EcM to AM root length colonized did not vary by depth ($p=0.45$). Dark

septate endophytes (hyphae or microsclerotia) were found to have colonized 27% of the shallow root length and 18% of the length of deep roots ($p = 0.12$) (Figure 2.1).

The shallow samples included both organic and mineral horizons in 34 of the 40 samples. We compared colonization rates of the organic and mineral portions of the shallow samples, and found greater colonization in the organic portion for EcM ($p=0.05$) and DSE ($p=0.04$) but not for AM roots ($p=0.29$). The ratio of EcM to AM roots did not differ significantly between organic and mineral horizons ($p=0.89$).

Since both root length density and mycorrizhal colonization rates declined with depth, the root length colonized by each mycorrizal group also declined with depth. (AM: $p<0.001$, EcM: $p=0.005$). This decline with depth was similar for the two types: the ratio of EcM to AM root lengths colonized did not differ with depth ($p=0.27$). The lack of significance was due to small differences between the two mycorrhizal types, rather than to high variability: a power analysis revealed that it would take a difference of at least 16% in these ratios to be detectable (alpha=0.05, beta=0.11, n=84).

The over 93,000 microscopic slide transects viewed in the study allowed for testing whether certain AM features were more common in the shallow or the deep soils. Vesicles, hyphal coils, and arbuscules were each found to be more abundant per unit length of colonized root in the shallow soils, though statistical significance was low (vesicles $p=0.12$, coils $p=0.10$, arbuscules $p=0.88$) Figure 2.3. There were few (< 100) observations of arbuscules. Dark septate endophytes were examined simultaneously with the AM observations; DSE were found to vary by depth, with DSE being more common in the shallow soils ($p=0.12$).

Discussion

These results indicate that regardless of mycorrhizal type, mycorrhizal colonization is strongest in the shallow soils, where annual input of nutrients from atmosphere and litter takes place. This is in agreement with prior observations on fungal distribution (Malajczuk et al. 1982; Read 1991; Dickie et al. 2002; Kuyper and Landeweert 2002; Koide and Mosse 2004; Baier et al. 2006b; Shukla et al. 2013).

Similarly, there were more fungal structures in the AM in the shallow soils. We had few observations of arbuscules, possibly because these are seasonal structures, and our sampling missed their peak. However, it is possible that our clearing protocol was too aggressive. Arbuscules are composed of hyphal tissue that maximizes surface area by proliferating extremely fine hyphae. These fine hyphae may have been damaged by the vigorous acid/base treatments.

Contrary to our expectations, we did not find vertical stratification of EcM and AM fungi. EcM and DSE significantly favor organic over mineral soils. Competition in the organic soils seems to favor EcM fungi and saprotrophic fungi because both EcM and saprotrophic fungi possess extracellular enzymes which target organics (Martin et al. 1998), but enough AM host roots are available as to provide a refuge for the AM fungi in the shallow soil, preventing complete dominance by EcM fungi and preventing the ratio of Ecm/AM to change across these horizons or depths.

Alternatively, each mycorrhizal group could have could have fungal members specializing in each horizon, and the lack of difference in percent colonization could accurately reflect similar colonization levels at each depth, but

this colonization could be by different species or individuals of each group specialized to a specific soil horizon. Lee Taylor (2013) cataloged fungi in Alaska and found that the shallow and deep soils were often colonized by different species within the same genus. If niche partitioning by depth were happening at this lower phylogenetic scale it would be undetectable by our methodology.

Mycorrhizal symbiosis depends upon the presence of both plant roots and fungi. Root density decreases with depth (Figure 2.2). It is reasonable to assume that fungal spores also decrease with depth. Mycorrhizas can, of course, also colonize from mycelium, but with lower root density, that mycelium would need to travel further before finding new roots to colonize. A worthy analysis could be the comparison of the rooting behaviors of plants to the groups of mycorrhizas with which they are associated, especially for plants that exist in settings multiple mycorrhizal groups co-occur.

There are no methodologies for looking at the proportion of roots colonized by mycorrhizas that can be applied to both AM and EcM samples. This is a critical drawback, as it makes it difficult to measure responses of each group using the same criteria. The development of a methodology that could be used with both these groups of fungi to score colonization would allow for more informative questions to be asked.

Percent root length colonization cannot be interpreted as a measure of the strength of the mutualistic interaction or a measurement of fitness for either partner in the symbiosis (Brundett et al. 1996). Within AM alone, two equally colonized roots could have major differences in the number of transfer structures.

Two roots that are 50% colonized, with one root being all hyphae traversing otherwise inactive root material, and the other a combination of hyphae, arbuscules, and vesicles are not equal contributors. Mycorrhizas have been shown to be seasonally variable (Rabatin 1979; Giovannetti 1985; Pringle and Bever 2002); temporal variation could be an alternative mechanism by which these mycorrhizal groups could avoid direct competition for soil resources. This mycorrhizal phenology may have caused us to miss the signal we were searching for.

The findings of this study are in agreement with much of what is known about mycorrhizal distribution with depth. Since the 1890's, when Frank first started observing the mycorrhizas, more activity was consistently found in the upper portions of the soil profile(Frank 1885; Dickie et al. 2002; Koide and Mosse 2004). Few studies have attempted to simultaneously observe the activity of both mycorrhizal groups. Our understanding of both plant and mycorrhizal behavior is limited by our knowledge of how roots and fungi behave at depth.

Acknowledgements

I would like to thank April Melvin and Natalie Cleavitt, who conducted all of our field sampling. Stephen Stehman deserves acclaim for his patience in discussing the statistics for our data. Lin Liu and Kikang Bae who initially scored colonization and helped store and catalog our samples (pencils always!). Craig See should be lauded for his excessive support, humor, and good nature, even if he gets confused between a goose and a grey duck. Sam Tourlette and Yazmin Rivera deserve praise as they established so many an odd traditions in the Horton lab. Jeremy Hayward, a compatriot, deserves acclaim for psychological consulting and damage control

during our time with Tom. I wish to thank Tony, for showing me that a tree is not too different from a horse, and Yang Yang for culinary inspiration. I wish to thank the rest of the Yanai and Horton labs for their feedback and support, and, of course, Dr. Yanai and Dr. Horton themselves. This work could not have been conducted without the support provided by McIntire-Stennis formula funding.

Figures

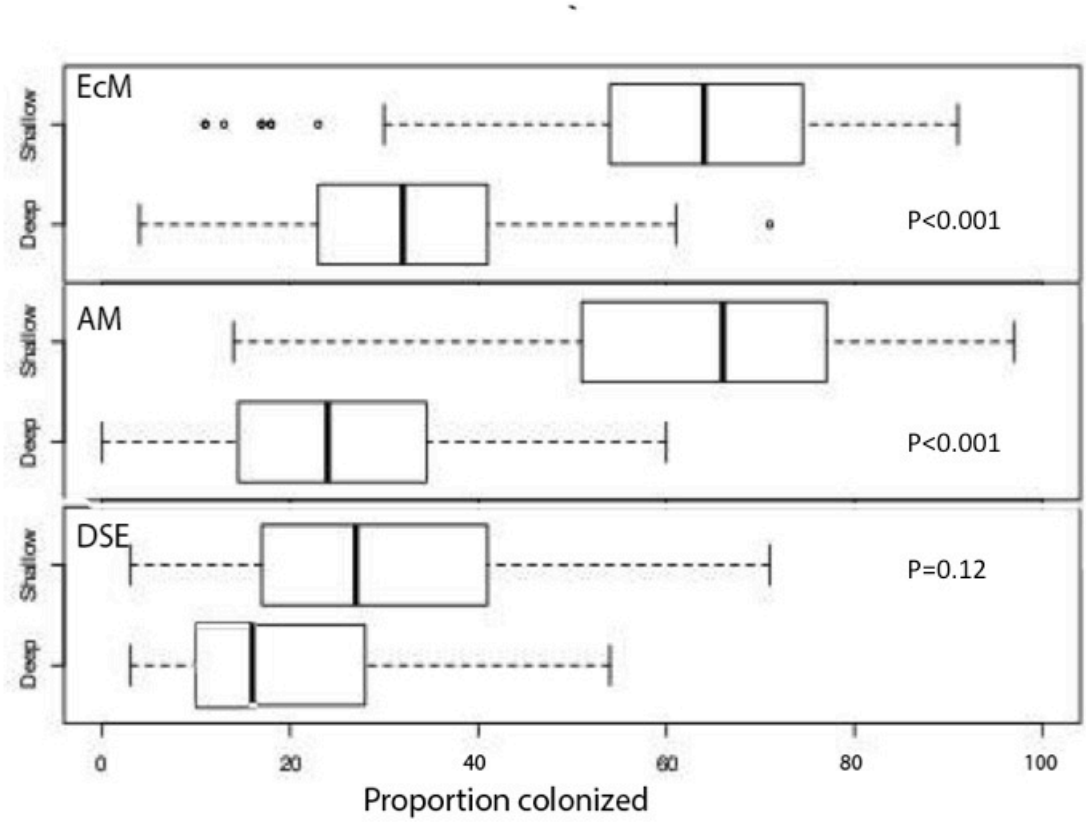

Figure 2. 1. Root colonization by three major root endophytes. The upper most plot shows colonization by ectomycorrhizas, the middle plot shows AM colonization and the final plot shows dark septate endophyte colonization.

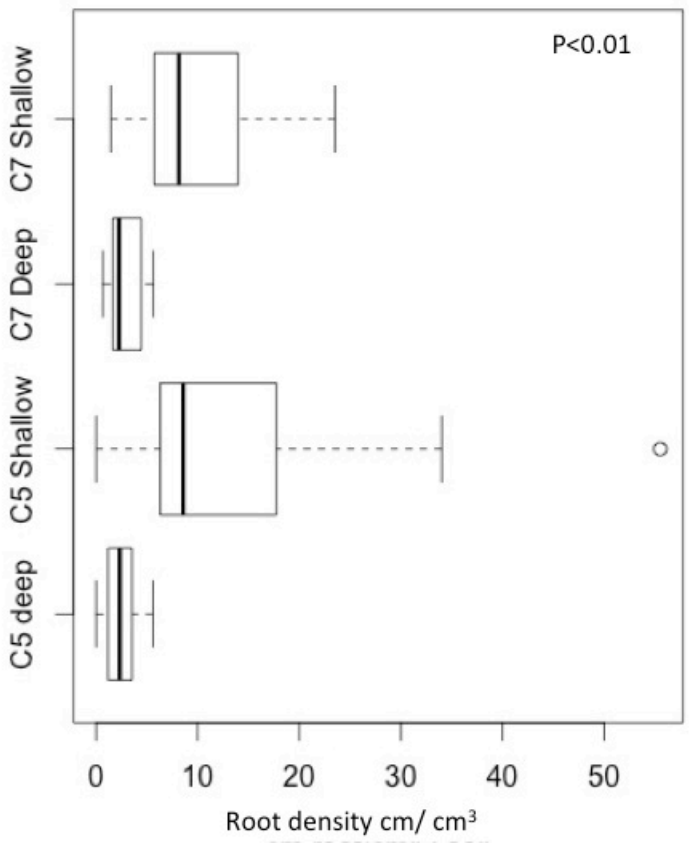

Figure 2. 2. Root length density in each of the stands by depth increment.

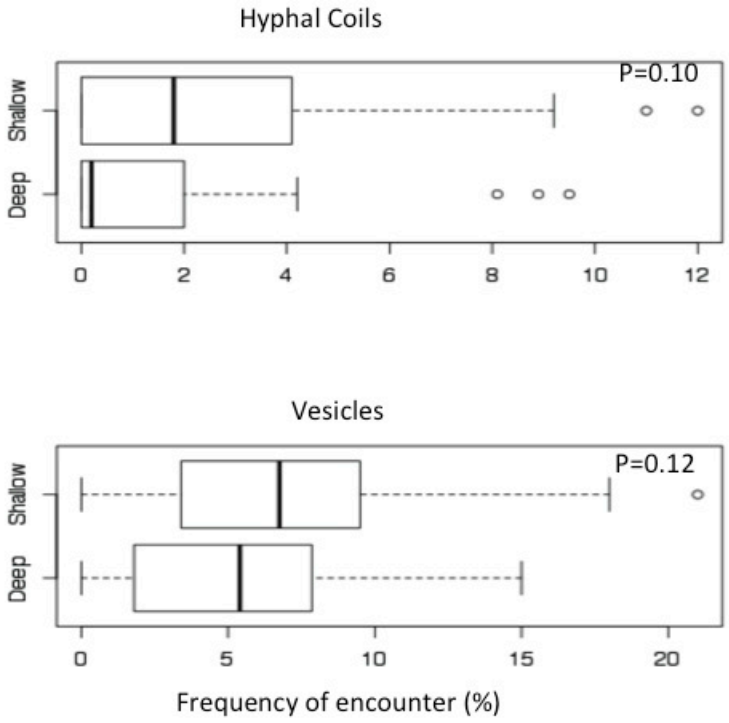

Figure 2. 3. Proportion of arbuscular mycorrhizal hyphal length in which hyphal coils or vesicles were encountered

Literature Cited

Affairs P, 14 UB| S, 2012 Fungus expert celebrates rare 10,000th citation. http://newscenter.berkeley.edu/2012/09/14/fungus-expert-celebrates-10000th-citation-for-1990-publication/. Accessed 25 Dec 2013

Agerer R (2000) Exploration types of ectomycorrhizae. A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114.

Allen M (1993) Mycorrhizal Functioning: An Integrative Plant-Fungal Process, 1st edn. Springer

Bacon CW, White J (2000) Microbial Endophytes. CRC Press

Baier R, Ingenhaag J, Blaschke H, et al (2006a) Vertical distribution of an ectomycorrhizal community in upper soil horizons of a young Norway spruce (Picea abies [L.] Karst.) stand of the Bavarian Limestone Alps. Mycorrhiza 16:197–206. doi: 10.1007/s00572-006-0035-z

Baier R, Ingenhaag J, Blaschke H, et al (2006b) Vertical distribution of an ectomycorrhizal community in upper soil horizons of a young Norway spruce (Picea abies [L.] Karst.) stand of the Bavarian Limestone Alps. Mycorrhiza 16:197–206. doi: 10.1007/s00572-006-0035-z

Berbee ML, Taylor JW (2007) Rhynie chert: a window into a lost world of complex plant–fungus interactions. New Phytol 174:475–479. doi: 10.1111/j.1469-8137.2007.02080.x

Biermann B, Linderman RG (2006) Quantifying Vesicular-Arbuscular Mycorrhizae: a proposed method towards standardization. New Phytol 87:63–67. doi: 10.1111/j.1469-8137.1981.tb01690.x

Brundett M, Bougher N, Dell B, et al (1996) Working with Mycorrhizas in Forestry and Agriculture. Australian Centre for International Agricultural Research

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37–77. doi: 10.1007/s11104-008-9877-9

Cannon HG (2011) VIII.—On Chlorazol Black E and some other new stains. J R Microsc Soc 61:88–94. doi: 10.1111/j.1365-2818.1941.tb00893.x

Catalfomo P, Trappe JM (1970) Ectomycorrhizal fungi: a phytochemical survey. Northwest Sci 44:19–24.

Cázares E, Trappe JM (1993) Vesicular endophytes in roots of the Pinaceae. Mycorrhiza 2:153–156. doi: 10.1007/BF00210584

Champely S (2012) pwr: Basic functions for power analysis.

DeVore JL, Berk KN (2007) Modern Mathematical Statistics With Applications. Cengage Learning

Dickie IA, Xu B, Koide RT (2002) Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. New Phytol 156:527–535. doi: 10.1046/j.1469-8137.2002.00535.x

Dighton J (1991) Acquisition of nutrients from organic resources by mycorrhizal autotrophic plants. Experientia 47:362–369. doi: 10.1007/BF01972078

Elewski BE (1996) Diagnostic techniques for confirming onychomycosis. J Am Acad Dermatol 35:S6–S9. doi: 10.1016/S0190-9622(96)90062-6

Eshel A, Beeckman T (2013) Plant Roots: The Hidden Half, Fourth Edition. CRC Press

Frank AB (1885) Uber die auf Wurzelsymbiose beruhende Ernlihrung gewisser Biiume durch Unterirdische Pilze. B. Eingegangen Am 17

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118. doi: 10.1111/j.1365-294X.1993.tb00005.x

George E, Marschner H, Jakobsen I (1995) Role of Arbuscular Mycorrhizal Fungi in Uptake of Phosphorus and Nitrogen From Soil. Crit Rev Biotechnol 15:257–270. doi: 10.3109/07388559509147412

Gerdemann JW (1968) Vesicular-Arbuscular Mycorrhiza and Plant Growth. Annu Rev Phytopathol 6:397–418. doi: 10.1146/annurev.py.06.090168.002145

Giovannetti M (1985) Seasonal variations of vesicular-arbuscular mycorrhizas and endogonaceous spores in a maritime sand dune. Trans Br Mycol Soc 84:679–684. doi: 10.1016/S0007-1536(85)80123-6

Harley JL (1969) The biology of mycorrhiza. Leonard Hill

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic Press

Innis MA, Gelfand DH, Sninsky JJ, White TJ (1990) PCR Protocols: A Guide to Methods and Applications. Academic Press

Jakobsen T, Erik Nielsen N (1983) Vesicular-Arbuscular Mycorrhiza in Field-Grown Crops. New Phytol 93:401–413. doi: 10.1111/j.1469-8137.1983.tb03440.x

John Brissette (2015) Bartlett Experimental Forest. United States Department of Agriculture, Bartlett New Hampshire

Katznelson H, Rouatt JW, Peterson EA (1962) The Rhizosphere Effect of Mycorrhizal and Non-Mycorrhizal Roots of Yellow Birch Seedlings. Can J Bot 40:377–382. doi: 10.1139/b62-037

Kessler KJ (1966) Growth and Development of Mycorrhizae of Sugar Maple (acer Saccharum Marsh.). Can J Bot 44:1413–1425. doi: 10.1139/b66-154

Koch R (1978) Untersuchungen uber die Aetiologie der Wundinfectionskrankeheiten.

Koide RT, Mosse B (2004) A history of research on arbuscular. Mycorrhiza 14:145–163. doi: 10.1007/s00572-004-0307-4

Kricher JC, Morrison G, National Audubon Society, et al (1998) A field guide to eastern forests, North America. Houghton Mifflin, Boston

Kuyper TW, Landeweert R (2002) Vertical niche differentiation by hyphae of ectomycorrhizal fungi in soil. New Phytol 156:323–325. doi: 10.1046/j.1469-8137.2002.00548.x

Lapeyrie FF, Chilvers GA (1985) An Endomycorrhiza-Ectomycorrhiza Succession Associated with Enhanced Growth of Eucalyptus Dumosa Seedlings Planted in a Calcareous Soil. New Phytol 100:93–104. doi: 10.1111/j.1469-8137.1985.tb02761.x

Levine CR, Yanai RD, Vadeboncoeur MA, et al (2012) Assessing the Suitability of Rotary Coring for Sampling in Rocky Soils. Soil Sci Soc Am J 76:1707. doi: 10.2136/sssaj2011.0425

Levy Y, Dodd J, Krikun J (1983) Effect of Irrigation, Water Salinity and Rootstock on the Vertical Distribution of Vesicular-Arbuscular Mycorrhiza in Citrus Roots. New Phytol 95:397–403. doi: 10.1111/j.1469-8137.1983.tb03507.x

Lindahl BD, Ihrmark K, Boberg J, et al (2007) Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytol 173:611–620. doi: 10.1111/j.1469-8137.2006.01936.x

Lohman ML Occurence of mycorrhiza in Iowa forest plants. Univ Iowa Stud Nat Hist 11:33–58.

Malajczuk N, Molina R, Trappe JM (1982) Ectomycorrhiza Formation in Eucalyptus. New Phytol 91:467–482. doi: 10.1111/j.1469-8137.1982.tb03325.x

Martin F, Selosse M-A, Di Battista C, et al (1998) Molecular markers in ecology of ectomycorrhizal fungi. Genet Sel Evol 30:S333. doi: 10.1186/1297-9686-30-S1-S333

McGonigle TP, Miller MH, Evans DG, et al (1990) A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. New Phytol 115:495–501. doi: 10.1111/j.1469-8137.1990.tb00476.x

Money NP (2006) The Triumph of the Fungi: A Rotten History. Oxford University Press, Oxford ; New York

Mosse B (1973) Advances in the Study of Vesicular-Arbuscular Mycorrhiza. Annu Rev Phytopathol 11:171–196. doi: 10.1146/annurev.py.11.090173.001131

Neville J, Tessier J., Morrison I, et al (2002) Soil depth distribution of ecto- and arbuscular mycorrhizal fungi associated with Populus tremuloides within a 3-year-old boreal forest clear-cut. Appl Soil Ecol 19:209–216. doi: 10.1016/S0929-1393(01)00193-7

Olson JS (1963) Energy Storage and the Balance of Producers and Decomposers in Ecological Systems. Ecology 44:322–331. doi: 10.2307/1932179

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–IN18. doi: 10.1016/S0007-1536(70)80110-3

Pickles BJ, Pither J (2014) Still scratching the surface: how much of the “black box” of soil ectomycorrhizal communities remains in the dark? New Phytol 201:1101–1105. doi: 10.1111/nph.12616

Pringle A, Bever JD (2002) Divergent phenologies may facilitate the coexistence of arbuscular mycorrhizal fungi in a North Carolina grassland. Am J Bot 89:1439–1446. doi: 10.3732/ajb.89.9.1439

R S, S AI de J da (1979) Litter decomposition and ectomycorrhiza in Amazonian forests, 1: A comparison of litter decomposing and ectomycorrhizal Basidiomycets in latosol-terra-firme rain forest and white podzol campinarana [Biologia do solo; Micorriza; Fungo; Floresta; Amazonia; Solo; Brasil]. Acta Amaz.

Rabatin SC (1979) Seasonal and Edaphic Variation in Vesicular-Arbuscular Mycorrhizal Infection of Grasses by Glomus Tenuis. New Phytol 83:95–102. doi: 10.1111/j.1469-8137.1979.tb00730.x

Read DJ (1991) Mycorrhizas in ecosystems. Experientia 47:376–391. doi: 10.1007/BF01972080

Redhead JF (1982) Ectomycorrhizae in the tropics. In: Dommergues YR, Diem HG (eds) Microbiol. Trop. Soils Plant Product. Springer Netherlands, pp 253–269

Shukla A, Vyas D, Jha A (2013) Soil depth: an overriding factor for distribution of arbuscular mycorrhizal fungi. J Soil Sci Plant Nutr 13:23–33. doi: 10.4067/S0718-95162013005000054

Smith SE, Read DJ (2008) Mycorrhizal Symbiosis, Third Edition, 3rd edn. Academic Press

Taylor DL, Hollingsworth TN, McFarland J, et al (2013) A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. Ecology. doi: 10.1890/12-1693.1

Tedersoo L, May TW, Smith ME (2009) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263. doi: 10.1007/s00572-009-0274-x

Trappe JM (2004) A.B. Frank and mycorrhizae: the challenge to evolutionary and ecologic theory. Mycorrhiza 15:277–281. doi: 10.1007/s00572-004-0330-5

Treseder KK, Cross A (2006) Global Distributions of Arbuscular Mycorrhizal Fungi. Ecosystems 9:305–316. doi: 10.1007/s10021-005-0110-x

Wang B, Qiu Y-L (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363. doi: 10.1007/s00572-005-0033-6

Warton DI, Hui FKC (2010) The arcsine is asinine: the analysis of proportions in ecology. Ecology 92:3–10. doi: 10.1890/10-0340.1

Went FW, Stark N (1968) Mycorrhiza. BioScience 18:1035–1039. doi: 10.2307/1294552

White, Thomas J, Bruns, Thomas, Lee, S, Taylor, JW (1990) amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Acad Pres 315–322.

Williamson GB (1975) Pattern and Seral Composition in an Old-growth Beech-Maple Forest. Ecology 56:727. doi: 10.2307/1935509

Yanai RD, Fisk MC, Fahey TJ, et al (2008) Identifying roots of northern hardwood species: patterns with diameter and depth. Can J For Res 38:2862–2869. doi: 10.1139/X08-12

Franklin Diggs

3049 Biber St Apt T8
East Lansing MI, 48823
(518)-645-1725
Pyrpoi@gmail.com

Education

SUNY(State University of New York) at Plattsburgh — Plattsburgh, NY
B.S. Environmental Science with concentration in Ecology
Applied Environmental Science Program, W.H. Miner Institute
Awarded Outstanding Graduating Senior in Environmental Science
Cum Laude — December 2009
GPA 3.67

SUNY College of Environmental Science and Forestry (ESF) — Syracuse, NY
M.S. Candidate in the school of Forest and Natural Resource Management
Focused in the role of mycorrhiza in forest ecosystems — December 2014

Relevant Coursework

Applied Forest Ecology and Management, Mycorrhizal Ecology, Advanced Mycology: Basidiomycetes, Linear regression, nonparametric statistics, Experimental Design and Analysis of Variance, Plant Ecology, Population and Community Ecology, Mathematical modeling (water quality), Organic Chemistry, General Biology, Fundamentals of Geographic Information Systems, Environmental Technology, and Applied Soil Science

Relevant Skills

Computer: ESRI ArcGIS software, Microsoft Office Suite, RUSLE, statistical programs (R, minitab), mathematical modeling software (STELLA, MAPLE)

Field: GPS field use and GIS integration, orienteering, use of basic forestry equipment and techniques (densiometer, DBH, increment borer), soil sampling, mark-recapture methods, radio telemetry, basic surveying, stream classifications (Rosgen), small mammal trapping, identification using dichotomies keys.

Laboratory: Fungal and mycorrhizal identification. DNA extraction, amplification, and analysis, Tree increment core analysis, agarose and salivary amylase cellulose acetate gel electrophoresis, familiarity with green house operations, soil texture analysis, spectroscopy, gas chromatography, water quality analysis, preparation of buffers, distillation, basic biology and chemistry laboratory maintenance.

Professional Experience

Teaching Assistant – SUNY ESF, Syracuse, NY — 12/2012-05/2013
Chemistry

Research Assistant – SUNY ESF, Syracuse, NY — 01/2012-08/2012;
Mycorrhiza in forest ecosystems; summer field crew — 07/2011-08/2011

Martin Nature Center – Oklahoma City — 07/2010-12/2010
Manual labor, Nature guide

Substitute teaching
Oklahoma City public schools — 02/2010-09/2010

Successful independent and team-based marketing in entertainment industry
(Fire Performance) — 2004- present
Fire poi performance and safety: Promoting, marketing, team organizing, safety training and compliance

Pano's – Buffalo NY — April-Sept 2007
Dishwasher: Washing dishes, cleaning equipment, overnight crew

Kilwin's – Sarasota FL — Summer 2006
Customer service: Serving food, cleaning, stocking

Clean Water Action - Tampa FL — Summer 2005
Canvassing