A FIELD STUDY OF SPECIFICITY IN THE ARBUSCULAR MYCORRHIZAL SYMBIOSIS

by

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Abstract

Host specificity in the arbuscular mycorrhizal (AM) symbiosis has been difficult to assess in the field. Here I report on a study designed to determine whether different plant species associate with unique communities of arbuscular mycorrhizal fungi (AMF), and how increasing plant diversity affects the composition of AMF communities. We sampled soil from a long-term common garden study – the biodiversity field experiment at the Cedar Creek Ecological Research Centre in Minnesota, USA. The soil samples were taken from plots with varying plant diversity (0, 1, 4, and 16 species) and AMF DNA was extracted from these samples for 454 sequencing to assess fungal community composition, host specificity, and plant diversity effects in the AM symbiosis. Results indicate that there is evidence for functional specificity in the AM symbiosis, particularly with grasses, which show distinct AMF communities from other plant functional groups, such as legumes and non-legume forbs. Due to loss of samples in the sequencing pipeline, little evidence could be garnered for host specificity at the plant species level. Though this study provides some evidence for host specificity at the functional group level, the results indicate that relative abundance of AMF taxa is affected by plant functional group, therefore also supporting the idea that AMF are more generalist in nature. Similarly, when investigating the impact of plant diversity on AMF community composition and phylogenetic composition, this study shows that that plant diversity has an effect on AMF community composition, but not on AMF species richness. Overall, the results of this study indicate that the maintenance of AMF community composition is affected by plant type and diversity above ground, and that other factors may be responsible for regulating the diversity of AMF communities in the soil. However, this study does provide a better understanding of belowground/aboveground interactions and community scale dynamics in the AM symbiosis.
Preface

The field site for this experiment was established at the Cedar Creek Long Term Ecological Research Site in East Bethel (MN, USA), primarily directed by Dr. David Tilman from the University of Minnesota, USA. Soil cores were collected from Biodiversity Experiment 120, which was established in 1993. Troy Meilke, a research associate at the University of Minnesota, carried out the soil sampling methods described in Chapter 2, while I was responsible for all the molecular and statistical analysis that followed soil sampling. 454 sequencing was performed at the UBC Prostate Centre (Vancouver, BC). I conducted the bioinformatic and statistical analyses that followed 454 sequencing. I was also responsible for writing this thesis with guidance from my supervisor, Dr. John Klironomos, and my supervisory committee: Dr. Miranda Hart and Dr. Melanie Jones from the University of British Columbia Okanagan Campus.
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List of Abbreviations

AM – Arbuscular Mycorrhizae

AMF – Arbuscular Mycorrhizal Fungi

ANOVA – Analysis of Variance

BLAST – Basic Local Alignment Search Tool

DNA – Deoxyribonucleic Acid

MUSCLE – Multiple Sequence Alignment with High Accuracy and High Throughput

OTU – Operational Taxonomic Unit

PCR – Polymerase Chain Reaction

PCO – Principal Coordinate Analysis

PERMANOVA – Permutational Analysis of Variance

QIIME – Quantitative Insights Into Microbial Ecology

RNA – Ribonucleic Acid

VT – Virtual Taxon
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To my Grandpa,
for being my very first
professor of ecology
CHAPTER 1: Introduction

1.1 General Introduction

The causes and consequences of biodiversity in ecosystems have been active topics in ecology for many decades. Not only have biodiversity studies informed policies on ecosystem preservation and species protection (Clavel et al., 2010; Cadotte et al., 2011), but fundamental research has also focused on how biodiversity contributes to ecosystem stability, productivity, carbon and nitrogen cycling in the soil, and trophic interactions (Schulze and Mooney 1993; Tilman 1997; Quijas et al., 2010). Specifically of interest is how diversity of plant communities impacts ecological processes. Observational studies focusing on plant diversity have shown that increasing biodiversity significantly increases community productivity. This pattern has been broadly explained by two possible mechanisms: a) niche complementarity, which suggests that as biodiversity increases, more niche space can be exploited, resulting in increased productivity, or b) the sampling effect, which postulates that high diversity ecosystems have a greater probability of containing one highly productive species (Lehman and Tilman 2000).

Experimental manipulations of terrestrial biodiversity illustrate how increasing species richness can drive productivity and stability patterns of communities (Tilman et al., 1998). Though plants have important roles in driving these relationships, ecological studies have traditionally taken a plant-centric focus, and have often neglected the potential contribution of soil microbial communities. For example, it has been shown that arbuscular mycorrhizal fungi, obligate root symbionts that form associations with plant roots (Smith and Read 2008), contribute to both productivity/biodiversity gradients and plant community assembly (van der Heijden et al., 1998a & b). Little is known, however, about how above ground plant diversity affects below ground microbial diversity, particularly in AMF communities. In this thesis, my goal is to explore how AMF communities assemble under different plant species. Understanding the degree of
specificity between plants and AMF is an important determinant of how plant diversity will affect AMF diversity. For example, if AMF show some level of host specificity, then one could expect AMF diversity to increase with plant diversity. The following sections will provide background information required to address and understand the objectives of this thesis and the hypotheses that have been developed.

1.2 Plant Diversity and Effects on Ecological Stability and Productivity

The importance of biodiversity in ecosystems has been a topic of research for decades, originating with Elton’s work on plant diversity in 1958 (Elton 1958). Since that time, biodiversity has been shown to affect stability and productivity across trophic levels in a variety of ecosystems (Schulze and Mooney 1993; Tilman 1997; Quijas et al., 2010; Haddad et al., 2011) and has become an important aspect of conservation biology (Balvanera et al., 2006; Clavel et al., 2010; Cadotte et al., 2011). Of these studies, the contribution of biodiversity to stability in ecosystems is of particular importance to ecologists, as it informs how well a system can respond to stress or disturbance-induced changes over time (Van der Putten et al., 2000). Of key interest is how biodiversity of plant communities affects the stability of terrestrial ecosystems. It has been hypothesized that ecosystem stability increases with biodiversity. As Lehman and Tilman (2000) postulate, stability increases linearly with increasing biodiversity. This relationship can be explained through competitive interaction effects (covariance effect), through statistical averaging (portfolio effect) and increased productivity with increasing diversity (over-yielding effect). With the combination of these three effects, stability of terrestrial ecosystems increases with increasing biodiversity at the community level (Lehman and Tilman 2000; Nestmann et al., 2011).

The relationship between stability and biodiversity is also important when considering the benefits of biodiversity on ecosystem functioning. Primary productivity of an ecosystem can
be defined as the total of all materials produced by the growth of plants over a period of time (Tilman 1999). Theoretically, it has been hypothesized that with increasing species richness in an ecosystem, there will be an increase in the amount of interspecific interactions, which in turn, will determine ecosystem functioning (Elton 1958, McNaughton 1977). It is also expected that ecosystems with high diversity should be more productive due to higher potential for alternative nutrient cycling and energy flow (Vitousek and Hooper 1993; Schulze and Mooney 1993). Furthermore, more diverse communities should also have the ability to better capture limiting resources as a result of differences in species morphology, life history strategies, and physiology (Lehman and Tilman 2000). While there are many possible patterns that describe the dependence of productivity on biodiversity, the most widely accepted is an increasing, asymptotic relationship (Tilman 1999). That is, as species richness increases, ecosystem functioning increases and then becomes saturated at some level (Tilman 1997; Tilman 1999). Here, as each new species is added to the community, it shares an increasing proportion of its traits with species that are already present, and thus contributes less to ecosystem functioning than it would at lower diversities (Lambers et al., 2004; Tilman 2011).

1.2.1 Ecological Mechanisms Behind Diversity/Productivity Relationship

To explain this relationship mechanistically, researchers have suggested two major reasons why increasing biodiversity contributes to increased productivity: the first is sampling effect, which suggests that the probability of a highly productive species being present in the community is greater with increasing plant diversity (Hector et al., 2001; Marquard et al., 2009). The second is complementarity, which suggests that different species have unique traits and complement each other with regards to resource utilization, creating saturation in ecosystem functioning at some level of diversity (Tilman 1996; Hector et al., 1999; Lambers et al., 2004; Schmid 2004; Montes et al., 2008; Schmidke et al., 2010). Other hypotheses to explain this
relationship are similar in nature. Tilman et al. (1997) described three different theoretical models predicting how productivity and community assembly respond to biodiversity. The first model involves the competition of plant species for one resource. This model suggests that the species with the lowest requirement for the limiting resource would dominate at equilibrium. In this case, total plant biomass increases with biodiversity because the best competitor produces more biomass, as it requires the least amount of a limiting resource, and the probability of having a superior competitor is increased with greater biodiversity. The second model involves competition between plant species for two limiting resources. This model predicts that no species is competitively superior and resources that deviate from the optimum range are left unconsumed. These unused resources provide an opportunity for new species to enter the ecosystem and persist, which would in turn reduce the availability of resources and increase productivity. Finally, the third model suggests that two or more factors limit species abundance as a result of interspecific interaction or functional tradeoffs within each species. Since each species is different, the combinations of these tradeoffs would allow different species to cover different portions of the habitat, but no single species would be able to fully exploit the entire range of environmental conditions. With greater exploitation of an area, comes greater resource utilization over time, leading to long term increases in ecosystem carbon and nutrient stores, which would further increase productivity.

1.2.2 Overview of Diversity/Productivity Studies

The various theoretical models of biodiversity/productivity relationships have been tested extensively. More recently, the long-term biodiversity experiments at the Cedar Creek Natural History Area in Minnesota, USA have produced many studies that provide evidence for the asymptotic dependence of productivity on diversity. For example, in 1996, Tilman completed a study that investigated variation in total community biomass over many years in 207 plots.
planted with different levels of plant diversity (Tilman 1996). This study found that biomass variation in low diversity plots was significantly greater than the variation in biomass within high diversity plots, suggesting that the higher diversity treatments had more consistent productivity over time, compared to the low diversity treatments.

In a similar study by Tilman et al. (1998), productivity and biodiversity were studied to determine whether sampling effect or niche complementarity had stronger effects on ecosystem functioning. In this study, abundances of 24 plant species were measured (abundance estimated by measuring percent cover) in both low diversity plots (monocultures and 2-species plots) and high diversity plots (16-species plots). The abundances of 24 species in low diversity plots were plotted against their abundances in high diversity plots. It was found that the most abundant species in low diversity plots was also more abundant in high diversity plots. However, at high diversity levels, the abundances of all species were greatly reduced, sometimes up to a 40-fold factor. This suggests that contrary to sampling effect, the presence of other species greatly reduced the percent cover of the most abundant species in low diversity plots, but the overall increase of plant species contributed to increased overall percent cover in the high diversity plots. This study provides support for niche complementarity as a mechanism that drives biodiversity/productivity relationships. Hector et al. (1999) also showed evidence for niche complementarity in a study investigating species loss in European grasslands. This study showed a linear relationship between reduction of biomass and species loss. Furthermore, communities with fewer functional groups were less productive, illustrating the role of complementarity in producing this relationship.

In another study, Tilman et al. (2001) measured productivity in high diversity plots versus monocultures. It was found that high diversity plots had 2.7 times greater biomass than monocultures. Furthermore, high diversity plots had greater productivity than the most productive monoculture, indicating again, that niche complementarity has more of an effect on
ecosystem functioning than sampling effect. Carbon stores were also measured, and a similar pattern was observed, showing increased carbon storage at high diversity levels.

Similarly, other bodies of research indicate that the presence of a particularly useful species, not necessarily the most productive species, drives the relationship between biodiversity and productivity, also supporting the niche complementarity model. In a study conducted by Fornara and Tilman (2009), it was suggested that improved nitrogen accumulation by the presence of certain plant species contributes to increased productivity at high biodiversity. Soil carbon and nitrogen were measured from 1m of soil in low diversity and high diversity plots. The results illustrate that high diversity treatments contained up to 500% more stored carbon and up to 600% more stored nitrogen, on average, than monocultures. This high level of carbon and nitrogen storage was attributed to the presence of C4 grass and legume functional groups, which individually increased soil carbon by 193% and soil nitrogen by 522%. This study illustrates the importance of functional complementarity in ecosystems, and how this can drive the relationship between biodiversity and productivity. In a similar study, Venterink (2011) proposed that the role of phosphorus accumulation could be a driver for productivity and species richness, even more so than nitrogen.

It is clear that biodiversity and productivity are linked, with productivity increasing as biodiversity increases. While many have postulated the mechanisms that drive this relationship, niche complementarity has garnered the most empirical support, with studies consistently illustrating that the most productive species in monoculture is not nearly as productive in high diversity treatments, and that this increase in productivity at high diversity is due to positive species interactions within plant communities (Tilman et al., 1998; Tilman et al., 2001; Fornara and Tilman 2009).
1.3 Soil Microbial Communities: Roles in Plant Community Productivity and Structuring

The idea that productivity increases asymptotically with biodiversity was first explored by focusing on plant-plant interactions (largely competition). However, research has also shown that microbial communities and plant-microbe interactions play an integral, or even essential role in driving this relationship (Schimel 1995; Dybzinski et al., 2008; Wagg et al., 2011). The accumulation of pathogens, fungal and microbial symbionts, and root herbivores can have a profound effect on what occurs above ground (Wardle et al., 2004), as it is known that pathogens and soil predators can significantly reduce plant productivity and biomass above ground (Bever et al., 1997). On the other hand, mutualisms such as the AM association, can serve to increase fitness of individual plant species or plant communities as a whole, by increasing access to limiting nutrients in soil (Smith and Read 2008), thus contributing to the biodiversity/productivity relationship. Beyond their role in promoting productivity of plant communities, soil microbial interactions with plants have been linked to changes in plant communities and overall structuring of terrestrial communities (Grime et al., 1987; van der Heijden et al., 1998 a & b; Bauer et al., 2012).

1.3.1 Plant Diversity and AMF Community Studies

Since the majority of terrestrial plants form some type of symbiosis with soil microbes, particularly mycorrhizal fungi (Smith and Read 2008), it is important to acknowledge the role that soil microbial communities play in influencing plant community structure and productivity. In particular, AMF communities seem to have a positive effect on promoting plant diversity (van der Heijden et al., 1998 a & b). Since there is a differential effect of AMF on plant species, AMF have the potential to limit or promote the presence of particular taxa aboveground, thus influencing plant community assembly and structure (Grime et al., 1987; Gange et al., 1990; van der Heijden et al., 1998 a & b; Hartnett and Wilson 1999; Bauer et al., 2012). It has been shown
that some plant species can have a much higher response to AMF, while others are less responsive (Klironomos 2002; Klironomos 2003; Jones and Smith 2004; Pringle et al., 2009), and their role as drivers of plant diversity and productivity may be determined by the mycorrhizal dependency of the species within the community.

Many studies have focused on the relationship between plant diversity and soil microbial communities and how this interaction can lead to changes in plant communities above ground. Looking from an arbuscular mycorrhizal paradigm, studies have focused on how manipulation of soil AMF communities can lead to observable and measurable changes in plant community structure and response above ground. This interaction between soil microbial communities and plant communities is important to understand, because it illustrates how microbial taxa interact with their plant hosts and how this interaction can have broad community effects above ground, leading to questions about selectivity and even host specificity in microbial symbioses.

The effect of AMF taxa on plant community structure was investigated in a study by van der Heijden et al. (1998a). Two experiments were set up to investigate if and how AMF species richness affected plant diversity. The first experiment manipulated the number of AMF species and measured plant response (biomass) to changes in AMF community composition. The second experiment tested the hypothesis that biodiversity and ecosystem productivity may increase as AMF species richness increases. This was tested using 70 experimental macrocosms, which manipulated the number and composition of AMF species present against a representative plant community. Above ground and below ground productivity were then measured, along with Simpson’s diversity index in order to determine the effects of AMF community change on plant community productivity. The results from both experiments illustrated that different AMF species can cause significant increases in host productivity, depending on the plant species. As well, this study also showed that AMF diversity played a key role in maintaining a base level of plant diversity, speaking to the importance of AMF communities in the maintenance and
promotion of plant diversity, and suggesting that the mycorrhizal symbiosis may also be an important driver of the observable relationship between biodiversity and productivity in plant communities.

In a similar study, Klironomos (2002) provided evidence that AMF communities and other soil microbes can contribute to the maintenance of plant diversity and can regulate the abundance of particular plant species. In this study, the effect of pathogen accumulation and AMF interaction in the soil, and how this accumulation and AMF interaction affected rare, native plants and invasive plants were studied. Here, rare plant species showed higher levels of negative feedback and increased pathogen accumulation when grown in their own soil, thus limiting their success. However, invasive plants showed strong positive feedback and greater interactions with AMF in foreign soil. These results indicate that differences occur in plant response to these AMF communities, which can potentially lead to the success of plants in the environment, influencing the development of community structure, while also illustrating their role in the structuring of plant communities.

More recently, Schnitzer et al. (2011) investigated the role of host-specific soil microbes in driving the positive relationship between plant diversity and ecosystem productivity. In this experiment, plant productivity and plant disease rates were measured in the presence of soil microbes. When plants were grown in the presence of soil microbes, disease prevalence significantly decreased with increasing plant diversity, while plant productivity increased by nearly 500%, compared to productivity in communities grown without soil microbes. The results of this study illustrate the importance of soil microbes in driving the relationship between productivity and biodiversity and contribute to the body of research that suggests soil microbial activity is an important factor in plant community productivity. A study by Maron et al. (2011) also showed the important role soil microbial communities play in the relationship between biodiversity and productivity. In this experiment, the role of soil fungal pathogens on plant
community productivity was investigated. Plots of varying plant diversity were either treated with fungicide or were left untreated and productivity was measured. In control plots, a positive, linear relationship was observed between plant diversity and shoot biomass. However, in plots treated with fungicide, this relationship disappeared. In low diversity plots treated with fungicide, productivity increased 131% over the growing period, while productivity only increased by 33% in high diversity plots treated with fungicide, again flattening the productivity/biodiversity relationship. These results indicate that soil fungi significantly influence the positive, asymptotic relationship between biodiversity and productivity in grassland ecosystems, which has been traditionally explained by niche complementarity and plant competitive interactions between plant taxa.

While patterns between biodiversity and productivity can be linked to competitive interactions between plant species and niche complementarity in communities, there is strong evidence to support the importance of soil microbial communities, particularly AMF communities, in establishing plant community structure, biodiversity, and productivity relationships in plant communities.

1.4 Determinants of AMF Community Structure and Host Specificity

Although both field and laboratory experiments have focused on how soil microbial communities can drive diversity and productivity above ground, little is known about how plant communities affect AMF community structure, and if there is some form of specificity in this relationship. Determinants of AM fungal community structure are not easy to study, as it is difficult to control for environmental variation in the field and difficult to recreate AMF communities in the lab, as some species of fungi are not culturable (Smith and Smith 2012). Furthermore, understanding how plant communities affect AMF community composition is limited by ambiguity surrounding specificity in the AM symbiosis. AMF are widely considered
to be generalist in nature, as they are globally distributed and found in diverse types of ecosystems (Brundrett 1991; Read 1991; Smith and Smith 2012). Despite this pattern, there are bodies of research that suggest particular species of AMF associate more often with certain plant species (Giovanetti et al., 1994; Kiers et al., 2000; Haussmann and Hawkes 2009; Pizano et al., 2011). The debate surrounding AMF host specificity is important to better understand how plant communities can affect composition of AM fungal communities. For example, if AMF are mostly generalist symbionts, then one may expect that changes in plant communities would have little effect on the composition of AMF communities. However, if there is high specificity between certain AMF and plant species, then one may expect to find a different assembly of AMF species in diverse plant communities. Therefore, understanding the role that plant communities have on the structure of AMF communities can help to elucidate patterns host specificity in the AM symbiosis.

1.4.1 Understanding AMF Host Specificity

In order to clearly understand host specificity in the AM symbiosis, it must first be defined. Host specificity can be thought of in many different ways, whether it is structural, functional, or genetic at the population, community or ecosystem level. If a particular AMF species can form arbuscules in the roots of a particular plant species, then there must be some degree of structural and genetic specificity between the plant and the fungal isolate. However, this structural specificity may be affected by other factors including plant physiology (Ali 1969; Smith and Read 2008), presence of other plants in the community (Alguacil et al., 2011), or environmental conditions of the soil (Grime et al., 1987). Assessing how AMF or plants establish an affinity towards one another is difficult to determine. When sampling in the field, variation in AMF community assemblages may result from a variety of factors, including differences in plant community composition, or edaphic factors in the soil. On the other hand,
differences in plant community composition may also be due to different AMF community assemblages. In order to understand how plant diversity and host changes affect variation in AMF communities, factors such as environmental heterogeneity must be controlled for experimentally, and ideally performed in a natural setting.

1.4.2 Laboratory Studies of AMF Host Specificity

The question of host specificity in the AM symbiosis has been researched thoroughly in both the field and the lab. Some studies have found that AMF can form associations with most plant hosts (Klironomos 2003; Aldriche-Wolfe 2007; Öpik et al., 2010); however, others have found that certain AMF will associate most often with particular plant species or plant groups (Dhillion 1992; Kiers et al., 2000; Mangan et al., 2010; Ying-Su et al., 2011), adding to the debate of whether AMF taxa, along with their plant hosts exhibit specificity. This debate has been approached from various perspectives, with some research focusing on creating representative AM fungal communities with a mix of cultured AMF isolates and inoculating a variety plant hosts with these communities in greenhouse experiments. Changes from the original AMF community composition are then documented under each host plant treatment.

This approach has many benefits, namely the high level of control and targeted manipulation of variables. Studying AMF host specificity in the greenhouse and laboratory allows for tight control of environmental conditions, eliminating problematic environmental variation. Notable studies that have taken this approach include Bever’s (2002) study, which aimed to measure AMF isolate fitness when grown with particular host species. In this study, 8 isolates of AMF were selected from the INVAM (International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi) collection, grown in culture from individual spores to ensure they were single isolates, and then mixed together to form the representative community. This community was then grown and trained with 4 different plant hosts (2 herbs and 2 grasses) for one growing
season. During this time the isolates colonized, proliferated, and sporulated. The spores from this first generation were grown again in the same laboratory microcosm in order to verify the differentiation observed from the first growing season. These results indicate that distinct assemblages of AMF spores were produced with specific genotypes of plants, suggesting that the host plant could influence changes in AMF communities. Eom et al. (2000) performed a similar experiment that explored the effect of grassland plant communities on sporulation and development of AMF communities. In one aspect of the study, five different types of grassland plants were grown with soil samples from the same area. AMF community composition was then determined for each different host plant. The results showed that AMF in the soils did display some degree of host specificity, as there were significant differences between the AMF communities under each host plant species. Other laboratory experiments have found similar results. When preselected AMF communities were established on different hosts, some species of AMF seemed to outperform other species, sporulate more readily, and were found in higher abundance depending on the host plant species (Klironomos 2002; Helgason et al., 2002).

Similarly, Hart and Reader (2002) also examined differential colonization in the AM symbiosis, illustrating fungal family differences in AMF colonization that may contribute to specificity with particular hosts and variation in the AMF community. The authors tested the colonization strategies of three families of AMF. Twenty-one different isolates were used and grown with four different host plants from two different functional groups: grasses and non-legume forbs. Over a 12-week growing period, the amount of colonization and colonization rate, along with fungal hyphal length and soil fungal biomass were measured. The results of this study indicate that isolates from the Glomaceae colonized most quickly and the most extensively. Isolates from Gigasporaceae and Acaulosporaceae tended to colonize more slowly, although Gigasporaceae did have significantly higher soil fungal biomass compared to the other two families. These results illustrate that different taxonomic groups of AMF have different
colonization strategies and that these colonization and life history strategies may reflect differences that lead to host specificity.

Finally, laboratory studies have also investigated host specificity at the individual level by exploring the effect of intraspecific genetic variability on plant response. Differential plant responses, even at the isolate level, could indicate that particular AMF individuals colonize plants differently, and suggests a mechanism for host specificity. In a study by Munkvold et al., (2004), the authors investigated the effect of 24 different *Glomus* isolates on plant host growth and phosphorus uptake, focusing on intraspecific genetic variability as a driver of host-fungal community interactions. The authors found that high genetic variability at the individual level of AMF taxa can cause significant differences in host plant response. Even with low fungal diversity, soil communities that contained AMF species with a high degree of genetic variability provided a wide range of functionally diverse effects on the plant community above ground. Similarly, Koch et al. (2006) found that genetically variable AMF isolates, in this case, *Glomus intraradices*, can cause differential plant growth of host plants, suggesting that it may not only be AMF communities that drives plant community structure and diversity above ground, but genetic variability within AMF taxa, as well. In this experiment, effects of genetically variant *G. intraradices* on host plant growth and performance were measured. Here, the authors measured the effects of different isolates on host root growth under normal and nutrient-deprived conditions, on host tissue weight, and the effect on plant growth under two watering treatments. The authors found that genetically variant isolates had different functional effects on each plant host. This illustrates that genetic variability within populations can influence host plant fitness and thus help drive plant community assembly and diversity, providing stepping stones to understanding differential responses of AMF isolates to different plant hosts. This study indicates that specificity and subsequent effects of host-AMF dynamics can be traced to the level
of the fungal individual, indicating that intraspecific genetic variability may drive plant community assembly.

Though many of these studies indicate that plant hosts may be able to select for particular AMF communities in the soil, and that there are distinct differences between AMF taxa that can lead to differential colonization, some aspects of studies like those described above do not directly answer the ambiguity surrounding AMF host specificity. Laboratory studies like the ones mentioned above address the problem of host specificity by inoculating a select group of host plants with either pre-mixed AMF species to simulate a natural AMF community or by taking field soil and training it with particular plant hosts. However, these manipulations only provide a snapshot of real community dynamics in the field, especially since AMF communities used are mostly culturable taxa, and plant hosts are chosen based on the ease and feasibility of growth in a laboratory over a short period of time. This makes it difficult to tease apart whether these mechanisms are unique to the lab setting, or if these are observable ecological drivers selecting for particular AMF assemblages at the community level. By testing AMF host specificity in laboratory experiments, community level inferences are difficult to make, as these experiments force particular AMF and plant host associations, and exclude potential AMF-host interactions that could be found in the field. Laboratory studies are also performed under ideal conditions, completely eliminating environmental changes and abiotic factors as potential factors in the assemblage of AMF communities, when abiotic factors could be a more influential driver of differences in AMF communities.

Overall, laboratory studies are useful in informing differential interactions between particular AMF taxa and corresponding plant hosts. As studies have illustrated, there is evidence for some level of specificity in the AM symbiosis, with significant variation in plant response to different assemblages of AMF taxa, and different responses of AMF to varying plant hosts.
However, drawbacks of the laboratory studies include artificial conditions, the use of mainly culturable fungi, and the issues of “forcing” AMF and plant associations.

### 1.4.3 Field Studies of AMF Host Specificity

In order to circumvent the drawbacks of laboratory studies, other studies have aimed to investigate host specificity in AMF communities using a field-based approach. This allows for extrinsic factors, such as environmental variation to be taken into account, while also sampling from natural communities. Some prominent studies that have taken this approach are described as follows. In the second part of the study by Eom *et al.* (2000), field-testing was done to determine differences in AMF assemblages under different species of grassland plants. Here, spores and species composition data were collected from patches of grassland that were dominated by one of five different plant hosts. With this approach, the authors were able to illustrate that under some plant hosts, AMF spore abundance and diversity was greater compared to other plant hosts. Furthermore, AMF species under different plant hosts showed significantly different sporulation patterns, suggesting that AMF assemblages are influenced by dominant plant hosts and can contribute to feedback mechanisms that regulate both soil and plant communities. In a similar study, Öpik *et al.* (2009) sequenced AMF communities to assess community diversity under different types of plants in a northern forest ecosystem. The authors were able to sequence and identify 47 AMF taxa in 10 different plant species. The plant species were split into two groups: forest specialist species, which were comprised of plants that only grew in forest habitat, and habitat generalist species, which consisted of plants that had a wide habitat range including both grassland and forested areas. When AMF communities were compared between the two groups of plants, there was a significant difference between AMF community composition associated with forest specialist species and AMF communities associated with habitat generalist species. In the forest specialist group, 22 AMF taxa were
consistently detected, while all AMF taxa were found in the habitat generalist group. These results indicate that AMF host specificity may be more prominent at the plant community level, as opposed to the host species level, but does illustrate evidence for host specificity nonetheless.

Aldriche-Wolfe (2007) also used a field-based approach to test AMF host specificity in tropical plant communities, however the author found little evidence for specificity in tropical soils. In this study, AMF communities in tropical tree species, pasture grass species, and tropical tree seedlings were compared. *Terminalia amazonia* (tropical canopy tree) seedlings were planted in three pasture sites adjacent to the rainforest and after two years, AMF communities under the seedlings were compared to *T. amazonia* parent plants in the rainforest and to two dominant pasture grass species. Aldriche-Wolfe predicted that if some type of host specificity in the AM symbiosis exists, then *T. amazonia* seedlings should have a more similar AMF community to their parent plant. If AMF host specificity was not present in *T. amazonia*, then the author predicted that the AMF community under the seedlings would be more similar to those of the pasture grass species. Interestingly, the author found a unique AMF community under the *T. amazonia* seedlings, significantly different from both the pasture grass and parent plant communities, suggesting that genetic specificity may not be a major mechanism that determines AMF communities in this system, and that some other mechanism (possibly competitive interaction or plant life stage), drives AMF community assemblage in tropical systems.

Field-based studies investigating AMF host specificity are beneficial because they address issues that cannot be addressed in laboratory studies, such as environmental variation, soil heterogeneity, and plant community composition (Grime *et al.*, 1987). As well, field studies also encompass more natural plant and AMF communities, allowing for more natural interactions between plants and AMF to be studied and compared. While field-based approaches are beneficial in order to more accurately represent what is happening in natural plant and soil
communities, one drawback is the number of confounding variables that could explain observable patterns. Typically, field experiments cannot control for soil heterogeneity, particularly in chemical and physical components. Factors such as pH and soil aggregate size have the potential to influence AMF communities and could contribute to observable differences in field studies and can influence AMF community assemblage (Grime et al., 1987; Johnson 2009). Although this may not be the sole driver for variation in AMF communities, these mechanisms cannot be ruled out as potential factors that could influence AMF community assembly, inhibiting some field studies from comprehensively answering specificity questions.

1.4.4 Other Types of Studies on AMF Host Specificity

Laboratory studies and field-based approaches have provided important insight into AMF host specificity, namely by illustrating AMF community patterns under different types of host plants/plant communities, while also showing differential plant response and AMF response with different plant hosts. However, research on AMF host specificity has also used other techniques in order to describe AMF host specificity. Ecological network analysis has proven useful in showing trends in host specificity within the AM symbiosis, identifying particular specialists and generalist species and showing their interaction with plant communities. In one important study, Chagnon et al. (2012) characterized the interactions between varying AMF species, identified from a pyrosequencing study (Öpik 2009), and differing plant communities. This study provided evidence for host specificity; AMF species ranged from generalists to specialists when interacting with certain plant species, suggesting that AMF taxa have the potential to form a higher degree of specificity with some plant hosts than others.

In Maherali and Klironomos (2007), phylogenetic patterns were tested to determine whether particular groupings of AMF families influenced AMF community diversity in the soil. In this experiment, three phylogenetically distinct families of AMF were inoculated into a
microcosm, using the host plant, Plantago lanceolata. In each treatment, the relatedness of fungal communities was manipulated and it was predicted that the fungal communities with more related species would display lower coexistence due to similar niche requirements, while those communities that were comprised of more divergent species would display higher coexistence. Here, the authors found that treatments that started with more divergent AMF species had greater realized species richness and stimulated higher plant productivity after one year than those treatments that started with more related AMF species. In this case, if host specificity was a major driver of AMF community assembly, then it is expected that despite the starting AMF communities, AMF communities should be more similar over time as each AMF community treatment was grown with the same plant host. However, the results of this experiment show that phylogenetic diversity of AMF community assemblages may also be an important factor in the assemblage of AMF communities in the soil.

Approaches like network analysis and analysis of phylogenetic patterns allow AMF interactions with their hosts to be categorized based on a variety of ecological functions along a continuum of generalist to specialist, and illustrate which AMF taxa form a more specialist relationship with plant hosts, providing evidence for specificity in some AMF taxa within this symbiosis.

1.5 Relationships Between Plant and AMF Community Diversity

1.5.1 Effects of Plant Diversity on Soil Microbial Communities

While the role of AMF and soil microbial communities in productivity/biodiversity relationships has been extensively researched, there are few studies that investigate the role of plant community diversity and composition on AMF communities in the soil. This is important to elucidate because if plant diversity has a significant impact on the diversity of AMF
communities, then mechanisms, like host specificity or competitive advantage, may describe patterns in the relationship between plant community and AMF community diversity.

*Zak et al.* (2003) investigated the effect of plant diversity on a diverse range of soil microbial communities, using phospholipid fatty acid analysis. The results indicated that microbial biomass, respiration, and fungal abundance significantly increased with increasing biodiversity. Furthermore, nitrogen mineralization rates also increased with increasing biodiversity. However, these effects were attributed to higher productivity at high plant diversity levels, not just biodiversity alone. In another study by Hausmann and Hawkes (2009), the role of plant community identity on AMF community composition was investigated. In this study, three species of grasses were grown in either monocultures or species mixtures. The results illustrate that both host plant species and community structure affected AMF community composition. In the monoculture experiments, unique AMF communities were observed between plant hosts. However, in the mixture soils, the different plant species influenced AMF communities through different mechanisms. This study suggests that plant community diversity and composition has effect on soil microbial communities, increasing microbial biomass, abundance, and nitrogen mineralization rates.

*Burrows and Pleger* (2002b) also performed a key study that aimed at better understanding the relationship between AMF communities and plant community diversity using a long-term common garden study. In this study, AMF sporulation was recorded in experimental plots with varying plant diversity (0, 1, 2, 4, 8 and 16 plant species), along with percent plant cover and soil NO$_3$ concentration. It was found that in plots with 16 plant species, AMF communities produced 30 to 150% more spores and 40 to 70% increased spore volume compared to those plots only planted with one species. Also, AMF species richness was positively correlated with increasing plant diversity. As well, plant diversity influenced AMF community composition, with the presence particular species, such as *Gigaspora* sp. and *Scutellospora* sp. (large-spored)
increasing with increasing plant diversity, though species with smaller spores varied in their response to plant diversity. This study illustrates that AMF communities respond to changes in plant diversity, and particularly highlights the benefits of increasing plant diversity in driving a more diverse and productive AMF community in the soil. Overall, while few studies have looked at the effect of plant diversity on AMF diversity, there has been evidence to show that plant diversity affects the diversity of AMF communities, which result from host specificity mechanisms.

1.6 Research Questions

Many studies have focused on how plant diversity affects above ground productivity or how soil microbes influence plant productivity relationships with biodiversity, as well as the potential for host specificity in the AM symbiosis. However, few studies have looked at how fungal communities are affected by plant diversity and if changes in AMF communities due to plant diversity can be described by a host specificity mechanism. Approaches to answering these questions in laboratory and field experiments have drawbacks that come at the cost of accurately answering questions about AMF host specificity and its relevance in plant community dynamics. While laboratory experiments cannot simulate all the extrinsic factors present in the field, field-based methods have drawbacks in that particular factors cannot be controlled for and therefore could also potentially explain changes in AMF communities. In order to better test the presence of host specificity in AMF communities, environmental variation, abiotic factors, and plant communities must be controlled for. These issues could be addressed through randomized, common garden experiments, where environmental heterogeneity can be better accounted for and eliminated as a confounding variable. The use of soil from Cedar Creek Biodiversity Experiment 120, a long-term common garden experiment at the University of Minnesota’s Cedar Creek Ecosystem Science Reserve provides a unique and powerful opportunity to address
questions of specificity in the AM symbiosis and the effect of plant diversity on AMF diversity. This common garden experiment is ideal for testing these questions due to the longevity of the experiment (20 years) and its highly controlled and randomized design of plant diversity. Using these soils to study AMF communities in grassland soils, the overall objective of my research was to investigate the potential for host specificity in the AM symbiosis, while also exploring whether plant diversity affects AMF community richness and diversity. Specifically, I asked the following questions:

1.6.1 Research Question 1: In the AM symbiosis, does plant species identity affect AMF species richness and AMF community composition in the soil? The specific hypotheses for the above research question are as follows:

- If AMF communities are influenced by plant species identity, then I expect to observe differing species richness and community composition depending on the corresponding host plant species present in the sampled plots (Figure 1.1). This pattern may be observed due to some degree of host specificity and specialist tendencies in the AM symbiosis, in that, particular plant hosts may acquire different AMF symbionts based on some sort of characteristic, such as species.

![Figure 1.1](image)

**Figure 1.1.** Hypothetical pattern showing the similarity between the AMF communities of two different plant species monocultures. Overlap between the circles represents taxa shared between AMF communities – in this case there is a high degree of difference between the AMF communities associated with each plant species, due to the minimal overlap.
• However, if AMF communities are not influenced by host plant species identity, I would then expect to see little difference in AMF species richness or community composition under different host plants. This pattern would be observed if AMF are more generalist organisms in nature, suggesting that other factors besides plant identity affect AMF community composition (Figure 1.2).

![Figure 1.2. Hypothetical pattern showing the similarity between the AMF communities of two different plant monocultures. In this case, there is a high degree of similarity in the AMF communities between two plant species, depicted by the large amount of circle overlap.](image)

1.6.2 Research Question 2: *In the AM symbiosis, does plant functional group affect AMF species richness and AMF community composition in the soil?* The specific hypotheses for the above research question are as follows:

• If AMF communities are influenced by plant functional group, then I expect to observe differing species richness and community composition depending on the corresponding plant functional group present in the sampled plots (Figure 1.3). This pattern may be observed due to some degree of host specificity and specialist tendencies in the AM symbiosis, in that, particular plant hosts may acquire different AMF symbionts based on some sort of characteristic, such as ecological functionality.
However, if AMF communities are not influenced by host plant functionality, I would then expect to see little difference in AMF species richness or community composition under different host plants. This pattern would be observed if AMF are more generalist organisms in nature, suggesting that other factors besides functional group affect AMF community composition (Figure 1.4).
1.6.3 Research Question 3: *Do plant species richness gradients affect AMF species richness and community composition in the soil?* My specific hypotheses for the above research question are as follows:

- If plant diversity affects the species richness of AMF in the soil, I expect to observe increasing AMF diversity with increasing plant species richness. This could be explained through a host specificity mechanism, as more plant species would provide a wider range of suitable hosts for a larger number of AMF species (Figure 1.5).

![Figure 1.5](Image)

**Figure 1.5.** Positive, asymptotic relationship between increasing plant species richness and AMF species richness, provided AMF show host specificity with different plant species.

- Alternatively, plant diversity could affect soil AMF communities by decreasing AMF species richness as plant diversity increases. This would suggest that increasing plant diversity promotes the proliferation of highly competitive species AMF species or species that are more generalist in nature and can colonize a wide variety hosts more rapidly than other species (Figure 1.6).
Figure 1.6. Negative, asymptotic relationship between increasing plant species richness and AMF species richness, provided increasing plant diversity fosters highly competitive or generalist AMF species.

- However, if plant diversity does not affect soil AMF communities, then I expect to observe little to no significant change in the composition of the AMF communities across plant diversity treatments. This would suggest that AMF species are more generalist in nature and are not significantly affected by changes in host plant species richness (Figure 1.7).

Figure 1.7. Relationship between increasing plant species richness and AMF species richness. This particular pattern could be described by an absence of strong host specificity, rendering AMF more generalist in nature.
CHAPTER 2: Methods

2.1 Field Site Description

Soil samples were collected from Biodiversity Experiment 120 (P.I. David Tilman) at the Cedar Creek Long Term Ecological Research site in East Bethel, MN, USA (45.3°N, 93.2°W). The Cedar Creek Long Term Ecological Research site is located on a sandy-outwash plain and can be described as a grassland, consisting mostly of C3 and C4 grasses and non-legume forbs, with some woody assemblages. This area is affected by a continental climate, consisting of cold, long winters with snowfall; short, warm summers with relatively high humidity and cool, wet springs and falls (Tilman 1997).

The Biodiversity II (Experiment 120) was established within the Cedar Creek Long Term Ecological Research site, (http://www.lter.umn.edu/research/exper/e120/e120.html). This 10-hectare experimental site was created in 1994 and was designed to test the effects of plant diversity on a variety of variables including productivity, soil fertility, and trophic effects in grasslands at the population, community, and ecosystem levels. To do this, the area was first sprayed with herbicide and subsequently burned in 1993 to clear all vegetation. Following this, the topsoil was removed (first 6-8cm) in order to reduce contamination from the seed bank. These plots were then harrowed and plowed. In 1994, it was then planted with a variety of native grassland plants, chosen randomly from a bank of 18 natively occurring grassland plant species. This plant bank included plants from 5 functional groups: 4 legume species, 4 non-legume forbs, 4 cool climate C3 grasses, 4 warm climate C4 grasses, and 2 woody species, as shown in Table A.1. The 10-hectare site was broken up into 150 9 x 9 m plots. Each plot was planted with a different plant diversity level. The diversity levels are as follows: barren plots (planted with 0 plant species), 1 plant species (monocultures), 2, 4, 8, and 16 plant species. Each plot was...
replicated approximately 30 times. Currently, these plots are actively maintained at the starting density by active weeding.

2.2 Soil Sampling

Soil samples were collected from all diversity levels from August 23 to August 27, 2010. Ten replicates from diversity levels 2 through 16, and 4 plots for diversity level 0 (barren plots), were sampled, and approximately 3 replicates from each type of diversity level 1 (monoculture) plot were also sampled. For the purposes of my project, only the soil samples collected from diversity levels 0 (n=4), 1 (n=30), 4 (n=10), and 16 (n=10) were used to establish a diversity gradient ranging from low, medium, and high, rendering a total of 54 samples. A 10 cm soil corer was used to sample soil from 6 randomly chosen spots from each 9 m x 9 m plot. After each soil core was taken, the soil core placed into a sampling bag and the soil corer was washed with an ethanol rinse. These soil cores were then pooled into a common container and homogenized. From the containers, the homogenized soil was transferred to 100mL falcon tubes and stored at -20°C.

2.3 DNA Extraction

A subsample of 100g was taken from each plot and was subjected to three DNA extractions using PowerSoil DNA Isolation Kits® according to the manufacturer’s instructions (MoBio Laboratories, Carlsbad, CA) and stored at -20°C. DNA from each extraction was pooled for use in further processing via PCR.

2.4 PCR Protocol and Pyrosequencing

DNA products were amplified according to the PCR procedure developed by Helgason et al. (1998) using the Glomeromycotan specific primers NS31
(5’ TTGGAGGGCAAGTCTGGTGCC ’3) (Simon et al., 1992) and AM1 (5’GTTTCCCGTAAGGCAGCGA’3) (Helgason et al., 1998), which target the small subunit (SSU) region of ribosomal RNA genes. This region has been shown to effectively represent the 4 major lineages of Glomeromycota, including Glomus group A, Glomus group B, Acaulosporaceae and Gigasporaceae (Simon 1993). 25μL reactions were used, containing 14.25μL of sterile water, 5μL of GoTaq Buffer (Promega), 2μL MgCl₂ (25mM) (Biolabs), 1μL (10mg/mL) BSA (Biolabs), 0.50μL (10mM) dNTPs (Amresco), 0.50μL (10μM) each primer (MID tagged NS31-F and AML1-R) and 0.25μL (5U/μL) GoTaq polymerase (Promega). An aliquot (1μL) of extracted soil DNA was added to the 19μL PCR mix and subjected to the following cycling conditions: 95°C for 3 minutes, 95°C for 30 seconds, 54°C for 30 seconds, and 72°C for 1 minute (35 cycles), followed by 10 min at 72°C and 4°C infinitely.

With the amount of microbial diversity in the soil, molecular methodology is often used to detect groups of organisms that cannot be found easily through more macroscopic techniques. Specifically, pyrosequencing is now more commonly used for phylogenetic community analysis of soil microorganisms. Currently, pyrosequencing is a better alternative to Sanger sequencing in that it provides similar accuracy (Huse et al., 2007), yet is processed more quickly, and is more cost effective per sequenced nucleotide (Kakirde et al., 2010). In order to process my samples using pyrosequencing technology, my forward primers (NS31) were tagged with a unique multiplex identification (MID) region: MID 1-29, excluding MID 9 and 12 (Roche Technical Bulletin 2009), along with Roche ligating adaptors, in order for samples to be resolved after pyrosequencing. DNA concentrations for each of the samples were quantified via Nanodrop (Nanovue Plus, GE Healthcare and Life Sciences) and replicate samples were pooled together. Samples were then standardized at 1.25ng/μL and purified for 454 sequencing using the SequaPrep® purification kit according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA). Samples were sent to the UBC Prostate Center (Vancouver, BC) for 454 pyrosequencing
using GS FLX+ Titanium chemistry in order to obtain AMF community data, using one-eighth of a standard PicoTiterPlate.

2.5 Bioinformatical Analyses

Quantitative Insights Into Microbial Ecology (QIIME) (Caporaso et al., 2010) was used to analyze sequences. The subsequent analyses on sequencing data were performed on reads that adhered to the following criteria: the sequences carried the proper MID barcode and the sequences had both Primer A and the proper forward primer NS31 with no allowable mismatches. All reads were trimmed to a minimum length of 400 base pairs and a maximum length of 700 base pairs, to capture only those reads that fell into the range of the forward primer length. A total of 295302 raw sequences were returned from the 454 sequencing process and after employing the above criteria, a total of 71662 sequences were removed from the data set. The remaining sequences were then analyzed using QIIME 1.3.0 software in order to generate operational taxonomic units (OTUs) for each treatment. In the pipeline, sequences were removed if they had a quality score lower than 25 (Huse et al., 2007) or contained over 6 homopolymers. Denoising and chimera checking were excluded from the analysis due to the stringent filtering settings. Rare OTUs resulting from errors are most common in long homopolymer runs, and since homopolymer length was capped at 6, and no primer mismatches were allowed, the instance of artifacts is expected to be insignificant (Caporaso et al., 2010). As well, OTUs were picked based on a 97% similarity using the MUSCLE algorithm (Edgar 2004), which generated 72 OTUs, representative of the known AMF taxonomy at this time. Due to the low number of sequences returned, samples were rarefied at 100 sequences per sample; samples with less than 100 sequences were excluded from the analysis, resulting in the exclusion of 14 of 54 samples. The resulting distribution of samples per treatment are shown in Table 2.1. OTUs were also filtered to include only those OTUs that had 5 or more sequences to ensure that identified OTUs
were not due to sequencing error. While this approach may bias against rare taxa, a more conservative approach was taken in order to ensure that the OTUs included in downstream analyses excluded as many PCR and sequencing artifacts as possible. In order to assign taxonomic names to the generated OTUs, the MaarjAM database was used (Öpik et al., 2010). This database contains representative sequences from the 18S region of AMF genomic DNA, and contains sequences that are complementary to regions amplified by NS31/AM1 primers. Sequences returned from 454 sequencing were compared against the MaarjAM database using the BLAST classifier in the QIIME pipeline and taxonomic identity and virtual taxon number (VT) were assigned to each OTU. After filtering and taxon assignment, 72 OTUs were identified. Multiple OTUs matched to the same AMF taxon in the MaarjAM database, resulting in 36 AMF taxa, all of which belonged to the phylum Glomeromycota. All 72 OTUs matched to a virtual taxon in the MaarjAM database and as such, no OTUs were removed from further analyses.

Table 2.1. Number of samples corresponding to plant functional group and diversity treatments after QIIME analysis.

<table>
<thead>
<tr>
<th>Functional Group Treatment</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grasses</td>
<td>7</td>
</tr>
<tr>
<td>Legumes</td>
<td>6</td>
</tr>
<tr>
<td>Forbs</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plant Diversity Treatment</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero (Bare Ground)</td>
<td>4</td>
</tr>
<tr>
<td>One (Monoculture)</td>
<td>21</td>
</tr>
<tr>
<td>Four</td>
<td>6</td>
</tr>
<tr>
<td>Sixteen</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
</tr>
</tbody>
</table>
2.6 Statistical Analyses

For the statistical analyses, virtual taxon (VT) identification was considered equivalent to an AMF species. In order to analyze AMF species richness in both functional group and plant diversity treatments, observed AMF species in each treatment were compared using one-way analysis of variance (ANOVA) tests (for plant functional group treatments) and linear regression (for plant diversity treatments) in SPSS statistical software (IBM SPSS Statistics for Windows Version 21.0). These statistical analyses were also repeated for different measures of alpha diversity including Shannon-Wiener Index and the PD whole tree statistic of assessing phylogenetic diversity (Chao et al., 2010) in both plant functional group and diversity treatments. The Shannon-Wiener Index (H’) measures both species richness and evenness in each treatment, though it is less sensitive to rarer species than other indices (Whittaker 1972). The PD whole tree statistic is sensitive to both species abundance and traditional phylogenetic branch length (Chao et al., 2010).

Community composition, relative abundance and turnover of AMF communities under different plant hosts and plant diversity treatments were analyzed using Type III PERMANOVA and PERMDISP statistical approaches in Primer E software (Clarke and Gorley 2006). The PERMANOVA test was used to test for differences in composition and relative abundance of taxa in AMF communities, while PERMDISP tests for differences in the dispersion of samples from group medians (Clarke and Gorley 2006; Anderson et al., 2008). The magnitude of this dispersion is related to changes in aspects of beta-diversity, where the greater the spread, the more turnover between samples. For the PERMDISP test, a significant value indicates mean turnover within groups, was different from other treatment groups. This was done using Bray-Curtis Index matrices, taking into account both presence/absence and relative abundances in AMF communities. The Bray-Curtis similarity index measures the relative distance between two samples, resulting in a metric of similarity, either 0 (no differences among samples) or 100
(maximum differences among samples) (Clarke et al., 2006), showing which communities are more similar to each other. For both PERMDISP and the PERMANOVA tests, 9999 permutations were used. In the PERMANOVA tests, due the asymmetric nature of the design, unrestricted permutation of residuals under a reduced model option and fixed effects sum to zero options were selected based on recommendations based from Primer-6 and PERMANOVA tutorial package. In PERMDISP tests, the deviations from the centroid option was retained.

Phylogenetic relatedness of AMF communities under different plant hosts and plant diversity treatments was also analyzed using PERMANOVA and PERMDISP, using unweighted and weighted UniFrac distances. Unweighted UniFrac is a measure of phylogenetic overlap of taxa in two samples, accounting for presence/absence of taxa, while weighted UniFrac measures community similarity based on the amount of phylogenetic overlap present between the taxa present in two samples, accounting for relative abundance (Lozupone and Knight, 2005).
CHAPTER 3: Results

3.1 AMF Host Specificity at the Plant Species Level

The first research question I set out to investigate was as follows: *In the AM symbiosis, does plant species identity affect AMF species richness and AMF community composition in the soil?* This question could not be completely answered, as many of the replicates for the monoculture plots were excluded from downstream bioinformatic and statistical analyses, due to the parameters that were set in the QIIME pipeline, particularly the way sequences were trimmed and rarefied. In this case, only three soil samples per plant species were collected. After sequencing and QIIME analysis, many of the samples contained fewer than 100 sequences, and as such were excluded from the analysis at the rarefaction step. This resulted in many of the monoculture plots containing less than three replicates per plant species, and as such, not enough power was present to perform meaningful statistical analysis. However, in order to observe any preliminary trends, the percentage of sequences belonging to the 10 most dominant AMF taxon were categorized with each plant species. Table 3.1 illustrates which plant species the 10 most dominant AMF taxa (accounting for approximately 86.6% of total sequences) occurred with. This matrix was created in order to elucidate preliminary patterns in AMF community assembly under particular plant host species and potentially describe basic patterns of host specificity in this system. Table 3.1 illustrates that AMF species, *Gigaspora margarita* (VTX00039), may be the most abundant AMF taxon, accounting for 19.7% of all sequences. This taxon occurred in all plant species and was also highly abundant. A *Glomus* species, deemed taxon *Glomus* Glo30 (VTX000126) was the third most abundant, accounting of 14.33% of all sequences and was found in most plant species. However, it was completely absent from the C3 grass, *Koeleria cristata* and the non-legume forb, *Asclepias tuberosa*. Interestingly, *Rhizophagus irregularis* (VTX00113), was found in very low abundance in most plant species (even absent in some), but
was found in high abundance in the grass species, *Koeleria cristata*. A similar pattern was observed for an *Acaulospora* sp. (VTX00044), which was absent from all grass species, and only found in forb and legume species at lower abundance. These preliminary patterns in AMF abundance under different plant species, this may indicate a signal for some level of host specificity of AMF taxa in North American grassland systems, though more exhaustive sampling efforts is required to statistically capture these patterns.
Table 3.1. A heat map depicting the ten most dominant AMF taxa that co-occur with a variety of native grassland species, organized by plant species. Values are given as the percentage of sequences belonging to a certain taxa out of the total sequences representing the AMF community for each plant species when grown in monoculture. Red squares indicate high percentage of sequences of particular AMF taxa, while green squares indicate low percentages of AMF taxa sequences. Letters in parentheses indicate associated functional group: (G): grass, (F): forb, and (L): legume.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Adropogon gerardi (G)</th>
<th>Koeleria cristata (G)</th>
<th>Schizachyrium scoparium (G)</th>
<th>Sorghastrum nutans (G)</th>
<th>Achillea millefolium (F)</th>
<th>Aesclepias tuberosa (F)</th>
<th>Liatris aspera (F)</th>
<th>Monarda fistulosa (F)</th>
<th>Lespedeza capitata (L)</th>
<th>Lupinus perennis (L)</th>
<th>Petalostemum purpureum (L)</th>
<th>Average Percent of Sequences</th>
</tr>
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<tr>
<td>Gigaspora margarita</td>
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</tr>
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<td>0.5</td>
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</tbody>
</table>
3.2 AMF Host Specificity and Community Diversity at the Plant Functional Group Level

The second research component aimed at expanding the focus of host specificity to include plant functional groups, as a different way to group plant hosts. When species richness (VT richness) was calculated, it was found that there was no significant difference in the observed number of AMF species associated with each plant functional group (p=0.25), as shown in Figure 3.1. A similar trend was observed when alpha diversity was calculated across plant functional groups using the Shannon-Wiener diversity index \( (H') \) (p=0.41) (Figure 3.2) and for phylogenetic diversity (PD whole tree metric), with no significant difference detected between plant functional groups (p=0.47) (Figure 3.3).

![Figure 3.1. Observed AMF species richness in three different plant functional groups: legumes, grasses, and non-legume forbs grown in monoculture plots. There was no significant difference between legumes, grasses, or non-legume forbs (p=0.25), n=21.](image-url)
Figure 3.2. Shannon-Wiener diversity index (H') in three different plant functional groups: legumes, non-legume forbs, and grasses grown in monoculture plots. There was no significant difference between legumes, grasses or non-legume forbs (p=0.41), n=21.

Figure 3.3. PD Whole Tree statistic measuring phylogenetic diversity in different plant functional groups: legumes, non-legume forbs, and grasses grown in monoculture plots. There was no significant difference between legumes, grasses, and non-legume forbs (p=0.47), n=21.
However, presence/absence of AMF taxa differed among the plant functional groups when measured using Bray-Curtis similarity index, in PERMANOVA. As shown in Figure 3.4, community composition of AMF taxa were different in AMF communities associated with grasses, clustering separately from both legumes (p=0.0008) and non-­legume forbs (p=0.002). AMF communities associated with legumes and non-­legume forbs did not significantly differ from each other (p=0.51). Also, PERMDISP tests showed that AMF community turnover was significantly different under grass hosts, turnover averaging 18.99, compared to turnover in AMF communities associated with legumes, averaging 24.6 (p=0.053) and non-­legume, averaging 25.03 (p=0.063), while AMF communities associated with legume and non-­legume forbs were not significantly different from each other (p=0.94).

![Figure 3.4](image.png)

Figure 3.4. AMF community composition associated with three different plant functional groups ordinated with presence/absence Bray-Curtis similarity index using PERMANOVA analysis detected a difference between plant functional groups (p=0.0008). Grasses clustered distinctly from both legumes (p=0.0008) and non-­legume forbs (p=0.002), however there was no difference between AMF community composition in non-­legume forbs and legumes (p=0.51), n=21. Using PERMDISP, it was found that turnover in AMF communities associated with grasses were significantly different from both legumes (p=0.053) and non-­legume forbs (p=0.064), but no significant difference was observed between legumes and non-­legume forbs (p=0.94).
Similarly, phylogenetic diversity (unweighted UniFrac distance) was found to be different in AMF communities associated with grasses, compared to AMF communities associated with both legumes (p=0.002) and non-legume forbs (p=0.006). However, AMF communities associated with legumes and non-legume forbs did not differ from each other (p=0.45) (Figure 3.5).

![Figure 3.5](image-url)

**Figure 3.5.** Phylogenetic distance of presence/absence in AMF communities associated with three different plant functional groups ordinated with unweighted UniFrac distance, using PERMANOVA analysis detected a difference between plant functional groups (p=0.0008). Grasses clustered distinctly from both legumes (p=0.002) and non-legume forbs (p=0.006), however there was no difference between AMF community composition in non-legume forbs and legumes (p=0.45), n=21. Using PERMDISP, it was found that turnover of AMF communities associated with grasses differed from legumes (p=0.085), but not from non-legume forbs (p=0.17). Similarly, non-legume forbs and legumes did not differ from each other (p=0.61).

As illustrated in Figure 3.6, a significant difference in relative abundance of AMF communities was found, with grasses significantly differing from both legumes (p=0.001) and non-legume forbs (p=0.003). AMF communities associated with legumes and non-legume forbs...
did not significantly differ from each other (p=0.52). However, turnover between samples using PERMDISP was found to be insignificant, with means differing less 0.1 of each other.

Figure 3.6. Community composition of AMF communities associated with three different plant functional groups ordinated with Bray-Curtis similarity index using PERMANOVA detected a difference between plant functional groups (p=0.001). Grasses clustered distinctly from both legumes (p=0.001) and non-legume forbs (p=0.003), however there was no difference between AMF community composition in non-legume forbs and legumes (p=0.52), n=21. Using PERMDISP, turnover of AMF communities among plant functional groups was not significantly different (p=0.41).

Furthermore, as Figure 3.7 illustrates, a signal for phylogenetic differences among plant functional groups (p=0.007) was observed. AMF communities associated with grasses were found to be significantly different from AMF communities associated with legumes (p=0.003) and non-legume forbs (p=0.03). However, AMF communities associated with legumes and non-legume forbs did not differ significantly from each other (p=0.51). When looking at the relative abundance of AMF species, it appeared that grasses contained a higher proportion of Glomus sp. 8 (VTX00126), Rhizophagus irregularis (VTX00113), Glomus sp. 4 (VTX00212) and Acaulospora longula (VTX00028), compared to AMF communities associated with legumes and forbs. While, Glomus sp.1 (VTX00197) was more abundant in non-legume forbs and legumes, compared to grasses (Figure 3.8).
Figure 3.7. Phylogenetic distance of relative abundance of AMF communities associated with three different plant functional groups ordinated with weighted UniFrac distance using PERMANOVA analysis (p=0.007). Grasses clustered distinctly from both legumes (p=0.003) and non-legume forbs (p=0.03), however there was no difference between AMF community composition in non-legume forbs and legumes (p=0.51), n=21. Using PERMDISP, turnover of AMF communities among plant functional groups was found to be insignificant (p=0.19).
Figure 3.8. Relative abundance of AMF taxa under three different plant hosts. A: Relative abundance of AMF taxa associated with grass hosts. Certain taxa have been bolded to highlight important differences between grass hosts and non-legume forb and legume hosts. B: Relative abundance of AMF taxa associated with non-legume forb hosts. C: Relative abundance of AMF taxa associated with legume hosts.
3.3 AMF Species Richness and Community Diversity with Increasing Plant Diversity

The third component of this research project aimed to uncover differences in AMF diversity and community composition under different treatments of plant diversity. As shown in Figure 3.9, plant diversity had no effect on AMF species richness (p=0.88), nor did plant diversity have any effect on alpha diversity, as measured by using the Shannon-Wiener diversity index ($H'$) (p=0.24) (Figure 3.10), or phylogenetic diversity (p=0.55) (Figure 3.11), calculated using PD whole tree metrics.

![Figure 3.9](image)

**Figure 3.9.** Observed AMF species richness across a plant species richness gradient. There was no significant difference with increasing plant diversity (Range: 0-16; Linear $R^2 = 0.001$; p=0.88), n=40.
Figure 3.10. Shannon-Wiener diversity index (H') across a plant species richness gradient. There was no significant difference with increasing plant diversity (Range: 0-16; Linear R² =0.034; p=0.24), n=40.

Figure 3.11. Phylogenetic diversity across a plant species richness gradient. There was no significant difference with increasing plant diversity (Range: 0-16; Linear R² =0.010; p=0.55,) n=40.
As Figure 3.12 illustrates, AMF communities (in terms of presence and absence of AMF species) associated with bare ground plots were significantly different from diversity treatments 1 (p=0.0002), 4 (p=0.005), and 16 (p=0.002). AMF communities associated with diversity level 16 plots also were significantly different from those associated with diversity level 1 plots (p=0.003), but not from diversity level 4 (p=0.13) plots. AMF communities associated with diversity levels 4 and 16 did not significantly differ from each other (p=0.19). AMF turnover between diversity treatments was not significant (p=0.73).

Furthermore, as Figure 3.13 depicts, the phylogenetic diversity of AMF communities (unweighted UniFrac distance) associated with bare ground plots differed significantly from diversity levels 1 (p=0.002), 4 (p=0.015), and 16 (p=0.003) plots. AMF communities associated
with diversity level 16 plots also differed significantly from those associated with diversity level 1 (p=0.004) and 4 plots (p=0.016), while AMF communities associated with diversity levels 4 and 16 did not differ from each other (p=0.23). Using PERMDISP, turnover in AMF communities did not significantly differ between plant diversity treatments (p=0.66).

![Phylogenetic distance of presence/absence in AMF communities across different plant diversity treatments](image)

**Figure 3.13.** Phylogenetic distance of presence/absence in AMF communities across different plant diversity treatments ordinated with unweighted UniFrac distance using PERMANOVA analysis (p=0.0001). Bare ground plots (diversity level 0) clustered distinctly from diversity levels 1 (p=0.0004), 4 (p=0.015) and 16 (p=0.003). Diversity level 16 plots also clustered distinctly from diversity levels 1 (p=0.0004) and 4 (p=0.016), however there was no difference between AMF community composition between diversity levels 1 and 4 (p=0.23), n=40. Using PERMDISP, turnover of AMF communities among plant diversity treatments did not differ significantly (p=0.66).

Similarly, as Figure 3.14 illustrates, the relative abundance of AMF taxa in bare ground plots (diversity level 0) was found to be significantly different from AMF relative abundance in diversity levels 1 (p=0.0002), 4 (p=0.004) and 16 (p=0.002). Relative abundance of AMF in high diversity plots (diversity level 16), also differed significantly from AMF relative abundance in plant diversity levels 1 (p=0.0001) and 4 (p=0.028), while diversity levels 1 and 4 did not show significant differences among each other (p=0.17). In terms of AMF community turnover,
AMF communities associated with different plant diversity treatments did not significantly differ from each other (p=0.46).

![Figure 3.1](image.png)

Figure 3.14. AMF community composition across different plant diversity treatments ordinated with Bray-Curtis similarity index using PERMANOVA analysis (p=0.0001). Bare ground plots (diversity level 0) clustered distinctly from diversity levels 1 (p=0.0004), 4 (p=0.004) and 16 (p=0.002). Diversity level 16 plots also clustered distinctly from diversity levels 1 (p=0.0001) and 4 (p=0.028), however there was no difference between AMF community composition between diversity levels 1 and 4 (p=0.21), n=40. Using PERMDISP, AMF community turnover associated with different plant diversity levels were not significantly different (p=0.46).

Finally, as shown in Figure 3.15, phylogenetic distance of AMF communities associated with bare ground plots showed distinct patterns compared to AMF communities associated with diversity levels 1 (p=0.005), 4 (p=0.06), and 16 (p=0.02). Similarly, high diversity plots also showed unique AMF community compositions patterns and significantly differed from diversity level 1 (p=0.002) and 4 (p=0.02) plots. Again, as with relative abundance, phylogenetic community composition of AMF did not differ between diversity level 1 and diversity level 4.
plots (p=0.46). Turnover of AMF communities among plant diversity treatments, using weighted UniFrac distances was found to be insignificant (p=0.41).

Figure 3.15. Phylogenetic distance of relative abundance of AMF communities across different plant diversity treatments ordinated with weighted UniFrac distance using PERMANOVA analysis (p=0.0004). Bare ground plots (diversity level 0) clustered distinctly from diversity levels 1 (p=0.005), 4 (p=0.06) and 16 (p=0.02). Diversity level 16 plots also clustered distinctly from diversity levels 1 (p=0.002) and 4 (p=0.02), however there was no difference between AMF community composition between diversity levels 1 and 4 (p=0.46), n=40. Using PERMDISP, turnover of AMF communities among diversity levels was found to be insignificant (p=0.41).

When looking at the actual composition and relative abundance of AMF taxa between different plant diversity treatments, it is clear that AMF communities associated with bare ground treatments and diversity 16 treatments were different from the rest of the plant diversity treatments (Figures 3.14, 3.16). Particularly, a large shift occurred between the bare ground treatment and plant diversity treatment 16, with the proportion of *Gigaspora margarita* (VTX00039) decreasing, while the proportion of *Glomus Glo30* (VTX00126), largely increasing and dominating in the plant diversity 16 treatment. Plant diversity treatments 1 and 4 showed more even distribution between AMF species, with *Glomus Glo30* (VTX00126), *Gigaspora*
margarita (VTX00039), *Glomus* sp. 1 (VTX00197), *Glomus* sp. 2 (VTX00093), and *Glomus indicum* (VTX00222) occurring in similar proportions.
Figure 3.16. Relative abundance of AMF taxa associated with different plant diversity treatments. Certain taxa have been bolded to highlight important differences between plant diversity treatments. A: Bare ground treatment (plant diversity = 0); B: Monoculture treatment (plant diversity = 1); C: Plant diversity treatment 4; D: Plant diversity treatment 16.
3.4 Summary of Results

In sum, when investigating host specificity in the AM symbiosis, it was found that the plant functional group and plant diversity had some impact on AMF presence/absence, relative abundance and phylogenetic diversity metrics. More specifically, it was found that AMF communities associated with grasses clustered separately from AMF communities associated with legumes and non-legume forbs, suggesting a potential functionality and phylogenetic component of host specificity. Furthermore, it was also found AMF communities in bare ground plots (0 plant species) and in high diversity plots (16 plant species) were significantly different from other plant diversity plots, although changes in AMF species richness were not significant across the plant diversity gradient.
CHAPTER 4: Discussion

4.1 General Discussion

These results clearly indicate that plant functional group and plant diversity influence the community structure of different AMF taxa in the soil. The fungal communities associated with grasses are distinct from those associated with forbs. Also, plant communities with high species richness support distinct AMF community patterns from plant communities with low species richness. These patterns illustrate that though there is little specificity in the symbiosis, in that most AMF taxa seem to associate with multiple plants, relative abundance of the fungi can be influenced by the identity of the plant hosts. This is to be expected in this system as the communities of soil fungi in a common garden experiment would likely be similar, but positive host interactions could influence different fungal species’ success in various plots, suggesting a biological driver for differences in AMF communities. As such, this study provides some evidence for host specificity determined by plant functional group and suggests that the framework of AMF host specificity should consider AMF as both generalist and specialist, in that they are compatible with most plant species and can form a symbiosis, but can be differentially promoted based on plant host. How plant host effects create these differences in AMF community composition may be a result of a variety of factors, including the life history strategies of fungal symbionts and plant hosts, as well as factors that modify soil and rhizosphere environments, including root exudates, plant host morphology, and investment of plant C to particular fungi in roots. In order to more clearly elucidate these patterns, more research should be done to better understand the functionality of AMF species and how this translates to host preference.
4.2 AMF Host Specificity at the Plant Species and Functional Group Level

It has long been thought that no specificity exists between AMF and host plant species (Magrou 1936, Gerdemann 1955). However the results of this study show that for this system, this is not necessarily the case. Results show that there is significant variation in AMF community composition among plant species and functional groups. This difference is mainly driven by changes in AMF species composition, not species richness or identity. Among these differences between plant species, there were high abundances of some fungal taxa across plant species, while other fungal taxa were only found in large abundance with one or two plant hosts (Table 3.1). This variation in AMF relative abundance at the plant species level is congruent with other studies. Studies that have focused on assessing AMF diversity associated with a variety of plant species have found that while there is little change in richness, the abundance of these taxa does change relative to the plant host (Öpik et al., 2003; Vandenkoornhuyse et al., 2003; Öpik et al., 2008; Dandan and Zhiwei 2007; Alguacil et al., 2010; Pellegrino et al., 2012; Lankau and Nodurft 2013). For example, when focusing on the AMF species, *R. irregularis* (which was found in low abundance in forbs, and higher abundance in grasses in the present study) Öpik et al. (2008) found a similar pattern in which *R. irregularis* was found in low abundance in forest herbs, compared to plant species found across many habitats. While the present study found no differences in AMF species richness across plant species and functional group, other studies have found larger differences in AMF richness across different plant species, suggesting that plant hosts may also affect presence or absence of AMF species (Douds et al., 1998; Bidartondo et al., 2002; Martínez-García and Pugnaire 2011; Stover et al., 2012; Brundrett and Ashwath 2013), and not just relative abundance. However, even these studies indicate that the majority of fungal taxa detected were found across plant species and groups, again suggesting that changes in AMF relative abundance, not species richness is most affected by plant host. Conversely, other studies have found little evidence for changes in AMF
communities based on plant species (Lugo and Cabello et al., 2002; Li et al., 2010; Davison et al., 2011; Alguacil et al., 2012; Lekberg et al., 2012; Schechter and Bruns 2013), suggesting that diversity observed in the field may be based more on environmental factors rather than host effects.

When plant species were organized by functional group, an even stronger pattern of specificity emerged, with grasses harboring distinct AMF communities, in terms of relative abundance, compared to forbs (includes legumes and non-legumes) (Figure 3.8). This pattern suggests that while there is AMF community variation at the plant species level in this system, much of the variation can be described by the functional group of the plant host. Studies have shown that AMF often associate with different groups of plants and that differential AMF community patterns are better described by plant functional group, as opposed to species (Burrows and Pleger 2002a; Öpik et al., 2006; Öpik et al., 2009; Hausmann and Hawkes 2010; Fedderman et al., 2010; McCain et al., 2011; Torrecillas et al., 2012; Koch et al., 2012; Lekberg et al., 2013). However, when looking at actual species composition and abundance in this data set, results are not as congruent with other studies that have taken similar approaches, in that certain Glomus species decreased in abundance in legumes and non-legume forbs, but have been detected in much higher abundances among forest herbs (Öpik et al., 2009), providing some grey area as to the breadth of specificity across different systems. As well, species including Glomus Glo30 and Acaulospora sp. have been found in higher abundances in tropical plants (Husband et al., 2002), contrasting the results in this study, in which these taxa were found in higher abundances in grass hosts. Similarly, other data sets have shown distinct AMF assemblages between legume and non-legume herbaceous functional groups (Scheublin et al., 2004), while this study found that legumes and non-legume forbs were more similar to each other and contained no significant differences in AMF community composition. As well, studies that have taken plant functional differences into account have shown weak differences in AMF
communities between different plant functional groups (Mohammad et al., 2003; Öpik et al., 2003; Li et al., 2010; Marshall et al., 2011). These discrepancies could be due to differential abiotic factors, as these studies were performed in the field, suggesting that abiotic factors may largely drive AMF community differences.

Specificity may also be approached through using phylogenetic information. In this study, plant species and functional groups can also be viewed as distinct phylogenetic lineages of plants. This suggests that AMF communities associated with more phylogenetically distant groups are distinct from AMF communities associated with more similar plant hosts. Literature has supported this idea with studies showing that the degree root colonization and plant response to AMF can be determined by phylogenetic signals when compared to null models and models including plant traits and taxonomy (Maherali and Klironomos 2007; Reinhart et al., 2012; Roger et al., 2013). In the case of the present study, it may be the phylogenetic patterns of particular plant families that lead to distinctive assemblages of AMF, or it may be the phylogenetic relatedness of AMF species that defines community differences under each plant host, a pattern that has also been indicated in the literature (Powell et al., 2009; Kivlin et al., 2011; Montesinos-Navarro et al., 2012).

Though the literature contains conflicting results regarding the existence of host specificity in the AM symbiosis, this study provides better insight into the role of plant hosts in determining AMF diversity, as it addresses limitations that have hindered past host specificity studies. While most studies mentioned have compared AMF communities from different field plots associated with different plant species or plant groups, leaving more variation explained by environmental heterogeneity, this study provides a key contribution as it was conducted in a long-term, common garden field experiment. Sampling from a long-term common garden provides a way to control for environmental variables that could affect AMF diversity, as well as providing a longer temporal scale allowing plant/fungal associations to establish over time. Since
environmental variables were controlled for in this experiment via randomization and full replication, the patterns that emerge more clearly support biological drivers, in this case, plant host identity and functionality. This study therefore shows that while AMF may be considered generalist in terms of colonizing potential, the promotion of particular AMF species is largely dependent on what hosts are present in the community. This idea has been hypothesized and shown in the literature, suggesting that associations between AMF and plants have evolved in part, based on partner choice, where plant hosts associate with fungal symbionts that are better cooperators, promoting their abundance (Sanders 2003; Kiers and van der Heijden 2006; Kiers et al., 2011; Montesinos-Narvarro et al., 2012; Hart et al., 2013; Sun et al., 2013).

However, specific mechanisms that cause the promotion of AMF species with different plant hosts still remain unclear. It may be that the genetic makeup of plant hosts limits colonization of particular AMF species to some degree due to different alleles in colonizing genes (Barker et al., 1998; Poulsen et al., 2005), or that mutualistic preference and evolutionary history of both the host and fungal symbiont dictate colonization patterns depending on plant communities or habitat (Martínez-García and Pugnaire 2011; Kiers et al., 2011). Recent papers also illustrate that host specificity between plants and AMF can occur at a cellular level, with specific signaling pathways specific to different AMF and plant species, allowing microbial symbionts, to range from pathogenic to mutualistic (Akiyama et al., 2005; de Bruijn and Scheublin 2013). The influence of host effects on the composition of AMF communities could also be explained by how different plant species modify the soil environment and create distinct niches for soil microbial habitants. Research has shown that this translates directly to changes in soil microbial communities, including AMF communities (Atkinson and Watson 2000; Becklin et al., 2012; Jordan et al., 2012). Therefore, differences in abundance of particular AMF taxa with the various plant species and functional groups could be characterized by how plant species
interact with and manipulate the rhizosphere through the release of root exudates, the investment of photosynthate into root versus shoot structures, and the root morphology of host plants. Studies have shown that differences in species-specific root exudates are able to alter arbuscular-mycorrhizal colonization (Tsai and Phillips 1991; Douds et al., 1998; Scheffknecht et al., 2006; Vranova et al., 2013), and increase hyphal growth, and increase spore density within the rhizosphere (Wang et al., 2012). Other studies have indicated that plants with different root morphologies and plant species that differentially allocate resources to shoot and root structures have distinct AMF communities compared to plants with alternate strategies (Collier et al., 2003; Sikes et al., 2009; Zubek and Blaszkowski 2009; Pagano et al., 2013).

Though host effects of plant species and plant functional group played a major role in determining AMF community composition, the variation in abundance of different AMF species could also be explained by fungal life history strategies and soil sampling effort. For example, the high abundance of G. margarita seen in this data set may result from traits associated with the Gigasporaceae. It has been documented that species of this family produce most fungal biomass outside of the plant root and sporulate more slowly (Hart and Reader 2002; Maherali and Klironomos 2007; Thornar et al., 2011). Since soil samples were exclusively analyzed, sampling efforts may have captured more Gigaspora species than if plant roots had been included in sampling. Similarly, the low abundance of Acaulospora sp. could also be due to family characteristics, as species of the Acaulosporaceae are known to produce low biomass both inside and outside the plant root (Hart and Reader 2002; Maherali and Klironomos 2007; Thornar et al., 2011). The low abundance and absence of Acaulospora sp. from many plant species and functional groups in this data set are congruent with other AMF sequencing studies, in which it has not been detected (Öpik et al., 2008; Öpik et al., 2009) and may suggest that it is a rarer species, complementing its life history strategy. Again, the low abundance of R. irregularis across most plant species, particularly forbs is interesting, as it has been characterized
as a fast growing and largely abundant species, which readily sporulates and colonizes plant hosts (Oehl et al., 2003; Öpik et al., 2006; Smith and Read 2008; Alguacil et al., 2009). It was therefore expected to appear in more plant species, if not all, though, as a member of the Glomeracaeae, it may have been detected at lower levels, as species in this group often produce most fungal biomass inside plant roots (Hart and Reader 2002; Maherali and Klironomos 2007; Thornar et al., 2011), which were excluded from sampling. It is important to note, however, that other Glomeracaeae species were detected in high abundance and that *R. irregularis* was detected in high abundance in grass plots, suggesting that this pattern is less likely due to low detection in the soil and more indicative of host effects.

Overall, these data show a signal for host specificity largely based on plant functionality, though the mechanism behind these differences is unclear. These data support studies that have investigated host specificity and plant functionality in the AM symbiosis, suggesting that there is a strong biological driver determinant of variation in the relative abundance of AMF taxa within communities.

### 4.3 AMF Communities and the Relationship with Plant Diversity

In this thesis, I also asked how plant diversity affects AMF community diversity in the soil, and results indicate that increasing plant diversity also affects relative abundance of AMF communities, but not species richness. Though few studies have directly explored the effect of plant diversity on AMF diversity, AMF relative abundance responses to plant diversity have also been shown in the literature, suggesting that greater host diversity selects for distinct AMF communities in the soil (Burrows and Pleger 2002b). Other patterns of AMF diversity have been largely attributed to changes in habitat, plant host community, environmental conditions or level of disturbance, however, studies that have investigated AMF diversity
patterns have revealed that ecosystems with higher plant diversity harbour distinct AMF communities (Allen et al., 1995; Burrows and Pleger 2002b; Carvalho et al., 2012; Davison et al., 2012; Gai et al., 2012; Brundrett and Ashwath 2013). This supports the observed pattern in this study that increased plant diversity can affect the relative abundance of different AMF in the soil. However, other studies have also illustrated the opposite pattern, where plant communities with the presence of specific trophic groups and thus low diversity, foster a more diverse AMF community. This indicates that diversity above ground does not always translate to more diverse AMF communities (Lekberg et al., 2013), though many studies of this nature have focused on invasive plant species, which have been known to manipulate AMF communities in different ways (Klironomos 2002; Klironomos 2003; Stinson et al., 2006; Mummey and Rillig 2006; Batten et al., 2008). While few studies have directly manipulated plant diversity and measured corresponding AMF diversity, studies that have investigated the reverse relationship (the effect of AMF diversity on plant diversity) have shown similar patterns in terms of changes to relative abundance. Studies typically report that more diverse AMF communities support more diverse plant communities, particularly affecting relative abundance of plant species over species richness (van der Heijden et al., 1998 a & b; van der Heijden et al., 2003; Hart et al., 2003; Wagg et al., 2011; Montesinos-Navarro et al., 2012; Sabais et al., 2012; Stover et al., 2012; Dostálek et al., 2013).

The mechanisms driving this variation in AMF relative abundance based on plant diversity may be dependent on the competitive and spatial interactions between fungal species. While host specificity in the AM symbiosis would suggest that more plant hosts could support a more evenly diverse AMF community, with increased niche space for rarer taxa, the data from this section suggests that when host diversity increases, more opportunities for superior competitors to establish in host plants (Janoušková et al., 2013; Roger et al., 2013) are provided. This competition paradigm has been described previously, where patterns of diversity can be
explained by through competitive interactions between neighboring species and how these species interact in space (Coffin and Lauenroth 1991; Tilman 1994; Pacala and Tilman 1994; Tilman 2011). This type of spatial-diversity maintenance may be applicable to AMF, where a variety of species exist in the soil and coexist on multiple host plants, resulting in a variety of species tradeoffs and patterns that could include, but are not limited to, investment in reproductive and vegetative structures, different requirements for particular nutrients and resources, and colonization abilities in different areas of the root (Hart et al., 2003; Cano and Bago 2005; Engelmoer et al., 2013). These different interactions between AMF species, along with spatial subdivision of the AMF communities, could provide a mechanism as to why an increase in plant host diversity may affect AMF diversity patterns. Despite the presence of host specificity, competitive strategies may be a more prominent driver of AMF diversity in highly diverse plant communities. However, more research is needed to more clearly understand the drivers of AMF diversity and species abundance in the soil in regards to plant diversity and how this relates to functional specificity in AMF.

Another explanation for the diversity pattern may have to do with fungal life history strategies (Hart and Reader 2002; Roger 2013). For example, since G. margarita and Glomus Glo30 belong to different AMF families with distinct life history strategies and characteristics. Studies have shown that G. margarita is a slower growing fungus and tends to remain in vegetative states for longer periods of time and has lower sporulation rates compared to Glomus species (Tiwari and Adholeya 2002). Glomus species on the other hand, tend to sporulate early and more quickly, while rapidly developing hyphal mycelium, allowing them to dominate more quickly (Douds and Becard 1993; Tiwari and Adholeya 2002). With these fungal traits in mind, it may make sense that bare ground plots are largely dominated by slow-growing, more vegetative fungal species, as there are no plant hosts to quickly colonize, and as such, a fungal species must remain dormant for longer periods of time. As more plant hosts become available,
Glomus Glo30, sporulating and developing hyphae quickly, would potentially be able to colonize plants more quickly, increasing its abundance in the soil.

In summary, increasing plant diversity has an effect on AMF community composition, but not on AMF species richness. While AMF species richness was unaffected by plant diversity, notable changes in the relative abundance of AMF species as plant diversity increased could be explained by fungal life history traits, or by competitive interactions of fungal species with different availability of plant hosts.
CHAPTER 5: Conclusion

5.1 General Conclusions

The goal of this thesis was to provide more insight into the role of host specificity in terms of plant species and plant functional group in the AM symbiosis, while also exploring the effect of plant diversity on AMF community composition. To do this, soil was sampled from a long-term common garden experiment, where plots were set up to contain varying levels of plant diversity ranging from bare ground to 16 plant species. Molecular techniques and 454 sequencing were used to determine species richness and relative abundance of AMF taxa under each plant treatment, yielding a profile of AMF taxa that co-occurred with particular types of plant species, plant functional groups, and plant diversity treatments. Support was garnered for two of the three hypotheses in question, of which are summarized below:

1) *In the AM symbiosis, do plant species affect AMF species richness and AMF community composition in the soil?*

   This question was unable to be fully answered due to lack of replication of monoculture samples after sequences were rarefied and filtered in the QIIME pipeline. However, the preliminary results indicated that particular plant species did contain AMF taxa that were not found in other plant species or in high abundances in only certain plant species. This question must be further explored to statistically illustrate a trend regarding changes in AMF community composition among different plant species.

2) *In the AM symbiosis, do different plant functional groups affect AMF species richness and AMF community composition in the soil?*

   The evidence gathered supported the idea that plant functional group has an effect on AMF community composition. However, no evidence was found to support the idea
that plant functional group affects AMF species richness. In this aspect of the study, it was found that grasses had distinct AMF communities compared to legumes and non-legume forbs, though species richness did not significantly differ between functional groups.

3) *Do plant species richness gradients affect AMF species richness and community composition in the soil?*

In the plant diversity treatment, it was found that while species richness of AMF taxa did not differ between plant treatments, AMF community composition did. It was found that plots with 0 plant species and plots with 16 plant species differed most significantly from each other and the other plant diversity treatments (1 and 4 plant species), particularly in the abundances of two species *Gigaspora margarita*, which dominated in bare ground plots and *Glomus Glo30*, which dominated in high diversity plots.

5.2 Methodology Discussion

While this study was able to elucidate patterns of AMF diversity under different host plants and diversity levels, there were some drawbacks that may have affected the patterns observed. The first of these drawbacks is the use of molecular techniques. While molecular methods are currently the most robust way to detect AMF diversity (Lumini *et al.*, 2010; Öpik *et al.*, 2010; Lindahl *et al.*, 2013), the use of these techniques may have caused discrepancies in AMF diversity patterns, depending on which molecular markers were used. In this study, the primer set NS31 and AM1 were used, which target the 18S region of the SSU ribosomal RNA gene. These primers are known to include most species in the Glomeromycota, though they exclude members of the Archaeosporaceae and Paraglomeraceae (Helgason *et al.*, 1998; Kohour *et al.*, 2014). The exclusion of these families could cause significant changes in observable
patterns, as species belonging to these families would be mostly excluded from any downstream community analysis. As such, there may be AMF taxa from these families that are largely dominant or change significantly among plant species, functional groups, and diversity treatments, and may provide a very different picture of how different plant treatments affect AMF community richness and composition in the soil, altering the results displayed here. While this is a drawback of molecular studies, the primer sets used here also cover a region that includes the highest diversity in Glomeromycota, allowing more AMF species to be resolved over this region compared to other primer sets available for AMF (Daniell et al., 2001; Ópik et al., 2009; Kohout et al., 2014).

Secondly, it should be noted that while the first aspect of my thesis addressed the effects of plant species on AMF community composition, this question was unable to be statistically analyzed due to the loss of samples corresponding to monoculture plots after sequencing analysis. In this aspect of the study, three replicates were collected from each of monoculture plots, however, during the sequencing process some samples were eliminated from downstream analysis after rarefaction and filtering because they contained sequence thresholds which were too low to be accurately analyzed. When these plots were sampled in the field, it was decided that due to the large number of monoculture plots, three replicates would be taken from each plot in order to minimize cost and maximize sampling efficiency, while still maintaining sufficient replicates to provide statistical power in further analyses. In hindsight, more replicates should have been sampled in order to account for potential sample loss in the sequencing analysis pipeline. This is an important consideration to keep in mind when moving forward with AMF sequencing studies, particularly when designing experiments and planning sampling methods. In this case, had more replicates been taken, there may have been sufficient samples retained in the end result of the sequencing pipeline, allowing a more in-depth analysis to be conducted on plant
species treatments. Moving forward with high-throughput sequencing studies, it should be kept in mind that more replicates are required to counteract the potential for sample loss during sequencing analysis and the minimum sample requirements should always exceeded if feasible.

Finally, this study did not include plant roots in the soil sampling, and focused solely on AMF community composition and diversity in soil. This is important to note because AMF diversity could significantly differ had plant roots had been included. The exclusion of root samples could have biased against AMF taxa that invest more in internal root structures and also rare taxa that may primarily reside in roots, with little investment in hyphae or sporulating structures. In the future, it would be beneficial to include roots and soil, homogenizing each sample and extracting DNA from a root/soil mixture in order to better capture real diversity patterns both inside and outside plant roots.

5.3 Future Directions

This study sheds light on how plant species identity, functional group, and diversity can affect changes in the AMF soil community, which could have large-scale impacts on various industries, particularly agriculture where manipulation of crop species could produce differential AMF community abundances and potentially more beneficial mycorrhizal inoculum in the soil. Furthermore, understanding how plant hosts affect AMF communities can also inform restoration and conservation projects, allowing researchers to better understand how mycorrhizal communities can be altered with the goal of yielding plant communities that are likely to succeed and persist throughout time.

The results of this experiment may also inform future research objectives concerned with increasing plant community productivity and nutrient cycling. It has been shown that
changes in AMF community composition contribute to increased mobilization, resulting in increased nutrient cycling within plant communities (Cavagnaro et al., 2012; Negrete-Yankelevich et al., 2013). As shown in this experiment, the significant impact of plant functional group and diversity on AMF community composition could be applied to promote increased nutrient cycling and usable nitrogen in infertile or degraded soils. As well, since AMF are known to increase nitrogen uptake in plants (Smith and Read 2008) and contribute to nutrient cycling in terrestrial ecosystems (Brussaard 1997; Coleman et al., 2004), potential manipulations of AMF communities via plant host manipulation could increase these desired effects and therefore contribute to healthier, more stable, and more diverse soils. Therefore, this study not only provides insight into the maintenance of AMF diversity in the soil and how plant functional group and diversity affect diversity in AMF communities, but also provides exciting avenues for further research into plant host effects and community diversity in the AM symbiosis.

Overall, the results of this thesis shed light on the complexities of the AM symbiosis, suggesting that there is a level of host specificity present at a plant functional group level. As well, the results of this thesis illustrate the effect of plant diversity on AMF community composition, informing how above ground diversity can affect the abundances of AMF taxa in the soil. This can have important implications in understanding how plants affect their soil microbial symbionts, providing invaluable information in order to better understand the ecological functionality and influence plant host and communities have on mycorrhizal communities.
BIBLIOGRAPHY


APPENDICES

Appendix A: Plants Used in Biodiversity Experiment 120

Table A.1. Bank of plant species and their corresponding functional group used in Biodiversity Experiment 120 at the Cedar Creek Ecological Research Station in MN, USA.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Functional Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achillea millefolium</td>
<td>Non-legume forbs</td>
</tr>
<tr>
<td>Agropyron smithii</td>
<td>C-3 Grass</td>
</tr>
<tr>
<td>Amorpha canescens</td>
<td>Legume</td>
</tr>
<tr>
<td>Andropogon gerardi</td>
<td>C-4 Grass</td>
</tr>
<tr>
<td>Asclepsias tuberosa</td>
<td>Non-legume forbs</td>
</tr>
<tr>
<td>Elymus canadensis</td>
<td>C-3 Grass</td>
</tr>
<tr>
<td>Koeleria cristata</td>
<td>C-3 Grass</td>
</tr>
<tr>
<td>Lespedeza capitata</td>
<td>Legume</td>
</tr>
<tr>
<td>Liatris aspera</td>
<td>Non-legume forbs</td>
</tr>
<tr>
<td>Lupinus perennis</td>
<td>Legume</td>
</tr>
<tr>
<td>Monarda fistulosa</td>
<td>Non-legume forbs</td>
</tr>
<tr>
<td>Panicum virgatum</td>
<td>C-4 Grass</td>
</tr>
<tr>
<td>Petalostemum candidum</td>
<td>Legume</td>
</tr>
<tr>
<td>Petalostemum purpureum</td>
<td>Legume</td>
</tr>
<tr>
<td>Petalostemum villosum</td>
<td>Legume</td>
</tr>
<tr>
<td>Poa pratensis</td>
<td>C-3 Grass</td>
</tr>
<tr>
<td>Quercus ellipsoidalis</td>
<td>Woody</td>
</tr>
<tr>
<td>Quercus macrocarpa</td>
<td>Woody</td>
</tr>
<tr>
<td>Schizachyrium scoparium</td>
<td>C-4 Grass</td>
</tr>
<tr>
<td>Solidago rigida</td>
<td>Non-legume forbs</td>
</tr>
<tr>
<td>Sorghastrum nutans</td>
<td>C-4 Grass</td>
</tr>
</tbody>
</table>
Appendix B: PERMANOVA Tables for Plant Functional Group and Diversity Treatments

Table B.1. PERMANOVA results for AMF presence/absence Bray-Curtis distances

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Pseudo F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional Group</td>
<td>2</td>
<td>4789</td>
<td>3.6812</td>
<td>0.0008</td>
</tr>
<tr>
<td>Diversity</td>
<td>3</td>
<td>12709</td>
<td>4.8169</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table B.2. PERMANOVA results for AMF relative abundance Bray-Curtis distances

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Pseudo F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional Group</td>
<td>2</td>
<td>5628.6</td>
<td>3.6125</td>
<td>0.0007</td>
</tr>
<tr>
<td>Diversity</td>
<td>3</td>
<td>14415</td>
<td>4.6455</td>
<td>0.0001</td>
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Table B.3. PERMANOVA results for AMF unweighted UniFrac distances.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Pseudo F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional Group</td>
<td>2</td>
<td>0.38925</td>
<td>2.7169</td>
<td>0.0008</td>
</tr>
<tr>
<td>Diversity</td>
<td>3</td>
<td>0.89991</td>
<td>3.6262</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table B.4. PERMANOVA results for AMF weighted UniFrac distances.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Pseudo F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional Group</td>
<td>2</td>
<td>0.46327</td>
<td>3.2307</td>
<td>0.0141</td>
</tr>
<tr>
<td>Diversity</td>
<td>3</td>
<td>1.074</td>
<td>4.1131</td>
<td>0.0005</td>
</tr>
</tbody>
</table>
Appendix C: PERMDISP Table for Plant Functional Group and Diversity Treatments

Table C.1. PERMDISP results for plant functional group and diversity treatments

<table>
<thead>
<tr>
<th>Factor</th>
<th>Diversity Metric</th>
<th>F</th>
<th>P (Perm)</th>
<th>df 1</th>
<th>df 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional Group</td>
<td>Presence/Absence</td>
<td>2.2811</td>
<td>0.1125</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Bray-Curtis</td>
<td>0.7542</td>
<td>0.4132</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Unweighted UniFrac</td>
<td>2.5746</td>
<td>0.1396</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Weighted UniFrac</td>
<td>1.5828</td>
<td>0.1852</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Diversity</td>
<td>Presence/Absence</td>
<td>0.7372</td>
<td>0.7097</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Bray-Curtis</td>
<td>0.588</td>
<td>0.7278</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Unweighted UniFrac</td>
<td>0.69387</td>
<td>0.6593</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Weighted UniFrac</td>
<td>1.0232</td>
<td>0.4082</td>
<td>3</td>
<td>36</td>
</tr>
</tbody>
</table>