Plant Mycorrhizal Feedback Patterns in the Context of Plant Life History and Phylogeny in the Prairie

By

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Abstract

My research bridges the general themes of plant species coexistence, mutualistic interactions, and microbial feedbacks. These mechanisms have implications for our understanding of plant community dynamics, for land management, and for restoration projects. I study these mechanisms within the context of plant – AM fungal feedbacks. AM fungi associate with the roots of the plant, forming symbiotic exchange sites inside the root cells. AM fungal composition influences plant community dynamics, and plant-AM fungal feedback provides a framework for understanding how the interactions between these two groups of organisms could drive patterns of plant biodiversity. My research extends the understanding of how plant-AM fungal feedbacks are shaped and influenced by plant life history, and phylogenetic relatedness. My research also examines how the environmental disturbance of phosphorus fertilization influences these interactions and how that might be mediated by plant host characteristics. My research demonstrated AM fungal communities differentiate in response to training plant characteristics and phosphorus treatments. These changes resulted in positive feedback effects that were functionally different between early and late successional hosts. I saw consistent effects of the phylogenetic structure of host plants shaped AM fungal communities, with closely related plant species having similar AM fungal composition. Within early successional hosts, these changes in AM fungal communities fed back positively plant fitness of closely related species, contributing to phylogenetically under-dispersed communities. AM fungal community composition changed due to phosphorus enrichment, with less beneficial AM fungi decreasing while non-beneficial AM fungi increased, thereby predicting degradation of mutualistic

quality of the AM fungal community. However, measured growth promotion of AM fungal communities with a legacy of phosphorus fertilization increased relative to AM fungal communities not exposed to fertilization. Overall, positive feedbacks between early and late successional plant species could result in alternative stable state. AM fungal dynamics could potentially constrain communities to dominance by early successional plant species, or they could accelerate successional turn over to dominance by late successional plant species. Additionally, the forces that maintain early successional dominated communities are expected to result in lower diversity.

Acknowledgements

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Chapter 1: Introduction

My research bridges the general themes of plant species coexistence, mutualistic interactions, and microbial feedbacks. I focus on perhaps the most common and most important mutualism on earth, that between plants and arbuscular mycorrhizal (AM) fungi. AM fungi form symbioses with roots of most plant species and improve plant resource uptake. I test the importance of the dynamics of AM fungi in plant community dynamics within the North American prairie ecosystem. I take advantage of the high diversity of this ecosystem and employ a highly quantitative approach that tests for consistent impacts of AM fungal dynamics on plant-plant interactions across many plant species.

Species Co-existence

The mechanisms contributing to the maintenance of biodiversity remain an important area of interest in ecology. These mechanisms have implications for our understanding of plant community dynamics, for land management, and for restoration projects. Several mechanisms have been proposed as contributing to the maintenance of biodiversity, including resource partitioning and abiotic facilitation (Kahmen et al. 2006; McKane et al. 2002; Wright et al. 2017; Barry et al. 2019). Plant-microbiome interactions is another framework which is being increasingly studied (Bever et al. 2010). While a growing body of evidence identifies plant pathogens as contributing to plant species coexistence (Bever, Mangan, and Alexander 2015), AM fungi can also influence plant-plant interactions.

The Importance of AM Fungi

AM Fungi form an ancient mutualistic association with most terrestrial plants. AM fungi are part of an ancient symbiotic relationship with terrestrial plants that date back 460 million years ago (Simon et al. 1993; Redecker, Kodner, and Graham 2000). AM fungi associate with the roots of the plant, growing inside the root cells. They aid in the uptake of water and nutrients, particularly phosphorus, to the host plant in exchange for carbohydrates from the host (Smith and Read 2008). The presence and diversity of AM fungi has been empirically shown to affect plant community diversity and structure (Bauer et al. 2012; Wilson and Hartnett 1997; Hartnett and Wilson 1999; Heijden et al. 1998; Vogelsang, Reynolds, and Bever 2006).

AM fungal composition influences plant community dynamics. AM fungal species are functionally different in in their average and specificity of plant growth promotion (Cheeke et al. 2019; Hoeksema et al. 2018), impact on plant defense (Bennett and Bever 2009; Middleton et al. 2015), and in ability to aggregate soil (Schreiner and Bethlenfalvay 1997; Wang et al. 2020). Because of the differences in function between AM fungal species, I expect changes in AM fungal composition to create feedback effects on host plant communities. There is empirical evidence supporting this expectation. Changes in AM fungal composition due to plant host identity has been shown to drive positive or negative plant soil feedbacks (Bever 1999; 2002a; Crawford et al. 2019; Koziol and Bever 2019; Mangan, Herre, and Bever 2010).

Plant Soil Feedbacks

Plant-soil interactions can provide a mechanism that could drive patterns of plant biodiversity. Previous research has shown links between positive soil feedbacks from mutualists leading to a loss of species diversity, and negative feedback effects, primarily from pathogens, driving a more diverse community (Bever, Westover, and Antonovics 1997; Bever 2003). This relationship can be explained by the reciprocal effects plant and soil community structure can have on each other. As a single plant species becomes dominant in a community, the structure of the soil community changes in response. Both beneficial and harmful organisms who associate with the dominant plant species will build up in the soil. If the change in the microbiome improves the fitness of the dominant plant species relative to its competitors, it dominates and excludes its competitors. This would lower the diversity of the plant community as a whole. If the inverse relationship is true, then stronger negative feedbacks would decrease the fitness of the dominant plant preventing competitive exclusion, and total diversity will increase.

Plant Life History and Species Turnover during Succession

Life history represents a tradeoff in life energy investment strategy. In the context of my work, I will be examining early and late successional plants. The traditional r-K continuum can be instructive in understanding the difference between these strategies (MacArthur and Wilson 1967; Pianka 1970). Species that are r-selected prioritize rapid growth and number of offspring which would describe the investment strategy of many early successional species. Late successional plants are a more K-selected group,

prioritizing increased investment into fewer offspring, and slower growth and maximizing competitive ability of adults. This framework was expanded in relation to plants in (Grime 1977). This framework establishes four theoretical environments for plants to inhabit resulting from two axes of external factors limiting growth, environmental stress and disturbance. Environments high in disturbance and stress are considered in-viable as high stress prevents recovery after disturbance. This leaves three viable strategies; low stress and low disturbance plants which must be competitive (C), high stress and low disturbance plants which must be stress-tolerant (S), and low stress and high disturbance specialists which are considered ruderal plants (R). In the mesic prairie system I will be working in, I am mostly considering early successional plants adapted to disturbance (R-selected), and late successional plants which invest in competitive ability (C-selected).

Early and late successional plants differ in their interactions with AM fungi and therefore plant AM fungal interactions can have important implications for succession. There is empirical evidence that the plants of different successional status are not similar in their degree of mycorrhizal responsiveness, the ratio of plant growth with AM fungal inoculation versus a control. Late successional plants have been shown to be more responsive to AM fungal inoculation (Koziol and Bever 2015). Late successional plants also have greater sensitivity to AM fungal species identity (Cheeke et al. 2019; Koziol and Bever 2016). Studies have shown that the strength of plant soil feedback effects correlate with plant successional stage (Bauer, Mack, and Bever 2015). AM fungi have been shown to increase plant diversity by increasing the fitness of late successional plant species (Koziol and Bever 2019). Low or high diversity treatments

provided benefits if they contained specific AM fungal species that promoted growth in late successional plants. These effects have been shown to advance succession in grassland restorations (Middleton and Bever 2012).

Tests of Consistency Across the Plant Phylogeny

Generalization in ecology requires tests for consistency of ecological interactions between species distributed across the plant phylogeny. Such a replicated, quantitative approach is particularly needed with regard to the dynamics of the AM fungal community, as to date the discipline has largely illustrated potential dynamics using individual plant-AMF study systems. Besides testing for consistency of effects, replicated tests across plant species permits tests of whether traits are evolutionarily conserved across the plant phylogeny (Ives and Helmus 2011; Rafferty and Ives 2013; Ives 2018). Throughout my work, I utilize phylogenetic mixed models that simultaneously test for consistency of mycorrhizal dynamics and feedbacks across environment and plant species, and for evidence of phylogenetic conservatism.

Previous research has shown varying degrees of phylogenetic conservatism in plant traits. For example, there is empirical evidence for phylogenetic conservatism in root diameter, specific root length, and branching intensity (Comas, Callahan, and Midford 2014). There is an important tension between phylogenetic conservatism of traits and rapid adaptive radiation in traits. Evidence exists showing variation in conservatism between traits, or between clades when considering a single trait (Ackerly 2009). Extending these considerations into questions of community ecology, it is important to consider the dueling forces of phylogenetic attraction and repulsion. To

the degree traits are conserved, I would expect similar species to inhabit similar habitats through niche filtering (Webb et al. 2002). Alternatively I could expect to see greater competition between species and therefore competitive exclusions of similar species (Gotelli and McCabe 2002). Importantly both of these forces can be at play simultaneously (Helmus et al. 2007). Given all the impacts phylogenetic relationships can have on plant traits and community composition, it is important to consider these relationships when looking at the effects of species interactions (Rafferty and Ives 2013).

Phosphorus Fertilization and the Degradation of Mutualism

AM fungi have been shown to be sensitive to disturbance in the form of fertilization. AM fungal composition has been empirically shown to change in response to fertilization and AM fungal species can vary in their fitness responses (Stover et al. 2012; Li, Li, and Zhao 2007; House and Bever 2018; Santos, Finlay, and Tehler 2006). Fertilization rates can also negatively impact AM fungal colonization, hyphal density and diversity (Lang et al. 2022; Emery et al. 2017; Santos, Finlay, and Tehler 2006). Multiple past studies have shown a degrading of AM fungal community function when exposed to high phosphorus environments. Growth rates of less beneficial AM fungi can also be promoted in high phosphorus environments (Johnson 1993). Fertilization has been empirically shown to decrease AM fungal colonization, hyphal density, and Shannon diversity (Lang et al. 2022; Emery et al. 2017). There has also been theoretical work expanding models of AM fungal function in the presence of fertilization that also

predicate phosphorus fertilization to favor non-beneficial AMF, allowing less beneficial mutualists to proliferate (Bever 2015; Ghosh, Reuman, and Bever 2021).

The Tallgrass Prairie as a Study System

North American prairies are an ecosystem strongly shaped by AM fungal interactions. The vast majority of prairie species form some relationship with AM fungi with varying degrees of responsiveness (Collins and Wallace 1990; Bragg and Hulbert 1976). Many studies have shown that the abundance and composition of AM fungal communities have significant impacts on prairie plant community composition (Wilson and Hartnett 1997; Hartnett and Wilson 1999; Vogelsang, Reynolds, and Bever 2006; Bauer et al. 2012). An area of particular concern is the effectiveness of restoration practices. Late successional plant species do not establish well under conventional restoration practices (Betz, Lootens, and Becker 1996). Plant adaptations often feature compromises in life history strategies between early and late successional plants. Early successional plants are generally faster growing and invest more in early reproduction than late successional plants. There is also evidence that the responsiveness of plants to AM fungi correlates more with successional stage than with phylogenetic relatedness (Bauer, Mack, and Bever 2015; Koziol and Bever 2015). Moreover, late successional prairie plant species are more sensitive to AM fungal community composition than early successional native or non-native plant species (Koziol and Bever 2016; Cheeke et al. 2019). AM fungal inoculation, when included as part of restoration efforts, has been shown to improve the establishment of late successional prairie plants (Bever et al. 2003; Middleton et al. 2015; Koziol and Bever 2016). For these reasons, I conducted a

series of experiments looking at questions of plant-soil feedbacks, the maintenance of above and below ground biodiversity, and the mechanisms that can explain the persistence of these complex interactions in the North American prairie system.

Objectives

The goals of my dissertation are to examine the impacts of AM fungi on plant diversity and coexistence, using the tallgrass prairie system as a model. This work extends a theoretical framework of plant-soil feedback driven coexistence. The questions this works seeks to answer are:

1. How does AM fungal community composition respond to host plant characteristics and phosphorus fertilizer?
2. What is the effect on mycorrhizal feedbacks resulting from the changes in AM fungi community composition due to host plant characteristics?
3. What is the effect of phosphorus fertilizer and plant phylogeny on AM fungal function and feedbacks?

Experimental Overview

Two-phase Experimental Design

As a framework to examine the plant fungal interactions in this system, my work is based on the two-phase conditioning experimental design (Figure 1) (Bever et al. 2010). In the first phase, different plants are grown in pots with soil that has a homogenous soil community. Time is allowed to pass – typically a growing season – so that the soil

community can differentiate in response to their association with the plant. This soil is then used in phase two for a growth assay. The soil "conditioned" on each plant species is now used as inocula for all plant species in a phase 2 study during the following growing season. This is similar to "home" and "away" treatments often used in ecology, where home would be a plant species growing in soil that was conditioned on the same species in phase 1. Away would be a plant species grown in soil conditioned on a different plant species in phase 1. Phase 2 plants are generally allowed to grow for a shorter period than in phase 1, with 6 to 8 weeks being ideal. This is to lessen the ability of the plants to recondition the soil to be similar to its home condition. Total dry weight biomass is then measured as a metric for the fitness response of the plants in phase 2.

The Experimental Timeline

In order to achieve my dissertation objectives, I conducted a series of investigations (Figure 2). For experiment 1, I conducted a greenhouse assay to characterize the fitness benefits that several known species of AM fungi receive from many native prairie plant species and common invasive plant species, conducted in phase 1 as described above. For experiment 2, I conducted a growth assay to determine the benefits conferred by the communities of AM fungi conditioned in the experiment 1 greenhouse assay to plants of every other species in the study (phase 2). For experiment 3, I conducted a growth assay with a single host plant species and all soil types including those with phosphorus additions to determine how fertilization affects the functioning and feedbacks of AM fungal communities (also occurring in phase 2).

Species Selection

I selected 38 prairie plant species for these series of experiments. Plants were selected from across three broad phylogenetic groups: lilies, grasses, legumes, milkweeds, mints, and asters (Figure 3). This was done to ensure there would be enough phylogenetic diversity among my species for a strong test of phylogenetic effects. I also chose early and late successional plants within each of these phylogenetic groups. This allows me to test for the effects of plant life history simultaneously with phylogenetic effects. Finally, I selected 7 AM fungal species to use as my common inocula; *Claroideoglomus lamellosum*, *Claroideoglomus claroideum*, *Entrophospora infrequens*, *Funneliformis mosseae*, *Racocetra fulgida*, *Cetraspora pellucida*, *Acaulospora spinosa*. These seven species were available as pure cultures from Bever-Schultz lab. These cultures were used in previous experiments which provide data on their effectiveness at promoting prairie plant growth (Koziol and Bever 2016; Cheeke et al. 2019).

Figures

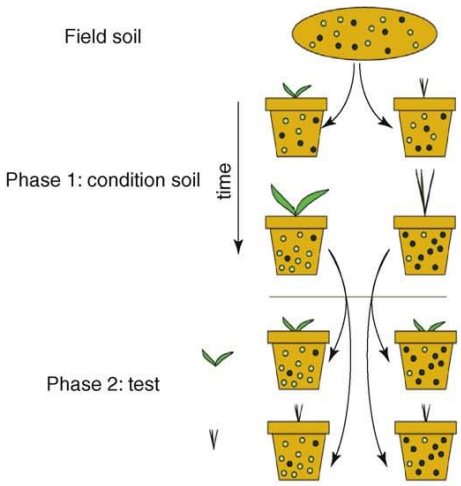


Figure 1: Two-Phase Experimental Design

In a two-phase experimental design, a started homogenous soil community is first conditioned in association with the plant hosts in phase 1. The soil communities differentiate due to the influence of the different host plants. In phase 2, a second generation of plants is now grown in soil conditioned in phase 1. Fitness measurements (typically biomass) is then measured for the response plants. Figure from (Bever et al. 2010).

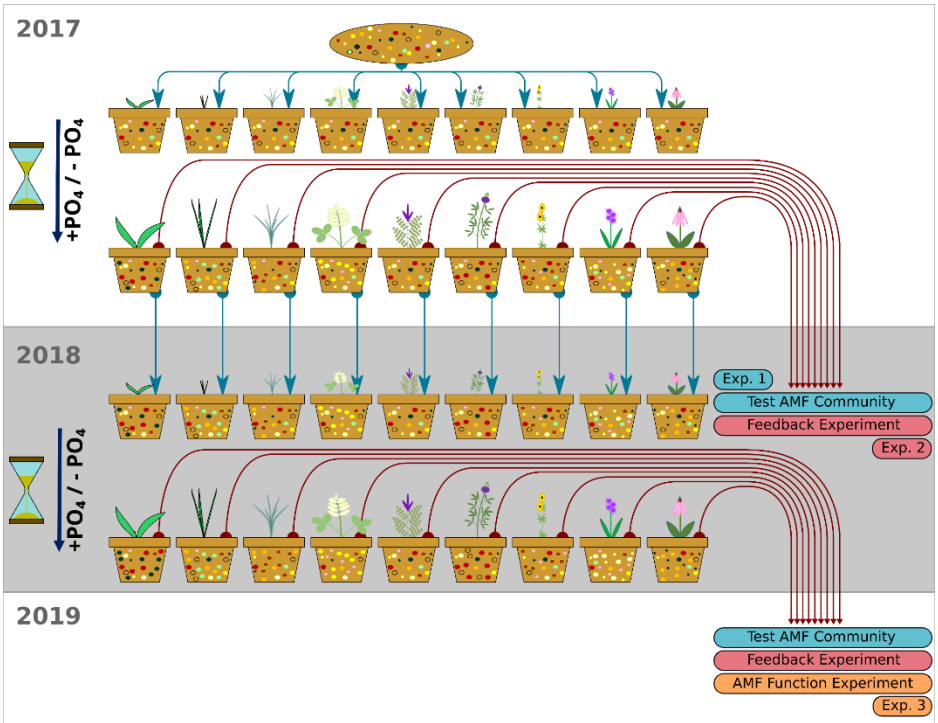


Figure 2: Dissertation Experimental Design

I conducted a series of experiments over the years of 2017, 2018, and 2019. Experiment 1 consisted of bioinformatics conducted on soil samples collected in 2017 and 2018 of my training communities. These communities had been allowed to differentiate over a growing season in association with different host plants in low and high phosphorus environments. Experiment 2 consisted of feedback assays in 2018 and 2019 using soil communities trained on all of my plant species and response plants which also represented all of my plant species. Experiment 3 was also a growth assay that was conducted in 2019. This experiment used soil communities trained on all of my plant species in high and low phosphorus environments and had a single response plant, the late successional grass *Schizachyrium scoparium*.

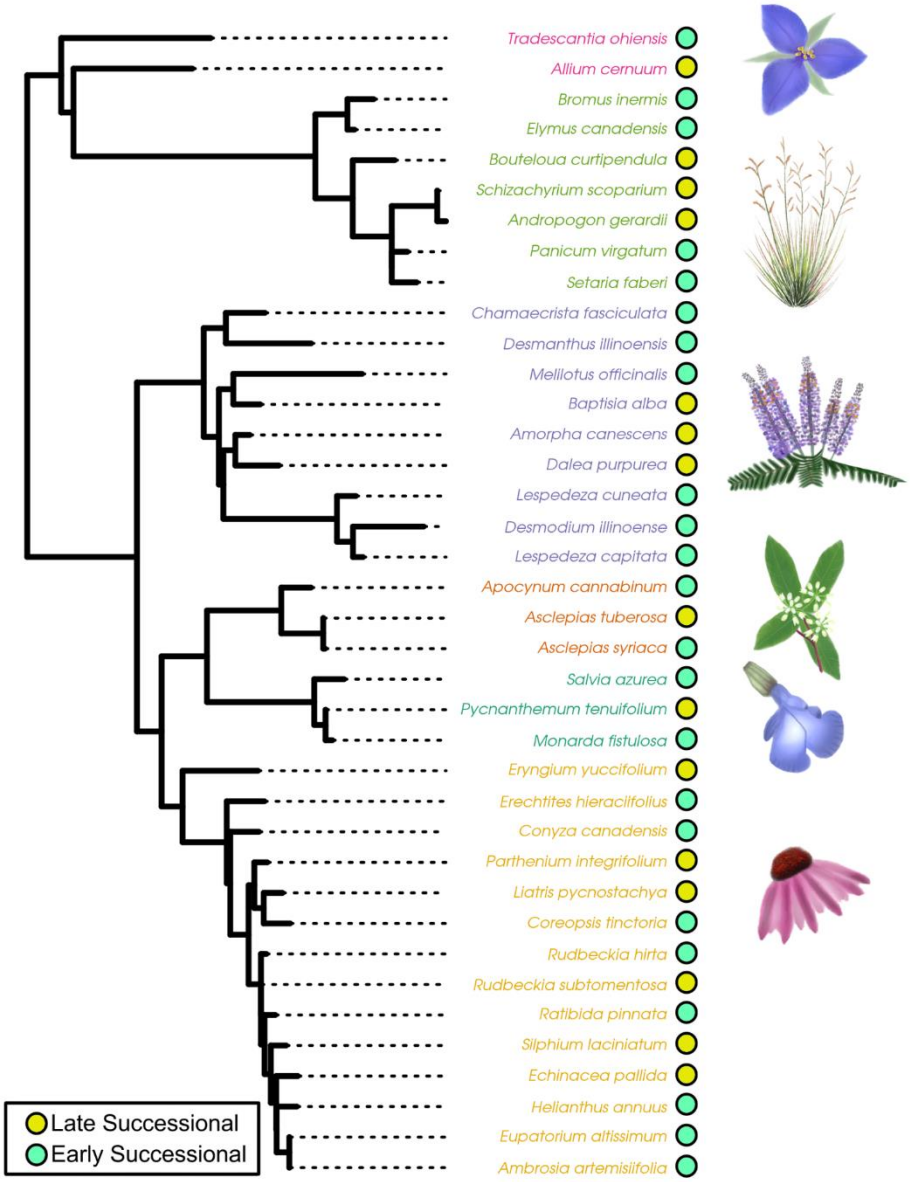


Figure 3: Species Selection

38 different prairie plant species were selected for these series of experiments. They span 6 broad phylogenetic groups: lilies, grasses, legumes, milkweeds, mints, and asters. Within each group early and late successional plant species were represented.

Chapter 2: Arbuscular Mycorrhizal Fungal Community Response to Plant Host Characteristics and Phosphorus Fertilizer

Abstract

The composition of AM fungal communities can create feedbacks on plant fitness, community composition, and terrestrial function. Plant hosts can have strong effects on AM fungal composition through the effects of plant host identity, phylogenetic relationships and plant life history characteristics. Environmental factors such as fertilization can also affect AM fungal composition and diversity. To test how all these factors combine to structure AM fungal communities a greenhouse experiment was designed to measure the growth of a known community of AM fungi on a phylogenetically diverse group of early and late successional host prairie plants with and without phosphorus enrichment. I found that phylogenetic distance was an important predictor of AM fungal community structure, and the growth rates of individual AM fungal species tended to be similar with phylogenetically similar plant species. The effects of phosphorus addition varied across AM fungal groups, with more beneficial groups experiencing a decline in growth rate when exposed to elevated phosphorus. Plant life history also had significant impacts on AM fungal growth rates with several groups benefiting from association with late successional host plants. Plant life history also had an important modulating effect on the outcome of phosphorus enrichment with my most beneficial AM fungal group showing a decrease in growth rate when raised on a late successional in high phosphorus, but an increase when grown on an early successional. Two other AM fungal groups, one beneficial and one neutral,

showed the opposite trend. My results show that for a full accounting of plant community dynamics driven by AM fungi, the factors influencing belowground dynamics must be taken into account.

Introduction

Arbuscular mycorrhizal (AM) fungi associate with and improve resource uptake of most plant species (Hoeksema et al. 2018), and the presence and diversity of the AM fungal community has strong effects on plant community diversity and structure (Bauer et al. 2012; Wilson and Hartnett 1997; Hartnett and Wilson 1999; Heijden et al. 1998; Vogelsang, Reynolds, and Bever 2006). AM fungal composition matters, because AM fungal species are functionally different in their average and specificity of plant growth promotion (Cheeke et al. 2019; Hoeksema et al. 2018), impact on plant defense (Bennett and Bever 2009; Middleton et al. 2015), and in ability to aggregate soil (Schreiner and Bethlenfalvay 1997; Wang et al. 2020). Therefore, changes in composition of AM fungal communities can create feedbacks on plant fitness, community composition, and terrestrial function. For example, changes in AM fungal composition due to plant host identity can drive positive or negative plant soil feedbacks (Bever 1999; 2002a; Crawford et al. 2019; Koziol and Bever 2019; Mangan, Herre, and Bever 2010) which can be important for plant species coexistence and species turnover during succession. AM fungal differentiation could also generate local adaptation (Johnson et al. 2010; Rúa et al. 2016) or mediate impacts of environmental perturbations such as fertilization, tillage, or climate change (House and Bever 2018; Schütte et al. 2019). Understanding the factors that impact AM fungal community

composition is essential to building a comprehensive understanding of the drivers of mycorrhizal feedbacks on the plants and ecosystem processes.

Plant hosts can have strong effects on AM fungal composition. Plant host identity can alter sporulation rates and AM fungal species richness both in greenhouse and field experiments (Bever et al. 1996; Eom, Hartnett, and Wilson 2000). AMF species' fitness impacts from plant host identity have been shown to feedback to host plant growth rates (Bever 2002b). Plant species could differentially impact plant fitness through variation in preferential allocation (Bever et al. 2009; Steidinger and Bever 2016; Grman 2012) or the competitive ability of AMF species could differ with host.

Phylogenetic relationships may also be important, with previous studies demonstrating that plant species may respond differently to different phylogenetic groups of fungi (Hoeksema et al. 2010; 2018). If AM fungal groups respond differently to plant groups that would be evidence for phylogenetically informed feedbacks. A recent meta-analysis found that plant species origin and relatedness were both important factors in plant-soil feedbacks (Crawford et al. 2019), and a 2018 study found effects were greatest for regionally rare species (Kempel et al. 2018). However, conflicting results do exist. A study examining the effects on tomato crops and crop rotation found no significant negative feedback signal, but curiously did find significant phylogenetic distance effects on soil microbial composition (Kaplan et al. 2020). Schroeder et al. found phylogenetic structure to fungal community composition in roots of tree species in tropical forests (Schroeder et al. 2019). However, this study of environmental patterns cannot distinguish causal influence of changes of microbiome

composition. To date there has been no test of the influence of plant phylogenetic influence on AM fungal fitness and composition.

Soil disturbance such as tillage and fertilization can have marked effects on AM fungal composition (Stover et al. 2012; Li, Li, and Zhao 2007; House and Bever 2018) and diversity (Su and Guo 2007). AM fungal composition has been shown to change in response to fertilization (Jumpponen et al. 2005) as well as negative effects on AM fungal diversity (Santos, Finlay, and Tehler 2006). Fertilization has also been shown to promote the growth of less beneficial AM fungi (Johnson 1993). Such shifts are expected from evolutionary theory. Plants can also preferentially allocate resources to more beneficial symbionts, thereby promoting beneficial mutualists' growth and proliferation (Bever et al. 2009; Kiers et al. 2011). In the absence of such a regulating mechanism, it has been shown that in mixture less beneficial AM fungi will often have a competitive advantage (Bever 2002a; Bennett and Bever 2009; Bever et al. 2009). The primary benefit of AM fungi to host plants is the acquisition of phosphorus and when plant's need to obtain phosphorus through their mutualism is lessened, as it would be under phosphorus fertilization, plants have been shown to reduce their rates of preferential allocation to the most mutualistic fungi (Ji and Bever 2016). Proliferation of less beneficial AMF with fertilization is predicted from models of plant preferential allocation moderated by plant need, and if plants are regulating their investment in the AM fungal community, phosphorus enrichment would be expected to degrade the AM fungal communities' mutualistic quality overall (Bever 2015; Christian and Bever 2018).

Understanding how plant host identity, plant host phylogenetic relationships, and nutrient enrichment all shape soil community structure is important to understanding the system of feedbacks that drives the great biodiversity of plant communities. In order to test the effects of host characteristics on AM fungal communities, a greenhouse experiment was designed to measure the growth of a known community of AM fungi on a phylogenetically diverse group of early and late successional host prairie plants. A phosphorus treatment was also included in this experiment to test the effect of phosphorus enrichment on these communities, their mutualistic quality, and if they modulate the effects of host plant characteristics on AM fungal community structure. To fully understand plant composition, particularly in the prairie, a robust understanding of AM fungal community dynamics is essential. By testing the effects of host plant species identity, phylogenetic relationships, and phosphorus enrichment together, I hope to gain novel insights into how these factors shape ecological patterns across the prairie landscape.

The prairie was the chosen study system due to the importance and prevalence of AM fungal relationships in that system. The majority of prairie species form relationships with AM fungi with varying degrees of responsiveness (Bauer, Koziol, and Bever 2018; Bragg and Hulbert 1976; Collins and Wallace 1990). Prairie plant diversity has been promoted by increasing AM fungal richness, with a large selection effect for individual AM fungal species (Vogelsang, Reynolds, and Bever 2006). Late successional plants have been shown to be more responsive to AM fungal inoculation (Koziol and Bever 2015). Late successional plants also have greater sensitivity to AM fungal species identity (Cheeke et al. 2019; Koziol and Bever 2016). By including early and late

successional prairie plants in this experiment I have a variety in plant AM fungal responsiveness and specificity to AM fungal species identity.

Methods

A common community of AM fungi was replicated into separate mesocosms that were allowed to differentiate in response to host species and fertilization over two growing seasons. The composition of the AMF community was sampled after each growing season, 2017 and 2018. 38 species of host plants (Table 1) were grown in association with AM fungi. My host plants were chosen to provide a high degree of both phylogenetic diversity as well as diversity in successional stage (Figure 1). To ensure I had a consistent community of known AM fungi as my starting soil community, I inoculated my pots with a common mixture of seven cultured species (*Claroideoglomus lamellosum*, *Claroideoglomus claroideum*, *Entrophospora infrequens*, *Funneliformis mosseae*, *Racocetra fulgida*, *Cetraspora pellucida*, *Acaulospora spinosa*). Liquid phosphorus was added to half of the pots each year. In both years plants were allowed to grow for the entire growing season and were harvested in the fall. For the second year experiment, soil from each replicate was retained from year 1 was used as inocula for year 2 pots with the same host plant species. AM fungal composition was measured using next-gen Illumina™ sequencing. I assume over-representation of sequences as indicative of positive fitness impacts in plant or fertilizer treatments and under-representation indicative of negative fitness impacts.

Greenhouse Methods

Plants were grown in two-gallon pots with four individuals of a species to a pot in the first year. Reproductive structures were regularly trimmed to minimize seeds falling into the soil. Half of the pots were subject to a phosphorus fertilization treatment. Those pots received 80 mg cm^{-3} of dissolved granular Triple Superphosphate (0-46-0) in eight applications. After the first year half of the soil in each pot was retained as inocula for year 2. In the second year 1 gallon sized pots were inoculated with 500 cm^3 of the retained soil. Phosphorus treatments in the second year received 80 mg cm^{-3} in four applications.

Meta-analysis Methods

The seven AMF isolates used in this experiment were used previously in three separate studies testing their impact on early and late successional plant species: one reported in (Koziol and Bever 2016) and two in (Cheeke et al. 2019). I conducted a meta-analysis of these studies to obtain estimates of the growth promotion for each isolate for early, non-native, and late successional plants. I calculated the log mycorrhizal responsiveness (LRR) using equation (1) where $\bar{x}_{inoc}$ and $\bar{x}_{ctrl}$ are mean plant biomass for the inoculated treatments and sterile controls respectively. I estimated the sampling variance ($\hat{\sigma}_2$) of LRR using equation (2) where SD_{inoc} and SD_{ctrl} are the standard deviations and n_{inoc} and n_{ctrl} are the sample sizes of the treatment and controls respectively (Hoeksema et al. 2018; Hedges, Gurevitch, and Curtis 1999).

$$1)\ LRR = ln\left[\frac{\bar{x}_{inoc}}{\bar{x}_{ctrl}}\right]$$

$$2)\ \ \hat{\sigma}_2 = \frac{SD_{inoc}^2}{n_{inoc} \times \bar{x}_{inoc}^2} + \frac{SD_{ctrl}^2}{n_{ctrl} \times \bar{x}_{ctrl}^2}$$

The analysis was conducted using the metafor package in R (Viechtbauer 2010). Moderators were the life history of the host plants, AM fungal isolate used and the interaction of the two terms. Random effects included individual experiment and plant host species. The model was estimated using the restricted maximum-likelihood ("REML") method. Marginal means were estimated for the interaction terms of the life history of the host plants and AM fungal isolate using the emmeans package (Lenth et al. 2021). The metafor model was converted to a reference grid for compatibility with the emmeans package using the qdrg() function.

Bioinformatics Methods

To measure the changes in relative abundances of AM fungal species 0.2 g of soil was retained from each pot for Illumina™ sequencing. Extraction was performed on 311 samples using the Qiagen DNeasy Powersoil Kit. One sample in both years were lost during storage. DNA was amplified using the LROR and FLR2 primers (Trouvelot et al. 1999; Vilgalys and Hester 1990). Volumes of 12.5μL of Phusion® Hot Start Flex DNA Polymerase master mix, 0.5μL of each primer, and 10.5μL of MilliporeSigma™ Direct-Q™ 3 type I water per sample were combined with 1μL of extracted DNA. PCR was conducted using one 5 minute cycle at 94.0 C, thirty-five cycles alternating between 94.0 C for 30 seconds, 48.0 C for 30 secs, 72.0C for 30 seconds and 72.0 C for 10 minutes.

Bioinformatics was conducted using the Dada2 and Qiime 2 pipelines as described in (Delavaux et al. 2021). Primers were removed using Cutadapt. Using Dada2 forward

reads were truncated to a length where 95% of base pair Phred quality scores (Q scores) had a greater than 99% base call accuracy, with forward reads truncated to a length of 190bp and reverse reads to a length of 140bp. Forward and reverse reads were merged using the "justconcatonate" flag in Dada2 and chimeras were removed. Final sequences were then loaded into Qiime 2 and clustered to OTUs at 97% identity using the vsearch plugin. Taxonomy was assigned by building a phylogenetic tree of my sequences using RAxML together with an included library of known AMF species (Callahan et al. 2016; House et al. 2016, 29; Stamatakis 2014; Delavaux et al. 2021). All background soil was sterilized. Therefore, the OTUs that were not identified as one of the seven inoculated species were assumed likely to be legacy DNA from dead organisms in the background soil and were not included in the statistical analysis.

OTU's were assigned a species taxonomy if they clustered within known species sequences from my reference library. OTU's could be identified for *Funneliformis mosseae*, *Racocetra fulgida*, *Cetraspora pellucida*, *Acaulospora spinosa*. I could not distinguish between OTU's for *Claroideoglomus claroideum*, *Claroideoglomus lamellosum*, and *Entrophospora infrequens*. *Entrophospora infrequens* clusters within *Claroideoglomus* and this group shows low rates of divergence and evidence of ancestral polymorphism in the rDNA (House et al. 2016). I therefore characterized those OTU's as a single *Claroideoglomus* group. As a final check all identified sequences were blasted against the reference library. Several OTU's that were placed inside AMF had unacceptably long branch lengths and were suspected of being non homologous gene regions that were erroneously being placed in the phylogeny (Delavaux et al. 2022). This filtering step eliminated one dubious *Claroideoglomus* OTU from the analysis.

Plant Phylogenetic Methods

A phylogeny was constructed for all plants in the study as well to determine phylogenetic distance. Sequences were obtained for the rbcL gene for all 38 species of plants from the NCBI GenBank database. A constraint tree was obtained using the Phylomatic "Tree of Trees" software using the silk megatree (Slik et al. 2018; Webb and Donoghue 2005). Tree construction was conducted using RaXML (Stamatakis 2014).

Statistical Methods

OTU abundances for my inoculated AM fungal species were centered log transformed and a PERMANOVA analysis was conducted using the adonis() function in the R package vegan (Oksanen et al. 2020). The predictors used were host plant life history, host plant phylogenetic group, phosphorus treatment, the interaction of phosphorus treatment with plant life history, the interaction of phosphorus treatment with host plant phylogenetic group, and the interaction of host plant life history with host plant phylogenetic group. Phylogenetic groups are characterized as lilies (Amaryllidaceae and Commelinaceae), grasses, legumes, milkweeds, mints (Lamiaceae and Plantaginaceae) and asters (Apiaceae and Asteraceae).

Using a categorical factor, such as phylogenetic group, to analyze the effect of phylogenetic relationships is a limited approach. I used a method to include phylogenetic distances as a random effect to preserve the information included in the full phylogenetic tree. When visualizing both the phylogenetic relationships and AMF fungal composition there are hints at relationships that would be difficult to test using more traditional statistical methods (Figure 2). A phylogenetic generalized mixed model

(PGLM) was fitted using the phyr R package (Ives et al. 2020; Ives 2018). Phosphorus addition, host plant successional status, and the interaction between them were modeled as fixed effects. Block and the log transformed sequences were included alongside phylogenetic distance and species as random effects. As a univariate analysis each AM fungal species' OTUs were both grouped and analyzed separately with their abundances logit transformed. I also assessed Shannon's' Diversity as well as the logit-transformed proportion of my inoculated AMF over the total number of AMF OTU's. My reason for analyzing this proportion is that as the legacy background DNA would not be growing in response to my treatments, I can use the proportion of my inoculated AMF as a proxy for changes in fungal density.

We also tested the influence of plant phylogeny on AMF composition as represented using the ratio of variance explained by the phylogeny over total variation between plant species as represented by phylogenetic heritability (Lynch 1991) using equation (3).

$$3) \quad H_P^2 = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_T^2}$$

In my analysis, $\hat{\sigma}_a^2$ was equal to the variance estimate for the phylogenetic distance matrix and $\hat{\sigma}_T^2$ was equal to the sum of the variance explained by phylogenetic distance and species effects.

Results

Meta-analysis

The meta-analysis of the growth promotion by AMF isolates used in this study showed distinct separation between more and less beneficial fungi (Figure 3). *Claroideoglomus lamellosum*, *Claroideoglomus claroideum*, and *Entrophospora infrequens*, are all highly beneficial to late successional prairie plants. These three species are the ones I could not identify to species but, as they are all beneficial species, I expect the *Claroideoglomus* group to behave as beneficial OTUs. *Funneliformis mosseae* was also a beneficial species to late successional species, leaving *Racocetra fulgida*, *Cetraspora pellucida*, and *Acaulospora spinosa* as less beneficial species. No species had a significant effect on growth of early or non-native plant species.

PERMANOVA Analysis of AM Fungal OTU's

We conducted a PERMANOVA on centered log ratio (CLR) transformed AM fungal OTUs from my inoculated AM fungal species; *Claroideoglomus lamellosum*, *Claroideoglomus claroideum*, and *Entrophospora infrequens*, *Racocetra fulgida*, *Funneliformis mosseae*, *Cetraspora pellucida*, and *Acaulospora spinosa*. This analysis found statistically significant effects from multiple factors (Table 2). AM fungal species groups varied significantly across host plant species. Plant species is the factor that explains the greatest variation with a pseudo-R^2 of 0.149 in year 1 ($p<0.001$) and 0.246 ($p<0.001$) in year 2. Host plant phylogenetic group was also a significant factor and explained the second greatest amount of variation with a pseudo-R^2 of 0.56 ($p<0.001$) in year 1 and

0.77 ($p<0.001$) in year 2. In year 2 I also saw a significant effect of plant life history with a pseudo-R^2 of 0.007 ($p=0.04$). In year 2 phosphorus additions also had a consistent significant effect with a pseudo-R^2 0.012 ($p=0.005$), it also had a marginally significant effect dependent on host plant phylogenetic group 0.019 ($p=0.075$).

AMF Diversity and Proportion of AMF Identified

Shannon's diversity was weakly affected by plant life history in year 1 ($p\leq0.1$), which became significant in year 2 ($p\leq0.01$) (Table 3). In both years, the late successional plant species had more diverse AMF communities. Across both years, plant species differed strongly in the diversity of AMF communities within their root systems ($p<0.01$). The phylogenetic signal in these species differences was weak in year one ($p<0.1$) and became stronger in year two ($p\leq0.05$).

In year 1, the proportion of inocula AMF, which should be correlated with overall density, was consistently decreased by phosphorus addition ($p\leq0.1$), and specifically when associating late successional host plants ($p\leq0.1$) (Table 3). Proportion of AMF identified differed between individual plant species ($p<0.01$), but these effects were not consistent with the plant phylogeny.

Plant Life History and Phosphorus Effects on AMF Composition

Overall, *Claroideoglomus* was the most common single group (Figure 2). This was also the group made up of 3 of my 4 most beneficial AM fungal species as predicted by my meta-analysis. *Racocetra fulgida* was my second most common group, representing a species containing 3 distinct OTUs. *Funneliformis mosseae*, my predicted third most

beneficial fungi, was also my third most common species. It also represents 3 distinct OTUs. *Cetraspora pellucida* and *Acaulospora spinosa* were my fourth and fifth common species, each represented by a single OTU.

Funneliformis mosseae saw significant positive effects ($p \leq 0.01$) of growing with late successional hosts (Figure 4) and marginally significant positive effects of the interaction of plant life history and phosphorus addition ($p \leq 0.1$). This means that when *Funneliformis mosseae* was grown with a late successional host it proliferated when treated with a phosphorus fertilizer. The *Claroideoglomus* group saw marginally significant negative ($p \leq 0.1$) effects of phosphorus (Figure 5) and significant ($p \leq 0.05$) negative effects of the interaction of plant life history and phosphorus addition in year 1 (Figure 6). These mirror the results of my percent inoculated results showing a negative fitness impact of fertilization. *Racocetra fulgida* had a marginally significant ($p \leq 0.1$) positive effect from plant life history. Similar effects to *Funneliformis mosseae* were seen with *Cetraspora pellucida* in year 1 ($p \leq 0.05$). *Acaulospora spinosa* only saw a marginally significant positive fixed effect with phosphorus addition in year 1 ($p \leq 0.1$).

Plant Phylogeny and Species Effects on AMF Composition

AM fungal composition responded strongly to plant phylogenetic structure, as reflected by the significant phylogenetic distance variance component in most analyses (Table 4). In fact, the matrix of plant phylogenetic distance significantly predicted abundance of every AMF OTU in at least one year for all but *Funneliformis mosseae* taxa 718 (Table 4). This indicates that phylogenetically proximate plant species have more similar AM fungal composition, reflecting more similar impacts of host on AM fungal relative

fitness, than more phylogenetically distant plant species. For example, *Funneliformis mosseae* showed high relative growth rates with legumes, particularly late successional legumes (Figure 2). Generally *Claroideoglomus* did worse among the grasses, while *Racocetra fulgida* tended to perform best with grass species. *Cetraspora pellucida* and *Acaulospora spinosa* grew best with the Apocynaceae (which includes the milkweeds). Phylogenetic heritability had a minimum of 14.12% for *Racocetra fulgida* taxa 182 with the minimum for entire *Racocetra fulgida* species being 15.64% (Table 5). The maximum was 100.00% for the *Claroideoglomus* group. The greatest year to year swing was experienced by *Cetraspora pellucida* with 34.17% phylogenetic heritability in year 1 and 99.60% in year 2.

Discussion

Previous studies have demonstrated that AM fungal growth rates varied between individual plant species (Bever 2002a). I affirm that this result is general, as I found that the AM fungal composition diverged significantly across the 38 plant species studied. Moreover, I found that host-specific differentiation of AM fungal composition had a strong phylogenetic signature. That is, the growth rates of individual AM fungal species tended to be similar on phylogenetically similar plant species. This phylogenetic influence was evident in the abundance of the *Claroideoglomus* group, *Racocetra fulgida,* and *Cetraspora pellucida* in both years. It was evident in *Funneliformis mosseae* during year 1 and *Acaulospora spinosa* during year 2 (Table 4). Phylogenetic distance was also weakly evident in measures of AM fungal diversity. While previous work has shown phylogenetic signature to plant species specific differentiation of soil

bacterial and fungal communities (Kaplan et al. 2020), my study provides the first demonstration of phylogenetic structure to host-specific differentiation of AM fungal communities.

Previous work showing that microbiome composition can be influenced by plant genotype used measures of “microbiome heritability" to represent the proportion of microbiome variation explained by plant genetics (Wagner 2021). I use an analogous metric of phylogenetic heritability (sensu Lynch 1991) of microbiome composition to describe the differential influence of plant species on microbiome composition as predicted by the plant phylogeny. My measures of “phylogenetic microbiome heritability” identify that a large proportion of variation, ranging from 15.64% for *Racocetra fulgida* to 100.00% for the *Claroideoglomus* group, in species impacts on microbiome composition that can be explained by plant phylogeny. I note that this influence on AM fungal composition does not appear to result from differential exclusion of AM fungal species from roots, as would be expected from incompatibilities of plant-AMF signaling, as inoculated AMF were found with all plant species. Rather, given that inoculated AMF had equivalent density at the beginning of the experiment, the change in composition is due to host-specific differences in AM fungal growth rates (i.e. fitness). The substantial phylogenetic microbiome heritability that I observed may reflect that phylogenetically conserved plant traits, such as root architecture , may moderate plant influence on the dynamics of AMF (Valverde-Barrantes et al. 2017).

The differentiation of the AMF community composition with plant species and plant phylogeny can have ecological consequences because AM fungal species have distinct ecologies. As AM fungi exert differential impacts on plant growth rates (e.g.

Figure 2, Hoeksema et al. 2018; Cheeke et al. 2019), host-specific differences can generate feedback on plant growth that can alter plant-plant interactions (Bever, Platt, and Morton 2012). The phylogenetic structure of AMF differentiation observed here, combined with the observation of significant phylogenetic signal in plant response to AMF (Hoeksema et al. 2018), would suggest that strength of feedback through AMF composition would on average increase with the phylogenetic distance between pairs of plant species. Interestingly, such a phylogenetic signature has been observed in strength of feedback through the whole soil community (Crawford et al. 2019). While both positive and negative plant-AM fungal feedbacks have been observed (Bever 2002b; Castelli and Casper 2003; Mangan, Herre, and Bever 2010; Koziol and Bever 2019; Vogelsang and Bever 2009), further work is required to test whether feedbacks through AM fungal communities are affected by phylogenetic distance between the plant species.

We saw consistent effects of plant life history on AM fungal community composition in year 2. Even when testing across the variation in plant species and across the plant phylogeny, I see consistent differences in AMF composition across plant species of different life histories. This differentiation could differentially feedback on early and late successional plant species, thereby potentially influencing the course of succession, given that early and late successional prairie plant species have been shown to differ in their response to AMF species (Koziol and Bever 2016; Cheeke et al. 2019). In fact, my meta-analysis of previous studies shows that the *C. claroideum*, *C. luteum*, *F. mosseae*, and *E. infrequens* isolates used in this experiment differentially benefit late successional prairie plant species (Figure 3). As I observed *F. mosseae* to increase

with late successional species, this would be predicted to advantage late successional plant species in future generations. Such positive feedbacks through AM fungal composition have been observed in mesocosm experiments (Koziol and Bever 2019) and in field inoculations with prairie inocula (Middleton et al. 2015; Middleton and Bever 2012; Koziol and Bever 2017). Such positive feedback could accelerate succession, once it was initiated (Michaels, Eppinga, and Bever 2020). While I could not differentiate the two *Claroideoglomus* species and *Entrophospora infrequens*, the entire clade was differentially beneficial to late successional species (Figure 4), but tended to differentially accumulate with early successional plant species (Figure 6), which would be expected to generate negative feedback. This dynamic could counteract the influence of *F. mosseae* accumulation. Such complexity in feedbacks within a single mutualistic guild could contribute to heterogeneity and context dependence in realized feedbacks in the field.

Consistent phosphorus fertilizer impacts on AM fungal composition were also seen in AM fungal community structure in year 2. This was also true when accounting for phylogenetic distance. As predicted from theory (Bever 2015; Ghosh, Reuman, and Bever 2021), the beneficial *Claroideoglomus* group saw a decrease in their abundances when exposed to phosphorus fertilizer after 2 years. This would be consistent with a reduction in preferential allocation from the host plants when phosphorus was more abundant as observed in experimental manipulations (Ji and Bever 2016). Also consistent with reduced sanctions, I saw an increase after 2 years of P fertilization in the less beneficial *Acaulospora spinosa*. *I* did not see any main effect of phosphorus on diversity in contrast to previous studies (Santos, Finlay, and Tehler 2006). I also did not

see main effects in any other AM fungal species though this is likely due to the importance of interaction effects between plant life history and phosphorus addition which were seen in many AM fungal species.

The effect on phosphorus addition conditional on plan life history was not a significant predictor of AM fungal composition, but when I examined individual species accounting for phylogenetic distance, there were several notable interactions, showing that the effects of phosphorus were sometimes conditional on plant life history. Late successional plants are expected to be more strongly preferentially allocating to the best mutualist given observations of positive feedback (Koziol and Bever 2019). However, I do not know whether to expect late successional species to modify their rates of preferential allocation with P fertilization more so than early successional species.

Conclusions

AM fungal diversity is a complex story that we have only begun to fully unravel. My results show that not only do individual species of AM fungi vary in their response to environmental stressors and plant host characteristics, but there is significant intra-species variation in several cases. The relationship between AM fungal and host plant phylogenetic distances is potential evidence for specialization across phylogenetic groups. This suggests that in order to gain a full accounting of plant community dynamics where AM fungi are significant mutualist, we must incorporate the community dynamics below ground into our understanding.

My predictions of AM fungal responses based on my meta-analysis saw mixed success. The multi-species nature of my *Claroideoglomus* group may have complicated matters, but the consistency of results between individual OTUs and the group seem to confirm that this group behaves distinctly from less beneficial fungi. The changes to AM fungal community composition, density, and diversity were driven by plant life history, fertilizer addition, and phylogenetic relationships. Looking at how my individual strains promote growth in a follow-up growth assay will help to confirm whether the differences I saw in this experiment will drive positive or negative feedback effects, and how context dependent those effects are.

Tables and Figures

Table 1: Plant Species Used

Species	Family	Status	Species	Family	Status
Allium cernuum	Amaryllidaceae	late	*Chamaecrista fasciculata*	Fabaceae	early
Eryngium yuccifolium	Apiaceae	late	*Desmanthus illinoensis*	Fabaceae	early
Apocynum cannabinum	Apocynaceae	early	*Desmodium illinoense*	Fabaceae	early
Asclepias syriaca	Apocynaceae	early	*Lespedeza capitata*	Fabaceae	early
Asclepias tuberosa	Apocynaceae	late	*Lespedeza cuneata*	Fabaceae	early
Ambrosia artemisiifolia	Asteraceae	early	*Melilotus officinalis*	Fabaceae	early
Conyza canadensis	Asteraceae	early	*Amorpha canescens*	Fabaceae	late
Coreopsis tinctoria	Asteraceae	early	*Baptisia alba*	Fabaceae	late
Erechtites hieraciifolius	Asteraceae	early	*Dalea purpurea*	Fabaceae	late
Eupatorium altissimum	Asteraceae	early	*Monarda fistulosa*	Lamiaceae	early
Helianthus annuus	Asteraceae	early	*Salvia azurea*	Lamiaceae	early
Ratibida pinnata	Asteraceae	early	*Pycnanthemum tenuifolium*	Lamiaceae	late
Rudbeckia hirta	Asteraceae	early	*Bromus inermis*	Poaceae	early
Echinacea pallida	Asteraceae	late	*Elymus canadensis*	Poaceae	early
Liatris pycnostachya	Asteraceae	late	*Panicum virgatum*	Poaceae	early
Parthenium integrifolium	Asteraceae	late	*Setaria faberi*	Poaceae	early
Rudbeckia subtomentosa	Asteraceae	late	*Andropogon gerardii*	Poaceae	late
Silphium laciniatum	Asteraceae	late	*Bouteloua curtipendula*	Poaceae	late
Tradescantia ohiensis	Commelinaceae	early	*Schizachyrium scoparium*	Poaceae	late

Table 2: PERMANOVA of Center Log-Transformed OTU Abundances

		Year 1			Year 2		
	Df	**R2**	**Pr(>F)**	**Sig**	**R2**	**Pr(>F)**	**Sig**
Host Plant Life History	1	0.005	0.125		0.007	0.040	*
Host Plant Phylogenetic Group	5	0.056	0.000	***	0.077	0.000	***
Host Plant Species	31	0.149	0.000	***	0.246	0.000	***
Phosphorus Treatment	1	0.002	0.692		0.012	0.005	**
Sequencing Depth	1	0.007	0.034	*	0.003	0.260	
Block	3	0.017	0.009	**	0.015	0.032	*
Host Plant Life History by Phosphorus Treatment	1	0.005	0.105		0.003	0.314	
Host Plant Phylogenetic Group by Phosphorus Treatment	5	0.017	0.234		0.019	0.075	·
Host Plant Species Group by Phosphorus Treatment	31	0.097	0.222		0.081	0.295	

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; · $p < 0.1$

Table 3: Pglmm of Diversity and Logit-Transformed Proportion Identified Separated by Year

Group	Year	Plant life history (late)			Phosphorus (added)			Plant life history by phosphorus			Phylogenetic Distance†			Species†			Block†	Log Sequencing Depth†	Intercept
		Est	S. Err	Sig	Est	S. Err	Sig	Est	S. Err	Sig	Var	S. Dev	Sig	Var	S. Dev	Sig	Sig	Sig	Sig
Shannon's Diversity by Species	**1**	0.0569	0.0294	*							0.0032	0.0562	*	0.0157	0.1252	***	**	**	***
	2	0.0831	0.0225	***							0.0012	0.0343	**	0.0114	0.1069	***		**	***
Shannon's Diversity by OTU	**1**	0.0582	0.0332	*							0.0043	0.1421	*	0.0202	0.1421	***	*	**	***
	2	0.1034	0.0272	***							0.0014	0.0377	*	0.0168	0.1297	***	*	**	***
Proportion Inocula vs Background	**1**				42.830	23.269	*	-40.025	22.046	(*)				61,410	247.82	***	***		***
	2													34,390	185.44	***			***

*** p < 0.01; ** p < 0.05; * p < 0.1; () = negative fixed effects

Table 4: Pglmm of Logit Transformed OTU Abundances Separated by Year

Group	Taxa	Year	Plant life history (late)			Phosphorus (added)			Plant life history by phosphorus			Phylogenetic Distance†			Species†			Log Sequencing Block†	Log Sequencing Depth†	Intercept
			Est	S. Err	Sig	Est	S. Err	Sig	Est	S. Err	Sig	Var	S. Dev	Sig	Var	S. Dev	Sig	Sig	Sig	Sig
Clariodeoglomus	Taxa 10	1							-0.254	0.119	(**)	0.634	0.796	***	6.8E-08	2.6E-04	***	***	***	
		2										1.556	1.247	***	1.6E-03	0.040	***	***	***	
	Taxa 116	1										0.822	0.907	***	0.254	0.504	***	***	***	(***)
		2				-0.506	0.184	(***)							2.497	1.580	***		*	(***)
	Taxa 1528	1				-0.202	0.099	(**)	-0.177	0.099	(*)	0.025	0.158	*	0.058	0.241	***	*	*	(***)
		2													0.345	0.587	***			(***)
	All Taxa	1							-0.313	0.147	(**)	0.763	0.873	***	0.000	0.001	***	***	***	
		2				-0.644	0.303	(**)				3.026	1.740	***	5.002	2.237	***	***	***	
mosseae	Taxa 62	1	0.658	0.248	***				0.417	0.226	*	0.213	0.461	***	0.070	0.264	***	***	***	(***)
		2													2.879	1.697	***			(***)
	Taxa 353	1										0.203	0.451	**	0.111	0.333	***	**	**	(***)
		2	0.364	0.199	*										1.166	1.080	***			(***)
	Taxa 718	1													0.084	0.290	***		*	(***)
		2	0.225	0.107	**										0.283	0.532	***			(***)
	All Taxa	1	0.656	0.252	***				0.423	0.229	*	0.217	0.465	***	0.081	0.285	***	***	***	(***)
		2													2.990	1.729	***			(***)
fulgida	Taxa 37	1										0.572	0.756	**	3.306	1.818	***	**	***	(***)
		2										1.336	1.156	***	4.539	2.131	***	***	***	(***)
	Taxa 119	1													1.791	1.338	***			(***)
		2										0.352	0.593	***	1.817	1.348	***	***	***	(***)
	Taxa 182	1										0.288	0.537	*	1.751	1.323	***	**	*	(***)
		2										0.878	0.937	***	4.2E-05	6.5E-03	***	***	***	(***)
	All Taxa	1										0.652	0.807	**	3.514	1.875	***	**	***	(***)
		2	0.835	0.502	*							1.380	1.175	***	5.563	2.359	***	***	***	(***)
pellucida	*Taxa 71*	1	0.682	0.346	**				0.467	0.208	**	0.870	0.933	**	1.676	1.294	***	***	***	(***)
		2										0.267	0.517	**	0.001	0.033	***	**	***	(***)
spinosa	*Taxa 236*	1													1.303	1.141	**			(***)
		2				0.090	0.050	*				0.012	0.109	**	1.2E-06	1.1E-03	**	**	***	(***)

*** p < 0.01; ** p < 0.05; * p < 0.1; () = negative fixed effects: †Significance for random effects are calculated with a Likelihood Ratio Test using the Chi square distribution

Table 5: Phylogenetic Heritability

Group	Taxa	Year	Phylogenetic Heritability	Sig
Claroideoglomus	Taxa 10	**1**	99.99%	***
		2	99.89%	***
	Taxa 116	**1**	76.38%	***
		2	14.62%	
	Taxa 1528	**1**	30.07%	*
		2	1.28%	
	All Taxa	**1**	100.00%	***
		2	37.69%	***
mosseae	Taxa 62	**1**	75.26%	***
		2	7.21%	
	Taxa 353	**1**	64.72%	**
		2	3.59%	
	Taxa 718	**1**	41.82%	
		2	1.25%	
	All Taxa	**1**	72.72%	***
		2	8.09%	
fulgida	Taxa 37	**1**	14.75%	**
		2	22.74%	***
	Taxa 119	**1**	12.77%	
		2	16.21%	***
	Taxa 182	**1**	14.12%	*
		2	100.00%	***
	All Taxa	**1**	15.64%	**
		2	19.88%	***
pellucida	*Taxa 71*	**1**	34.17%	**
		2	99.60%	**
spinosa	*Taxa 236*	**1**	0.00%	
		2	99.99%	**

*** p < 0.01; ** p < 0.05; * p < 0.1

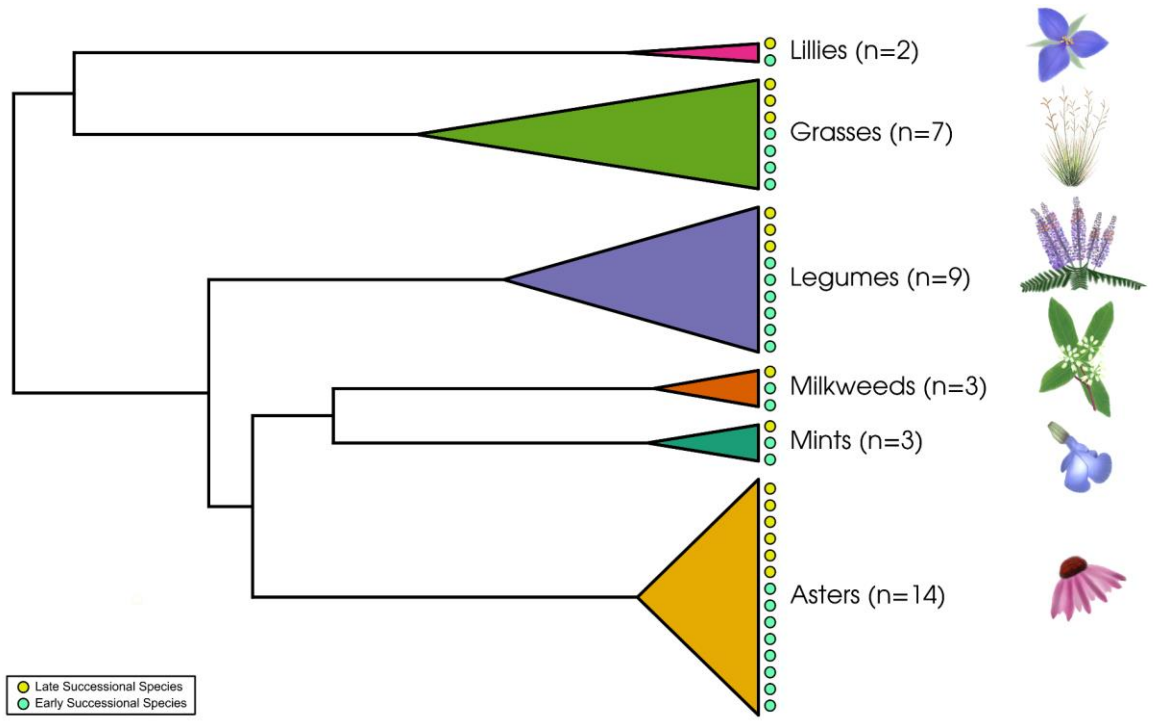


Figure 1: Host Plant Characteristics

Plant species were selected to represent a phylogenetically diverse group of early and late successional plants. Plants were selected from 5 large phylogenetic groups; grasses (*Poaceae*), legumes (*Fabaceae*), asters (*Asteraceae* and *Apiaceae*), mints (*Lamiaceae* and *Plantaginaceae*), milkweeds (*Apocynaceae* and *Asclepiadaceae*), and lilies (*Liliaceae* and *Commelinaceae*).

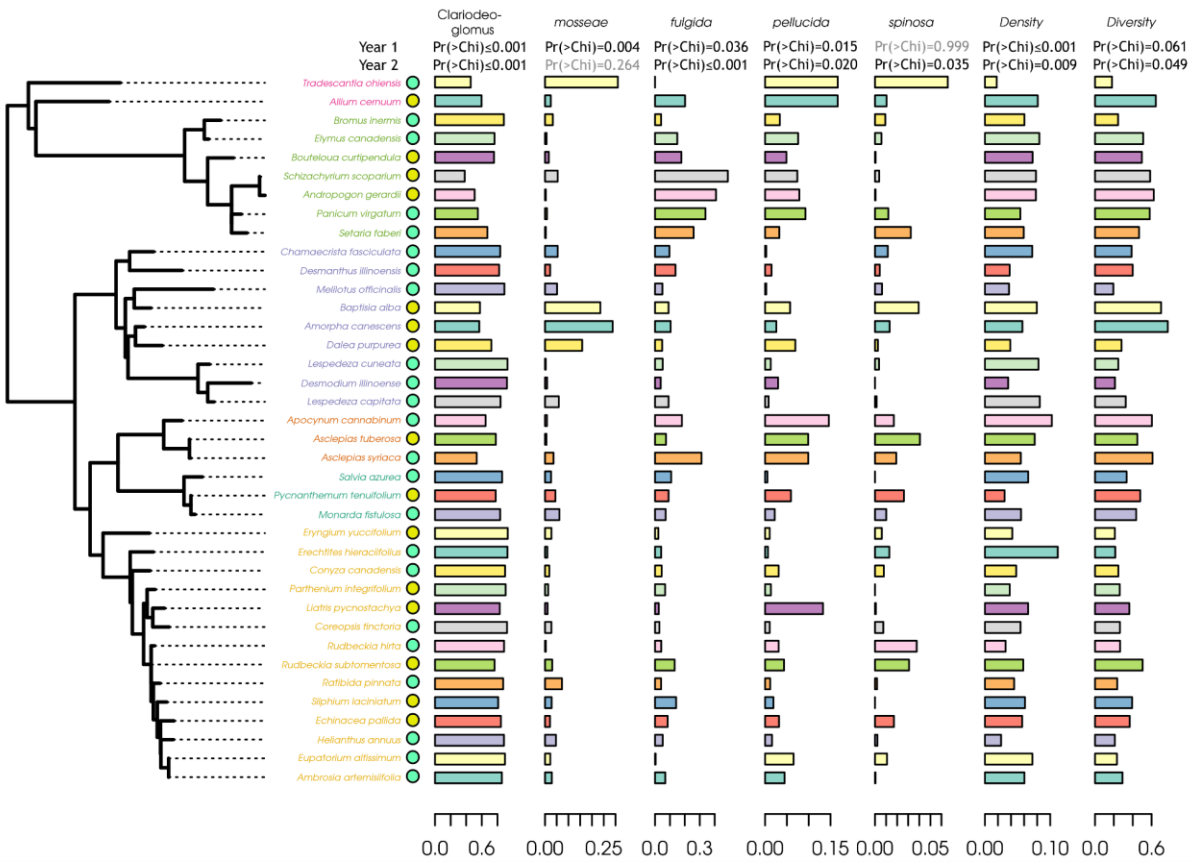


Figure 2: OTU Relative Abundances Across Plant Phylogeny

AM fungal Relative abundances for the *Claroideoglomus* group (*Claroideoglomus lamellosum*, *Claroideoglomus claroideum*, *Entrophospora infrequens)*, *Funneliformis mosseae*, *Racocetra fulgida*, *Cetraspora pellucida*, and *Acaulospora spinosa* are presented alongside estimated abundance (proportion of OTUs identified as inoculated over total AM fungal OTUs) and Shannon's Diversity. Results are by host plant species arranged phylogenetically. Significance effects are calculated with a Likelihood Ratio Test using the Chi square distribution.

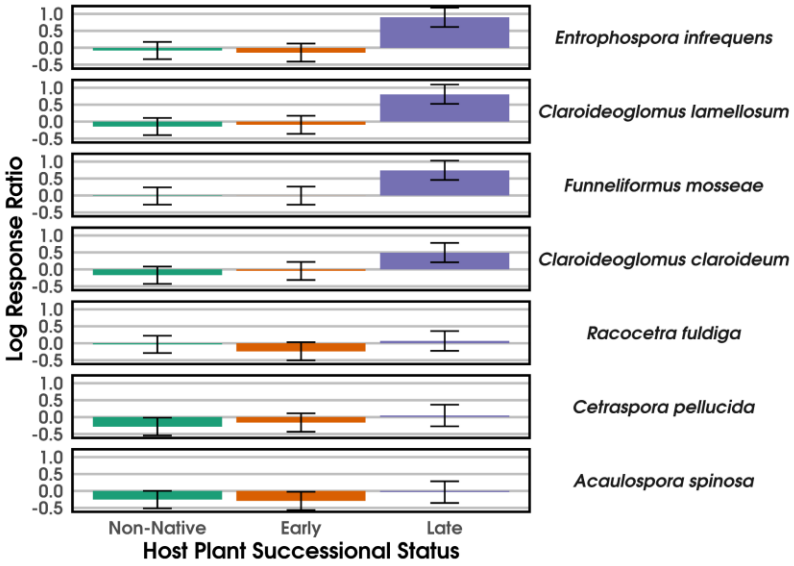


Figure 3: Meta-Analysis Results

The marginal means for the log response ratio's (LRT) of each of the seven AM fungal species used in this study. The LRT is the natural log of the ratio between how large plant species grew with AM fungal inoculation versus how they grew in a sterile control. These are derived from a meta-analysis of three previous experiments using these same cultures. Late successional plants showed benefits when grown in association with *Entrophospora infrequens*, *Claroideoglomus lamellosum*, *Funneliformis mosseae,* and *Claroideoglomus claroideum*.

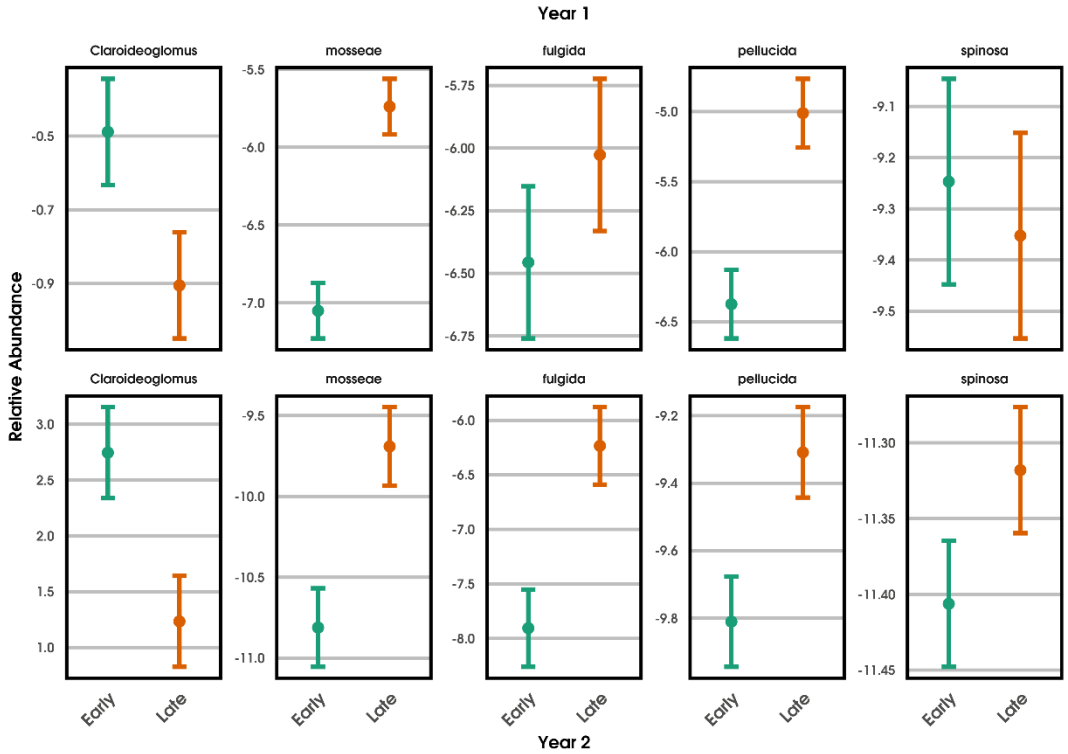


Figure 4: Marginal Means of Relative Abundance by Plant Life History

These are the estimated marginal means of AM fungal relative abundances when host plants were early or late successional. AM fungal species are arranged left to right in order of most to least beneficial. Significant differences were seen for the relative abundance of *Funneliformis mosseae*, and *Cetraspora pellucida* in year 1, and *Racocetra fulgida* in year 2.

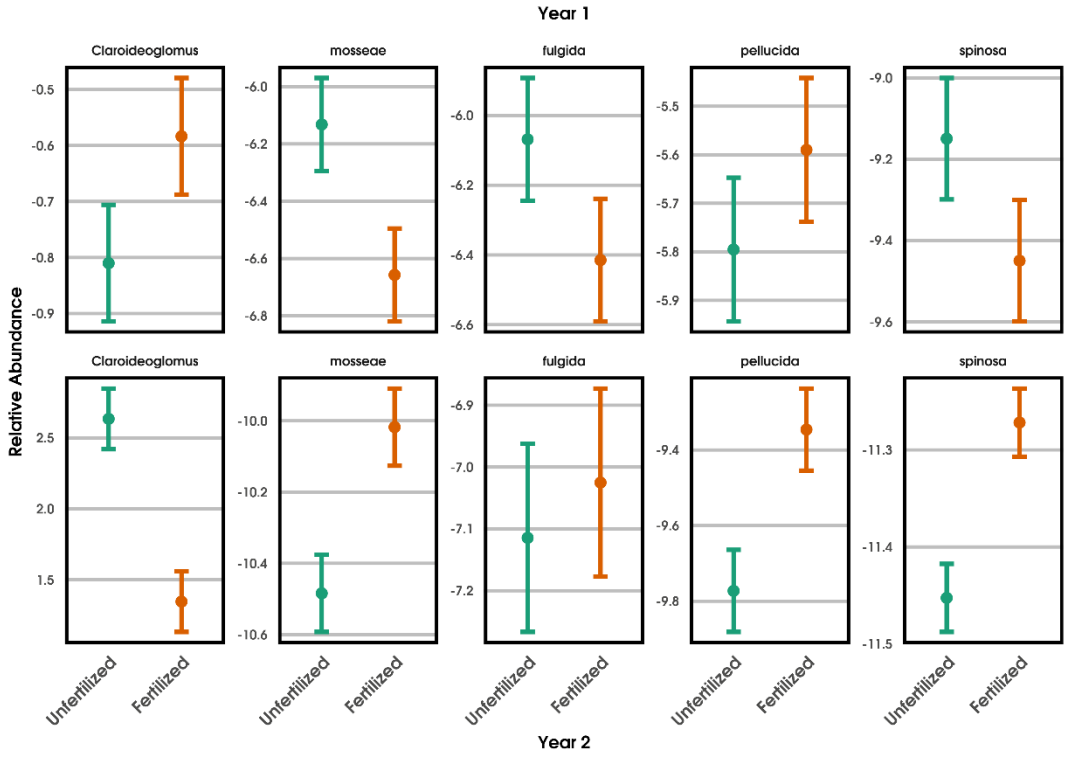


Figure 5: Marginal Means of Relative Abundance by Phosphorus

These are the estimated marginal means of AM fungal relative abundances when exposed to high or low phosphorus treatments. AM fungal species are arranged left to right in order of most to least beneficial. The *Claroideoglomus* group and *Acaulospora spinosa* had significant differences in relative abundances in year 2.

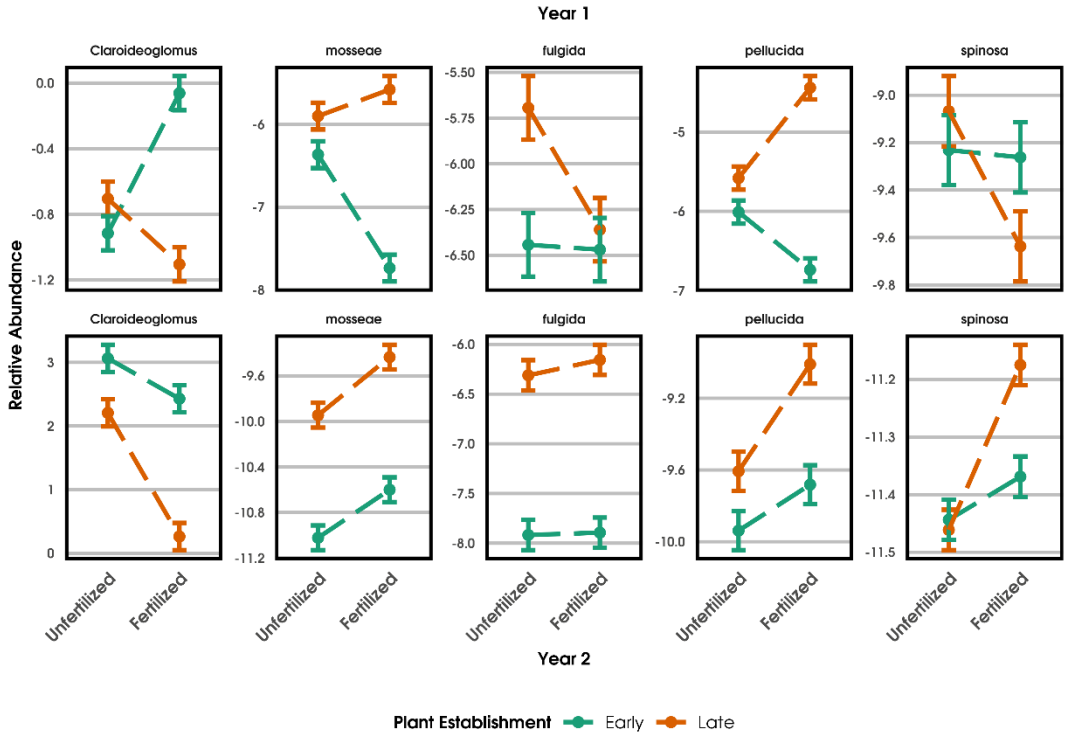


Figure 6: Marginal Means of Relative Abundance by the Interaction of Plant Life History and Phosphorus

These are the estimated marginal means of AM fungal relative abundances when exposed to high or low phosphorus treatments conditional on host plant life history (early vs late successional). AM fungal species are arranged left to right in order of most to least beneficial. The Claroideoglomus group, Funneliformis mosseae, and Cetraspora pellucida saw significant effects in year 1. Claroideoglomus relative abundances increased with fertilization when the host plant was an early successional but decreased when the plant was a late successional. Funneliformis mosseae and Cetraspora pellucida had decreased relative abundance when the host plant was an early successional. Cetraspora pellucida also had increased relative abundances when the host plant was a late successional.

Chapter 3: The Impact of Phylogenetic Relationships and Life History on Mycorrhizal Feedback Effects

Abstract

The mechanisms contributing to the maintenance of biodiversity have implications for our understanding of plant community dynamics, for land management, and for restoration projects. Plant microbiome feedbacks are driven by reciprocal fitness impacts between plants and their microbiomes, and differential impacts of mutualists and competing hosts can create scenarios where positive feedback is no longer the outcome of mutualistic relationships. Specific AM fungal species can promote growth in late successional plants, and these effects have been shown to advance succession in grassland restorations. Phylogenetic relationships are also important and plant species may respond differently to different phylogenetic groups of fungi. Two growth assays were conducted to assess the host fitness impacts of communities of AM fungal species on a phylogenetically diverse selection of 38 early and late successional plant species trained by association with the same set of plant species in the previous growing season. When looking at pairwise feedback effects, feedbacks ranged from significantly positive to significantly negative, with positive feedbacks being most likely to be seen between early successional plant species, or between an early successional and a late successional plant species. By the second year I saw a significant improvement in biomass accumulation for plants grown in soil trained by late successional plants when the host plant itself was a late successional. Early successionals favored soil communities trained by the host plant's close relatives in both years. Early

successionals promote less phylogenetically diverse communities, and late successionals promote the growth and retention of late successionals which has implications for succession. Late successional plants needed at least two generations before their effects were strongly felt. This is evidence that late successionals are slower at developing positive feedback effects then early successionals.

Introduction

The mechanisms contributing to the maintenance of biodiversity remain an important area of interest in ecology. These mechanisms have implications for our understanding of plant community dynamics, for land management, and for restoration projects. Several mechanisms have been proposed as contributing to the maintenance of biodiversity, including resource partitioning and abiotic facilitation (Kahmen et al. 2006; McKane et al. 2002; Wright et al. 2017; Barry et al. 2019). Plant-microbiome interactions is another framework which is being increasingly studied (Bever et al. 2010). Previous research has shown that positive feedbacks, such as those that can come from mutualistic interactions, can lead to a loss of species diversity, while negative feedbacks often driven by host specific pathogens can lead to a stabilization of community diversity (Bever, Westover, and Antonovics 1997; Bever 2003).

Plant microbiome feedbacks are driven by reciprocal fitness impacts between plants and their microbiomes. As a single plant species becomes dominant in a community, the structure of the soil community changes in response. Both beneficial and harmful organisms who associate with the dominant plant species will build up in the soil. If the change in the microbiome improves the fitness of the dominant plant

species relative to its competitors, it dominates and excludes its competitors. This would lower the diversity of the plant community as a whole. If the inverse is true a buildup of detrimental soil organisms causes the fitness of the associated plant to decrease relative to competitors at higher plant densities. This effect can be observed through the phenomenon of conspecific negative density dependence. This is where juveniles of a plant species can often be found at the greatest density not where propagule pressure is highest, nearest its conspecifics, but at the far extents of the dispersal range of conspecific adults, where juveniles can escape host specific pathogens (Janzen 1970; Connell, Den Boer, and Gradwell 1971; Johnson et al. 2012; Packer and Clay 2000; Mangan, Herre, and Bever 2010). Traditionally the effects of mutualists are expected to contribute to positive feedbacks, but it is possible for mutualists to contribute both to positive and negative feedbacks though differential fitness impacts (Bever, Westover, and Antonovics 1997; Bever 1999).

The differential impacts of mutualists can create scenarios where positive feedback is no longer the outcome of mutualistic relationships. Previous research has established a model for understanding how negative feedback could be exerted in a mutualistic system through interguild frequency dependence (Bever 1999). This framework predicts that depending on the symmetry of fitness relationships between multiple hosts and their mutualists, there can be either positive or negative feedbacks (Figure 1). During traditional positive feedback, plant A gains the greatest fitness benefit from soil mutualist α. Likewise, mutualist α has the greatest fitness when associating with plant A. Under such symmetric fitness relationships, the system will experience positive feedback and either plant A or plant B will be competitively

excluded. However, If the relationships are not symmetrical other outcomes are possible. If mutualist ß grows best in association with plant A but confers the greatest fitness benefit to plant B, then an increase in the density of plant A results in a greater density in mutualist ß and a relative increase in the fitness of plant B. This can create a dynamic similar to what is experienced under host specific pathogen load. No one plant species can become too dominant, because as it proliferates the plant increases the relative fitness of its competitors.

Host-specific changes in AM fungal growth rates have been demonstrated to generate feedback on plant fitness empirically. Both positive and negative feedback through AM fungal communities have been observed (Bever 2002b; Middleton et al. 2015; Garcia-Parisi and Omacini 2017). There is evidence of positive feedback driven by degradation of mutualisms during invasion (Vogelsang and Bever 2009; Burke 2008). There is also a growing body of evidence of positive feedback between early and late successional prairie plant species which have implications for succession.

Late successional plants have been shown to be more responsive to AM fungal inoculation (Koziol and Bever 2015). Late successional plants also have greater sensitivity to AM fungal species identity (Cheeke et al. 2019; Koziol and Bever 2016). Studies have shown that the strength of plant soil feedback effects correlate with plant successional stage (Bauer, Mack, and Bever 2015). AM fungi have been shown to increase plant diversity by increasing the fitness of late successional plant species (Koziol and Bever 2019). This effect was not driven by AM fungal diversity, but by composition. Low or high diversity treatments provided benefits if they contained specific AM fungal species that promoted growth in late successional plants. These effects have been

shown to advance succession in grassland restorations (Middleton and Bever 2012). By including early and late successional prairie plants in this experiment I have a variety in plant AM fungal responsiveness and specificity to AM fungal species identity.

Phylogenetic relationships may also be important with previous studies demonstrating that plant species may respond differently to different phylogenetic groups of fungi (Hoeksema et al. 2010; 2018). A recent meta-analysis found that plant species origin and relatedness were both important factors in plant-microbial feedbacks (Crawford et al. 2019), and a 2018 study found effects were greatest for regionally rare species (Kempel et al. 2018). Additionally phylogenetic distance can help test plant-microbial feedback effects. If a host plant grows better in a microbial community trained by a previous plant with a low phylogenetic distance, then close relatives are creating more favorable soil communities and I am seeing positive feedback effects. If I see better growth when there is greater phylogenetic distance to the training plant, then close relatives have less favorable soil communities, and I am seeing negative feedback effects.

During the summers of 2018 and 2019, two growth assays were conducted to assess the host fitness impacts of communities of AM fungal species on a selection of 38 plant species trained by association with the same set of plant species in the previous growing season. A series of factorial experiments were conducted to measure the performance of my plant species in their own conspecific soils and those of their competitors. I selected a phylogenetically diverse group of host plants for this assay which will allow me to simultaneously test the impact of phylogenetic distance and life history characteristics on these fitness relationships.

Methods

In 2017 38 plant species were grown in sterile 50/50 soil sand mix and inoculated with a common mixture of seven AMF cultures (*Claroideoglomus lamellosum*, *Claroideoglomus claroideum*, *Entrophospora infrequens*, *Funneliformis mosseae*, *Racocetra fulgida*, *Cetraspora pellucida*, *Acaulospora spinosa*). They were allowed to grow for an entire growing season and harvested in the fall. Soil from each pot was retained and then used as inocula for a second growing season in 2018. In 2018 two growth assays were conducted using soil from the 2017 training experiment. In 2019 a third growth assay was conducted using soil retained from the 2018 training experiment. To limit the overall size of the experiment plant species were not grown in soil trained by every other plant species in the experiment. The exact design differed between 2018 and 2019. In 2018, Experiment One used 8 species in a fully reciprocal design, experiment two prioritized crossing the major plant families reciprocally. In 2019, the experiment had host plant species subsetted into 5 fully reciprocal combinations. These Above and below ground biomass was collected from all the plants in 2019 while only above ground was taken for all the plants in 2018.

Greenhouse Methods

In 2018, two separate experiments were conducted. Experiment One had eight species of plants each paired with all the soil communities associated with the seven other plant species and itself. Experiment Two was designed so that the principle plant families (Asteraceae, Fabaceae, and Poaceae) were fully reciprocal with early and late successional species of each family. Above and below ground biomass was collected

from all the plants in the year 1 part 1 experiment. Due to time and labor constraints only above ground biomass was collected for some of the species in the year 1 part 2 experiment.

In 2019 an additional growth assay was conducted. An alternative design was used that emphasized estimation of pairwise feedbacks, where 5 nested experiments were conducted as separate blocks. Each block was a fully reciprocal experiment where each plant species was grown in its own soil and soil conditioned with every other plant species in the same block. The blocks were designed so that one of the principle plant families would be over represented so that the phylogenetic relationships within and outside of each family could be analyzed. In 2019 I increased the number of phylogenetic groups under consideration into grasses, legumes, asters, mints, milkweeds, and lilies. Lilies were the smallest group and did not get their own block in the experimental design.

Statistical Methods

We used a method to include phylogenetic distances as a random effect to preserve the information included in the full phylogenetic tree. A phylogenetic generalized mixed model (PGLM) was fitted using the phyr R package (Ives et al. 2020; Ives 2018). Host plant life history, training plant life history, and the interaction between them were modeled as fixed effects as well as the phylogenetic distance of host plant to the training plant. The log transformed initial host plant size (height multiplied by leaf count) for each plant species was included in both years data as a covariate. Total days grown were included as covariates in 2019 but could not be included in 2018 as harvest

dates were not collected for several host species. Block and species were included alongside the host and training phylogenetic distance matrix as random effects. In the end, for the 2018 data I decided to combine log transformed above ground biomass measurements from experiment one and two in order to include all the plants in my study. For 2019, I included log transformed total biomass measurements as my response variable.

Phylogenetic distance is useful to test the effect of relatedness between host and training plant species, but it does not easily separate out the effects of being raised in soil trained by a conspecific — which would have a phylogenetic distance of 0. A second model was constructed to attempt to ascertain the special effect of being raised in soil trained by a conspecific plant. I used a three level factor to divide pairings into Conspecifics, Same Family, and Different Family groups. This factor was analyzed for both consistent effects as well as differing effects when the host plant was an early or late successional species. This model also analyzed the consistent effects of host and training plant life history, as well as their interaction.

In the 2019 experiment, I purposely included full factorial tests of plant species and training plant species for subsets of the 39 plant species. Within these subsetted pairs of plant species I am able to estimate pairwise feedbacks that govern the influence of mycorrhizal fungal dynamics on plant-plant interactions. Pairwise feedbacks are measured by the interaction coefficient (I), as derived from the models of interguild frequency dependence and host-microbiome feedback and is calculated according to equation (1) (Bever, Westover, and Antonovics 1997; Bever 1999).

$$1) \quad I = F_{A\alpha} - F_{A\beta} - F_{B\alpha} + F_{B\beta}$$

Where $F_{A\alpha}$ is the fitness of plant A in soil α, $F_{A\beta}$ the fitness of Plant A in soil β, $F_{B\alpha}$ the fitness of plant B in soil α, and $F_{B\beta}$ the fitness of plant B in soil β (Figure 1). If this coefficient is positive then the plants are experiencing positive feedback, and if the coefficient is negative the plants are experiencing negative feedbacks. I used a type III ANOVA analysis to test the effect on the interaction coefficient from host plant and training plant life history and the interaction between them.

Results

Host Relatedness to Training Plant Factor Model

In my model which examined host relatedness to training species as a three level factor I saw significant differences between 2018 and 2019 results. Host relatedness to training species was not a significant predictor of total biomass in 2018, but by 2019 I saw a significant ($p=0.052$) effect of relatedness (Table 1). This was driven by a consistent increase in total biomass when host plants were grown in soil trained by a conspecific (Figure 3). In 2019 I also saw an additional marginally significant effect of host plant relatedness to the training plant that was conditional on host plant life history ($p=0.065$), as the benefit of growing in conspecific trained soil was particularly strong for early successional plant species (Figure 4). There were no significant effects of host or training plant life history nor their interaction, in either year. In 2018 I saw significant random effects of both host and training species ($p\leq0.01$), but not of the phylogenetic distance between host plants or for phylogenetic distance between training plants. By 2019, I do see a significant effect on total biomass of the phylogenetic distance between training plants, as well as species effects for both host and training plants ($p\leq0.01$).

Host Phylogenetic Distance to Training Plant Model

When I examined the effect on total biomass using a model that included phylogenetic distance between plant species and training plant species, I gained additional insights into my data. Notably, the effect of training plant life history, conditional on host plant life history, was a significant predictor in 2019 (p=0.039) (Table 2). I generally saw positive feedback between plant species of different successional stages, as early and late successional species grew better in soils trained by other species of a similar life history category. This effect was strongest for late successional plant species (Figure 5). The interaction of plant life history by phylogenetic distance from host to training plant species was marginally significant in 2018 (p=0.076). When the host was an early successional species, total biomass decreased with increasing phylogenetic distance, with late successional species showing the opposite pattern (Figure 6). In year 2 the overall interaction was not significant, but when testing only early successional host plants I found a similar negative slope to be marginally significantly different than zero (p=0.095).

Pattern of Pairwise Plant-AM Fungal Feedback

For data collected in 2019, I was able to estimate pairwise feedback between a large number of species pairs. These individual pairwise feedback values varied from significantly positive to significantly negative (Figure 7). Signed rank test of all pairs of plant species showed a significant positive median value for pairwise feedbacks (p = 0.003). In comparing only pairs of early successional plant species, the median pairwise feedbacks tended to be positive (p=0.069), and the mean feedback was significantly

positive (t_{41}=2.654, p=0.01, Figure 8). When considering only feedback between early and late successional plant species, both the median (p=0.009) and mean values (t_{68}=3.055, p=0.003) were significantly positive. When both plant species were late successional, neither the median (p=0.488) nor the mean (t_{14}=-0.009, p=0.993) feedback were significantly different from 0.

Discussion

By testing across 38 plant species and across two years of training, this study provides the most comprehensive test of feedback on plant growth through the AM fungal community to date. Consistent with pairwise mutualism theory (Bever 1999; Umbanhowar and McCann 2005; Jiang et al. 2020; Abbott et al. 2021), both positive and negative pairwise feedback were observed, confirming the potential for AM fungal dynamics to contribute to both plant species coexistence and competitive exclusion. Across all pairs of species tested, the median value of feedback through AM fungal community was positive, consistent with the fitness effects of plants and AM fungi generally being correlated. However, observations of significant negative feedback between individual pairs of species, as is consistent with previous studies (Bever 2002b), identifies that the fitness relations are not always positively correlated. Given this range of dynamics and underlying fitness, caution should be used in generalizations from theory based on assumptions of positive correlations of fitness between mutualist partners (e.g. Bascompte and Jordano 2013). My study provides guidance to build a predictive framework for understanding the direction and strength of plant-AM fungal community feedbacks.

Across 38 plant species, I generally find positive feedback between early and late successional plant species, both between any species in these life history categories (as presented in Fig. 4b) and between specific pairs of plants (as represented in Fig. 7). Late successional species have previously been shown to be generally being more responsive to AM fungi (Koziol and Bever 2015; 2016; Bauer, Koziol, and Bever 2018) and more sensitive to AM fungal identity (Cheeke et al. 2019) than early successional species. Monitoring of the change in AM fungal composition gave an ambiguous signal of feedback, as it showed *F. mosseae*, which differentially benefits late successional plant species, to increase with late successional hosts, but it also showed Claroideoglomeraceae species, which also favor late successional plant species growth, to decrease (Chapter 2). My results show that the net effect of these AM fungal dynamics is to favor late successional species when late successional species are hosts. This result is consistent with mesocosm studies showing positive frequency dependence of late successional plant species when beneficial AM fungi are present (Koziol and Bever 2019), as expected from positive feedback through AM fungal composition. My observation of positive feedback between early and late successional species are also consistent with the accumulated evidence that inoculation with AM fungi from undisturbed prairies improves establishment and growth of late successional plant species when inoculated with AMF from unplowed (hence late successional) prairies (Koziol et al. 2021; Koziol and Bever 2017; Koziol et al. 2018; House and Bever 2020).

Positive pairwise feedback effects were also likely to be seen when both hosts were early successional plant species. While early successional species have been shown to be less variable in their responses to AM fungal isolates than late successional species

(Cheeke et al. 2019), my results show that there is still sufficient variation in response to AM fungi among early successional species to generate feedback. Moreover, my results show that relatively responsive early successional plant species also tend to be better hosts for AM fungi. Conversely, for pairs of late successional species which have been shown to be more sensitive to AM fungal identity, AM fungal feedbacks were less likely to deviate from neutral. Perhaps this reflects late successional species being more consistently good hosts for AM fungal species. In the absence of differences in "hostiness" of plant species there will be no net feedbacks (Abbott et al. 2021).

Across all species, AM fungal feedbacks were also structured by phylogeny. This is evident in year 2 plants generally grew better with AM fungal communities trained by conspecifics compared to other species from their same plant family (Figure 3b). This effect was particularly significant in early successional plant species (Figure 4b). Considering phylogenetic distance more continuously, I find that the growth benefits that early successional species receive from their soil community decreases with phylogenetic distance and that this effect was strongest after the first growing season (Figure 6). An inverse trend could be seen late successionals after the first year of training, with late successional plants growing best when the soil community was trained by a more distant relative. These phylogenetically structured AM fungal feedbacks could drive early successional plant communities to be less phylogenetically diverse than late successional plant communities.

More generally, this study provides the most comprehensive test to date of the variation in plant species as hosts for AM fungal communities. I find strong and consistent differences between host plant species in their impact on the AM fungal

community's average growth promotion, as evidenced by the highly significant plant host species variance component across both years (Tables 1 and 2). Moreover, by year 2, the variation between plant species in their impact on AM fungal average function was significantly structured by plant phylogeny. That is, species that are phylogenetically related tended to have similar average effect on AM fungal average function, i.e. plant impacts on AM fungal function is phylogenetically heritable (Lynch 1991). This result is a logical expectation from the observation that AM fungal composition was phylogenetically heritable (Chapter 2).

Some of the feedback effects of early successional host plants seem to be measurable in 2018, but not the effects of feedback between late successionals. Late successional plants needed at least two generations before their effects were strongly felt. This is evidence that late successional species are slower at developing their positive feedback effects then early successionals. This result could be one driver of the benefits of inoculation during restoration (Middleton and Bever 2012; Koziol and Bever 2017; 2019), as late successional plants and their associated mycorrhizal communities may be slower to re-establish after disturbance, and inoculation could accelerate this process.

Importantly, observations of positive plant-AM fungal feedback set up an expectation of alternative stable states. Positive feedback between early and late successional plant species, for example, could both accelerate or inhibit plant succession. Soon after disturbance, a degraded, poorly functioning AM fungal community dominated by early successional plants could inhibit the establishment of late successional species, thereby inhibiting succession. However, once late

successional plant species and the AM fungi with which they grow best are established, succession can proceed at an accelerating pace. As plants and AM fungi interact and disperse at a local level, spatial processes can facilitate transitions from the early successional to the late successional state through the process of nucleation (Michaels, Eppinga, and Bever 2020).

Conclusions

Though I find that plant-AM fungal feedbacks vary from strongly negative to strongly positive, I also find that plant-AM fungal feedbacks generally tend toward positive. Positive feedbacks are particularly likely to be observed between early and late successional species, thereby likely influencing the course of succession. Positive feedbacks were also likely to be observed between early successional species, and between species in the same family. Dynamics generated by such feedbacks have implications for our understanding of plant community dynamics, for land management, and for restoration projects.

Tables and Figures

Table 1: Log Total Biomass - Host Relatedness Factor

	2018			2019		
Fixed Effects	**F.ratio**	**p.value**	**Sig**	**F.ratio**	**p.value**	**Sig**
Host Plant Life History	0.049	0.824		0.345	0.557	
Training Plant Life History	1.786	0.181		2.635	0.105	
Host Relatedness to Training Species	0.828	0.437		2.961	0.052	·
Host Plant Life History by Training Plant Life History	0.355	0.551		1.153	0.283	
Host Relatedness to Training Species by Host Plant Life History	0.872	0.418		2.739	0.065	·
Log Initial Plant Size (Height in cm × Leaf Count) by Host Species	2.371	0.000	***	1.746	0.003	**
Replanted (Y/N) or Growing Period (Days)	2.991	0.084	***	7.225	0.007	**
Random Effects	**Variance**	**Pr(>Chisq)**	**Sig**	**Variance**	**Pr(>Chisq)**	**Sig**
Host Plant Species	0.067 (1.450)	0.000	***	0.238 (2.027)	0.000	***
Host Plant Phylogenetic Distance	0.006 (0.418)	0.571		0.000 (0.003)	1.000	
Training Plant Species	0.000 (0.061)	0.000	***	0.000 (0.085)	0.000	***
Training Plant Phylogenetic Distance	0.001 (0.174)	1.000		0.002 (0.184)	0.001	***
Block	0.000 (0.121)	0.437		0.000 (0.062)	1.000	

p<0.001***, p<0.01**, p<0.05*, p<0.1·

Table 2: Log Total Biomass - Host Phylogenetic Distance to Training

	2018			2019		
Fixed Effects	**F.ratio**	**p.value**	**Sig**	**F.ratio**	**p.value**	**Sig**
Host Plant Life History	0.159	0.690		0.225	0.635	
Training Plant Life History	1.660	0.198		0.765	0.382	
Host Phylogenetic Distance to Training Species	0.169	0.681		2.266	0.132	
Host Plant Life History by Training Plant Life History	0.573	0.449		4.242	0.039	*
Host Phylogenetic Distance to Training by Host Plant Life History	3.138	0.076	·	0.236	0.627	
Log Initial Plant Size (Height in cm × Leaf Count) by Host Species	2.363	0.000	***	1.754	0.003	**
Replanted (Y/N) or Growing Period (Days)	3.038	0.081	***	6.923	0.009	**
Random Effects	**Variance**	**Pr(>Chisq)**	**Sig**	**Variance**	**Pr(>Chisq)**	**Sig**
Host Plant Species	0.060 (1.378)	0.000	***	0.247 (2.056)	0.000	***
Host Plant Phylogenetic Distance	0.007 (0.461)	0.655		0.000 (0.003)	1.000	
Training Plant Species	0.000 (0.028)	0.000	***	0.000 (0.083)	0.000	***
Training Plant Phylogenetic Distance	0.001 (0.173)	1.000		0.002 (0.186)	0.001	**
Block	0.000 (0.001)	0.527		0.058 (0.242)	1.000	

p<0.001***, p<0.01**, p<0.05*, p<0.1·

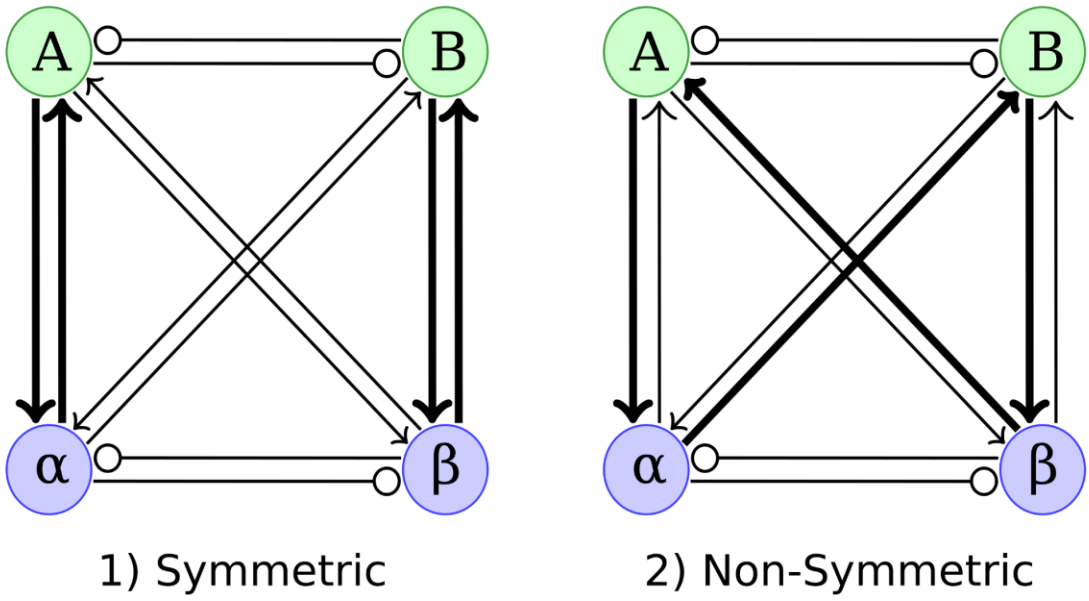


Figure 1: Interguild Frequency Dependence

This model predicts that when there are non-symmetric fitness relationships between hosts and their mutualists, there can be either coexistence or competitive exclusion. During traditional positive feedback (1), mutualist α has higher fitness in association with host A, and host A receives the greatest benefit from mutualist α. The system will experience positive feedback and one of the competitors will be competitively excluded. However, if the relationships are not symmetric (2), such as when mutualist β has higher fitness in association with host A but confers the greatest benefit to host B, then an increase in Host A will indirectly increase the fitness of its competitor.

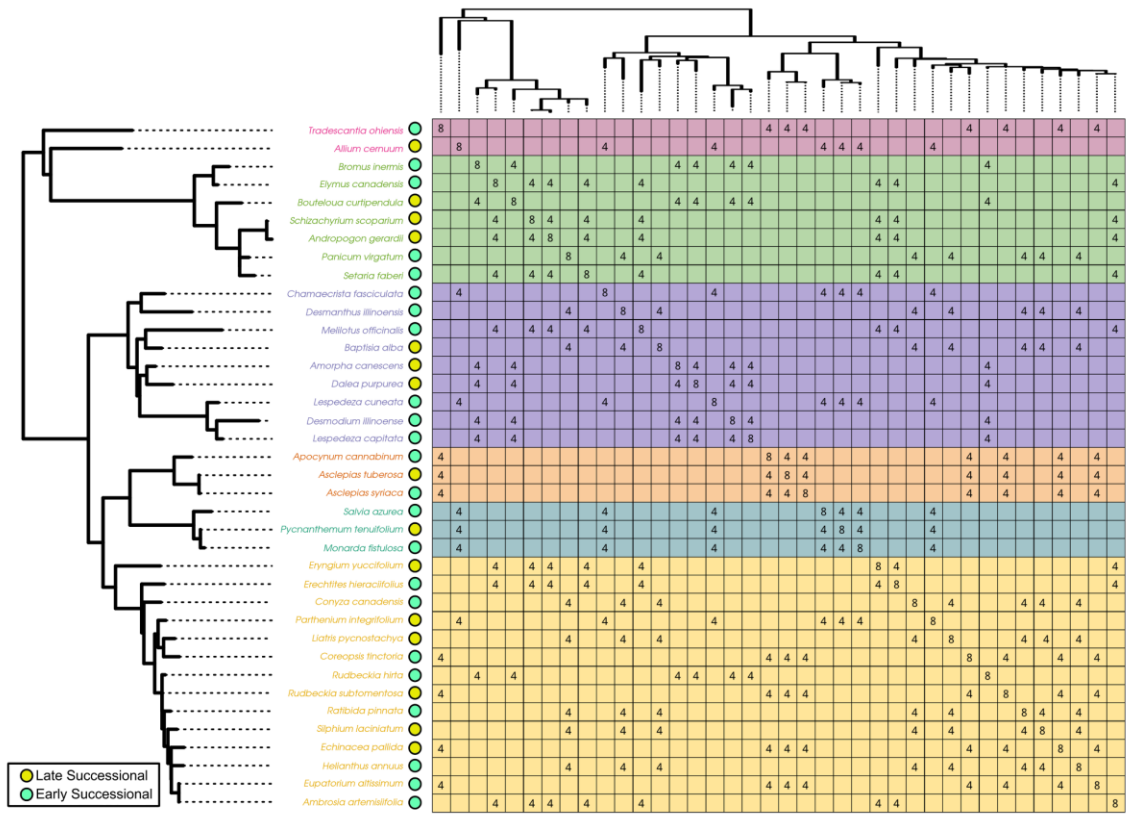
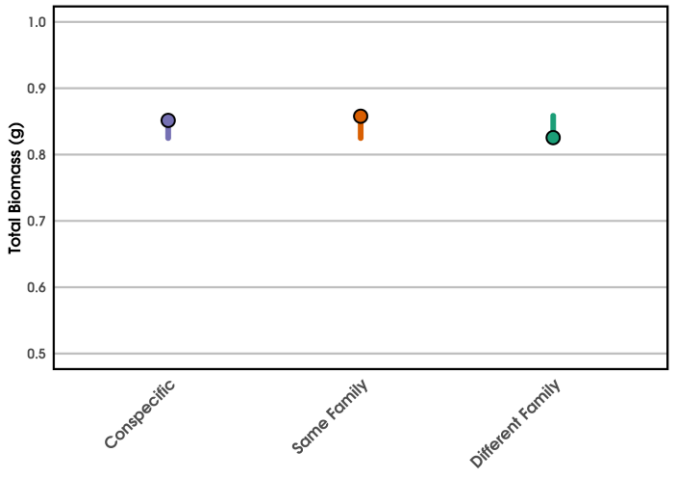


Figure 2: Experimental Design

The experimental design for the 2019 feedback experiment included 5 subset groups of plants where all plant pairings were made in a fully factorial design. Each group also included early and late successional plants. When these pairing are arranged phylogenetically it becomes clearer that we also have a good representation of species pairs across the plant phylogeny. This will allow us to test pairwise feedbacks, host plant characteristics, and plant phylogeny effects in a single experiment.

A. 2018



B. 2019

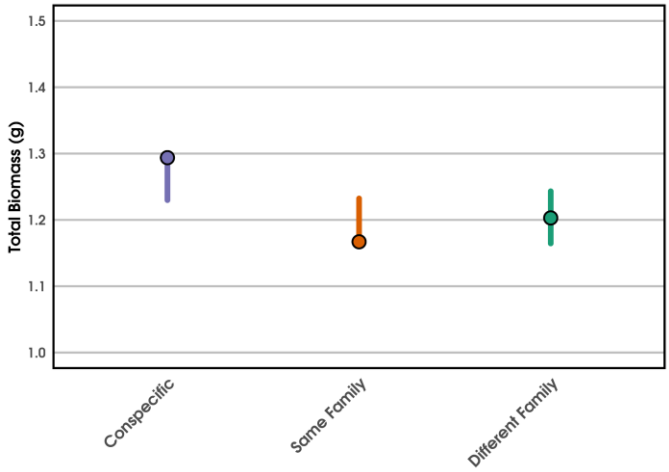
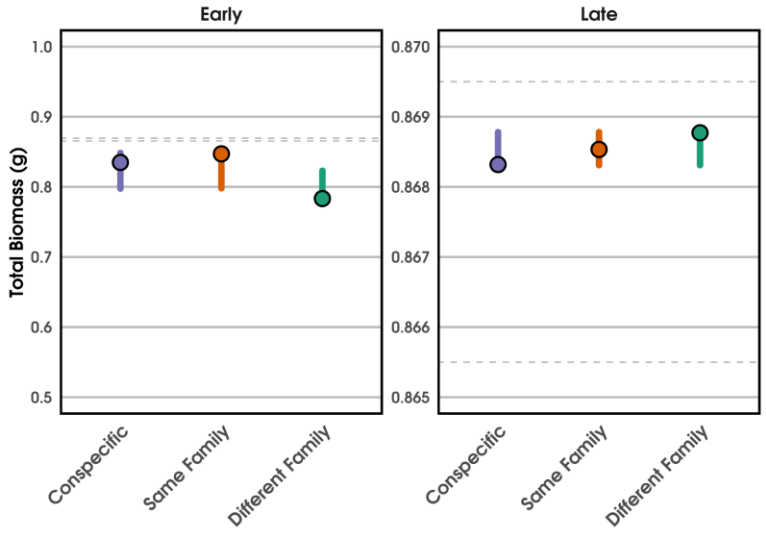


Figure 3: Total Biomass by Host Plant Relatedness to Training Plant

These are estimated marginal mean biomass for host plants grown in AM fungal communities trained on conspecifics, training plants from the same family as the host plant, or training plants from a different family. There was a significant mean increase in biomass for plants grown in conspecific trained soil in 2019.

A. 2018



B. 2019:

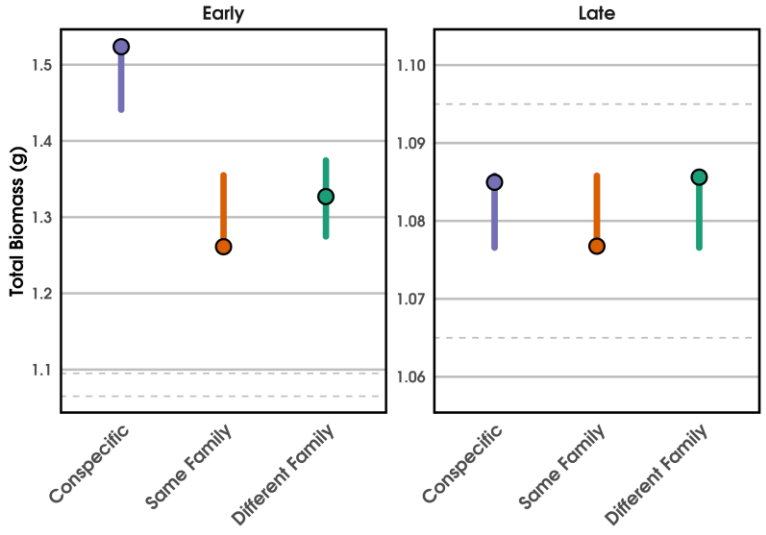
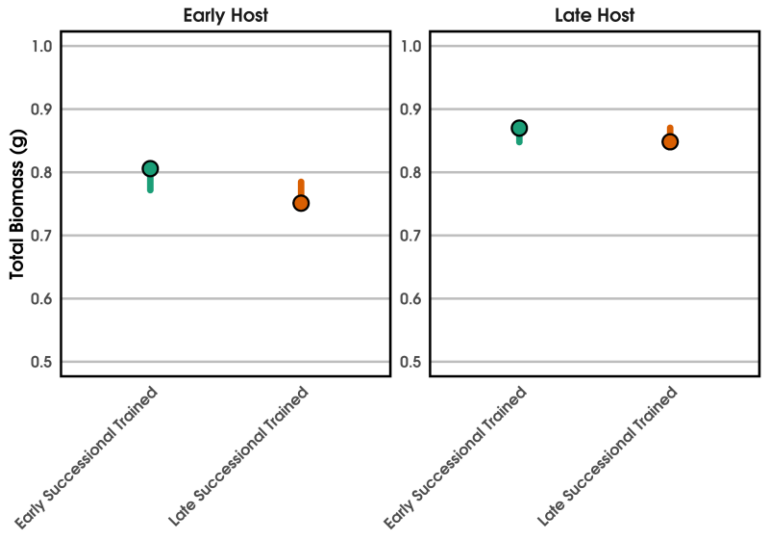


Figure 4: Total Biomass by Host Plant to Training Plant and Plant Host Life History

These are estimated marginal mean biomass, conditional on host plant life history (early or late successional), for host plants grown in AM fungal communities trained on conspecifics, training plants from the same family as the host plant, or training plants from a different family. There was a significant mean increase in biomass for plants grown in conspecific trained soil in 2019 when the host plant was an early successional. There was less variation in means for late successionals, as can be inferred from the differences in y-scales. Dotted horizontal lines denote the same span of biomass values for early and late plants.

A. 2018



B. 2019

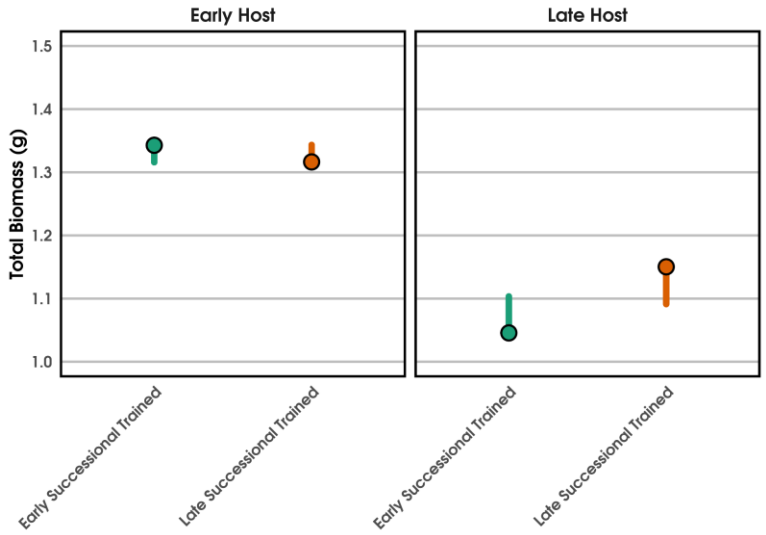
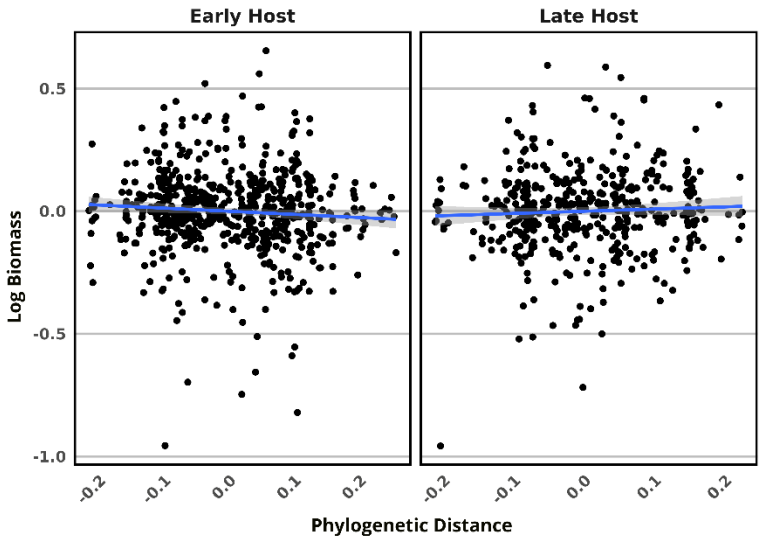


Figure 5: Total Biomass by Host Plant Life History and Training Plant Life History

These are estimated marginal mean biomass, conditional on current host plant life history (early or late successional), for host plants grown in AM fungal communities trained on early or late successional training plants. In 2019 late successional hosts grown in soil trained by a late successional saw a significant increase in mean biomass.

A. 2018



B. 2019

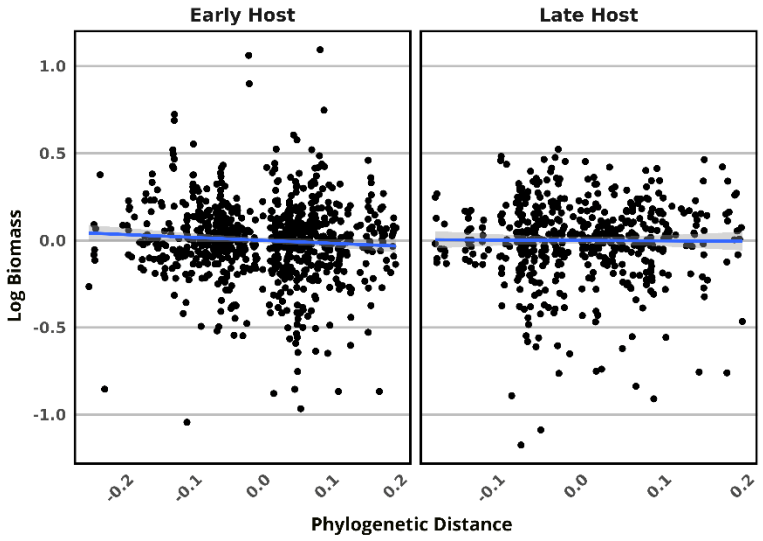


Figure 6: Partial Regression Plot of Phylogenetic Distance by Host Life History

These are partial regression plots of the effect on phylogenetic distance, conditional on host plant life history (early or late successional), on plant biomass. Residuals of the statistical model without phylogenetic distance (Phylogenetic Distance) are plotted against the residuals of the model with phylogenetic distance as the response variable (Log Biomass). These plots reveal a significant negative relationship with increasing phylogenetic distance (training species are more distantly related to the host species) and biomass, when the host plant is an early successional.

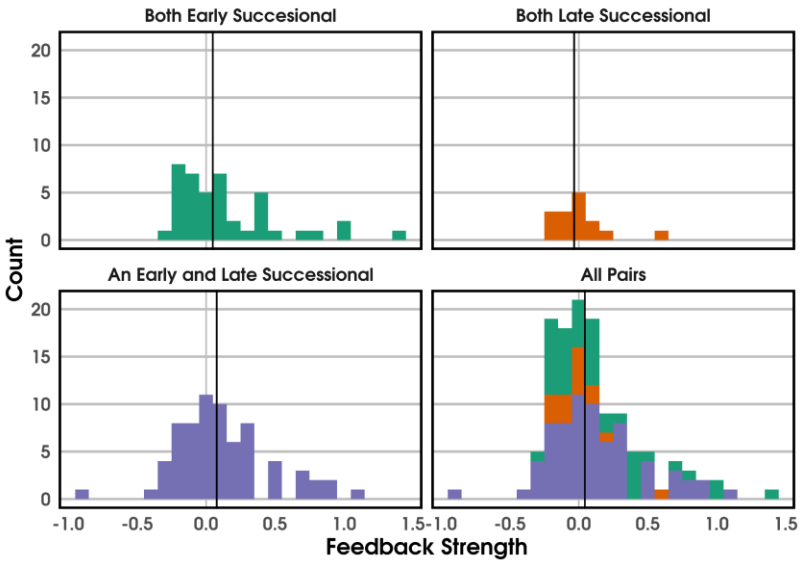


Figure 7: Histogram of Interaction coefficients

Interaction coefficients were calculated for all unique pairs of host plants with reciprocal training treatments. Negative interaction coefficients denote negative feedback, and positive values denote positive feedback. Counts were bin at 0.1 increments and totals are presented for all pairings as well as where both host plants are early successionals, both are late successionals, and one host is an early and one a late successional. The vertical line denotes the median value.

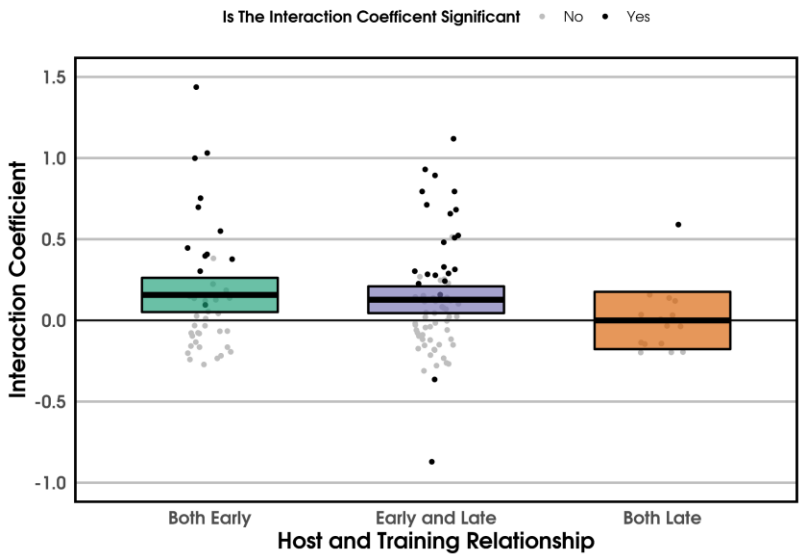


Figure 8: Feedbacks by Host and Training Life History Relationship

Interaction coefficients were calculated for all unique pairs of host plants with reciprocal training treatments. Negative interaction coefficients denote negative feedback, and positive values denote positive feedback. Estimated marginal mean interaction coefficients were calculated for pairs where both host plants are early successionals, both are late successionals, and one host is an early and one a late successional. Dots represent individual interaction coefficients. Dots are grayed out if they are not significantly different from zero.

Chapter 4: Impacts of Phosphorus Fertilization and Plant Phylogeny on Arbuscular Mycorrhizal Fungal Function and Feedback

Abstract

Arbuscular fungal composition matters, because AM fungal species are functionally different, and changes in composition of AM fungal communities can create feedbacks on plant fitness, community composition, and terrestrial function. Fertilization is a widespread modern practice with the potential to drastically affect AM fungal community structure and function. Phosphorus fertilization is expected to favor non-beneficial AMF, thereby degrading mycorrhizal function. This, in addition to host-specific differences in AM fungal growth rates, can generate feedback on plant fitness. Differential impacts of AM fungal composition changes that are tied to life plant history characteristics can affect succession dynamics and grassland restoration success. Differential impacts of AM fungal composition changes tied to plant phylogeny can influence phylogenetic diversity of plant communities. I test AM fungal dynamics within the North American prairie, which is an ecosystem strongly shaped by AM fungal interactions and heavily impacted by fertilization practices tied to agriculture. I assess the functioning of AM fungal communities grown in high and low phosphorus environments for two growing seasons with each of 38 plant species by inoculating and monitoring growth of *Schizachyrium scoparium*, a late successional prairie grass. The legacy of phosphorus fertilization impacted the functioning of AM fungi. AM fungal communities that were exposed to high phosphorus levels improved the fitness of their host plant more than those communities that were not. I hypothesize that the positive

effect on plant fitness from AM fungal communities trained in high phosphorus environments could be driven by a form of nutrient specialization. When reared in a high phosphorus environment, AM fungal communities raised on early successional plants improved in their ability to infect roots. Early successional plants are promoting very infectious, though not necessarily more beneficial, AM fungal communities.

Introduction

Arbuscular mycorrhizal (AM) fungi are a mutualistic soil fungi which form relationships with most terrestrial plants and improve their hosts ability to obtain resources (Hoeksema et al. 2018). Improvement to phosphorus uptake is the main benefit provided to plants from AM fungi, and in exchange the fungus receives carbohydrates from the plant (Smith and Read 2008). AM fungal species vary in their ability to promote plant growth (Cheeke et al. 2019; Hoeksema et al. 2018). Changes in AM fungal community composition is then expected to change AM community function. Changes in AM fungal composition due to the plant characteristics of previous hosts are also expected to create feedbacks on plant fitness. Understanding the factors that impact the change in the functioning and feedbacks of the AM fungal community over time is essential to building a comprehensive understanding of the drivers of mycorrhizal impacts on ecosystem processes.

Fertilization is a widespread modern practice with the potential to drastically affect AM fungal community structure and function. Agricultural disturbance such as tillage and fertilization can have marked effects on AM fungal composition (Stover et al. 2012; Li, Li, and Zhao 2007; House and Bever 2018). Previous research has shown

that the effects of long term fertilization can alter microbial assemblages, including Arbuscular Mycorrhizal (AM) fungi (Wang et al. 2022). Fertilization rates can decrease AM fungal colonization, hyphal density, and Shannon's diversity, and it has been shown that individual AM fungal species will have varying fitness impacts of phosphorus fertilization (Lang et al. 2022; Emery et al. 2017; Santos, Finlay, and Tehler 2006). Fertilization has also been shown to promote the growth of less beneficial AM fungi (Johnson 1993). Forms of phosphorus also have an effect, with organic fertilizers often increasing fungal density or diversity over mineral phosphorus treatments (Dai et al. 2013; Bedini et al. 2013). Generalization of the impact of fertilization on AMF function from previous studies, however, is limited by the overrepresentation studies focused on a few annual crop species. Domesticated annual crops are less likely to be highly mycorrhizal responsive (Hoeksema et al. 2018). Generality necessarily relies on tests of consistent impacts across many plant species.

Eco-evolutionary theory can provide guidance on expectations for the impact of phosphorus fertilization on AMF function. Specifically, phosphorus fertilization is expected to favor non-beneficial AMF, thereby degrading mycorrhizal function (Bever 2015; Ghosh, Reuman, and Bever 2021). Non-beneficial AM fungi who receive the benefits associated with host plants—energy rich carbohydrates—but do not provide phosphorus to the host plant in return, have been called "cheaters". It is difficult for plants to avoid cheater microbes, as they share signaling pathways within beneficial AM fungi. AM fungi associate with multiple hosts in the soil which decouples their fitness outcomes from that of any individual host (Steidinger and Bever 2014). Cheaters tend to have a fitness advantage over more beneficial AM fungi when in mixture (Bennett

and Bever 2009; Bever et al. 2009; Bever 2002a; Hart et al. 2013), and cheaters are therefore expected to proliferate in the absence of a stabilizing mechanism. Plants are able to utilize the preferential allocation of resources for the maintenance of their mutualism (Bever et al. 2009; Kiers et al. 2011; Bever 2015). This strategy entails the host plant granting more resources to beneficial AM fungi, which improves the beneficial symbiont's fitness and proliferation. Since increased access to phosphorus is one of the major benefits AM fungi provide to their host plants, phosphorus fertilization would be expected, and has been shown, to reduce their rates of preferential allocation to beneficial symbionts (Ji and Bever 2016). As a result of this plastic response, mutualistic quality of the AM fungal communities is expected to decline with phosphorus fertilization (Bever 2015; Ghosh, Reuman, and Bever 2021). Understanding how mechanisms of preferential allocation shape soil community structure is important to understanding the system of feedbacks that drives the great biodiversity of plant–microbial systems.

Plants vary in the degree they will benefit from association with AM fungi. Late successional prairie plants have been demonstrated to be both more responsive to AM fungal association and more sensitive to AM fungal species identity (Koziol and Bever 2015; 2016; Cheeke et al. 2019). Studies have shown increases in plant community diversity attributed to AM fungal inoculation driven by increases in the successful establishment and retention of late successional species (Koziol and Bever 2019). Multiple studies have also extended these observations to improve the success of grassland restorations, by increasing the establishment and retention of late successional species (Middleton and Bever 2012; Koziol and Bever 2017).

The effects of phylogenetic relationships can also not be ignored. Many plant traits important to belowground resource acquisition have been shown to have varying degrees of phylogenetic conservatism. There is empirical evidence for phylogenetic conservatism in root diameter, specific root length, and branching intensity (Comas, Callahan, and Midford 2014). Research has shown that plant species relatedness was an important predictor of plant-microbial feedbacks (Crawford et al. 2019). Additionally phylogenetic distance can help test plant-microbial feedback effects. When a plant grows better in a microbial community that was associated, then those close relatives are creating more favorable soil communities. This would be evidence for positive feedback effects. If the opposite relationship is observed and a plant does best in a AM fungal community trained by a distant relative, that is evidence of negative feedback.

My work was conducted using the North American prairie as the study system. These grasslands are in large part shaped by AM fungal interactions, with AM fungal associations very common among prairie plant species (Bragg and Hulbert 1976; Collins and Wallace 1990). Phosphorus addition, as well as a host of other practices tied to agriculture, has caused a sharp decline in the diversity and services provided by soil communities. Many studies have shown that the abundances and compositions within AM fungal communities have significant impacts on prairie plant community composition (Wilson and Hartnett 1997; Hartnett and Wilson 1999; Vogelsang, Reynolds, and Bever 2006; Bauer et al. 2012). There is evidence that the responsiveness of plants to AM fungi correlates with successional stage and phylogenetic relatedness, with late successional plants being more responsive overall (Bauer, Mack, and Bever 2015; Koziol and Bever 2015).

We test the influence of two growing seasons of phosphorus fertilization on mycorrhizal fungal community functioning. Replicate mesocosms with a common initial AM fungal community composition were grown in fertilized and unfertilized replicate pots with 38 different plant species that varied in life history and plant family. Previous work identified that AM fungal composition was consistently altered by the fertilization treatment (Chapter 2). Here, I assay whether the legacy of fertilization altered mycorrhizal functioning by conducting a growth assay testing the effects of these altered AM fungal communities on the growth of *Schizachyrium scoparium*, a late successional grass. As a late successional prairie species, I expect *S. scoparium* to be highly responsive to AM fungal inoculation (Cheeke et al 2019) thereby making it a good test species for mycorrhizal function. I also test whether the legacy effect of phosphorus fertilization on the mutualistic quality of AM fungal communities depends upon the life history or phylogenetic relationships of the plant host.

Methods

In 2017, 38 plant species were grown in sterile 50/50 soil sand mix and inoculated with a common mixture of seven AM fungal cultures (*Claroideoglomus lamellosum*, *Claroideoglomus claroideum*, *Entrophospora infrequens*, *Funneliformis mosseae*, *Racocetra fulgida*, *Cetraspora pellucida*, *Acaulospora spinosa*). Four replicates of each plant species were fertilized with phosphorus and four were not. The mesocosms were allowed to grow for an entire growing season in a randomized block design and harvested in the fall. Soil from each pot was retained and then used as inocula to restart pots with each of the same plant species and fertilization treatment for a second

growing season in 2018. In 2019, a growth assay was conducted using soil retained from 2018. To limit the overall size of the experiment, only one test plant was used for my growth assay. *Schizachyrium scoparium* was chosen as a mycorrhizal responsive plant that would also be easy to work with in large numbers. *S. scoparium* replicates were inoculated with the individual conditioned AM fungal communities grown with phosphorus fertilized and unfertilized treatments of each species. Above and below ground biomass were collected from all plants. Root samples were also collected and stained to look at percent infection rates.

Greenhouse Methods

The training phase was conducted as outlined in Chapter 2, 38 different plant species (Fig. 1) were grown in 2 gallon pots with soil that have a common homogeneous AM fungal community. To ensure I had a consistent community of known AM fungi as my starting soil community, I inoculated my pots with a common mixture of seven cultured species (*Claroideoglomus lamellosum*, *Claroideoglomus claroideum*, *Entrophospora infrequens*, *Funneliformis mosseae*, *Racocetra fulgida*, *Cetraspora pellucida*, *Acaulospora spinosa*). During the training phases, plants were grown in two-gallon pots with four individuals of a species to a pot in the first year. Reproductive structures were regularly trimmed to minimize seeds falling into the soil. Half of the pots were subject to a phosphorus fertilization treatment. Those pots received a total of 80 mg cm^{-3} of dissolved granular Triple Superphosphate (0-46-0) in eight applications. After the first year, half of the soil in each pot was retained as inocula for year two. In the second year, 1 gallon pots were inoculated with 500 cm^3 of the retained soil and the same host

and fertilizer treatments were reinitiated. Phosphorus treatments in the second year received a total of 80 mg cm^{-3} in four applications. Each plant species and phosphorus treatment had four replicates in a randomized block design.

AM fungal communities were used as inocula in a subsequent growth assay. The growth assay was conducted in 1 liter pots planted with one individual seedling of S. *scoparium*. Soil-root inocula from the phosphorus fertilized trained soil treatment and unfertilized trained soil treatment were mixed with equal volumes of sterilized soil from the opposite treatment (Figure 1). This was done to equalize any residual phosphorus levels or other soil characteristics in the inocula so that any differences in plant growth could be more definitely ascribed only to changes in the AM fungal community and not to the phosphorus enrichment itself (Bever 1994).

We tested a subset of inocula to ensure that there would be no unintended phosphorus enrichment. A total of 40 inocula samples were sent for phosphorus testing at the Soil Agronomy Lab at Kansas State University. 1 replicate pair of 20 species were chosen: 3 early and 3 late successional asters, 2 early and 2 late successional grasses, 2 early and 2 late successional legumes, 1 early and 1 late successional lily, 1 early and 1 late successional milkweed, and 1 early and 1 late successional mint. I found no significant effect ($p=0.42$) of phosphorus treatment on detected phosphorus concentrations (Table 1) with live high phosphorus inocula having a mean between 18.072 and 20.298 ppm and live low phosphorus inocula having a mean between 17.452 and 19.678 ppm.

After thirteen weeks, plants were harvested. Above ground biomass was clipped just above the roots. If any reproductive structures were present, those were clipped

and stored separately. Roots were washed, and three 1 cm2 subsamples were taken for later root staining. Belowground, aboveground, and reproductive biomass were then dried in a 60 ℃ drying oven for a minimum of one week.

Root Staining and Scoring Methods

Sections from the roots of each plant were put into cassettes for staining. Samples were taken from three areas of the root, approximately 1 cm2 in size. Roots of control plants were noticeably smaller, and only one sample was feasible on them. A solution of 10% KOH was boiled to 100 ℃, and the cassettes soaked in the 10% KOH for 10-20 minutes to clear. Afterwards, they were rinsed in running water for 5 minutes. Next, cassettes were soaked in 2% HCl for 30 minutes. After draining, cassettes were added to a boiling Trypan Blue solution and soaked for at least 20 minutes. Roots were de-stained by placing them into a 1:1 glycerol:diH20 mixture in the refrigerator for at least 2 days. Roots were then transferred to 70% ethanol for short term storage.

Ten root segments for each sample were mounted and fixed on slides. Slides were scored using four transects. While traversing the field of view on a compound microscope, the total number of visible structures were counted every time the center of view passed over a root fragment. If no structures were seen that event was also tallied. For most root samples, this resulted in 20 total passes, though if roots shifted during mounting or were crossed, fewer passes may have been possible.

Statistical Methods

We used a method to include phylogenetic distances as a random effect to preserve the information included in the full phylogenetic tree. A phylogenetic generalized mixed model (PGLM) was fitted using the phyr R package (Ives et al. 2020; Ives 2018). Phosphorus addition, training plant successional status, and the interaction between them were modeled as fixed effects as well as the phylogenetic distance of *S. scoparium* to the training plant. The phylogenetic distance between *S. scoparium* and the training plant species will allow me to test feedback effects. We also tested the interaction between phylogenetic distance and phosphorus environment to see if feedback effects varied between my phosphorus treatments. However, this interaction was not significant in any of my analyses and was dropped in the final model. Log transformed initial plant height*plant species and total days grown were included as covariates in the model. A matrix of training plant phylogenetic distance, training plant species' identity, and block were identified as random effects. Either a significant variance component for training plant phylogenetic distance or training plant species' identity indicates that AM fungal function depended on the identity of the plant host. A significant plant phylogenetic variance component indicates that the influence of plant species on AM fungal function is phylogenetically conserved. I tested both log transformed total biomass and logit transformed percent infection as response variables.

Previous studies have shown that the strength of plant soil feedback effects correlate with plant successional stage (Bauer, Mack, and Bever 2015), and that AM fungi can disproportionately improve the fitness of late successional plant species

(Koziol and Bever 2019) with my meta-analysis showing that my beneficial fungi on average only improved the fitness of late successional plants (Chapter 2). To test if certain effects of phosphorus enrichment are only present when the training plant was late or early successional I also conducted separate contrasts of the effects of the phosphorus by successional stage using the by statement in the emmeans joint_tests() function (Lenth et al. 2021).

Results

Total Biomass

I saw a statistically significant legacy effect of phosphorus fertilization ($p = 0.017$) (Table 2). The significance in the phylogenetic mixed model, indicates that the change in AMF function with a legacy of phosphorus fertilization was consistent across the 38 host plant species. The AMF communities with a legacy of phosphorus fertilization resulted in greater plant biomass (Figure 2). There was no significant consistent effect of training plant life history. The interaction of historic phosphorus levels by training plant life history was also not significant. When I tested contrasts of historic phosphorus levels by each training plant life history characteristic, I found that historic phosphorus levels were significant but only when the training host plant was a late successional ($p = 0.02$) (Figure 3). There was also a significant random effect of training host plant species ($p = 0.01$), indicating that the species of plant host impacted AM fungal functioning. I saw no significant random effects from the phylogenetic distances between the training plants. The effect of phylogenetic distance conditional on the phosphorus environment was also not significant ($p=0.490$).

Percent Infection

There is a significant effect of phosphorus (p = 0.025) (Table 2). The significance in the phylogenetic mixed model, indicates that the change in AMF infectivity with a legacy of phosphorus fertilization was consistent across the 38 host plant species. A history of phosphorus fertilization resulted in an AM fungal community that was more infectious (Figure 2). Similar to the results for total biomass, the interaction of historic phosphorus levels by training plant life history was again not significant. Contrasts of historic phosphorus levels by each training plant life history characteristic found that historic phosphorus levels were only significant when the training species was an early successional (p = 0.01). There was no significant consistent effect of training plant life history. There was also no significant random effect from training plant species. The phylogenetic distance between the training species did have a marginally significant (p=0.082) effect, with training plants more closely related to *S. scoparium* corresponding to increased AM fungal infectiousness. The effect of phylogenetic distance conditional on the phosphorus environment was also not significant (p=0.581).

Discussion

We find strong evidence that the legacy of two years of phosphorus fertilization altered the function of AM fungal communities. By testing this effect across 38 training plant species, my results present a uniquely robust test of the fertilization impact on AMF function. These results resonate with, and amplify, previous studies showing fertilization impacts on the functioning of AM fungal communities (Dai et al. 2013; Lang et al. 2022; Santos, Finlay, and Tehler 2006). The experimental approach of mixing

sterile and live inocula serves as a control for potential confounding impacts of direct effects of fertilizer in my test experiment and gives me confidence that I am seeing changes in plant fitness driven by changes in AM fungal community composition. Indeed, I was unable to detect differences in P levels between my AMF inoculation treatments. In addition, I have previous confirmation that the AM fungal composition was altered by fertilization (Chapter 2). Fertilization-driven changes in AM fungal communities have measurable impacts on mycorrhizal function in subsequent generations.

AM fungal communities that were exposed to high phosphorus levels improved the fitness of their host plant more than those communities that were not. This result is counter to prior expectations from theory (Bever 2015; Ghosh, Reuman, and Bever 2021) and counter to previous experimental results (Johnson et al. 2010). Moreover, this result is also not consistent with observations of AM fungal species composition from environmental sequencing, as the beneficial AM fungi in the Claroideoglomeraceae decline while the non-beneficial AM fungus *A. spinosa* increased with P fertilization (Chapter 2). I hypothesize that the positive effect on plant fitness from soil communities trained in high phosphorus environments could be driven by specialization on phosphorus forms. AM fungal communities in phosphorus fertilizer treatments could be specializing in environments rich in inorganic phosphorus, and as all pots in my test experiment have equal amounts of soil from the P fertilizer treatment, my test soil environment may well be enriched in inorganic P. Evidence of differential growth promotion of individual AMF when paired with different P forms, an essential assumption of this hypothesis, has been found using AMF isolated from an old field (Reynolds et al. 2006). Studies of the differential effects of mineral and organic

fertilizers have found that fungal density or diversity can be greater when AM fungi are grown on organic fertilizers (Dai et al. 2013; Bedini et al. 2013). A 2018 study found that the benefits from two AM fungal species varied with the form of mineral phosphorus (Pel et al. 2018). However, an indirect test of differential access to phosphorus of different forms using AMF isolated from prairies yielded ambiguous results (Vogelsang, Reynolds, and Bever 2006).

While changes in AM fungal species composition does not appear to explain the improved growth promotion in communities with a history of P fertilization (Chapter 2), it is possible that evolution within individual AM fungal populations drove the change in function. AMF are unusual organisms in that they house a high level of genetic variation within multinucleate cells (Hijri and Sanders 2005; Bever and Wang 2005; Bever et al. 2008). Evolution of functional traits has been shown to occur within a growing season, a rapid response consistent with selection on segregating nuclei (Angelard et al. 2010; 2014; Bever and Morton 1999; Bentivenga, Bever, and Morton 1997). Moreover, genetic variation in impact on plant hosts under high phosphorus was observed within a single population of a species of AM fungus (Ehinger, Koch, and Sanders 2009). More work is necessary to test whether P form specialization accounts for my results, and whether such specialization is due to genetic changes within one of the AM fungal species.

Treatments trained by early and late successional training plant species differed in their response to historic phosphorus levels. When I considered total biomass, only the AM fungal communities associated with late successional training plants showed a significant improvement in their mutualistic quality when exposed to high phosphorus

levels. Conversely, when reared in a high phosphorus environment, only AM fungal communities raised on early successional plants had a statistically significant improvement in their ability to infect roots (Figure 3). I believe this is evidence for late successional hosts promoting strong mutualists in their soil community, and this promotion is even greater when it occurs in a high phosphorus environment. In contrast, in high phosphorus environments, early successional plants promoted more infectious, though not necessarily more beneficial, AM fungal communities.

We find evidence that plant species differ in their impact on the growth promotion of the AM fungal community, as evidenced by the significant plant host species variance component (Table 2). The variation between plant species in their impact on AM fungal average function, however, was not structured by plant phylogeny. This result is in contrast to results from a separate test of plant host impacts on AM fungal community functioning (Chapter 3) and to the evidence of phylogenetic structure to AM fungal composition (Chapter 2). Overall then we did not see any consistent phylogenetically structured feedback effects, nor any effects contingent on phosphorus environment. Interestingly, I do find a tendency for infectiousness of the AM fungal communities to increase with increasing distance from *S. scoparium*, the test species (Table 2), which is consistent with observations of phylogenetic structure in AM fungal composition. If the more infectious community indeed is a weedier less beneficial one, this would then be evidence of a consistent positive feedback effect for closely related species.

Conclusions

In this study, I confirmed that there are consequences on AM fungal function from a legacy of phosphorus fertilization. Both AM fungal growth promotion and infectiousness increased in communities trained in high phosphorus environments, but there are noteworthy differences between the effect of exposure to a high phosphorus environment between AM fungal growth promotion and infectiousness. I see evidence that increased infectiousness does not result in a more beneficial AM fungal community. I hypothesize that the improvement in host growth promotion in my AM fungal communities could be due to specialization on forms of phosphorus, but more work needs to be done to determine if my AM fungal species are specializing on different sources of phosphorus and through what mechanism.

Tables and Figures

Table 1: Nutrient Analysis for Inocula

Type II ANOVA - P (ppm)	Df	F value	Pr(>F)	sig
Training Plant Species	19.00	1.94	0.08	·
Phosphorus Treatment	1.00	0.67	0.42	
pH	1.00	0.32	0.58	
Marginal Mean P (ppm) for Phosphorus Treatment	**df**	**emmean**		**SE**
Live High Phosphorus Inocula	18.00	19.19		0.53
Live Low Phosphorus Inocula	18.00	18.57		0.53

Table 2: PGLMM Results for Log Total Biomass and Logit Percent Infected

	Log Total Biomass	Logit Percent Infected
High Phosphorus AMF Community	**0.0453 (0.0190)** **p-value=0.017**	**0.1811 (0.0810)** **p-value=0.025**
Plant Life History	0.0138 (0.0258) p-value=0.593	0.0721 (0.0849) p-value=0.396
High P AMF by Plant Life History	0.0228 (0.0190) p-value=0.231	-0.1003 (0.0805) p-value=0.213
Relatedness to Training Species	0.3519 (0.2483) p-value=0.156	**1.7025 (0.9778)** **p-value=0.082**
Species†	**0.0105 (0.3159)** **Pr(>Chi)=0.014**	0.0113 (0.0925)) Pr(>Chi)=0.152
Phylogenetic Distance†	3.28E-08 (5.59E-04) Pr(>Chi)=1	0.0017 (0.0362) Pr(>Chi)=1
Block†	0.0014 (0.1173) Pr(>Chi)=0.322	0.2640 (0.4464) Pr(>Chi)=0.445
Residual†	0.1049 (0.3239)	1.3243 (1.1508)
Log Initial Plant Size (Height in cm × Leaf Count)	0.3454 (0.0515) p-value=0.000	-0.0354 (0.2018) p-value=0.861
Growing Period (Days)	0.0164 (0.0022) p-value=0.000	-0.0159 (0.0092) p-value=0.084
Intercept	-1.8660 (0.2911) p-value=0.000	1.5062 (1.2302) p-value=0.221

†Significance for random effects are calculated with a Likelihood Ratio Test using the Chi square distribution

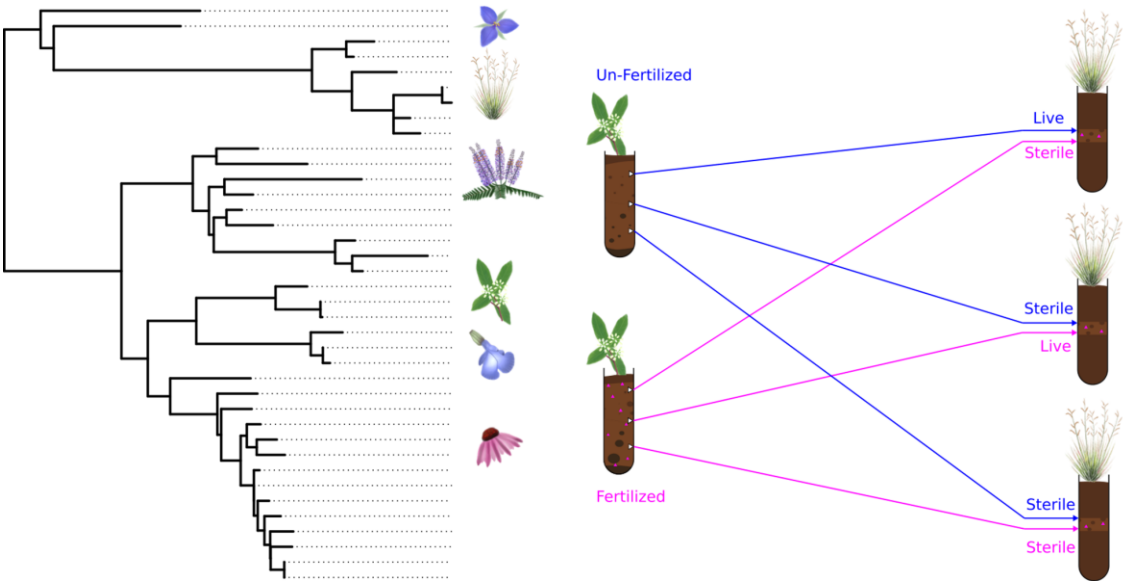


Figure 1: Feedback Phase Design

This experiment took AM fungal communities which had been trained on a phylogenetically diverse group of 38 plant species and used them as inocula for my test plant *Schizachyrium scoparium*. Each training plant had two treatments: a high and low phosphorus treatment. I seek to test differences in feedback effects due to changes in AM fungal communities exposed to differing levels of phosphorus. To eliminate any legacy effect on the phosphorus fertilizer itself, all inocula was a mixture of equal parts high phosphorus and low phosphorus soil. I alternated autoclave sterilization: sterilizing high phosphorus soils in my low phosphorus community treatments, sterilizing low phosphorus soil in my high phosphorus community treatments, and sterilizing both in my controls.

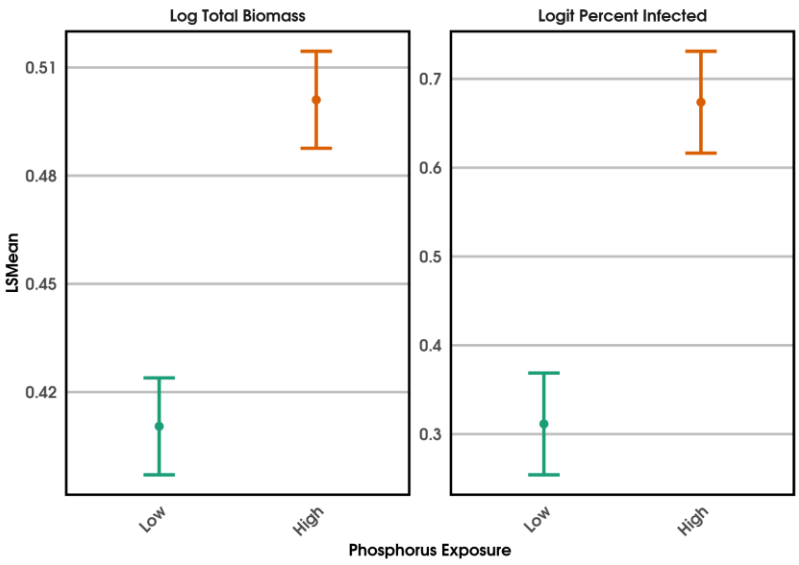


Figure 2: Mean Response to Soil Community Phosphorus Exposure

These are estimated marginal means for log total plant biomass and percent root infection for my low phosphorus and high phosphorus AM fungal community treatments. When grown with AM fungal communities that had been exposed to high phosphorus levels both mean log total biomass and percent infection were greater.

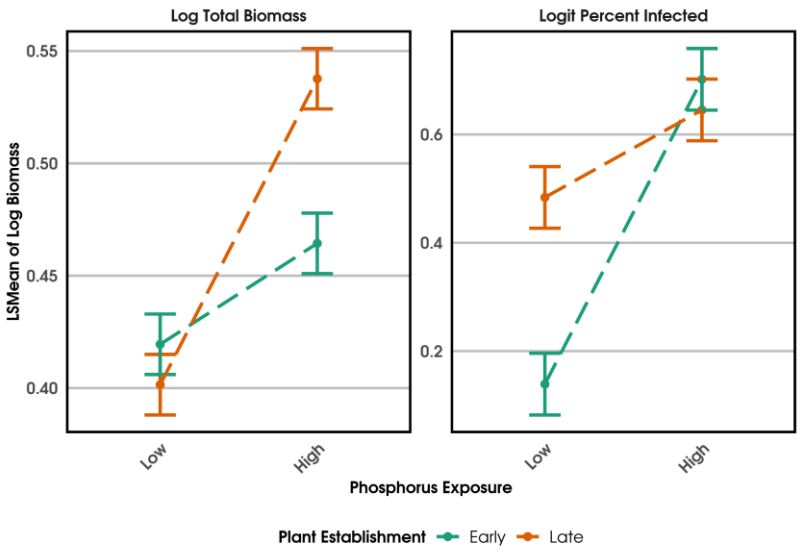


Figure 3: Mean Response to Soil Community Phosphorus Exposure by Training Plant Life History

These are estimated marginal means for log total plant biomass and percent root infection conditional on host plant life history (early or late successional) for my low phosphorus and high phosphorus AM fungal community treatments. When grown in an AM fungal community trained by a late successional, *Schizachyrium scoparium* grew larger in communities exposed to high phosphorus. When grown in an AM fungal community trained by an early successional, *Schizachyrium scoparium* had a greater proportion of its roots infected when grown in soil communities exposed to high phosphorus.

Chapter 5: Conclusions and Implications

In Chapter 2, I found that AM fungal communities differentiated in response to training plant characteristics and phosphorus treatments. The phylogenetic structure of host plant species influenced estimates of AM fungal density, AM fungal diversity, and the relative proportions of our individual AM fungal species. After controlling for phylogenetic non-independence, the life history of the 38 plant species significantly shaped the composition of the AM fungal community. This change in composition could generate feedbacks that could alter plant species turnover during succession. However, the change in AM fungal composition gave ambiguous signals as to the sign of this feedback. AM fungal composition also changed in response to phosphorus fertilization, with beneficial AM fungi decreasing while non-beneficial AM fungi increased with phosphorus enrichment, thereby predicting degradation of AM function. I was then able to test our predicted feedback effects based on changes in AM fungal community composition directly.

When I tested feedbacks for host plant characteristics, I observed feedback effects with clear implications. Fitness of early successional plant species declined with increasing phylogenetic distance of the host plant of the AM fungal community. This effect was evident in our first-year feedback experiment and this positive phylogenetic feedback would tend to reduce phylogenetic diversity in early successional plant species. A saw a similar effect in our second-year feedback experiment but only when we ran a separate contrast for each life history type. When I tested the effects on conspecific trained soil, I saw evidence of positive feedbacks in the second year results

when host plants were early successional. Late successionals showed a different pattern, with late successional hosts showing positive feedbacks when grown in soil trained with late successional soils, but not experiencing strong feedback effects based on relatedness, or the effects of conspecific trained soil. I also did not see pairwise effects when both plants are late successionals, which would be consistent with the positive feedback effects experienced by late successionals being determined more by life history traits, then species specific interactions.

AM fungal communities grown in high phosphorus environments were more beneficial and more infectious. These effects were conditional on training plant life history. Early successional training plant species generated more infectious AM fungal communities in high P environments. Late successional training plant species generated more beneficial AM fungal communities in high P environments. I hypothesize the higher infection in AM fungal communities with early successional species with P fertilization reflects increase in weedier, less beneficial AM fungi. I would then predict negative feedbacks for early successionals as they are promoting AM fungal communities with poorer mutualistic quality. I would also predict positive feedbacks for late successionals due to the legacy of P fertilization. The overall effect of phosphorus fertilization seemed to have been an intensification of the forces driving feedbacks, not a degradation of mutualistic quality as theory and previous studies led me to expect.

The positive feedbacks between early and late successional plant species leads to the expectation of alternative stable states (Bever, Westover, and Antonovics 1997; Scheffer et al. 2001; Suding, Gross, and Houseman 2004; Bever, Platt, and Morton 2012; Kéfi, Holmgren, and Scheffer 2016). A plant community rich in early successional plants

would have feedbacks promoting conspecifics. Not only would this maintain the dominance of early successionals, but it would depress the diversity of the community overall. Early successional species are often the first to colonize after disturbance, and as they are less dependent on AM fungi on average, would be less impacted by degraded soil communities from disturbances such as tillage (Koziol and Bever 2015; Middleton and Bever 2012; Koziol et al. 2018). This makes early successional plants highly likely to establish and dominate when restorations occur in land that had previously been used in agriculture. Late successionals also can experience positive feedbacks, but as these forces are operating at the life history level, I would not expect to see the same kind of depression in diversity in those communities. Also, late successionals are more mycorrhizal responsive overall and would not initially establish well in sites with disturbed soil communities, such as those who had recently been tilled (Koziol and Bever 2015). However if the positive feedbacks associated with late successionals can be established, through the restoration of the soil community, I would expect succession to proceed to a high diversity, late successional dominated system (Bauer, Mack, and Bever 2015).

Early successional dominated communities, and late successional dominated communities are two states that are self-reinforcing, but there is an energetic barrier separating them. Techniques to lower this energetic barrier, such as spatial processes like nucleation, could facilitate this transition (Michaels, Eppinga, and Bever 2020). Traditional restoration practices in prairies often struggle to establish many late successional species (Betz, Lootens, and Becker 1996). Inoculation has been shown to improve the establishment rate of these late successional species and increase diversity

overall (Bever et al. 2003; Middleton et al. 2015). My research increases the understanding of the feedback effects that drive these successional patterns, and how disturbance events like fertilization can change those underlying forces in surprising ways.

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