DISSERTATION

EVALUATING SOIL MICROBIAL COMMUNITY ASSEMBLY TO UNDERSTAND PLANT-SOIL DIVERSITY FEEDBACKS

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ABSTRACT

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The integral role of soil biological relationships in ecological restoration is widely acknowledged as critical for vegetation establishment and primary ecosystem functions. In the era of rapid land degradation, soil restoration will likely become a reoccurring need across global conservation and restoration efforts. By increasing our understanding of the relationship between aboveground plant communities and belowground soil communities, we can begin to include the restoration of soil biological communities. Within the rhizosphere, plant roots and arbuscular mycorrhizal fungi (AM fungal) form intimate associations, by which their diversity and functioning are inherently linked. Increasing our understanding of AM fungal interactions with other soil microorganisms in the rhizosphere microbiome can help us explore how changes to plant-soil feedbacks contribute to plant community restoration success. To achieve this goal, I evaluated how changes in plant diversity impacts AM fungal and bacterial interactions in mixed grass prairies using amplicon-based sequencing techniques, diversity metrics, and microbial network analyses.

To understand how plant diversity influences soil microbes, I conducted an observational field study using replicated plots across an experimental plant diversity gradient (low, medium, high plant diversities). Soils were sampled and processed for amplicon-based sequencing, which revealed the coupled nature between aboveground plant communities and soil bacteria, but not fungi. A microbial network analysis of the data showed that high plant diversity had the least

nodes and edges, but high modularity and positive interactions. These results suggest that soil microbial communities associated with high plant diversity developed complex interactions that increased the stability of soil microbial communities and their interactions.

This led me to create an experimental greenhouse study to evaluate microbial community response to changes in plant diversity. Using conditioned soils from the observational field study as inoculum (from high and low plant diversity plots), I created mesocosm plant communities of high (30 species), medium (15 species), and low (5 species) diversities with prairie species. Pots were inoculated with the high and low diversity field-conditioned soils. Inoculated mesocosm plant communities grew in the greenhouse for six months. Soils were then sampled and processed using amplicon-based sequencing methods as well as diversity metrics and network analyses to evaluate how the relationship between AM fungi and bacteria change with shifts in plant diversity. Overall, we found that AM fungi dominated in contributions to network interactions in all field inoculum treatments. Furthermore, AM fungi also dominated as the hub taxa for most treatments. Positive interactions outweighed negative interactions in the high greenhouse-established plant diversity, high field-conditioned inoculum treatment. Along with the high alpha diversity of AM fungal and bacterial communities in these treatments, the data inferred that these networks are self-maintaining and stable. Bacteria played a minor role in the stability of microbial interactions in field-conditioned inoculum treatments but became the dominant hub taxa in the uninoculated control treatment.

Lastly, I explored how the microbial community responds to aboveground disturbance. To answer this question, I used mesocosm pots from the previous experiment to initiate a disturbance on the greenhouse-established plant community by clipping above-ground biomass once every two weeks for two months, followed by a one-month recovery period. Soil samples

were then collected and processed using amplicon-based sequencing methods to evaluate soil microbial diversity and network analyses post-disturbance. I found that alpha diversity metrics showed little difference across greenhouse-established plant diversity and inoculum treatments. When evaluating beta diversity, bacteria showed differences across all treatments and AM fungi showed different microbial ordinations across high and low field-conditioned inoculum treatments. Under disturbance, negative interactions outweighed positive interactions, which is a common finding for stressed systems. In addition, high plant diversity treatments showed greater modularity, or stability of interactions, which is likely due to AM fungal contributions to network interactions.

Overall, this research explored some controversial assumptions often made in plant-soil feedback studies and addresses the diverse use of methodologies to better understand linkages between plant community diversity and soil microbial community dynamics. Plant community diversity is not necessarily a direct reflection of soil microbial diversity and was correlated with bacterial diversity. This finding indicates that members of the soil microbial community have different relationships with the plant community. Despite changes in AM fungal diversity across treatments, AM fungi play a major role in interactions within the rhizosphere microbiome, which was confirmed through hub taxa analyses. In the face of disturbance to aboveground communities, dynamics in the rhizosphere shift based on the composition of the plant community, with AM fungi contributing the most in high greenhouse-established plant communities. AM fungi and bacteria differentially contributed to plant-soil feedbacks and their contributions are likely to shift as plant stressors limit the functioning of plant-soil feedbacks.

Collectively, this dissertation shows that alpha and beta diversity metrics do not reveal much pertaining to soil microbial interactions and stability. However, they are still a valuable

tool when used in conjunction with network analyses to understand the complex relationships between soil microbial communities and changes in plant community dynamics.

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DEDICATION

For my mother and father,

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Words cannot describe how much you have given me. I am so grateful to be like each of you.

This is for you, 엄마 and 아빠.

And for Lyla and Lucas Egas.

The most that I can relay is that I wish this gives you hope and a cloud to dream on.

Love you, 입이.

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The key to the root microbiome: incorporating bacteria and arbuscular mycorrhizal fungal community assembly to understand interactions and functionality

1.1.Introduction

Arbuscular mycorrhizal (AM) fungi are a valued fundamental taxonomic group in the rhizosphere because of their intimate relationship within the plant root, their role in plant nutrient acquisition, and their contributions to plant success under unfavorable environmental conditions (van der Heijden et al. 1998, Jeffries et al. 2003, Powell and Rillig 2018). As a prospect for agricultural and ecological restoration applications, AM fungal community assembly has long been debated (Horn et al. 2017) and recent studies have yet to elucidate the interdependent complexities behind its composition (Kokkoris et al. 2020). Within the rhizosphere, AM fungi interact with many microbes and have collectively adapted beneficial relationships that influence the fitness of their host plants (Filion et al. 1999, Artursson et al. 2006). The entire functional entity, known as the holobiont (*see Box 1. for term glossary*), is based on the co-evolutionary history between a host plant and its associated microbes, as well as interactions amongst microbes (*see Box 1. for term glossary*) (Vandenkoornhuyse et al. 2015, Sánchez-Cañizares et al. 2017). The complexity behind AM fungal community assembly begs the question of how soil microbes and interactions within the holobiont help shape the function of the rhizosphere microbiome and its contributions to ecosystem dynamics.

1.1.1. Past and current perspectives on AM fungal community assembly

Over the past 20 years, there have been ongoing debates about the drivers of mycorrhizal community assembly. Historically, scientists have viewed the drivers of AM fungal assembly from the plant perspective (the *Passenger hypothesis*) (Newsham et al. 1995) or the fungal

perspective (the *Driver hypothesis*) (Hart et al. 2001). Neither of these hypotheses fully represent the determinants of mycorrhizal community assembly because they fail to incorporate a holistic view of this symbiotic relationship and the factors that contribute to the holobiont microbial community assemblage. More recently, ecologists have developed the *Codependency hypothesis* where community assembly of both host plant and fungus are linked (Horn et al. 2017) and rely on each other for the sustainability of their respective community structures (Kokkoris et al. 2020). Various methodological and experimental approaches have increased our understanding of the AM fungal-plant relationship, allowing for the untangling of this complex relationship to be achieved from the holobiont perspective.

One evolutionary hypothesis that contributes to the holobiont view of community assembly is partner fidelity feedback (PFF), by which the evolutionary cooperation of one individual contributes to the fitness of the other individual, its partner (Fredrickson 2013, Vandenkoornhuyse et al. 2015). This feedback requires the mutualistic coevolution of two individuals for the symbiotic partner to receive benefits from this feedback and in turn optimize their host partners. Another example of community assembly through the conceptual holobiont can be explained at the transcriptional and metabolic levels. The host genome represents the persistence of host evolutionary processes, along with the genetic contributions of its evolutionarily selected microbiota that benefit from host evolutionary processes (Guerrero et al. 2013). Therefore, it is imperative that AM fungal scientists consider the holobiont concept as a system of microbial interactions to gain insight into how AM fungi influence the function of the plant microbiome.

The plant holobiont concept considers the coevolutionary history between plant hosts and their associated microorganisms, a process that is likely shaped by the composition and assembly

of AM fungal communities. Throughout this paper, I will refer to the holobiont concept as the assemblage of microorganisms that occupy the space in and around the host, influencing host fitness and survival through interdependent and complex plant-soil feedback dynamics (Fig 1.1) (Guerrero et al. 2013, Vandenkoornhuyse et al. 2015, Sánchez-Cañizares et al. 2017, Hassani et al. 2018). When studying mycorrhizal symbiosis, it is important that we increase our understanding of the interactions between AM fungi and bacteria to extend the plant-mycorrhizal holobiont concept to include other interacting microbial groups that contribute to the functioning of a plant as a whole. Early ancestors of AM fungi co-evolved with plants, along with other plant-associated microbiota, to adapt to historic high carbon levels in the environment (Helgason & Fitter 2009). Furthermore, the host genome and its associated microbiome, or the hologenome (*see Box 1. for term glossary*), includes the coevolutionary dynamics that lead to a 'genomic reflection' that is evident in host or microbial genomes during these interactions (Guerrero et al. 2013, Tipton et al. 2019). Despite co-evolutionary knowledge behind the processes that have led to our present-day plant and AM fungal relationship, the breadth of AM fungal species diversity is not as great as its relative, ectomycorrhizal fungi. However, the narrow taxonomic scope of AM fungal diversity is a common evolutionary product of other endophytic microorganisms, indicative of the functional plasticity across AM fungal taxonomic groups (Vandenkoorhuyse et al. 2015, Knapp and Kovács 2016, Powell and Rillig 2018).

In this review, I argue that within root microbiome studies, arbuscular mycorrhizal fungi should be studied in conjunction with soil microbes due to similarities in scale, niche occupation, and plant relationships that can shift coexistence dynamics between microorganisms in the root microbiome. I also provide some insights this knowledge gap by delving into pattern-based analyses, network analysis and core microbiome research, that have been effective in

communicating data trends throughout other disciplines over the past 10+ years. I intend for this paper to bring light to interdisciplinary research and cross-disciplinary collaborations that can help push AM fungal research toward understanding the potential, functionality (*see Box 1. for term glossary*) and shifts that lead to the assembly of AM fungal communities. Research on microbiomes have shown progress in understanding a plant's associated organisms by focusing on how microorganisms from different phyla interact with the host. In this review, I highlight how bacteria and arbuscular mycorrhizal fungal associations sustain functionality in the root microbiome by incorporating knowledge from multiple disciplines.

1.2.The coevolution and ecology of AM fungal community assembly

1.2.1. Assembly within the holobiont

Researchers have come to recognize that different mycorrhizal types are governed by different soil microbial traits based on plant-soil relationships (Neuenkamp et al. 2018). However, it has been difficult to discern factors that govern mycorrhizal community assembly due to contradicting methodologies, lack of consensus across the literature, and lack of studies that go beyond AM fungi's morphological and physiological intricacies. While environmental factors, soil characteristics, host associations and dispersal potential all can influence where and how certain communities become established, we have yet to establish why certain AM fungal groups persist within certain plant communities. Studying interactions between AM fungi and their hosts have further limited our understanding AM fungal interactions in the rhizosphere microbiome, but the incorporation of the holobiont concept may lead to insights about biological factors that could contribute to community assembly and interactions with other microorganisms in the rhizosphere.

To understand factors that contribute to AM fungal community assembly, it is helpful to reexamine factors that govern community assembly within the broader field of ecology. Community assembly is the process that shapes a species' correspondence to a local community of organisms (HilleRisLambers et al. 2012). Thus, the definition of assembly rules must include the exploration of patterns that determine the occurrences of species assemblages (Weiher et al. 1998). A better evaluation of AM fungi would include a more holistic evaluation with the consideration of plant-symbiont co-evolution and AM fungi's role in the holobiont system. It is important to evaluate AM fungi in the context of the holobiont because the plant host is a major contributor to the interactions within the rhizosphere. The greatest factor that defines a holobiont-influenced system is its interactions between organisms associated with the host (Wipf et al. 2019). Therefore, by understanding the plant host and AM fungal interactions in the rhizosphere we will be able to gain a better understanding of patterns that contribute to the formation of AM fungal communities.

1.2.2. AM fungal and bacterial cooperation in the rhizosphere

At each trophic level of interacting organisms, microbial and symbiotic co-evolutionary processes support the establishment and persistence of the plant host and the success of plant host microbial communities. These coevolutionary processes are evident in many forms within the rhizosphere including the production of antimicrobial and antifungal compounds, the high biomass of mycorrhizal hyphae, and host plant exudations that inhibit pathogens that influence the root microbiome.

While bacteria and AM fungi occupy the similar spatial niches to benefit their host plants, the distinct, yet collaborative roles of bacterial-fungal interactions in the rhizosphere have only recently been studied (Vályi et al. 2016, Yuan et al. 2021). Bacteria play a protective role in

the persistence of mycorrhiza through the inhibition of antagonistic AM fungal pathogens, promotion of hyphal growth, and the protection of mycorrhizal associations by endophytic processes (Igiehon and Babalola 2018). Physiologically, we know that AM fungi grow to occupy spaces beyond the rhizosphere by delving into crevices and aggregates through hyphal extension to acquire and expose pockets of nutritional hotspots (Leake et al. 2004, Peay 2016). On the other hand, bacteria occupy a much smaller space and have evolved various mechanisms for movement due to their limited spatial occupation in the rhizosphere. To provide functionally different benefits to the plant host under various conditions, bacteria and fungi are most abundant in the rhizosphere where metabolites are exuded by plants as a communicative bridge between soil microbes and plants (Boer et al. 2005, el Zahar Haichar et al. 2008). Recent research suggests that collaborative efforts between fungi and bacteria contribute to plant optimization due to their physiological differences which can be advantageous to host plants under different environmental conditions (Bonfante and Anca 2009, Bergmann et al. 2020).

Due to its intimate association within plant roots, AM fungi have been known to influence the development of the soil microbial community (Zhang et al. 2018, Chen et al. 2019). AM fungi benefit soil microbes by encouraging the growth of plant beneficial bacteria, such as plant-growth-promoting-rhizobacteria (PGPR) and mycorrhiza helper bacteria, which synergistically prevent antagonistic prokaryotic infections in the rhizosphere (Arturrsson et al. 2006, Frey-Klett et al. 2007, Scherlach et al. 2013). Mycorrhiza helper bacteria increase AM fungal spore germination and symbiosis establishment with the host plant (Giovannini et al. 2020). Isolates of actinobacteria (within the genera *Streptomyces*, *Corynebacterium*, and *Pseudomonas*, amongst others) have likely co-evolved with AM fungi because of their ability to decompose insoluble biopolymers that make up AM fungal spore walls, enhancing AM fungal

spore germination under the appropriate conditions (Turini et al. 2018). In utilizing *in vivo* and *in vitro* techniques, researchers have found that co-inoculation of AM fungi and bacteria increase the success of host plants with bacteria, playing an important role in plant-AM fungal symbioses (Kameoka et al. 2019, Emmett et al. 2021). Studies using PGPR have shown that the synergistic effect of co-inculation with both AM fungi and *Pseudomonas* enhances host plant defenses (Pérez-de-Luque et al. 2017), increases host plant salinity tolerance (Pan et al. 2020, Moreira et al. 2020), and alleviates host plant stress from drought (Ghorchiani et al. 2018, Begum et al. 2019). In all cases co-inoculation was more effective than inoculation with either microbial group alone. Therefore, the interactions between fungi and bacteria provide more for the rhizosphere microbiome than each kingdom alone.

Not only do fungi benefit bacteria, but fungi also act as a selective force in the rhizosphere. Bacteria's coevolution with fungi is evident in bacteria's resistance to antibacterial products produced by fungi, allowing bacteria to colonize near fungi (de Boer et al. 2005). Researchers have found that mycorrhizal-associated bacteria inhibit fungal pathogens through the production of antibiotics or by secreting siderophores that outcompete pathogenic bacteria for iron (Garbaye 1994, Turrini et al. 2018). There are also specific AM fungal characteristics that have coevolved with bacteria. For example, the surface of AM fungal hyphae selects for particular bacteria that excrete extracellular polymers to adhere to the hyphal surface (Bianciotto et al. 2001, Artursson et al. 2006). On the other hand, the extraradical mycelium of AM fungi have shown to play a different role in the relationship between fungi and bacteria. Parts of the extraradical mycelium are known to be areas where active nutrient absorption occurs (Kameoka et al. 2019).

These synergistic interactions between mycorrhizal fungi and bacteria help provide necessary nutrients for plant growth, such as phosphorus, which are mobilized by bacteria and taken up by AM fungal hyphae (Wang et al. 2019, Sharma et al. 2020), where it is transported to the host plant for uptake. From the plant-to-bacteria perspective, plant-assimilated photosynthates are transferred indirectly to bacteria by travelling through AM fungal hyphae from plant roots (Kaiser et al. 2015). This likely increases the number of nutritional hotspots by stimulating bacterial communities with labile C in an environment that contains mostly non-labile (recalcitrant) forms of carbon (Jansa et al. 2013). There is accumulating evidence that AM fungi do not act alone in contributing to the root microbiome. Other than root metabolite utilization, bacterial contributions to ecosystem processes are likely to be indirectly (or directly) influenced by other biotic factors and interactions that have shown reproducible results (Emmett et al. 2021).

It is well established that AM fungi act as a major conduit of carbon transfer between plants and soil microbial communities (Drigo et al. 2010), which could have a substantial impact on nutrients available to the bacterial community, influencing bacterial composition and structure (Wang et al. 2019). To access these nutritional hotspots, bacteria adhere to hyphal surfaces enabling them to spread throughout the soil environment (Hassani et al. 2018). These mycelial networks, or 'fungal highways', mobilize bacteria thus increasing their exposure to nutrients that are spread out in the bulk soil environment (Kohlmeier et al. 2005, Worrich et al. 2016). AM fungal hyphae also recruit specific bacteria that enhance nutrient mineralization and harbor distinct bacterial communities near AM fungal hyphae that differ from bulk soil (Zhang et al. 2018). These interactions between bacteria and mycorrhizal fungi indicate the distinct

physiological and ecological advantages that AM fungi contribute to the rhizosphere microbiome and how mycorrhizal fungi enhance accessibility of critical nutrients for plants.

Other synergistic interactions between bacterial consortia and fungi are evident in the formation of biofilms on ectomycorrhizal hyphae. Ectomycorrhizal fungi release exudates such as trehalose and organic acids that attract fungal-associated bacteria (de Boer et al. 2005). These bacteria utilize fungal exudates, much like their utilization of root exudates, as energy sources. While the bacterial biofilms are evident across mycobiomes, some fungi in the Ascomycota class are not able to provide an environment conducive to hyphal biofilm formation (Miquel Guennoc et al. 2018). The presence of beneficial plant-biofilm associations may also enhance aspects of hyphal network resilience to ecological stressors and promote the adhesion and motility of other beneficial soil bacteria (Motaung et al. 2020). There is a highly facilitative effect between mycorrhizal species and multiple bacterial strains, however, little is understood about plant-associated fungal biofilms due its non-medical application.

1.2.3. AM fungal influence on plant molecular processes

Beyond physical and biochemical bacteria-fungal interactions, AM fungi influence plant metabolic processes that concomitantly have effects on plant-bacterial interactions. AM fungi play a direct role in regulating hormone levels and plant genes via molecular crosstalk with plants (Scherlach et al. 2013). AM fungi trigger host plant genes involved in immunity response (Aseel et al. 2019), allowing for the priming of plant defenses to be initiated before a threat, or disease, has made contact. These shifts in plant gene expression boost host plant immune systems and communicate molecular information to multiple plants tapped into the shared AM hyphal network.

Associations with AM fungi alter gene expression in host plants, some of which regulate symbiosis with AM fungi and others that influence plant metabolic activity via differential gene expression. When colonized with AM fungi, 362 plant genes have been shown to be up-regulated, with most genes being associated with primary and secondary metabolism, response to stimuli, and protein modification (Fiorilli et al. 2009). Other studies have found that 80% of plant genes were up-regulated with many differentially expressed genes influencing transcription factors, which may be involved in the transcriptional regulation during symbiosis (Handa et al. 2015). More recently, Vangelisti et al. (2018) found that 694 genes were over-expressed in *Helianthus annuus* roots during the late stage of mycorrhizal colonization, with many differentially expressed genes influencing plant metabolic processes when associated with AM fungi.

Due to AM fungi's influential role in host plant transcriptomes, it is likely that AM fungal-influenced plant transcriptional changes leads to changes in the expression of plant metabolites. In a study using wheat roots, inoculation with AM fungi stimulated plant root exudation and the production of secondary metabolites (Lucini et al. 2019). The idea of microbes stimulating changes in root exudation is a concept that has gained substantial attention in the rhizosphere research community as the 'cry-for-help' hypothesis (Rolfe et al. 2019). This hypothesis suggests that stressed plants release exudates that recruit beneficial microbes while simultaneously discouraging the development of plant pathogens in the root zone (Rolfe et al. 2019). While the 'cry-for-help' hypothesis is not highly cited by the AM fungal research community, AM fungi influence bacterial recruitment outside of the rhizosphere through molecular interactions in the hyphosphere (*see Box 1. for term glossary*) (Schueblin et al. 2010). Signaling communications through the AM fungal hyphosphere confirm that plants influence

metabolic activity within the hyphosphere, changing microbial composition within the AM fungal hyphosphere (Cabral et al. 2019). The mechanisms behind neighbor-induced triggers to increase plant defenses deserves more investigation. Nonetheless, AM fungi are heavily involved in processing communications from host plants to the soil environment. This communication between AM fungi and other microbes throughout the soil environment warrants more attention in AM fungal research.

Primary metabolites exuded by plant roots are not likely to be the sole contributor to changes in microbial assembly within root microbiomes. Secondary metabolites are often induced by biotic stressors and consist of signaling or antimicrobial communications that are less likely to be metabolized by microbes and persist longer in the root microbiome (Rolfe et al. 2019). Transcriptional changes that occur with plant association to mycorrhiza have been found to be in both primary (nitrogen, protein, and carbohydrate pathways) and secondary metabolic pathways (Sbrana et al. 2014). Therefore, AM fungal interactions with the host plant provide a pathway for the indirect regulation of microbial communities (in the hyphosphere) and through their direct influence on plant secondary metabolites that are released as exudates, interfering with microbe-microbe crosstalk in the rhizosphere.

While the effect of plant secondary metabolites on rhizosphere bacteria are obscure, there have been several studies that have investigated the production of secondary metabolites in plants associated with AM fungi. Associations with AM fungi change the amount of phenolic acid exudates released by plants, which contain antimicrobial properties (Wu et al. 2021). Specific AM fungal interactions, between two species (*Funneliformis geosporum* and *Acaulospora laevis*), reduced phenolic acid production in associated host plants while all other combinations of mycorrhizal inoculum increased phenolic acid levels (Wu et al. 2021). While

AM fungal-induced changes in plant secondary metabolite production could indirectly influence bacterial communities, AM fungal-induced increases in plant phenolic acid levels have been presumed to attract other bacteria to the rhizosphere, imposing direct competition with the existing microbial community (O'Banion et al. 2018).

The production of secondary metabolites due to plant associations with mycorrhizal fungi may also play a role in metabolic mutualism, or cross-feeding, amongst other microorganisms in the rhizosphere (D'Souza et al. 2021). Rhizosphere bacteria have been known to synthesize their own secondary metabolites for microbial communications including anti-fungal, anti-bacterial, pigments that provide protection, and siderophores involved in scavenging iron (Dror et al. 2020). While the functionality of AM fungal taxa has yet to be elucidated, it is evident that AM fungi indirectly influence host plant function, plant metabolite production, and communication with soil bacteria in ways that bacteria do directly. Nonetheless, AM fungi's high biomass throughout the soil environment lend to ecological advantages that increase their interactions both within and outside the rhizosphere.

1.2.4. AM fungal interactions contribute to microbiome functional diversity

While the direct effects of AM fungi's contributions to rhizosphere microbiome function are limited, it is likely that AM fungi's presence and role as a foundational organismal group in the rhizosphere have many indirect interactions that increase holobiont functional diversity (*see Box 1. for term glossary*) and resiliency. AM fungal communities likely contribute to increased rhizosphere microbiome functional diversity when groups of mixed AM fungal taxa are incorporated into inoculum (Ceccarelli et al. 2010). Inoculation with mixed AM fungal communities enhance host plant secondary metabolite production and metabolic plasticity increasing plant adaptations to environmental stressors (Albrechtova et al. 2012, Hart et al. 2015,

Avio et al. 2018). Furthermore, plants associated with different AM fungal strains have been observed to have different plant metabolic plasticity that enhance plant tolerance to stress (Rivero et al. 2018). The contents of these secondary metabolites are thought to play a fundamental role in recruitment of plant health-promoting bacteria and increasing functional diversity of the rhizosphere microbiome (Agnolucci et al. 2015, Turini et al. 2018, Agnolucci et al. 2020).

Further work incorporating experimental manipulations of soil microbial communities is needed to reach a better understanding of how microbial interactions influence AM fungal communities and in turn influence host plant secondary metabolic exudation. For example, mock, or synthetic, bacterial communities could be constructed and implemented in the rhizosphere with the addition of AM fungi and without. Here we would be interested in how the presence of AM fungi influences plant secondary metabolic production and if these metabolites change bacterial community structure. By studying how AM fungi influence plant secondary metabolic production and indirectly influence bacterial communities, we may begin to understand the holobiont system more clearly.

1.3. Exploring AM fungal community assembly through microbiome functionality

1.3.1. Impact of AM fungal functioning on host plants and ecosystems

The functional capacity of AM fungal communities exists on a spectrum based on different host plant and ecosystem qualities that determine the functional potential of plant-AM fungal relationships. AM fungi benefit from nutrient deficient, or low-phosphorus soil, because phosphorus-limitations encourage plants to make associations with AM fungi (Smith et al. 2011, Johnson et al. 2015). AM fungi have also been shown to enhance decomposition and acquire

nitrogen from patches of organic materials in direct competition with other microbes (Hodge et al. 2001). AM fungi have been found to alleviate salt stress through a series of molecular, proteomic, and biochemical reactions (Evelin et al. 2009, Porcel et al. 2012, Jia et al. 2019). Furthermore, Augé et al. have spent over a decade studying how AM fungi are beneficial under drought stress to find that AM fungi influence stomatal conductance (Augé 2001, Augé et al. 2015). While AM fungi have an influence on the plant transcriptome and metabolic pathways during drought (Begum et al. 2019), it is likely that the interactions between bacteria (Rubin et al. 2017) and fungal network stability (de Vries et al. 2018) promote plant success under drought conditions. Current research has only begun to uncover how interactions between fungal symbionts and beneficial bacteria contribute to plant survival under stressed conditions. On the other hand, plant pathologists have evaluated how AM fungi alter plant-pathogen interactions and have found that the presence of AM fungi negatively affects plant-pathogen growth (Borowicz 2001, Sikes et al. 2009, Sikes 2010). However, much of the research covered only investigates questions directed from one perspective of the entire soil biotic environment within the rhizosphere.

While many studies have described the beneficial impact that AM fungi have on plants, AM fungal contributions to plant fitness are highly variable (Johnson et al. 1997). There are likely many abiotic factors that influence AM fungal influence on plant fitness, but the biological processes that contribute to plant fitness in associated plant hosts are unclear. Conflicting, context-dependent results further convolute the impact that AM fungi have on plant fitness (Hoeksema et al. 2010, van der Heyde et al. 2017, Ryan & Graham 2018). Plant association with AM fungi does not always contribute to greater plant benefit, but plant benefit is highly dependent on the relatedness of the AM fungi shared within plant networks (van't Padje et al.

2020). Differences in biotic and abiotic variables also change the strength in symbiotic relationships where some AM fungal taxa receive greater recognition and benefits from plant partners based on their contributions to plant fitness (Kiers et al. 2011). While many researchers have debated how morphology and phylogeny contribute to AM fungal functionality, neither incorporate the holobiont perspective and lack the consideration that the relationship between AM fungal-plant host relationships are not strictly exclusive.

An additional factor that needs to be accounted for in AM fungal studies is the interactions between microbes within the biological marketplace, as represented by a series of hyphal networks (Kiers et al. 2011, Fellbaum et al. 2012, Noë & Kiers 2018), as hyphal networks provide a niche for bacterial establishment. For example, Bahram et al. (2020) found that soils dominated by AM fungi experience more nutrient turnover and cycling compared to ectomycorrhizal dominated soils suggesting that plant benefits from AM fungal associations are reliant on the function of the entire holobiont and its associated microbiota.

1.3.2. AM fungal functionality: why phylogenetic and morphological solutions are inadequate

Collections of morphological and phylogenetic data pertaining to AM fungal assembly have shown to be insufficient in understanding AM fungal functionality. In addition, the relationships between AM fungal morphology and phylogeny are elusive and limited in its interpretation of functional roles. AM fungal morphology is rather cryptic with single species forming multiple spore morphs, unclear genetic repercussions of anastomosis, and constant systematic reconfigurations (Morton & Msiska 2010, Schüßler et al. 2011, Krüger et al. 2012). As obligate symbionts with ambiguous reproductive strategies, understanding the life-history traits that could indicate functionality have been a challenge. While there is an extensive analysis

that has provided a phylogenetic basis for the classification of *Glomeromycota* (Krüger et al. 2012), linkage of phylogeny to AM fungal functionality has not been extensively confirmed in the literature.

Many mycorrhizal studies have focused on evaluating AM fungal phylogenetic and morphological differences in hopes of bringing insight into functional roles. Life history classification of AM fungal traits was also considered in the context of Grime's C-S-R model (Grime 1977) where physiological AM fungal traits, like hyphal growth, were characterized by family (Chagnon et al. 2013). While this gave researchers a better idea of the physiological attributes that occur in AM fungal families, it could be improved with the incorporation of the holobiont (plant and bacterial evolutionary traits) alongside the model. More recently, spore morphologies were evaluated (Chaudhary et al. 2020) using life-history traits to understand AM fungal dispersal since dispersal is a mechanism that is independent of the holobiont. Nonetheless, life-history traits and morphological differences are not sufficient in determining the functionality of AM fungi within the holobiont. Since bacteria likely co-evolved along with the plant-fungal symbiosis, these inter-kingdom interactions deserve more attention in order to develop a fuller understanding of AM fungal functionality and its correlation with AM fungal taxonomy.

Phylogenetic and taxonomic attributes should not be the only evidence collected when studying AM fungal functionality. The complex relationship between AM fungal morphology and function suggests that phylogenetic classification is far from being established (Sbrana et al. 2014). While taxonomy and phylogeny may lead to indications of functionality, neither consider plant host associations and interactions that likely define AM fungi these roles. It is still unclear how AM fungal functionality and AM fungal families are related. While there have been

attempts to elucidate these patterns (Chagnon et al. 2013), it is possible that AM fungal community diversity cannot solely be used to predict community functionality. As Munkvold et al. found in (2004), AM fungal communities with low species diversity may still have considerable heterogeneity in their functional representation and contributions to the rhizosphere. Therefore, it would be more effective for researchers to evaluate soil bacterial and saprophytic fungal interactions with AM fungi as context clues for understanding the metabolic benefits that AM fungi indirectly (or directly) influence.

While there is extensive evidence in the literature that hypothesize the particular function of AM fungal groups, there has been less evidence indicating that AM fungal associations work independently from other microbial groups. AM fungi interacts with most organisms in the rhizosphere, which indicates its high importance in connecting different microbial communities and maintaining the functioning of root systems (Banerjee et al. 2016). While linkages between community assembly and metabolic attributes in the root microbiome are unknown, the assembly of AM fungal communities has been found to be based on both niche and neutral processes (Dumbrell et al. 2010). AM fungal community assembly may be driven by either neutral or niche processes depending on how the filters of assembly rules affects either the plant or AM fungal communities (Chagnon et al. 2015). AM fungi communicate and interpret external stressors to the plant, dictating their neutral assembly, which may not directly affect the community assembly of AM fungal communities. As external stressors are recognized as stressors, signaling are translated by the plant host to the soil microbial community through molecular communications, dictating niche processes that determine the soil microbial functional roles needed by the plant begin to take hold. We may be able to better understand how plant communications with AM fungi facilitate further microbial interactions in the rhizosphere by

understanding how these molecular communications dictate the functional capacity and stability of the rhizosphere microbiome.

1.3.3. The Drawbacks and Feedbacks that Influence AM Fungal Community Assembly Studies

Identifying the factors that contribute to AM fungal community assembly remains elusive due to lack of research on the contributions of specific taxa to holobiont and ecosystem processes, which may have resulted from discipline differences across the AM fungal research community. For example, some researchers may think of community assembly from a particular perspective (ie. from the plant or fungal perspective). Furthermore, scale plays a factor in many of the discrepancies in our communication about AM fungal community assembly, which has been acknowledged in other areas of microbial ecology research (Nemergut et al. 2013). As such, microbial assembly processes are distinct due to the biological features and biogeographical patterns that make microorganisms unique, ie. size, dormancy, and energy acquisition. Thus, we should reconsider how we use the term "community assembly" when referring to AM fungi and assure that its use is in the context of microbial and symbiotic functioning to make the most of emerging datasets and cross-system meta-analyses.

Patterns of AM fungal community composition have been studied using a variety of methods and molecular primers. For example, a common method in the early 2000s was using T-RFLP (terminal restriction fragment-length polymorphism) (Johnson et al. 2004) and 454-pyrosequencing (Öpik et al. 2009, Dumbrell et al. 2011), which have now been replaced with more precise methods like amplicon-based high-throughput sequencing. Throughout soil bacterial ecology, the use of standard primers has proven consistent results in capturing the conserved regions of bacterial rRNA due to consensus in soil microbiology to use these established universal primers (Head et al. 1998). On the other hand, AM fungi have a series of

primers that have been tested and utilized in a range of studies. Primer pair ITS1-ITS4 were created to capture the diversity of all fungal taxa and may skew results with an overrepresentation of other fungal groups due to relatively low AM fungal frequencies (Suzuki et al. 2020). The most widely used primers for sequencing AM fungi remain to be AM1 and NS31. NS31 was designed as a universal eukaryotic primer (Gorzelak et al. 2012) and has been shown to amplify non-AM fungi (Helgason et al. 1999). Nonetheless, when considering AM fungal taxa at the family and genus levels, it is imperative that there is consensus in primer selection with low sequence variability, such as WANDA and AML2 (Egan et al. 2018). The lack of primer consensuses poses a major barrier for AM fungal research and for understanding AM functionality. If we cannot consistently identify AM fungi using a set of standard primers, then patterns pertaining to AM fungal composition and community assembly will remain elusive.

Confusion surrounding AM fungal community assembly may also be due to lack of distinction between AM fungal studies performed in standard conditions in controlled environments versus interactive conditions in field environments. At this point in AM fungal ecological research, we still lack an understanding of AM fungal functionality in standard conditions within controlled environments, therefore contributing to inconsistent and irreproducible results. Novel efforts to produce a trait-based framework incorporating the holobiont perspective in mycorrhizal studies are imperative for clarifying the communication of these symbioses, but many baseline aspects of AM fungal communities have yet to be explored (Dawson et al. 2021). Other fields of study have resolved these discrepancies by developing tools to define standard conditions in which traits can be measured (Pérez- Harguindeguy et al. 2013, Moretti et al. 2017), allowing for standard measurements of traits to be applied to all AM fungal taxa across all biomes. Efforts have been made to provide consensus in data and metadata

management (Tedersoo et al. 2015), but no consensus has been developed on experimental design or data collection methods used to elucidate AM fungal community patterns under standard conditions, resulting in further confusion in the interpretation of feedback processes (Krause et al. 2014).

Plant-AM fungal associations can influence long term plant-soil dynamics. Fungi with ruderal traits can influence long term plant-soil feedbacks and later plant successional trajectories (Duhamel et al. 2019). Due to the co-evolutionary holobiont nature of the system, changes in AM fungal communities have a domino effect on bacterial communities resulting in emergent ecosystem properties. The functional complementarity across both soil fungal and bacterial taxa promotes different aspects of ecosystem function and stability, where fluctuations in microbial richness increase the functional complementarity of the microbiome and the stability of the system (Fig 1.1) (Wagg et al. 2021). In addition, multiple studies have found that soil conditioning plays a significant role in the recovery of plant communities after disturbance and indicate that previous plant community spatial structure (soil conditioning) influence AM community assembly (Bittebiere et al. 2020). Therefore, soil conditioning and plant-soil feedbacks may play a significant role in influencing assembly of AM fungal communities, but a lack of evidence remains on the mechanisms that are responsible for these patterns. A possible solution to this lack of knowledge can be found through the incorporation of synthetic communities of AM fungi and bacteria under standard conditions to understand how soil microbial interactions can infer trait-based microbial functions (de Souza et al. 2020, Toju et al. 2020).

1.3.4. Beyond structure: interactions influence microbiome community assembly

Examining community structure can be an important tool in understanding AM fungal community assembly by exploring the interactions that lead to rhizosphere microbiome formation. The coevolutionary history of AM fungal community assembly show that AM fungi and bacteria are influenced by different filters, biotic in AM fungi (Neuenkamp et al. 2018, Davison et al. 2020) and abiotic filters in bacterial communities (Fierer & Jackson 2006, Delgado-Baquerizo et al. 2018). Understanding interactions between microbes can indicate how the microbiome functions as a whole and which taxa influence the structure of the microbiome. For example, we can determine if AM fungi provide a supplementary role to bacterial functions or if bacterial and fungal roles are independent by developing experiments examining interactions between interkingdom microbial groups.

Although there has been much controversy in using community assembly data to interpret functionality, it has shown to be a helpful tool in identifying repeated and repeatable patterns in community structure that can characterize AM fungal taxa (van der Heijden & Scheublin 2007, Van Diepen et al. 2011). Nonetheless, AM fungal community structure is an important tool but is best used in conjunction with other 'omic' based techniques to provide insight into the mechanisms contributing to assembly. The incorporation of network interactions and predictive tools, like machine learning, have seldom been used in microbiome studies, but have the potential to give insight on microbial contributions to ecosystem functionality (Thompson et al. 2019). Through the coupling of experimental approaches and modeling, we can likely resolve many of the methodological and technological challenges that face soil microbial studies and translate 'omics'-based datasets into functional predictions (Trivedi et al. 2020). After establishing community-level structural compositions, we may then be able to elucidate patterns

that contribute to community structural patterns, which can then guide experimental design for the elucidation of functional roles.

1.3.5. Quantifying synergistic properties and drawing inferences from networks

Microbe-microbe interplay has proven to consist of important selective forces in forming complex microbial assemblages impacting resource acquisition for host plants (Hassani et al. 2018). Much of the research investigating microbial interplay has been the result of careful experimental design with synthetic (or mock) communities (Liu et al. 2019). Much of this research has ignored AM fungi, which is unfortunate given its keystone role in the rhizosphere (Jeffries et al. 2003). Recently, a number of studies analyzed the metabolic facilitation of AM fungi and bacterial interactions in acquiring nutrients for the host plant (Nacoon et al. 2021, Jansa & Hodge 2021, Jiang et al. 2021). Much more research on AM fungal-bacterial interactions are needed to quantify how AM fungi play a keystone role in the rhizosphere and to utilize the complementarity between AM fungi and bacteria effectively in an applied setting. At the moment, the most efficient way to study these interactions will be through understanding patterns in microbial networks and using inferred data to dictate the questions and experiments that we design.

Network analyses performed on microbial communities are often evaluated as co-occurrence networks where multiple correlations and models indicate influential taxa, core taxa, and the types of relationships (synergistic or antagonistic) predicted amongst microbial consortia. In 2018, de Vries et al. found that soil bacterial co-occurrence networks were destabilized by drought in grassland systems, whereas fungal networks were more stable. Along with networks, de Vries et al. found that shifts in bacterial communities had greater effects on ecosystem

functioning than fungi. Here, co-occurrence networks are used in conjunction with other analyses to understand the stability of microbial communities under stress as well their recoveries. Furthermore, networks are used to understand the linkages between taxa. Scientists have found that fungal-bacterial networks provide insight into cooperative and competitive interactions (Zheng et al. 2018). Therefore, the utilization of network analyses can help scientists understand the types of interactions that occur between soil microorganisms, and under which circumstances they shift. The culmination of multiple network analyses may lead to changes in the way that we think about and evaluate relationships between soil organisms and the spatial scale at which they operate.

Fungal-bacterial co-occurrence networks also have an important application in the understanding of soil functioning. For example, Banerjee et al. (2016) used network analysis to find that organic matter decomposition rates were associated with keystone microbial taxa in bacterial and fungal communities. Despite being contextually inferential, the application of network analyses could elucidate functional roles and potential impacts on ecosystem functioning by building predictive models. Mathematical models, such as dynamic network modelling, characterize aspects of microbial community interactions and reveal quantitative insights into complex dynamics utilized in microbiome studies (Garcia & Kao-Kniffin 2018, Garcia & Kao-Kniffin 2020). These particular network models bring important inferences to microbial interactions that are crucial to ecosystem functioning (Zhu & Penuelas 2020). Nevertheless, there are certain limitations in the use of network analyses and their associated models, particularly with sample size. To maximize the robustness of inferred networks, studies should have a large number of replicates and should therefore (in all cases) aim to have a large

collection of samples to improve the predictive power of these models (Barroso-Bergadá et al. 2020).

Due to the complex nature of the rhizosphere, it is difficult to determine to what extent host-symbiont interactions or microbe-microbe interactions influence microbial community dynamics. Other studies have found that plant-AM fungal networks are stochastic in nature (Encinas-Viso et al. 2016) because they lack information pertaining to the holobiont that could give insight into AM fungal community assembly (Ryan & Graham 2018, Johnson & Gibson 2021). An often overlooked, but important aspect to consider is how interactions between AM fungi and soil microbes influence rhizosphere microbiome community assembly (Hassani et al. 2018, Ryan & Graham 2018). Certain functional dynamics can be interpreted from network analyses that are useful for modeling or other downstream analyses. For example, the quantification of network complexity can indicate how specialized the interactions are in that community (Mendes et al. 2014). Furthermore, community abundance distributions can be used to discern the factors that contribute to community assembly. Nonetheless, experimental design remains an important factor in testing the subjective results of network analyses and community abundance datasets. However, the use of large datasets in conjunction with machine learning algorithms has proven to be more powerful in creating predictive models than network analyses alone (Ramirez et al. 2018). The predictive power of machine learning analyses can be used to identify potential 'indicator' taxa that have a strong influence on maintaining community structure of microbiomes.

1.3.6. Core microbiomes and hierarchical scales of plant-associated microbes

With the advancement of sequencing technology and computationally dense datasets, it has become increasingly possible to explore datasets in ways that elucidate microbial processes

in ecology. The identification of a "core" microbiome is a useful step in reducing the complexity within intricate microbial datasets and can be useful in generating novel hypotheses for studies that reconstruct aspects of the rhizosphere microbiome (Trivedi et al. 2021). Nonetheless, the context in which core microbiota data is interpreted has resulted in discrepancies across some disciplines and consensus among others.

While we can define core microbes that are evident in samples and across treatments, it is important to understand where the concept of 'core microbes' comes from and how it is used to make ecological inferences in each field of study. Since hub microbiota in network analyses are such an important tool in identifying which organisms provide the structure of network interactions, identification of the core microbiota can be used in conjunction with network analyses to get a better idea of which taxa has the most influential presence in the studied microbiome and where core microbiota contributes to network structure. Nonetheless, it is important to recognize that core taxa are not necessarily hub or connector taxa (Stopnisek & Shade 2021). Within the context of the plant holobiont, the core microbiota refers to microorganisms that are consistent across samples for a given host plant species (Vandenkoornhuyse et al. 2015). However, the definition of core microbiota is highly dependent on the context of the study (Risely 2020).

Core taxa can be used to identify key microorganisms that regulate microbiome structure in the rhizosphere. In ecology, taxa that contribute to the maintenance of ecosystem dynamics have been coined as 'keystone organisms' (Banerjee et al. 2019, Risely 2020). Often, core microbiome analyses decipher common taxa across treatments that infer importance to microbiome assembly. The incorporation of metagenomic and transcriptomic tools in conjunction with the identification of core microbiomes are helpful in assessing microbiome

functioning and may help decipher which taxa are crucial to host survival under various levels of abiotic stressors (Shade & Handelsman 2012). Recent advances in network analyses and metagenomics can be useful in reducing the complexity of the microbiome by identifying the keystone taxa that contribute to microbiome functions through the deconstruction of the microbial community. These communities are then reconstructed with and without proposed keystone taxa (through the use of synthetic communities and 'omic' techniques in experimentally manipulated environments), which will help delineate the hierarchical importance of each taxon to microbiome function (Toju et al. 2020, Trivedi et al. 2021).

1.4. Conclusions and Future Directions

The field's latest advances in whole genome sequencing of the AM fungal model organism, *Rhizophagus irregularis*, has been a crucial first step in understanding AM fungal gene function (Tisserant et al. 2013). As a result, many more studies have utilized this promising work to further our understanding of AM fungal genetics and bacterial contributions to AM fungal genetic diversity (Tamayo et al. 2014, Chen et al. 2018, Li et al. 2018, Masclaux et al. 2019). By investigating gene expression, we could have a better idea of AM fungal nutrient transporter genes, which could then be used to identify AM fungi for use as agricultural inoculum (Giovannini et al. 2020). Many avenues could be explored with AM fungi using molecular-based techniques that tell us more about function rather than composition. Nonetheless, compositional data is fundamental in cataloguing which taxa are associated with particular plant species or found in particular systems. While abundance data is useful in relation to other data, it is necessary to collect pattern-based information to be used for hypothesis

generation. These community patterns could be critical in exploring the community assembly of AM fungi.

Much more inter-disciplinary research is needed in order to develop a better understanding of AM fungal community assembly and the role that AM fungi play within the rhizosphere microbiome. Some of the challenges that face the field of AM fungal ecology have the potential to be overcome by incorporating a combination of techniques (like amplicon-based sequencing, transcriptomic, and proteomic methods) from the field of microbiome science and other disciplines. Due to similarities in scale and niche occupation, the field of AM fungal and soil microbiome research should include and promote research by each other's fields to broaden tools and increase cross-disciplinary collaborations.

To better understand AM fungal community assembly and functionality, research that includes both field studies and controlled environment studies is an appropriate first step to take. Furthermore, pattern-based analyses, like network analyses, could help shed light on the interactions and functional roles that allow these microbes to persist in the rhizosphere microbiome. By manipulating key taxa that contribute to holobiont function, using synthetic or mock communities, we can experimentally tease apart these interactions and build knowledge pertaining to microbial function using controlled environment studies (Egan et al. 2018). The use of synthetic communities has a great advantage over exclusionary treatments, like fungicide because chemical applications may have adversary effects that change the overall chemistry within soil microbial communities. By utilizing the technologies, like synthetic communities, and bringing tools together from different disciplines, we can overcome many of the obstacles pertaining to the study of AM fungal community assembly and ecology.

In this dissertation, I aim to discuss how plant community diversity, AM fungal community assembly and bacterial interactions contribute to plant-soil feedback dynamics over three chapters. My second chapter examines patterns of AM fungal, bacterial and overall fungal communities in a mixed-grass prairie to gain an understanding of the microbial communities established under low, medium, and high levels of plant community diversity in an observational field study. Throughout this chapter, I investigate if aboveground diversity mirrors belowground diversity and observe the microbial interactions that sustain these plant communities. In my third chapter, I explore how soil conditioning and plant diversity influence microbial community composition, structure and interactions in an experimental greenhouse study. In my last chapter, I seek to understand if plant stress by clipping contributes to changes in microbial composition, structure, and interactions by using the experimental design established in Chapter 3. By using network analyses to reduce the complexity of large datasets obtained from genetic sequencing, I explore microbial taxa co-occurrence to identify patterns of their community assembly and decipher plant-soil feedbacks through experimental manipulation. Through the utilization of plant-soil feedback experiments, I aim to understand questions on the community scale that can give insights into community assembly and expand the knowledge basis contributions to management and restoration practices.

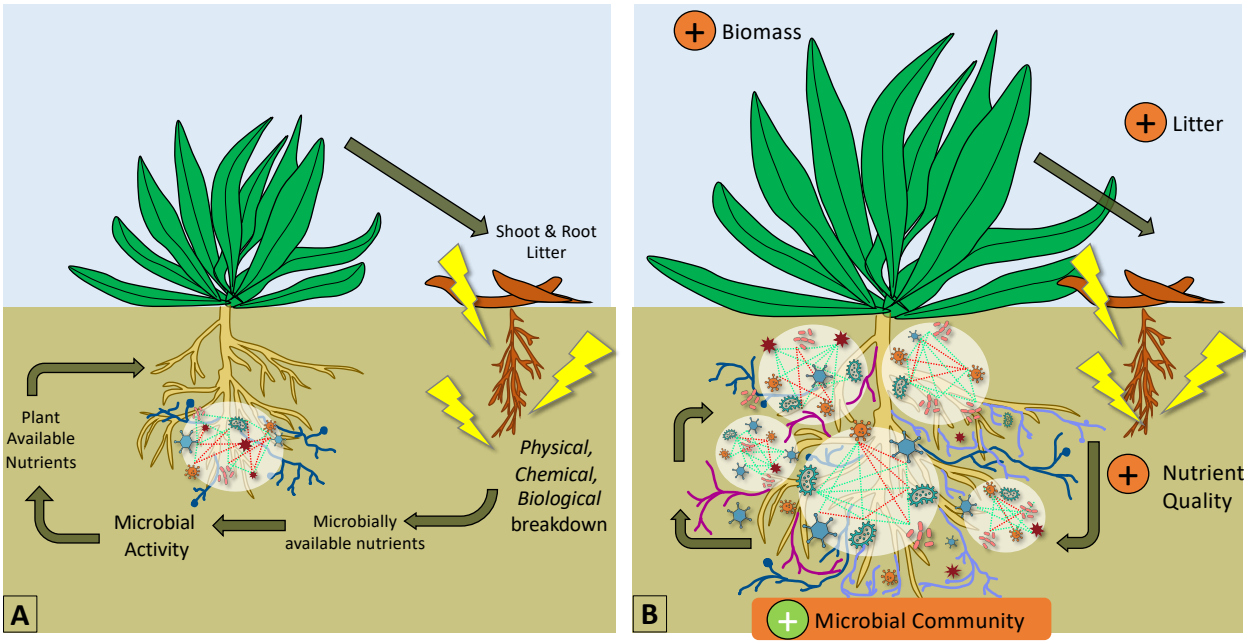

Figure 1.1. Feedback dynamics between plant and soil biogeochemical processes that shift soil biological network interactions to increase functional complementarity in the rhizosphere microbiome. Panel A represents a structurally and compositionally simple network of microbial interactions with feedbacks that sustain its existing microbial root community. Panel B represents a positive plant-microbial feedback loop, consisting of structurally and compositionally complex network formations of microbial interactions that contribute to higher microbial diversity, increased microbial connections, increased plant biomass, and increased diversity of nutrient qualities returning to the soil.

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CHAPTER 2.

Understanding plant and soil microbial community diversity through the lens of network interactions

2.1. Introduction

Although much of our knowledge about plants and their associated microbiota is based on aboveground dynamics, microbial interactions within the plant microbiome may lead to above and belowground feedback dynamics that become obstacles to post-restoration ecosystem recovery (Wall et al. 2020, Zenni et al. 2020). Understanding how microbial interactions influence plant feedbacks will help restoration practitioners implement microbial tools to restore plant community success, while supporting the long-term development of microbial interactions that can support restoration goals. This has substantial implications for the restoration of plant communities and their recovery at sites that struggle with multiple ecological barriers that impair the system's functioning.

The restoration of ecosystem processes is often related to the assembly and functioning of the microbial community associated with high aboveground heterogeneity (Lange et al. 2015; Schmidt et al. 2018). Belowground microbial interactions can stimulate feedback mechanisms that contribute to ecosystem functions and influence aboveground plant community composition (van der Putten et al. 2013). By utilizing cooccurrence networks, we can computationally quantify interactions across microbial groups to understand how this may affect the ecology of a system (Coyte et al. 2015). As a result, these belowground interactions have direct and indirect repercussions on ecosystem stability and functioning. For example, plant communities with low diversity undergo an accumulation of pathogens (Mommer et al. 2018) and an imbalanced utilization of resources (Eisenhauer et al. 2012), which can have compounding negative impacts on plant-soil feedbacks. On the other hand, plants that are microbially obligate contribute to

ecosystem processes by increasing nutrient retention and soil fertility leading to positive feedbacks that structure diverse plant communities (Lange et al. 2015; Eisenhauer et al. 2012; Semchenko et al. 2018).

The aim of this study was to determine how aboveground plant diversity impact: (a) the structure of the microbial community and (b) the network of interactions across the most linked soil microbial communities. We hypothesized that high diversity plant communities will have higher microbial diversity and more complex network properties (between soil microbial groups) compared to medium and low diversity plant communities in mixed-grass prairie restorations.

To test our hypothesis, we collected soil samples from Platte River Prairies in southeastern Nebraska within tallgrass prairie experimental plots (Appendix Figure 1). The site consisted of 12 plots that measured 60-x60-meters. Plots were seeded at three different diversity levels in 2010: monoculture, low and high diversity plots. Each diversity treatment (monoculture, low, high plant communities) was replicated four times across the site. Plots were not managed during the time of the experiment and plant composition in monoculture plots increased because of plant dispersal and plant community succession. There were approximately 16 plant species within the monoculture plots, 32 plant species in the low diversity plots, and 44 plant species in the high diversity plots (Gholizadeh et al. 2019). From here forward, we will refer to the diversity treatments as low, medium, and high due to changes in plant community composition over time.

We used amplicon-based sequencing to profile the bacterial, fungal, and arbuscular mycorrhizal (AM) fungal communities associated with different plant diversity treatments (Gholizadeh et al. 2019). We used co-occurrence network analyses to examine interactions and structural shifts between soil microbial taxonomic groups. Network approaches have become an

increasingly common tool used to tease apart complex interactions between plant-soil microbial communities and inter-kingdom microbial communities (Banerjee et al. 2018; Toju et al. 2018; deVries et al. 2018, Banerjee et al. 2021, Xue et al. 2022).

2.2. Materials and Methods

2.2.1. Study site

Our study site was located in south-central Nebraska at Platte River Prairies in Wood River, NE (40°43'48.8"N 98°35'35.0"W). We sampled from a long-term plant diversity experimental field. The site consisted of 12 plots that measured 60 x 60 meters. Plots were seeded at three different diversity levels in 2010: monoculture, low and high diversity plots (Appendix Figure 1). Each diversity treatment (monoculture, low, high plant communities) was replicated four times across the site. Since plots were not managed for the maintenance of diversity levels from the inception of the experiment, plant compositions shifted (likely due to natural seed dispersal) over the 8 years from the time when plots were established to the point of soil collection in 2018. Previous studies have measured the change in plant richness over time using the line-point intercept method to estimate plant diversity. In 2017, there were approximately 16 plant species within the monoculture plots, 32 plant species in the low plots, and 44 plant species in the high plots (Gholizadeh et al. 2019). In this manuscript, we refer to the diversity treatments as low, medium, and high due the changes in plant composition over time (Fig. 2.1).

2.2.2. Sample collection

Soil samples were collected in August 2018 within a 50- x 50-m area, excluding a 10-m^2 buffer at the perimeter, for each plot based on established plant diversity levels (Appendix Figure

2). Transects were laid out in a grid-like pattern (50-m vertical and 50-m horizontal) and samples were collected every 10-m across each 50-m transect. Five transects were laid out per plot with a 10-m distance between each transect. We collected 5 samples per transect with a total of 25 soil cores sampled per plot using a T-corer at a 5 to10-cm depth. All soil samples from one plot were composited and homogenized into one bag and transported on ice to the lab where they were stored at 4° C. We pre-processed samples by drying each sample in a sterile laminar flow hood for 48 hours and sieved each sample two times using a 2-mm sieve. Samples were then stored at -80° C prior to DNA extraction.

2.2.3. Molecular methods

From each of the 12 homogenized soil samples collected from each plot, we selected five random 0.5-g subsamples, by which DNA was extracted using DNeasy Powersoil kit, (Qiagen, Germantown, MD, USA) as per manufacturer's instructions. In preparing samples to be sequenced, extracted DNA from our soil samples had an average of 40 ng/µl of genomic DNA per sample, as quantified using a Qubit dsDNA BR Assay Kit to run the Qubit fluorometer according to manufacturer's protocol (Thermo Fisher Scientific Inc., Waltham, MA, USA). Library preparation differed between bacteria/overall soil fungi and AM fungi due to the complexity in amplifying AM fungal DNA. Therefore, we amplified bacteria and overall soil fungi using a one-step PCR library preparation, whereas AM fungal DNA was amplified using a two-step PCR library preparation protocol, as detailed below.

For our bacterial dataset, we amplified the V3 and V4 regions of the small sub-unit bacterial ribosomal RNA gene using universal prokaryotic primers 314F and 806R (Muyzer et al. 1993, Caporaso et al. 2011, Takahashi et al. 2014). To amplify overall fungal DNA, we

amplified the ITS region using the primers ITS1 and ITS2 (Blaalid et al.2013). Our bacterial and our overall soil fungal datasets underwent a one-step PCR library preparations step, using Earth Microbiome Project (EMP) adapters. After PCR amplification, samples were cleaned using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) and pooled using Quant-iT™ PicoGreen™ dsDNA Assay Kits (Thermo Fisher Scientific Inc., Waltham, MA, USA) following standard manufacturer's instructions.

Amplification of AM fungi DNA was concentrated in the small subunit region of the ribosomal RNA gene (SSU rRNA) by using primers WANDA (Dumbrell et al. 2011) and AML2 (Lee et al. 2008). Amplicon library preparation consisted of a standard 2-step polymerase chain reaction (PCR) commonly used for AM fungal library preparations for amplicon-based sequencing (Egan et al. 2018). PCR amplifications were conducted using a Bio-Rad PCR system (Bio-Rad, Hercules, CA, USA).

The forward primers in the first PCR reaction consisted of a CS1 forward tag, heterogeneity spacer, and the sequencing primer WANDA (5' CAGCCGCGGTAATTCCAGCT 3'). The reverse primers in the first PCR reaction consisted of the sequencing primer AML2 (5' GAACCCAAACACTTTGGTTTCC 3'), heterogeneity spacer, and the CS2 reverse tag. The forward primers for the PCR2 reaction consisted of an Illumina adaptor (P5), forward barcode, and CS1 tag while the reverse primers consisted of a CS2 tag, reverse barcode, and Illumina adaptor (P7). Primers for PCR2 were provided by the sequencing facility at iBEST (Moscow, ID).

PCR reactions were carried out in 50µl volumes using 1µl of template DNA and 49µl of a master mix (0.1µM WANDA, 0.1µM AML2, 25mM $MgCl_2$, BSA 20mg/ml, 10mM dNTP mix, 10xPCR Buffer-standard Taq Reaction Buffer, 5000U/ml Taq DNA polymerase). Conditions for

all PCR were as follows: initial denaturation at 95° C for 2 minutes followed by 30 cycles of 95° C for 1 minute, 58° C for 1 minute, and 72° C for 1 minute, and a final elongation for 10 minutes at 72° C (Egan et al. 2018). Products from all PCR reactions were visualized to confirm amplification using 1.5% agarose gel electrophoresis and 0.1% TBE buffer. PCR products contained approximately 600 base pairs per sample. Samples were pooled, cleaned, sequenced, and demultiplexed by the sequencing facility at iBEST (Moscow, ID) using an Illumina MiSeq 2x300 run.

2.2.4. Bioinformatics

AM fungal raw sequences were processed through the QIIME2 bioinformatics pipeline using DADA2 (Bolyen et al. 2019, Callahan et al. 2016). Low abundance and poor-quality reads were filtered out using the QIIME2 pipeline. Amplicon sequence variants (ASVs), as calculated by DADA2, were not identified taxonomically or identified as non-*Glomeromycota* were excluded from downstream analyses. After filtering, the remaining sequences were taxonomically identified by training feature classifier with the MaarjAM reference database (Öpik et al. 2010; *accessed* June 2019). ASVs were taxonomically identified as virtual taxa (VT) according to alignment with the MaarjAM database.

All bacterial and overall fungal reads were processed using USEARCH. Bacterial (16S) and fungal (ITS) raw sequences were processed using the USEARCH v10 software (Edgar 2010). The USEARCH pipeline included features such as *cutadapt* (Martin 2011) and *UPARSE* (Edgar 2013) for demultiplexing and OTU construction. All *Glomeromycota* OTUs were bioinformatically removed from the ITS/overall soil fungal pipeline to assure that *Glomeromycota* taxa were not being accounted for in our samples more than once. Reads were

blasted against the SILVA_132 reference database (Quast et al. 2012) for 16S reads and UNITE_v8.2 database for ITS reads (Nilsson et al. 2019).

2.2.5. Statistical analyses

All statistical analyses were performed using R (version 3.6.1; R Core Team, 2019) within R studio (version 1.2.5001; R Studio Team, 2019).For statistical analyses that require one biological replicate per sample, samples were chosen for each plot based on the median of the technical replicates sampled. For analyses using richness, alpha diversity, and beta diversity, only one technical replicate was chosen to represent one plot. Community composition was analyzed using both alpha and beta diversity in Calypso (Zakrzewski et al. 2017). Alpha diversity metrics were calculated using Shannon diversity index and represented using a boxplot. Bray-Curtis distance was calculated to plot beta diversity between samples on an ordination plot using NMDS (non-metric multidimensional scaling).

Microbial cooccurrence networks were calculated using Fastspar (Watts, Ritchie et al. 2019), a fast version of the SparCC algorithm (Friedman and Alm 2012), and then laid out in Gephi (Bastian, Heymann et al. 2009). Prior to calculation, OTUs and ASVs with less than three occurrences and 10 reads were filtered out to minimize spurious correlations of rare taxa. All the edges in the network indicate strong ($r > 0.7$) and significant ($p<0.01$) correlations between microbial taxa. The Benjamini-Hochberg (BH) multiple test with 100 bootstraps was used to control the false positive rate (FDR, $p < 0.01$). Topological properties of the resultant network including node degree of connectivity were also calculated in Gephi.

2.3. Results and Discussion

Many studies have found limited support for coupling between above and belowground alpha-diversity patterns (Wardle 2006; McElroy et al. 2012; Li et al. 2015; Prober et al. 2015; Delgado-Baquerizo et al. 2019). In accordance, with these studies we did not observe significant differences in the alpha-diversity (measured using the Shannon diversity index) of overall soil fungal communities in different plant diversity treatments (Appendix Fig 1). AM fungal alpha-diversity within medium plant diversity treatments were significantly different from the high and low plant diversity treatments, but not significantly different when associated with high or low plant diversity treatments (Appendix Fig 1). However, the alpha-diversity of bacterial communities mirrored aboveground diversity wherein high plant diversity treatments had significantly higher bacterial diversity ($P < 0.05$) as compared to low diversity plots. Bacteria and fungi differ in their metabolic requirements and cellular capabilities (Mille-Lindblom and Tranvik, 2003). By rapidly recycling simple structured nutrient-rich organic compounds such as root exudates (Gessner et al., 2007; Krauss et al., 2011), bacteria can maintain their shorter turnover and higher metabolic activities (Bardgett et al., 1999; Attermeyer et al., 2013). In contrast, fungi have a lower metabolic nutrient demand and higher capacity for production of enzymes for mineralizing low-quality substrate (Danger et al., 2016; Güsewell and Gessner, 2009). It is likely that higher bacterial diversity is due to availability of a more diverse resources pool maintained by root exudates within the high diversity plots.

In contrast to alpha-diversity, our beta-diversity analysis demonstrated consistent patterns in response to plant diversity within all three microbial groups studied. Redundancy analysis (RDA) showed clear separation between bacterial (Fig 2.2A), overall fungal (Fig 2.2B) and AM fungal (Fig 2.2C) communities of low, medium, and high plant diversity treatments ($P < 0.001$).

Previous reports have suggested that plant diversity can predict patterns in soil microbial community composition at the local (Navarro-Cano et al. 2014) and global scales (Prober et al. 2015). Nonetheless, other studies have indicated that the relationship between soil microbial communities and plant diversity are strongest at intermediate scales (Liu et al. 2020). Regardless, these studies do not incorporate different organismal groups that make up the soil microbiome, often excluding AM fungi that are locally adapted (Bahram et al. 2015).

Variations in plant traits and functional groups, including root exudates and architecture, can modify key soil properties (Gould et al. 2016) and directly influence the presence of specific belowground microbial communities (Carney & Matson 2006; Broeckling et al. 2008; Davison et al., 2020). In turn, interactions between AM fungal and soil microbial communities can lead to shifts in feedback dynamics that drive changes in plant community composition (Vályi et al. 2016). It is known that AM fungi can drive grassland succession by mediating positive plant-soil feedbacks with their hosts (Koziol and Bever 2019). Within the rhizosphere microbiome, AM fungi are considered a key taxonomic group due to their high biomass in the soil, contributions to soil engineering, facilitation of plant community success, and influence in plant-soil feedback dynamics (Bauer et al. 2017; Horn et al. 2017). These plant-soil feedbacks can perpetuate dynamics amongst low diversity plant communities that exacerbate unstable biotic conditions, species dominance over time, and changes in ecosystem function over time (Maron et al. 2011).

In the medium plant diversity treatments, AM fungal communities showed greater evenness compared to the low plant diversity treatments (Appendix Figure 3). More evenness in the medium plant diversity treatments could indicate that the functional roles of AM fungi may overlap more and contribute to greater competition between taxa. Therefore, AM fungal communities may have a lesser impact on the plant community due to increased competition

between AM fungal taxa as a result of limited realized niche capacity within medium diversity plant communities. Nonetheless, this could allow for other soil microbes to interact more often with the plants, creating more associations with bacteria.

The presence of different fungal strategists within the high plant diversity treatments could suggest that AM fungal communities that have more families or greater phylogenetic breadth amongst genera that allow the AM fungal community to collectively supply broader functional capability than other soil microbial communities. Other studies have found that the response of plant communities to changes in AM fungal communities is dependent on the identity of plant hosts present in the community (van der Heijden & Scheublin 2007). However, different AM fungal taxa contain different P-transporter genes (Benedetto et al. 2005; Harrison 2005) and therefore provide a very specific role to the plant community that is present. Beyond P-transporter genes, AM fungi provide roles for the plant and soil community that require more insight about microbial interactions to understand.

Within the bacterial community, we observed an increase in bacterial beta diversity as plant diversity across plots increased (Fig 2.2). In addition, bacterial Shannon diversity was significantly greater in plant communities with high diversity compared to low diversity plant communities (Appendix Figure 3). It is likely that higher bacterial diversity is associated with high plant diversity due to plant attributes that influence the soil community through diverse exudates that maintain a diverse bacterial structure and consistent nutrient sources for fungal evenness. Overall, microbial community composition is more similar between plant communities with high and low richness compared to low diversity communities. Belowground nutrient processing and differential root exudates have been found to contribute to the differences seen in low diversity systems (Wang et al. 2012).

The overall soil fungal community showed higher fungal evenness in the high diversity plots compared to the low richness plots (Appendix Figure 3). This could be an indication that there are distinct factors within high diversity plant systems that could be contributing to shifts in bacterial and fungal communities. High plant richness has been associated with protection against soil-borne pathogens, increased soil microbial activity, and soil carbon storage (Latz et al. 2012, Lange et al. 2015). However, high diversity plant systems offer a variety of leaf litter and root exudates that influence microbial community structure. A diverse availability of root exudates released within high diversity plant systems has been shown to provide a crucial link between plant diversity and soil microbes (Steinauer et al. 2016).

Our analysis also revealed differences in the relative abundances of various microbial groups between different plant diversity treatments (Appendix Tables 1, 2, 3). Interestingly, for bacterial community we observed that various genera involved in nitrogen fixation (e.g. *Burkholderia*, *Rhizobium*, *Bradyrhizobium, Bosea*) and turnover (e.g. *Rhizomicrobium*, *Nitrosopira*) were significantly higher ($P < 0.05$) in the high as compared to low diversity plant treatments (Appendix Table 1). We also observed the greater abundance of genera belonging to Actinobacteria, Beta-Proteobacteria, and Bacteroidetes ($P< 0.05$) in high as compared to low plant biodiversity treatments (Appendix Table 2). Members within these groups are classified as copiotrophs (Trivedi et al. 2013) and might have taken advantage of the greater and diverse resource availability in the high plant diversity treatments for maintaining rapid growth. In addition to Actinobacteria and Beta-Proteobacteria, we also observed a significant increase in the relative abundance of various groups belong to phylum Firmicutes (including *Bacillus* and *Paenibacillus*) (Appendix Table 2). The members of these groups play a pivotal role in soil

nutrient cycles by generating extracellular enzymes and possess various traits related to plant growth promotion (Lee et al. 2021).

For AM fungi, we examined strong evidence supporting decreased ($P<0.05$) relative abundance of genera *Acaulospora* and *Claroideoglomus* in higher diversity plant treatments in relation to low diversity treatments (Appendix Table 3). Interestingly, these two genera were also described as indicators of crop monocultures in a recently published study (Guzman et al. 2021). While we still have limited understanding on the functional traits of individual members of AM fungi (e.g., Chagnon et al., 2013), differences in the relative abundances of taxonomic groups in different plant diversity treatments, could indicate differences in community functionality with implications for plant performance and ecosystem processes (van der Heijden, 2002; Šmilauer et al., 2020).

In co-occurrence networks analyses, interactive microbial taxa are linked together based on positive or negative correlations indicating mutualistic or antagonistic co-occurrence patterns (Russel et a. 2017, Hernandez et al. 2021). Although such interactions do not indicate true, physical physically interfering, network analyses have the potential to infer inter- and intra-kingdom correlations that can be applied to understand the ecological principles guiding community assembly (Lupatini et al. 2014, Agler et al. 2016, Banerjee et al. 2018) and ultimately, practices in ecological restoration. Furthermore, microbial interactions in co-occurrence networks may give insight into the ecological processes governing community structure, such as niche filtering due to interspecific competitive exclusion (Legras et al. 2019).

Our network analyses revealed that different plant diversity treatments impacted mutualistic (positive correlations) and antagonistic co-occurrence interactions (negative correlations) (Fig 2.3). For instance, there were considerably more positive correlations in the

high diversity (92.30%), compared with medium diversity (48.20%) or low diversity (43.10%) treatments. From the perspective of microbial networks, several ecological interpretations can be made based on the degrees (number of linkages per node) of negative and positive associations. Higher proportion of negative correlations in low diversity treatments may originate from a wide range of mutual exclusion mechanisms, including direct competition, toxin production, and environmental modification. Negative associations can also reflect intense competition that it is expected to be more common under a homogeneous scenario of low biodiversity treatments wherein microbes with similar ecological requirements will compete for similar resources. In contrast, low proportions of negative associations in higher diversity treatment suggest a prevalence of collaboration or niche sharing, in which heterogeneous microenvironments could reduce direct competition and provide a diverse set of benefits to the plant community. Our analyses represent one of the first studies that have empirically linked multi-group (bacterial-AM fungal-overall soil fungal) networks to plant community diversity processes that could lead to insights in plant community restoration success. Future studies that utilize network comparisons across inter-kingdom ecological systems, will allow for the development, exploration, and generation of new ecological hypotheses.

Within our co-occurrence network analyses, the number of nodes (or OTUs/ASVs) and edges (computationally generated linkages between nodes based on the correlation of occurrences) decreased with increasing plant diversity (high < medium < low) (Fig 2.3). Therefore, our networks show that there was more network complexity (higher number of nodes and edges) in low diversity plant communities and less network complexity in high diversity communities. Studies in other systems have shown that increases in the number of nodes is reflective of increased species diversity and could indicate a decrease in interactions between

species due to the larger availability of microbial groups (Larson & Claasen 2018). Within a low microbial biodiversity ecosystem (ie. human gut), there are generally more nodes within microbial networks, which was proportional to symbiotic associations (Larson & Claasen 2018). However, our results demonstrated that when soil microbial communities show higher biodiversity, the greater number of nodes can reflect competition represented by greater negative interactions between microbial groups. While bacterial communities tend to dominate in microbial networks across all plant richness levels, fungal taxa were proportionally higher in the high plant richness plots. Overall, our network analyses show that interactions between AM fungi and soil bacteria may have shifted AM fungal community composition and interactions into competitive niches under lower diversity plots that have allowed for a distinct AM fungal community to thrive in high diversity plots. Furthermore, AM fungi may be an important contribution to more positive correlations in high diversity plant systems that enable positive interactions between soil microbial groups.

The reestablishment of complex belowground interactions is critical in rebuilding ecosystem resilience and achieving a functional stable state (Calderon et al. 2017). Arbuscular mycorrhiza develop below-ground mycelial networks, where multiple AM fungal species colonize one or more plants (Selosse et al. 2006). Although we understand that multiple AM fungal species colonization is beneficial for host plant growth (Koziol & Bever 2016) and resilience (Allen et al. 2003), the development and species composition in mycorrhizal networks is largely not understood. AM fungi are ubiquitous microorganisms that facilitate the interface between plant roots and the soil through an intimate intracellular composition within plant root cells. Furthermore, mycorrhizal fungi occupy more space, biomass, and surface area than other soil organisms between rhizospheres due to hyphal extensions (Ekelund et al. 2001).

To the best of our knowledge, our study is the first to explore the impact of plant biodiversity on the multi-group (bacterial-AM fungal-soil fungal) interactions of belowground microbial communities. Together, the higher number of cooperative microbial interactions in response to higher plant diversity is indicative of the positive relationship between plant diversity and belowground microbial community composition. In fact, we observed enrichment of various microbial groups that are reported to have a positive impact on plant growth and ecosystem functions in the higher plant diversity treatments. Our study thus highlights the fact that aboveground diversity is essential in harnessing microbial interactions to enhance plant production and microbial community stability and suggests that microbial communities may be central to understanding the emergent properties of complex plant-soil systems. This insight encourages a deeper look into the mechanisms causing shifts between AM fungal, soil fungal and bacterial communities at different plant richness levels. By beginning to understand the interactions between soil microbial communities and shifts in plant community structure, we can begin to explore how soil microbes can influence the stability of high diversity plant communities and increase restoration success.

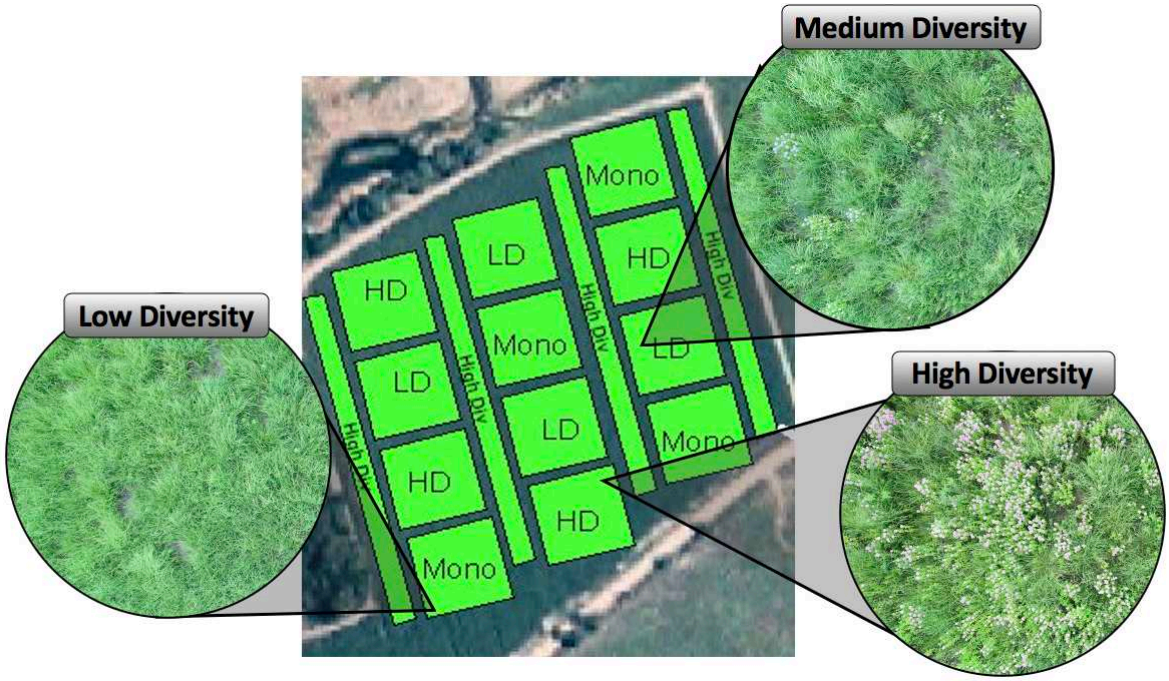


Figure 2.1. Aerial view of experimental diversity plots in Platte River Prairies, NE.

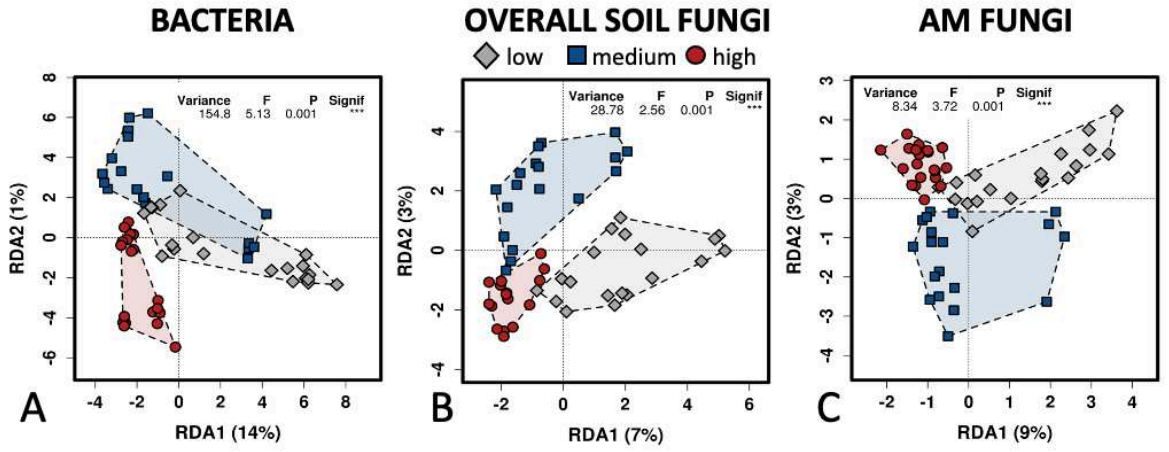
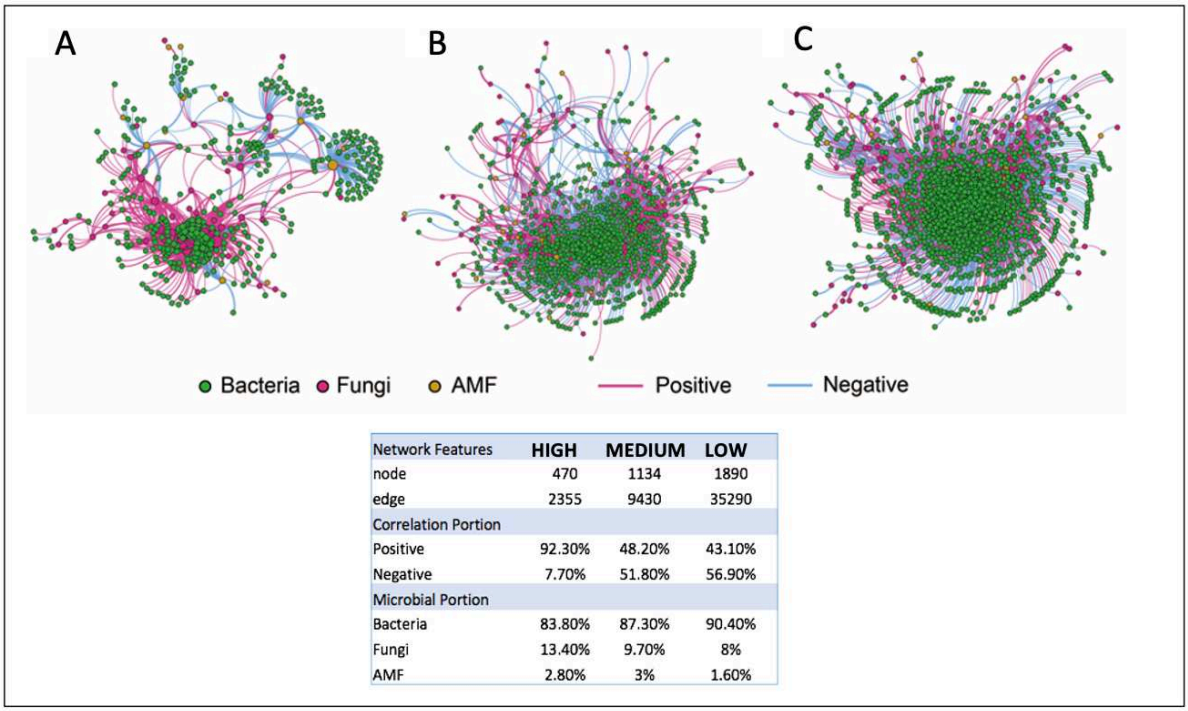


Figure 2.2. RDA plot showing constrained ordination showing the variation in data based on explanatory variables across high, medium, and low diversity plant communities based on bacterial (A), overall soil fungal (B), and AM fungal (C) communities.



Network Features	HIGH	MEDIUM	LOW
node	470	1134	1890
edge	2355	9430	35290
Correlation Portion			
Positive	92.30%	48.20%	43.10%
Negative	7.70%	51.80%	56.90%
Microbial Portion			
Bacteria	83.80%	87.30%	90.40%
Fungi	13.40%	9.70%	8%
AMF	2.80%	3%	1.60%

Figure 2.3. Network co-occurrence across bacterial, fungal, and AM fungal groups shows the linkages between microbial taxa in plant communities with high plant diversity (A), medium plant diversity (B), and low plant diversity (C). Each node represents a bacterial or overall soil fungal OTU, whereas the AM fungal nodes represent an ASV. This network shows that high diversity plant communities had more positive linkages between bacteria and saprophytic fungi, while AM fungi had negative interactions with bacteria as well as a more prominent role in the network through the formation of hubs. Overall, positive linkages increased with an increase in plant diversity between overall soil fungi, AM fungi, and bacteria.

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CHAPTER 3.

Influence of plant community diversity and field-conditioned inoculum on soil microbial community structure and network interactions

3.1. Introduction

The maintenance of biodiversity is critically important for the functioning of ecosystems and the services they provide. Loss in biodiversity influences multifunctionality of an ecosystem, limiting its chances to sustain itself and recover from stressful events (Duffy 2009, Maestre et al. 2012). The relationship strength between biodiversity and ecosystem functioning is dependent on multiple factors including scale, biome, and environmental gradients in aboveground systems, causing variations in the strength of the relationship based on these factors (Naeem et al 1994, Tilman et al. 1998, Thompson et al. 2018). Changes in biodiversity can lead to shifts in species dynamics and community processes which affects the range of functions provided by an ecosystem (Bowker et al. 2010, Cardinale et al. 2012, Tilman et al. 2014). Due to the immense species richness in belowground systems, loss in soil biodiversity could mean loss of important ecosystem services like nutrient cycling, decomposition, and mineralization that support aboveground systems (Jeffrey et al. 2010). Therefore, understanding the relationship between aboveground diversity and belowground interactions is crucial in evaluating the functional relationships needed to restore ecosystem functioning in degraded ecosystems.

Plants play a leading role in biodiversity-function relationship, but the significance of their role is dependent on the multifaceted microbial relationships that sustain plant functioning (Eisenhauer et al. 2012, Bever et al. 2015, Teste et al. 2017). An early study on the relationship between aboveground and belowground diversity suggests that arbuscular mycorrhizal (AM) fungal diversity is correlated with plant diversity in calcareous grasslands (van der Heijden et al. 1998). However, positive relationships between plant and AM fungal diversity have not been

evident in all systems. Antoninka et al. (2011) found that AM fungal diversity was higher in lower plant diversity communities that were established over a seven-year timespan. The relationship between aboveground and belowground diversity is often seen as a coupled relationship due to the complexity of soils as a habitat, which is expected to vary across ecosystems and environmental stressors (Hooper et al. 2000). Recently, researchers have found that conservation of aboveground biodiversity does not reduce the threats facing belowground systems (Cameron et al. 2019), which suggests that belowground systems should be treated as a distinct entity from aboveground systems. The variability in aboveground-belowground coupling strengths may not be a good indicator of ecosystem processes, unlike soil microbial communities and interactions that can determine the functioning of ecosystem services (Hooper et al. 2000, De Deyn & Van der Putten 2005, Scheiter & Higgins. 2013).

The biological structure of microbial communities is important for understanding how changes in soil biodiversity influences the multifunctionality of a system (Bardgett and Van Der Putten 2014, Delgado-Baquerizo et al. 2016). While alpha and beta diversity are important metrics in understanding soil biodiversity, these tools are limited in unraveling complexities across soil microbial communities because they fail to address the interactions that contribute to microbiome function (Shade 2017). More specifically, interactions within the rhizosphere microbiome between AM fungi and bacteria exhibit cooperative associations that are responsible for plant nutrient acquisition (Fitter & Garbaye 1994, Bianciotto et al. 2003, Taktek et al. 2017, Emmett et al. 2021). There is evidence showing that microbial community composition and structure reflect microbial adaptation to environmental disturbance more efficiently than multiple types of alpha-diversity indices (Pereira et al. 2014, Li et al. 2017). Furthermore, strong evidence using network analyses in Canadian prairies was used to identify hub taxa found in the canola

rhizosphere under different levels of cropping system diversities but did not find any differences in alpha-diversity, or community composition (Floc'h et al. 2020). Other studies have held alpha diversity at a constant to understand the microbial interactions and network structure that contribute to methane production, giving better insight to the functional role behind microbial interactions (Lin et al. 2017). Network interactions between microbes provide important insight into cooperative and competitive dynamics that influence soil functions (Ma et al. 2015). Network analyses that include fungal interactions often find its structure to be complex but often linked to greater adaptability and resistance to environmental perturbations compared to bacterial networks (deVries et al. 2018, Wan et al. 2020). Therefore, understanding fungal-bacterial networks can help tease apart complex microbial interactions that give insight into function.

The plant microbiome is a dynamic modular entity formed by co-evolutionary processes that are interwoven across multi-kingdom microbial network interactions (Dini-Andreote & Raajmakers 2018, Getzke et al. 2019). Utilizing microbial networks, we can better understand the ecological associations, microbial interrelationships, and keystone taxa that drive microbiome function and identify (Banerjee & Schlaeppi 2018, Wagg et al. 2019). As a critical keystone microbial guild in the rhizosphere microbiome, arbuscular mycorrhizal fungi play a key role in ecosystem processes and have a major impact on soil microbial community dynamics, but little is understood about their interactions with other rhizosphere microbiota (Nuccio et al. 2013, Banerjee & Schlaeppi 2018, Jiao et al. 2020, Wang et al. 2021). Through co-occurrence network analyses, we can identify hub taxa that have a strong biotic signature influencing microbial community structure in order to break down complex ecological patterns that lead to microbiome formation (Agler et al. 2016). The identification of hub microorganisms can be used to evaluate

the relationships between interacting microbes and give a better understanding of the traits that contribute to biodiversity and ecosystem functioning (Trivedi et al. 2020).

One concept that is unclear in rhizosphere microbiomes is how microbial priority effects and plant species effects on the soil environment, or soil conditioning, change microbiome and plant-soil feedback dynamics. While it is known that the AM fungi can lead to shifts in plant community structure (Wubs et al. 2016) and alter plant succession (Koziol et al. 2021), it is still unclear how microbial additions influence hierarchical microbial interactions that support plant community diversity. It has been shown that changes in aboveground diversity alone does not support soil microbial communities and that historic plant community structure influences AM fungal persistence, which could have a great impact on the restoration of plant communities (Bittebiere et al. 2020, Wall et al. 2020). Nonetheless, throughout the literature it is unclear how changes to the aboveground community influences soil microbial interactions. Furthermore, there is a lack of clarity on how historic plant community structure influences AM fungal and bacterial persistence over time. Therefore, understanding how soil conditioning influences the microbial interactions between AM fungi and bacterial communities could be crucial in restoring plant diversity and rehabilitating degraded ecosystem function.

To evaluate how soil microbial interactions influence plant community restoration, we used high throughput sequencing and network analyses to understand how plant diversity influences the AM fungal and bacterial interactions within soils conditioned by different plant richness levels. We used an experimental approach with 2 levels of soil conditioning by collecting field soil from plant communities of high and low diversity and using this field-conditioned soil inoculum in a greenhouse study with mesocosms containing high, medium and low levels of plant diversity. Our hypotheses asked: (1) Is there a link between soil microbial and

plant diversity, (2) Do AM fungal and bacterial community composition change according to plant diversity manipulations, (3) Do AM fungal and bacterial co-occurrence network structure change according to plant diversity manipulations? Our research looked at AM fungal and bacterial alpha diversity, beta diversity, microbial community enrichment, and network analyses under varying levels of plant diversity to identify shifts in microbial community and network structure. By exploring network properties, we identified hub taxa and identified potential AM fungal and bacterial interaction dynamics that were influenced by soil conditioning and that supported the plant community. These findings can change the way that we consider plant restoration efforts and give insight into key microbial interactions that contribute to the complexity and functioning of the rhizosphere microbiome.

3.2. Methods

3.2.1. Greenhouse Study

Soil inoculum for our greenhouse study was collected from October 18-20, 2018 at experimental grassland diversity plots (located at UTM zone 14 E 534572.55 and N 4510395.93) established in 2010 with three levels of plant diversity (low, medium, high). Twenty-five soil samples were collected in a grid-like pattern every 10 m (excluding a 10m buffer) in the high (4 plots) and low (4 plots) diversity plots. Soil samples were composited per plot replicate for a total of 8 soil inoculum samples. Soil inoculum was transported in coolers on ice and transferred to 4°C storage for preprocessing. Samples were air dried in a sterile laminar flow hood for 48 hours before being dry sieved (0.25mm sieve) and stored in -18°C until used as inoculum in the greenhouse study. Previous efforts to understand the microbial ecology of high and low field conditioned plant diversity treatments at this site showed that bacterial communities had

significantly greater diversity and richness in high plant diversity plots compared to low plant diversity plots (*See Chapter2*). AM fungal communities did not show significant difference in diversity or richness between high plant diversity plots and low plant diversity plots (*See Chapter2*).

Throughout this paper, we use the term 'field inoculum treatments' as soil inoculum that was collected from high and low diversity field plots (along with an uninoculated control treatment). We used these field inoculum treatments in a greenhouse experiment and planted various levels of plant diversity in replicated mesocosm pots. We refer to these greenhouse-established plant diversity treatments as greenhouse conditioned soils, or greenhouse conditioned plant diversity treatments (Fig 3.1). Our greenhouse conditioned plant diversity treatments included a selection of 5, 15, and 30 native tallgrass prairie plant species chosen based on their presence in Nebraska and Kansas tallgrass prairies. To randomize the selection of plant species in each pot, we selected plant species based on a pool of 88 different native species. The plant species used per pot were selected by random. Each plant species was assigned a number and subsequently selected for each plant diversity replicate by using a random number generator. Seeds were purchased from regional seed suppliers. Seeds were stratified in a 75% sand 25% perlite mixture using plastic containers with drainage holes and germinated according to commercial seed provider instructions to ensure at least 70% germination. Stratified seeds were sprayed with water every 4-5 days to mimic outdoor moisture conditions.

All seed germination trays (30- x 60-cm) and were UV-radiated for 2 minutes on each side. Trays were filled with 2.5 cm of General-Purpose Premiere Pro-Mix BX potting-medium (without mycorrhizal treatment) that had been autoclaved 2 times (within 48 hours) for 1 hour at 375°C. In starting the seeds, a 2.5 cm layer of autoclaved potting mix was added to each tray and

sprayed with water. Next, we added approximately 110-180 grams of seed to each tray. For some species, we cold-stratified seed in moist sand for 2-3 months prior to this step. One final layer of autoclaved potting-medium was added to each tray (amount varied based on seed size) and sprayed down with water. Each tray contained one plant species, and 2-3 trays per plant species were prepared for germination. Trays were watered every 48 hours and grew for 30 days in the greenhouse before emerged seedlings were transplanted into mesocosms.

Large pots (38 L, diameter of 50-cm and height of 43-cm) were used to prepare mesocosm communities. Pots were filled with 30-L of General-Purpose Premiere Pro-Mix BX potting-medium (without mycorrhizal treatment) and watered before transplanting. Pots that received inoculum treatments were topped off with a 1-cm layer of field soil (either low or high diversity field conditioned soils) followed by a cap of 5 cm of Pro-Mix BX potting-medium and were watered prior to transplanting. Pots designated as control treatments did not contain inoculum from field conditioned soils but were prepared using only the Pro-Mix BX potting medium.

Each plant species used for the greenhouse conditioning treatment was given a designated number (through the use of a random number generator) to select species to be included in each pot based on the diversity treatment: 5 species in low plant diversity pots, 15 species in medium plant diversity pots, 30 species in high plant diversity pots. Each pot contained a total of 60 individual plants. As a result, pots designated as low plant diversity contained a higher representation of each plant species than others. For example, pots designated as low plat diversity had 5 plant species with 12 individuals transplanted per species. Pots designated as medium plant diversity had 15 plant species with 4 individuals per species. Pots designated as high plant diversity had 30 plant species with 2 individuals per species. Pots were watered 3-4

times a week and grew in the CSU greenhouse for 6 months. Seedlings that did not survive initial transplanting were replaced during the first 2 weeks of the study.

In total, our study included three greenhouse conditioned plant diversity treatments (low, medium, high diversity) and three inoculum treatments (control, low-conditioned field inoculum, high-conditioned field inoculum). With nine plant-soil treatment combinations and 10 replicates per treatment combination, we had a total of 90 mesocosm pots.

After 6 months of growth in the greenhouse, mesocosm communities were harvested by clipping all aboveground biomass. Soil samples were collected from each mesocosm after biomass removal using a 5-cm wide by 10-cm deep soil corer. Three samples were collected at the soil surface of each pot approximately 10 cm from the center from the pot, with each collected soil core being 20 cm from the other two cores collected. The three cores from each pot were composited to yield one sample per pot, homogenized with large roots removed from the soil, stored in plastic bags, and transported on ice to the lab. At the laboratory, sample were air dried under a laminar-flow hood for 48 hours before being dry sieved (0.25mm sieve) and stored at -18°C prior to DNA extraction.

3.2.2. Molecular Methods and Bioinformatics

Using 0.2 g of air dried mesocosm soils, we extracted DNA from each of our samples using the DNeasy PowerSoil Kit. Due to the absorptive nature of the potting-medium soils, we altered the standard protocol using the following modifications: (1) Powerbead tubes consisted of 120µl of C1 solution and 600 µl of Powerbead solution, (2) after the first centrifuge, supernatant was pipetted into a new tube, (3) Powerbead tube was used again with an additional 200µl of Powerbead solution, vortexed briefly and centrifuged at 10,000 rpm for 1 minute. AM fungal

libraries were prepped using a two-step PCR process. We cleaned the samples after the first PCR using Sera beads and cleaned again using a column cleaning procedure after pooling to equimolar concentration.

For our bacterial dataset, we amplified the V3 and V4 regions of the small sub-unit bacterial ribosomal RNA gene using universal prokaryotic primers 314F and 806R (Muyzer et al. 1993, Caporaso et al. 2011, Takahashi et al. 2014). The primers for the AM fungal dataset, WANDA and AML2, were selected based on its wide use in AM fungal community surveys, its SSU-based marker diversity, and its balanced amplification of AM fungal families (Egan et al. 2018, Vasar et al. 2021).

Our bacterial dataset and our AM fungal dataset underwent two different methods of library preparation due to the difficult nature of amplifying AM fungal samples. A one-step PCR library preparation was implemented on our bacterial dataset using Earth Microbiome Project adapters. We used a two-step PCR process for our AM fungi samples, with a Serabead cleanup step between each PCR. Library preparation was performed using KAPA 3G plant PCR kit and polymerase. With a total reaction volume of 25µl, our first PCR reaction contained 12.5µl of buffer, 0.75µl of the forward primer, 0.75µl of the reverse primer, 0.2µl of Taq Polymerase, 1.5µl of $MgCl_2$, and 9.3µl of template DNA. The following conditions were used to amplify the SSU region of AM fungal DNA: 95°C for 2 minutes, 30 cycles of 95°C for one minute, 64°C for 1 minute, and 72°C for 1 minute courses of 30 cycles. After the cycling period was over, each sample was amplified at 72°C for 1 minute and kept at 0°C until removed.

All samples were then pooled at equimolar concentration and assessed using the PicoGreen assay. Pooled samples were then sequenced at the Colorado State University Sequencing Core with Illumina Miseq (paired-end 2x300). All reads were processed using

USEARCH v10 software (Edgar 2010), using *cutadapt* (Martin 2011) and *UPARSE* (Edgar 2013) for demultiplexing and OTU construction. Reads were blasted against the SILVA_132 reference database (Quast et al. 2012) for 16S reads and the MaarjAM database for AM fungal reads (type sequence V.05/06/2019) (Öpik et al. 2010).

3.2.3. Statistical Analyses

After completing our bioinformatic analyses, we had 20,082 OTUs and 86 samples. We took three steps into consideration when filtering our dataset. The first step was to include OTUs that had at least 50 reads across our sample dataset. For our 16S dataset, this step filtered out 15,311 OTUs. Then, we filtered to include all samples in the dataset that had more than 5,000 reads across all OTUs. For our 16S data set, we filtered out 4 samples (1A, 2A, 14A, and 78A), leaving us with a total of 82 samples. Finally, we calculated occupancy by OTU to filter out OTUs that had less than 20% occupancy. This filtering step allows us to only include OTUs that are present in 20% of the samples in order to avoid making inference about taxa that occur in low numbers across all samples. This step filtered 269 OTUs from the 16S dataset, with a total of 4,502 OTUs and 82 samples before rarefication.

For our AM fungal dataset, we had a total of 4,198 OTUs and 87 samples after our bioinformatic analyses. We retained OTUs that had aminimum of 50 reads, leaving us with 3, 473 OTUs after filtering those OTUs out. Next, we filtered to include only samples that had a minimum of 5,000 reads across all OTUs. This step filtered out 10 samples (3A, 17A, 29A, 41A, 44A, 49A, 53A, 68A, 81A), leaving a total of 77 samples. Finally, we calculated 20% occupancy by OTU, filtering out 2,549 OTUs, with a total of 923 OTUs and 77 samples before rarefication.

Each OTU table was rarified using the package *vegan* in Rstudio (Oksanen et al. 2007). CAPS analyses were performed for beta diversity ordinations using the Bray-Curtis dissimilarity metric in *Biodiversity R* package (Kindt & Kindt 2019). Permanovas were run using the *adonis* function in the *vegan* package (Oksanen et al. 2007). Alpha diversity was calculated with Shannon Diversity Index metrics using the *vegan* package (Oksanen et al. 2007). To determine statistical significance of alpha diversity plots, the *agricolae* package was used to calculate Tukey's Multiple Comparisons test (de Mendiburu & de Mendiburu 2019). Ternary plots were created using the *ggtern* package (Hamilton & Ferry, 2018). All data wrangling and tidying was performed using the *tidyr* package and all figures were plotted using the *ggplot2* package (Wickham 2011, Wickham & Wickham 2017). In making the network analyses, we utilized the app CoNet (Faust & Raes 2016) within Cytoscape v3.7.2 (Shannon et al. 2003). Network visualization was performed using the platform Gephi 0.9.2 (Bastian et al. 2009). Nodes with a degree of less than 10 were eliminated to reduce noise in the network visualizations. Hub taxa were classified as such based on high degree (>120) and closeness centrality (0.2) based on co-occurrence network metrics (Agler et al. 2016, van der Heijden and Hartmann 2016).

3.3. Results

3.3.1. Beta Diversity

Overall, beta diversity across bacterial and AM fungal communities showed different ordinations based on greenhouse conditioned plant diversity treatments. Both bacteria and AM fungal communities exhibited variation according to field inoculum treatments. Across the low and high field inoculum treatments, we observed distinct groups of 16S (bacteria) community composition clustering associated with each greenhouse conditioned plant diversity treatment

(low, medium, high) within field inoculum treatments. (Fig 3.2). Bacterial communities in high field inoculum treatments clustered by greenhouse conditioned plant diversity treatments and occupied a distinct space in the ordination (p-value <0.00001) (Fig 3.2A). The low field inoculum treatments showed similar trends with distinct clusters, however there was less separation between clusters (Fig 3.2B). The uninoculated control treatment, which consisted solely of potting-medium without field inoculum, (Fig 3.2C) showed a clear separation between low and medium/high greenhouse conditioned plant diversity treatments. These results in the uninoculated control treatments were rather surprising considering that greenhouse conditioned plant diversity treatments show similar separation in high and low field inoculum treatments. Nonetheless, the separation between greenhouse conditioned plant diversity treatments in the uninoculated control treatments were significantly different ($p = < 0.00001$).

Overall, beta diversity of AM fungal composition across different field inoculum treatments showed different trends compared to bacteria composition, with more overlap across greenhouse conditioned plant diversity treatments. In the high field inoculum treatment, there was distinct separation between the greenhouse conditioned high plant diversity and greenhouse conditioned medium/low plant diversities. Based on p-value (0.0046), there was significant separation between AM fungal composition across greenhouse conditioned plant diversity treatments; however, NMDS ordination showed that there was substantial overlap between medium and low greenhouse conditioned plant diversity treatments (Fig 3.1A). Surprisingly, the low field inoculum and uninoculated control treatments showed similar results, where AM fungal community composition across all three-greenhouse conditioned plant diversity treatments had substantial overlap, as represented by their p-values (Fig 3.1B-C). The

uninoculated control treatments likely contained AM fungal communities due to contamination from the greenhouse environment.

There were significant differences between bacterial communities in the various treatments based on beta diversity metrics, particularly between inoculum treatments (uninoculated control vs. field inoculum treatments) (Table 3.1A). The majority of variance calculated for bacterial communities was due to the greenhouse conditioned plant diversity treatments, based on field inoculum treatments (Table 3.1A). Since field inoculum treatments shows the most significant p-value, it is likely that the greenhouse conditioned plant diversity treatments are significant based on the residual effects from field inoculum treatments. The beta diversity of the AM fungal communities showed significant differences between field inoculum and greenhouse conditioned plant diversity treatments (Table 3.1B). Unlike the bacterial beta diversity, the AM fungal beta diversity is different for the wild soil treatment, indicating no significant differences between the uninoculated control and field inoculum treatments. The overall results from AM fungal beta diversity show a strong effect from the field inoculum.

3.3.2. Alpha Diversity

The general trends in alpha diversity show that microbial diversity varied with greenhouse conditioned plant diversity treatments, but greenhouse conditioned plant diversity trends were different based on the field inoculum treatments. Both the bacteria and AM fungi in high field inoculum treatments showed significantly different microbial diversities across greenhouse conditioned plant diversity treatments. Bacterial alpha diversity was greatest in the greenhouse conditioned high plant diversity treatments and lowest in the greenhouse conditioned low diversity plant treatments (Fig 3.4B). However, the greenhouse conditioned medium plant

diversity treatments did not statistically differ from the greenhouse conditioned high and low plant diversity treatments. On the other hand, AM fungi in the high field inoculum treatments showed the highest alpha diversity in the greenhouse conditioned high plant diversity treatments and lowest diversity in both the greenhouse conditioned low and medium plant diversity treatments (Fig 3.5A). Unlike bacteria diversity, AM fungal diversity did not differ in greenhouse conditioned low/medium plant diversity treatments within the high field inoculum treatments.

The low field inoculum treatment was different between bacterial and AM fungal diversity. Bacterial communities in low field inoculum treatments showed the highest alpha diversity in the greenhouse conditioned low/medium plant diversity treatments and the lowest alpha diversity in the greenhouse conditioned high plant diversity treatments (Fig 3.4B). There was no statistical difference among AM fungal diversity in low field inoculum and uninoculated control treatments (Fig 3.6B-C). In addition, similar trends were observed within the uninoculated control treatments for bacteria, which showed that greenhouse conditioned plant diversity treatments did not influence bacterial diversity (Fig 3.4C).

Bacterial community alpha diversity showed significant differences across all three greenhouse conditioned plant diversity treatments. Similar to the beta diversity PERMANOVA table (Table 3.1A), we saw significant differences between the uninoculated control and field inoculum treatments (Table 3.2A). Unlike beta diversity, the ANOVA for alpha diversity shows that the majority of the variance is due to the field inoculum treatments (Table 3.2A). The second highest level of variance is due to the residual effect of the greenhouse conditioned plant diversity treatments. This residual effect is likely due to the greenhouse conditioned plant diversity treatments combined with the effect of the field inoculum source. The AM fungal alpha

diversity showed very similar results to the AM fungal beta diversity PERMANOVA (Table 3.1B). The AM fungal alpha diversity shows significant differences among field inoculum treatments and greenhouse conditioned plant diversity nested treatment (Table 3.2B). AM fungal alpha diversity did not show significant differences across the uninoculated control and field inoculum treatments. The addition of the greenhouse conditioned plant diversity treatments contributed to the majority of the variance calculated.

3.3.3. Co-occurrence Networks

To investigate how soil microbial interactions change with soil inoculum source (low versus high diversity plant community) and plant diversity, we analyzed bacterial-fungal interkingdom networks by utilizing co-occurrence metrics. Of the nine treatments tested in this experiment, all nine co-occurrence networks showed different patterns in network topology. Overall, the treatments that included soil inoculum (from either high or low diversity plant communities in the field) show a higher percentage of AM fungal contribution to network co-occurrence (Fig 3.6A-C, 3.7D-F). Within the control soils (Fig 3.6G-I), we found that the percentage of co-occurrence contributions from AM fungal and bacterial communities generally stay the same across the plant diversity treatments with the highest contribution from AM fungal communities in the low plant diversity treatment (69.57%) as compared to the high plant diversity treatment (59.88%). Alternatively, within the control soils, we observed the highest contribution from bacterial communities to the co-occurrence network is in the high plant diversity treatment (40.12%) compared to the low plant diversity treatment (30.43%). Within the field inoculum treatments, we generally see similar trends with AM fungal contributions to co-

occurrence networks increase with plant richness and bacterial communities decrease with higher plant richness.

The structure of different network components also varies based on field inoculum source and plant diversity treatments. The low plant diversity treatments in the uninoculated control soils showed the highest number of nodes, where each node represents one operational taxonomic unit (OTU) (Fig 3.6I), along with the highest number of edges, which represents the number of 'interactions' (as derived by correlation) between nodes. The lowest number of nodes and edges of the nine networks were found in greenhouse conditioned high plant diversity and high field inoculum treatments (Fig 3.6A). Within the high field inoculum (Fig 3.6A-C) and uninoculated control treatments (Fig 3.6G-I), we generally observed network components (nodes and edges) decrease with more greenhouse conditioned plant diversity increases. On the other hand, low field inoculum treatments show the opposite trend, an increase of nodes and edges with as greenhouse conditioned plant diversity increases.

After establishing our correlation matrices, interaction values were calculated for each node based on the node's degree, which represents the number of edges (or linkages) to other nodes (Faust & Raes 2012). Degree values can have a positive or negative value indicating the type of interaction: copresence or mutual exclusion, respectively (Faust & Raes 2012). For simplicity, we refer to these values as positive (indicating copresence) or negative (indicating mutual exclusion) edges. In the control and monoculture conditioned soils, negative edges were generally twice as prevalent as positive edges. More specifically, low plant diversity treatments show the highest proportions of positive edges and medium plant diversity treatments show the highest proportion of negative edges. Overall, the most even proportions of positive and negative edges occurred in the high soil conditioning treatments within the low and medium plant

diversity treatments (Fig 3.6B-C). The highest proportion of positive edges occurred in the high soil conditioning treatments within the high plant diversity treatments (Fig 3.6A) by a substantial margin (1.8 times higher than the treatment with the second highest proportion of positive edges as seen in Fig 3.6B).

Hub taxa within each network were evaluated as nodes that exceeded a degree of 120 and a closeness centrality value of 0.2 (Fig 3.7). Overall, most of the hub taxa across all treatments are AM fungi. The most common families associated with these AM fungal hub taxa are Glomeraceae and Gigasporaceae. The high soil conditioning treatments (Fig 3.7A-C) had the most diverse array of AM fungal hub taxa including Acaulosporaceae, Pacisporaceae, Diversisporaceae, and Archaeosporaceae. Other treatments that had only AM fungi hub taxa include mono soil conditioning within the low plant diversity treatment and control soils within the high plant diversity treatment (Fig 3.7F-G). Monoculture soil conditioning within medium plant diversity was the only treatment to show both bacterial and AM fungal hub taxa (Fig 3.7E). Those hub taxa were in the bacterial subphylum Alphaproteobacteria, bacterial phylum Acidobacteria, and AM fungal family Gigasporaceae. Of the nine treatments, monoculture soil conditioning within high plant diversity and control soils within low and medium plant diversity exhibited bacterial hub taxa (Fig 3.7D, 3.7H-I). Within the monoculture conditioned soils, the bacterial hub taxa were in the Flavobacteria and Planctomycetes phyla (Fig 3.7D). Within the control soils, the bacterial hub taxa belonged to the subphylum Deltaproteobacteria, phylum Spirochaetae, phylum Bacteroidetes, and phylum Planctomycetes (Fig 3.7H-I).

3.3.4. Ternary Plots

To assess the differences that we saw in network structure in greater detail, we analyzed groups of enriched taxa based on each soil conditioning and plant diversity treatment using

ternary plots based on the relative abundances of taxa that had greater abundances of one plant diversity level compared to the others (Fig 3.9, 3.10). Overall, these ternary plots show a shift in enriched taxa composition based on soil inoculum conditioning and the influence of plant diversity. These ternary plots show that particular bacterial phyla and AM fungal families are enriched in some treatments, but not enriched for others. In addition, these plots show that some treatments are dominated OTU with high relative abundance (as seen in the monoculture soil conditioning/high plant diversity) (Fig 3.9B) treatment, while other treatments are dominated by multiple OTUs at high relative abundances (control soils/medium plant diversity) (Fig 3.9C). Overall, these plots show approximately how many taxa were enriched for each soil condition and plant diversity treatment and that no two treatments have the same number of enriched OTUs, the same enriched families or phyla, or the same differences in relative abundances.

To take a closer look at phylogenetic families that were present and absent in each treatment, we plotted each OTU from the ternary plots to a heatmap and grouped them based on bacterial or AM fungal family (Fig 3.8D, 3.9D). In the bacterial dataset, we saw that bacterial families that are enriched in the ternary plots show consistent grouping patterns based on soil conditioning (Fig 3.8D). Overall, plant diversity treatments within the same soil condition showed similar trends across bacterial families. High plant diversity treatments in control and monoculture conditioned soils show lower relative abundance compared to medium and low plant diversities in the same soil condition. In the high soil conditioning treatments, we saw similar relative abundances across plant treatments, but different bacterial families being enriched in the high diversity soils compared to the low and medium diversity soils.

Within the AM fungal dataset, we do not see enrichment of AM fungal families based on soil condition or plant diversity treatments (Fig 3.9D). The treatment with the most abundant and

most diverse AM fungal families was high soil conditioning within high plant diversity treatments. We saw strong enrichment of Diversisporaceae, Pacisporaceae, Glomeraceae, Gigasporacea, and Acaulosporaceae within the high soil conditioning/high plant diversity treatments. The least abundant and least diverse AM fungal families were evident in the uninoculated control and greenhouse conditioned medium plant diversity treatment. The high diversity plant treatment within the monoculture soil conditioning also showed low abundance and low AM fungal family diversity, however, it did show the enrichment of Archeosporaceae.

3.4. Discussion

Based on our hypotheses, we found that our treatments differed in their relationship between belowground and aboveground diversity. Both AM fungal and bacterial diversity increased in greenhouse conditioned high plant diversity treatments with high field inoculum treatments, showing evidence for coupled changes in aboveground-belowground diversity. Low field inoculum and uninoculated control treatments did not show coupled changes between aboveground-belowground diversity. Bacterial diversity was primarily dependent on inoculum treatments (field inoculum vs. uninoculated controls), while AM fungal community diversity was solely dependent on field conditioned plant diversity treatments, or the legacy of historically conditioned plant communities in the field. Soil microbial composition shifted based on the enrichment of particular AM fungal and bacterial taxa, exhibiting changes in community composition with as greenhouse conditioned plant diversity changes. Network analyses showed that AM fungal and bacterial co-occurrence network structure shifted based on field inoculum and field conditioned plant diversity treatments.

3.4.1. Bacteria and AM fungi Respond Differently to Field Inoculum Source and Plant Diversity

Overall, we find that field inoculum treatments influenced both bacterial and AM fungal diversities in different ways. Bacterial communities showed distinct patterns based on soil inoculum source (field inoculum vs. uninoculated control) (Fig 3.2D, 3.4D), similar to findings in other studies evaluating live soil inoculum sources (Ishaq et al. 2017, Schmid et al. 2019). Across all inoculum treatments, greenhouse conditioned plant diversity treatments had a greater influence on bacterial communities (Fig 3.2A-3.2C) than AM fungal communities (Fig 3.3A-3.3C), due to distinct clustering in bacterial beta diversity ordination patterns based on greenhouse conditioned plant diversity. Similar studies have also found that bacterial communities shift with changes to plant diversity (Schlatter et al. 2015, Guerrero-Ramirez et al. 2019). Alternatively, AM fungal communities only responded to greenhouse conditioned plant diversity treatments in high field inoculum treatments (Fig 3.3A).

In high field inoculum treatments, we saw an exponential increase in bacterial alpha diversity with an increase in plant diversity (Fig 3.4A). This increase in diversity suggests that high field inoculum treatments contained more specialized bacterial taxa due to higher alpha diversity, which could have allowed for greater functional niche space with higher greenhouse conditioned plant diversity (Xun et al. 2019). High plant diversity allows for high resource and plant exudates diversity, creating more opportunities for bacterial taxa due to the increased availability of resources (Schlatter et al. 2015). These results infer that increased plant diversity enables coevolutionary niche differentiation within rhizosphere microbial community that compete when plant diversity is low, indicating that higher plant diversity sustains high diversity across microbiome members (Kinkel et al. 2011).

Bacterial communities in the low field inoculum treatments (Fig 3.2B), likely experienced a microbial bottleneck in their community diversity, where a higher number of bacterial generalists were able to survive due to the inherent lack of litter diversity in field conditioned low plant diversity plots. Our previous results have also shown that field conditioned low plant diversity plots had less alpha diversity than field conditioned high diversity plots *(See Chapter2).* Microbial bottlenecks are commonly discussed in plant-soil feedback studies where the plant community plays an active role in structuring the microbial community via litter inputs (Chapman et al. 2006, Martin et al. 2021).

In our study, the legacy effects captured by the soil community in the field were likely driven by litter inputs, indicating that bacterial communities were limited by the litter quality contributing to organic matter in the soil (Demoling et al. 2007). The microbial bottleneck observed in our study is likely due to the interactive effect of the soil litter input legacy and the filtering capabilities of the plant diversity treatments. This indicates that the different field inoculum treatments (low vs. high) had different effects on its associated microbial community, where litter inputs from monoculture soils lowered bacterial alpha diversity (Fig 3.4B), refining the associated bacterial community and constraining community functions (Xun et al. 2019) as plant diversity increased.

Inoculum treatments had a greater impact on AM fungal communities compared to plant diversity. Across all inoculum treatments, AM fungal beta diversity showed differential clustering based on field inoculum treatments (Fig 3.3D). Here, it is important to note that communities under high inoculum treatments had a wider range in variance (greater spread in the ordination of experimental replicates) and minimal variance across low field inoculum and uninoculated control treatments. High AM fungal community variance is also evident within the

high field inoculum treatments, specifically within high plant diversity treatments, which show distinct clustering pattern compared to medium and low plant diversity treatments (Fig 3.3A).

The high field inoculum treatments consisted of AM fungal communities that historically sustained high diversity plant communities. Therefore, high field inoculum treatments consisted of microbial taxa that would be associated with a wide range of plant species, indicating that high variance across the AM fungal community associated with high diversity plant communities could indicate that the AM fungal communities differed based on the different plant identities present in each high diversity plant replicate. On the other hand, in the field, AM fungal community plasticity was more discrete, and had less variance when plant communities were similar across replicates (Fig 2.2C).

Interestingly, the AM fungal beta diversity within the high field inoculum treatments showed distinct clustering in high plant diversity treatments but overlapped in clustering for medium and low plant diversity treatments. It is likely that a microbial bottleneck based on plant identity limited the proliferation of AM fungal diversity within low and medium plant diversity treatments, providing for suitable hosts for more generalists AM fungi, unlike the high plant diversity treatment. The lack of significant differences across plant diversity treatments in low field inoculum and uninoculated control treatments, also confirms that plant diversity had less influence on AM fungal community structure than field inoculum treatments (Fig 3.3B-C).

Alpha diversity trends reinforced that the AM fungal community had a greater response to field inoculum treatments (high vs. low) than to changes in plant diversity treatments. High field inoculum treatments showed greater alpha diversity than low field inoculum and uninoculated control treatments (Fig 3.5D). Recent studies on AM fungal community assembly showed that historic plant composition, or soil conditioning, has a greater influence on the AM

fungal community compared to present-day plant community structure (Bittebiere et al. 2020). However, in our study AM fungal communities did respond to plant diversity treatments under high inoculum treatments, where alpha diversity was greater in high diversity plant treatments (Fig 3.5A). This indicates that AM fungal communities are influenced by plant diversity, but that soil legacies are more important to how AM fungi respond to plant diversity. Soil legacies, known as the residual biological imprints left behind by plant-soil feedbacks over time, are produced by plant community conditioning of the soil and can be a driver of plant community dynamics and succession (Kostenko et al. 2012, Heinen et al. 2020). Low initial AM fungal beta and alpha diversity within low field inoculum treatments (Fig 3.3B, 3.5B) and uninoculated control treatments (Fig 3.3C, 3.5C), did not shift with changes in greenhouse conditioned plant diversity. Within the low field inoculum treatments, the pool of available AM fungi was limited despite the range of host plant diversities that were conditioned in the greenhouse.

Bacterial and AM fungal communities differed in their responses to inoculum treatments due to the bacterial response to field inoculated and uninoculated control treatments. Since field soils had an established microbial community that was field-conditioned with various soil characteristics (structure, chemical composition, and pH) and not evident in the potting-medium that made up the uninoculated control treatments, the field conditioned soils had a drastically different bacterial composition (Fig 3.2D, 3.4D). On the other hand, uninoculated control treatments did not show significant separation for AM fungal beta diversity (Fig 3.5D). This is likely because our uninoculated control treatments did not contain enough AM fungi to make substantial associations for success with its hosts, but likely experienced some sort of contamination in the greenhouse environment. Unlike bacterial communities, AM fungal community diversity was not driven by field inoculum or uninoculated control treatments.

However, field conditioning of the inoculum may have been a stronger driver for AM fungal communities. Previous studies also show that plant composition has a stronger legacy effect on AM fungal communities compared to soil abiotic properties (Semchenko et al. 2018, Bittebiere et al. 2020). Higher AM fungal alpha diversity in high field inoculum treatments indicates that field conditioning in high plant diversity communities contributed to the stronger legacy effect evident in high versus low field inoculum treatments (Fig 3.5D).

3.4.2. Networks Properties and Enriched Abundances Indicate Shifts in Biological Interactions

Cooccurrence network analyses are a widely used tool in understanding the complexity behind soil microbial interconnectivity and relationships between microbe-microbe interactions to interpret niche space allocation and competition (Toju et al. 2016, Li & Wu 2018, Wagg et al. 2019). Network analyses are also useful in linking aboveground-belowground relationships to provide insight on species interaction within multiple communities (Ramirez et al. 2018).

In this study, we use network analyses to explore the relationship between bacterial and AM fungal communities using greenhouse mesocosms varying in plant diversity that had been inoculated with field soil from high and low diversity plant communities. Network structures (nodes and edges) and properties (positive/negative correlations, bacterial /AM fungal proportions contributing to network structure) along with ternary plots that mapped enriched microbial abundances were used to understand how soil microbial interactions influenced the rhizosphere microbiome.

Within our cooccurrence network analyses, we observed a high proportion of negative interactions and a high proportion of AM fungi (relative to bacteria) that contribute to network structure and dynamics (Fig 3.6). Other studies have found that network interactions between

microbes in different kingdoms tend to be negatively correlated, particularly in soil microbial networks (Faust et al. 2015, Agler et al. 2016). In network analyses, computational correlations made between two taxa are categorized into positive and negative correlations, which are indicative of copresence or mutual exclusion, respectively (Faust & Raes 2012). While many studies have evaluated rhizosphere microbial cooccurrence networks from the field or plant communities dominated by few species (Barberán et al. 2012, Ma et al. 2016, de Vries et al. 2018, Van Nuland et al. 2020), this is the first study to compare rhizosphere microbial networks from plant communities with experimentally controlled and replicated levels of plant species diversity, specifically looking at interactions between two kingdoms (bacterial and AM fungal).

Within high field inoculum treatments, higher proportions of AM fungi contributed to network structure compared to bacteria. This high proportion of AM fungi increases with plant diversity (Fig 3.6A), along with the proportion of positive correlations. Previous studies have shown that soil microbial networks tend to contain more negative edges than positive edges (Faust et al. 2015). Therefore, the higher percentage of positive edges in the high plant diversity treatment could indicate microbial cooperation, as seen in other studies. The high field inoculum treatment also has less nodes (taxa) and edges (significant correlations) that decrease with greater plant diversity, which is indicative of microbial niche specialization. Plants in high diversity communities likely have a greater chance of making mycorrhizal associations due to the high number of plant species with a greater reliance on mycorrhizal associations (Koziol & Bever 2017, Neuenkamp et al. 2019). Along with higher alpha and beta diversity under high field inoculum and greenhouse conditioned high plant diversity treatments, the decrease in nodes contributing to network dynamics indicates a selective pressure imposed by the surrounding environment influencing the interactions amongst microbial taxa. This is direct evidence for the

plant community refinement and niche specialization of the microbial community (Goss-Suoza et al. 2017).

Interestingly, the proportion of AM fungi to bacteria was not the determining factor in the positive contributions to network formation in our study. In the high field inoculum treatment, medium and low plant diversity have a higher proportion of AM fungi but more negative edges contributing to network structure. Here, we saw evidence that the host community had a direct influence on the organisms available in the species pool and direct evidence that AM fungal persistence is dependent on positive feedback mechanisms associated with the historic plant community, similar to trends that have been observed in other studies (Bittebiere et al. 2020, Guzman et al. 2021). Kokkoris et al. (2020) speculated that such feedback mechanisms are evidence of codependency theory that structures AM fungal community assembly.

Codependency theory explains that AM fungal and plant communities are causally determined by one another (Kokkoris et al. 2020). Our study shows that causal relationships between plant and AM fungal communities are evident, but only under high field inoculum with greenhouse conditioned high plant diversity treatments. At the lower levels of greenhouse conditioned plant diversity, we did not see the same trend.

Within the low field inoculum treatment, proportions of AM fungi and bacteria were similar to those observed in the high field inoculum treatment; however, there was a greater proportion of negative edges that contributed to network formation across all plant diversity treatments. In addition, the number of nodes and edges did not drastically change, indicating that plant diversity was less of an influence on network structure within the low field inoculum treatment. In conjunction with the relative abundance data of enriched communities, bacteria could have outcompeted AM fungi in greenhouse conditioned high plant diversity treatment.

Other studies have indicated that AM fungi can influence bacterial community assembly and particular bacterial groups in terms of the types of nitrogen made available to plant hosts, making AM fungal associations more taxing than beneficial to plant function (Nuccio et al. 2013). While AM fungi maintained high contributions to network formation in the greenhouse conditioned high plant diversity treatment, the enriched AM fungal abundance was low (Fig 3.9D).

Bacteria may have had a greater influence in low field inoculum treatments due to priority effects and positive feedback cycles that sustained bacterial populations. The field conditioned low plant diversity plots (Gholizadeh et al. 2019) may have consisted of more early successional grassland species are less responsive to AM fungal associations (Cheeke et al. 2019). Therefore, bacterial communities dominated in soil microbial interactions (*See Chapter2*), giving way to high abundances in enriched bacterial communities for low plant diversity treatments (Fig 3.D) which became more specialized with increased plant diversity and outcompeted AM fungi for hosts (Fig 3.8D, 3.9D). Chloroflexi, Actinobacteria, Bacteroidetes, Alphaproteobacteria, and Betaproteobacteria were highly enriched in the greenhouse conditioned high plant diversity and low field inoculum treatments. Since there was no difference in bacterial alpha diversity between high and low field inoculum treatments (Fig 3.4D), bacterial communities may have outcompeted AM fungal communities for rhizosphere resources, by mineralizing organic nitrogen for plant uptake (Nuccio et al. 2013), which could limit plant community need for AM fungal associations, subsequently decreasing AM fungal alpha diversity (Fig 3.5D). Ternary plots showed enriched AM fungal communities in low and medium plant diversities were not prevalent in high plant diversity treatments, suggesting competitive exclusion of AM fungal groups (Fig 3.9D).

Network dynamics of the uninoculated control treatment indicated a more equal proportion of bacteria to AM fungi contributing to network formation, unlike the field inoculum treatments. Contributions from AM fungi to network formation generally decrease with higher plant richness levels. In addition, the medium plant diversity treatment had the highest proportion of negative edges throughout the entire study (Fig 3.6H). This likely indicates high levels for the competitive exclusion of AM fungi considering that there was a low abundance of enriched AM fungi in the uninoculated control and greenhouse conditioned medium plant diversity treatments (Fig 3.9D). Nonetheless, abundance of Actinobacteria, Verrucomicrobia, Alphaproteobacteria, and Gammaproteobacteria were highly enriched in this treatment (Fig 3.8C).

3.4.3. Networks Reveal Predominant Hub Taxa as AM Fungi

Microbial hubs are the few taxa that are highly interconnected to other taxa and have strong, central weight in microbial network structure. Perturbations to hub taxa can have severe impacts on the structure, connections, and relationships in microbial communities (Toju et al. 2018). It is important to recognize that these changes in microbial hubs can cascade through connected community members altering community assembly patterns (Agler et al. 2016). We found that AM fungal taxa were the leading hub taxa across 66% of treatments (Fig 3.7). Other studies have also found that AM fungi play a central role in metacommunity networks and are often designated as hubs in more temperate communities (Toju et al. 2018). In restored and remnant plant communities, AM fungal 'keystone taxa' (Banerjee et al. 2018) had not fully recovered AM fungal communities in restored plots that were present in remnant plots (Wall et

al. 2020). This indicates the loss of particular AM fungal taxa can be detrimental to the restoration of soil microbial interactions and AM fungal community composition.

In the high field inoculum treatment, AM fungi in the family Diversisporaceae appeared to be influential in network formation as they were the taxa with the highest degree in both high and medium plant diversity treatments (Fig 3.7A-B). Diversisporaceae hub taxa were also among the enriched taxa that were highly abundant in high field inoculum, along with Pacisporaceae, which is known for its fast growth and high carbon demands (Fig 3.9D) (Dudinszky et al. 2019). Diversisporaceae as hub taxa (Fig 3.7A-B) likely play a role in the cooperative enrichment of taxa in other AM fungal families (Fig 3.9D), Alphaproteobacteria, Bacteroidetes, and Nitrospirae phyla (Fig 3.7D). In the high field inoculum and greenhouse conditioned low plant diversity treatments, hub taxa were in the family Acaluosporaceae, which have been described as counter-complimentary to taxa in the Glomeraceae and Gigasporaceae families (Maherali & Klironomos 2007). However, the enrichment and coexistence of taxa in Acaluosporaceae, Gigasporaceae, and Glomeraceae in all high field inoculum treatments in our study suggest the contrary (Fig 3.9D). It could be possible that the hub taxa in the Acaulosporaceae family (Fig 3.7C) persisted in low abundances amongst enriched taxa (Fig 3.9D) to shape network dynamics and prevented generalist taxa, in the Glomeraceae and Gigasporaceae families, from taking over.

Within high field inoculum treatments, AM fungi outcompeted bacteria as hub taxa in medium and low plant diversity treatments as supported by the increase in negative edges. In high plant diversity treatments, bacteria and AM fungi likely cooperated due to higher AM fungal alpha diversity and increased positive interactions. This suggests that more collaborative, specialist AM hub taxa are present in high field inoculum and greenhouse conditioned high plant diversity treatments. Here, niche specialization likely occurred within AM fungal communities

due to: (1) higher alpha diversity of AM fungi means more specialized taxa are able to work cooperatively with bacteria, (2) shift to specialist hub taxa, Diversisporaceae, which have a particular investment in extraradical mycelial networks (Varela-Cervero et al. 2016), (3) the enrichment of multiple, diverse AM fungal families (Fig 3.9D).

The dominating hub taxa in low field inoculum treatments were Gigasporaceae and Glomeraceae, due to its high degree in greenhouse conditioned medium and low plant diversity treatments (Fig 3.7E-F). Bacterial hub taxa, Flavobacteria, were the most connected taxa in greenhouse conditioned high plant diversity treatments. Flavobacteria degrade highly complex compounds in soil systems, represent large fractions of root-associated microbiota and contribute to enzymatic activity directly correlated with plant growth promotion (Maimaiti et al. 2007, Kolton et al. 2016). In our study, bacteria alpha diversity was greater in greenhouse conditioned high plant diversity treatments. Soil bacteria in the genus Flavobacteria are known to be highly abundant on root surfaces, physically outcompeting AM fungi by preventing its molecular crosstalk with host plants and AM fungal penetration of plant cell walls (Kraut-Cohen et al. 2021). The other predominant hub taxa in greenhouse conditioned high plant diversity and low field inoculum treatments was Planctomycetes, which forms symbiotic associations with multispecies bacterial assemblages (Kaboré et al. 2020). AM fungal hub taxa in medium and low diversities were Gigasporaceae and Glomeraceae, which are AM fungal generalists (Higo et al. 2020, Malicka et al. 2020). These taxa were likely outcompeted by specialist bacterial taxa that persisted due to diverse root exudates in greenhouse conditioned high plant diversity treatments.

Within uninoculated control treatments, hub taxa were dominated by bacteria within greenhouse conditioned medium and low plant diversities (Fig 3.7H-I). However, there were multiple AM fungal taxa that were hubs in the high diversity treatment (Fig 3.7G). In the

greenhouse conditioned low plant diversity treatment, there were high abundances of enriched bacteria and AM fungi, but within the greenhouse conditioned medium plant diversity treatment there were only high abundances of enriched bacteria. Hub taxa in medium plant diversity included Spirochaetae, which is involved in plant litter decomposition (Maynowska et al. 2020) and Bacteroidetes, which degrades chitin (Wieczorek et al. 2019). Bacterial communities in the medium plant diversity treatment showed evidence of direct negative competition with AM fungi due to high enriched relative abundances of bacterial communities (Fig 3.8D), high degree and bacterial hub taxa designation (Fig 3.7H) within the treatment showing the highest percentage of negative interactions throughout the entire study (Fig 3.6E).

The highest enriched relative abundance in the uninoculated control and greenhouse conditioned medium plant diversity treatments was the class Gammaproteobacteria (Fig 3.8D). Therefore, the bacterial communities in uninoculated control treatments and greenhouse conditioned medium plant diversity treatments appeared to directly compete with AM fungal communities, as evidenced by the lack of enriched AM fungal abundances in that treatment (Fig 3.9D). Low plant diversity hub taxa in control soils were in the class Deltaproteobacteria (Fig 3.7I), which also showed high abundance as enriched taxa (Fig 3.8D) in the low plant diversity treatments. Deltaproteobacteria are oligotrophic and produce enzymes like chitinase (Trivedi et al. 2013), a function that would directly compete with AM fungi. Furthermore, the oligotrophic Deltaproteobacteria taxa are typically able to persist in low diversity environments due to lack of carbon and diverse root exudates that would sustain more diverse, copiotrophic microbial communities with high nutrient turnover (Fierer et al. 2007, Lladó et al. 2017).

3.5. Conclusions

Results from our study suggest that not all cooperative networks weaken the stability of a system. It is thought that cooperation leads to less species, which leads to redundancy and weakened ecological interactions (Coyte et al.2015). However, this study shows that greenhouse conditioned high plant diversity and high field inoculum treatments maintain high alpha diversity of AM fungal and bacterial communities leading to less taxa that influence network formation, but more taxonomic diversity overall. When observing a network, we are capturing a time stamp of how that network has been shaped, but networks are constantly shifting and subject to change as major players in the network shift.

Overall, our findings indicate:

- Enriched or highly abundant taxa do not correlate with strong contribution to cooccurrence and network interactions but indicate shifts in biological composition.
- Studies that look into multi-kingdom interactions in the holobiont provide insight into microbial interactions that could be dependent upon one another.
- Future studies should include adding particular taxa to known microbial communities to see how microbial community dynamics are influenced with the introduction of different microbial taxa.

Our study is unique because it teases apart field conditioned and greenhouse conditioned effects on the soil microbial community due to changes in plant community structure. This reveals new insights about the residual effects of long-term plant-soil feedbacks on restoring plant diversity and the roles of specific microbial taxa in promoting and sustaining diversity in plant communities. Through the manipulation of microbial communities to increase diversity levels, restoration practitioners restoring sites with diminished soil biodiversity could better

understand what microbial interactions need to be present to increase plant community diversity and ecosystem functioning. Understanding how soil conditioning influences plant community diversity could an important first step in implementing restoration practices that contribute to plant-soil feedback dynamics. It is important to evaluate how historic changes to plant community diversity (like our field conditioned treatments) have shaped the soil microbial community and implement restoration strategies that address both aboveground and belowground processes to ensure for plant community success.

Table 3.1. PERMANOVA tables for bacteria (16S) and AM fungi for beta diversity (community ordination). Table 3.1A represents bacterial beta diversity and Table 3.1B represents AM fungal beta diversity. Treatments listed in the table include: inoculum.trt (which tested the variance between field inoculum and uninoculated controls), field.inoculum (which tested the variance between low and high field inoculum treatments), and plant.div (which tested the variance between greenhouse conditioned low, medium, and high plant diversity treatments.

A) Bacteria

Treatment(s)	Df	Sums of Squares	F value	R^2	P value
inoculum.trt	1	464250	5.6672	0.06011	0.001 ***
inoculum.trt : field.inoculum	2	415536	2.5363	0.05380	0.003 **
inoculum.trt : field.inoculum : plant.div	5	1027579	2.5088	0.13305	0.003 **

B) AM Fungi

Treatment(s)	Df	Sums of Squares	F value	R^2	P value
inoculum.trt	1	54419	2.2167	0.02673	0.062
inoculum.trt : field.inoculum	2	112982	2.3011	0.05551	0.034 *
inoculum.trt : field.inoculum : plant.div	5	296956	2.4193	0.14589	0.003 **

Table 3.2. GLM/ANOVA tables for bacteria (16S) and AM fungal community alpha diversity (Shannon diversity). Table 3.2A represents bacterial alpha diversity and Table 3.2B represents AM fungal alpha diversity. Treatments listed in the table include: inoculum.trt (which tested the variance between field inoculum and uninoculated controls), field.inoculum (which tested the variance between low and high field inoculum treatments), and plant.div (which tested the variance between greenhouse conditioned low, medium, and high plant diversity treatments.

A) Bacteria

Treatment(s)	Df	Sums of Squares	F value	R^2	P value
inoculum.trt	1	1.3870	11.2150	0.17569	< 0.01 **
inoculum.trt : field.inoculum	2	3.9169	15.8356	0.49617	< 0.00001 ***
inoculum.trt : field.inoculum : plant.div	5	2.5903	4.1889	0.32813	< 0.01 **

B) AM Fungi

Treatment(s)	Df	Sums of Squares	F value	R^2	P value
inoculum.trt	1	0.05462	3.3471	0.09559	0.0721
inoculum.trt : field.inoculum	2	0.14227	4.3595	0.24899	0.0169 *
inoculum.trt : field.inoculum : plant.div	5	0.37449	4.5900	0.65541	< 0.01 **

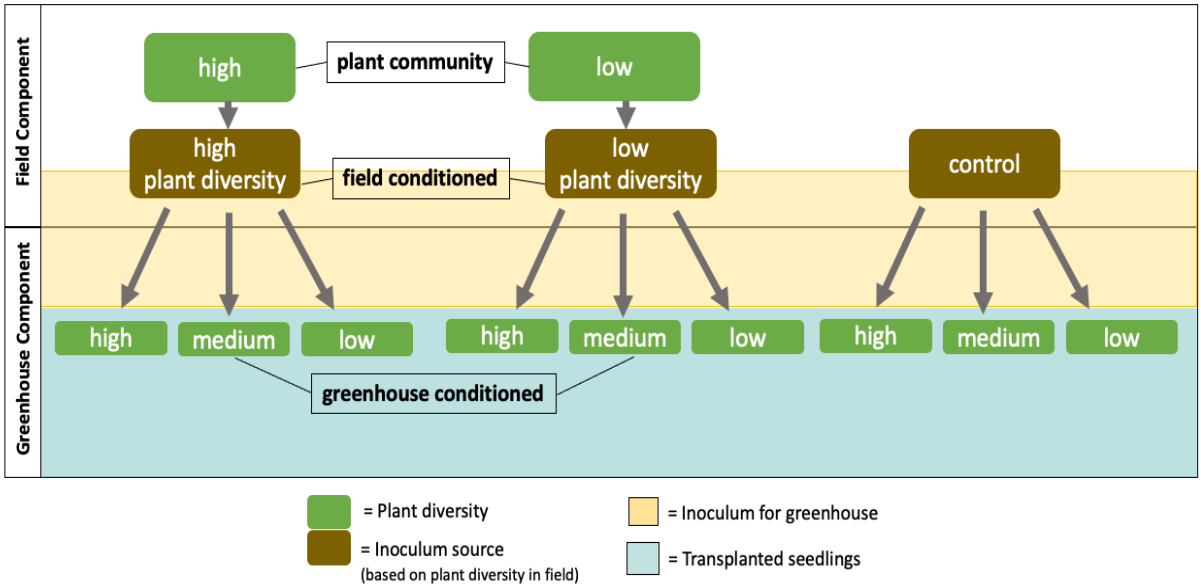


Figure 3.1. A graphical representation of our field and greenhouse studies showing how field conditioned plant diversity treatments were used to collected inoculum for use in greenhouse study. Field soils were used to inoculate mesocosm pots in the greenhouse, which were then planted with native tallgrass plant species as our greenhouse conditioned plant diversity treatments

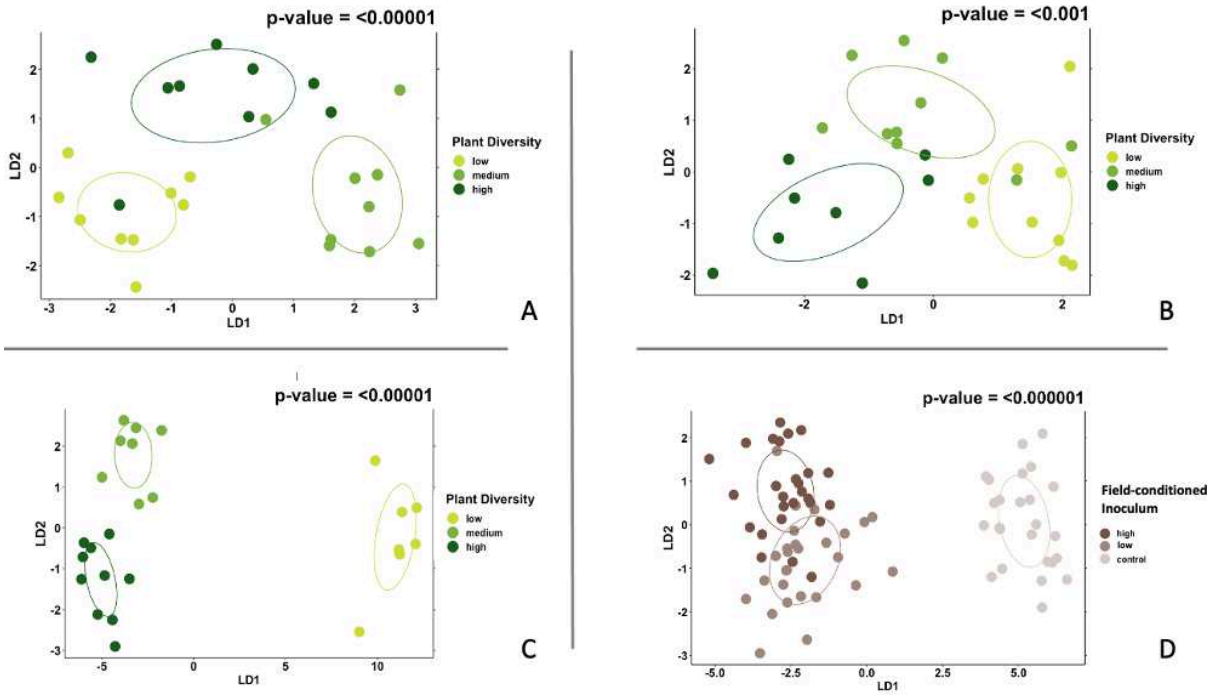


Figure 3.2. NMDS ordination representing the bacterial community within different field inoculum treatments: (A) high field inoculum (B) low field inoculum (C) uninoculated control, and (D) bacterial community grouped by field inoculum treatments. The different colors represent greenhouse conditioned plant diversity treatments with yellow-green representing low plant diversity (5 species), light-green representing medium plant diversity (15 species), and dark-green representing high plant diversity (30 species).

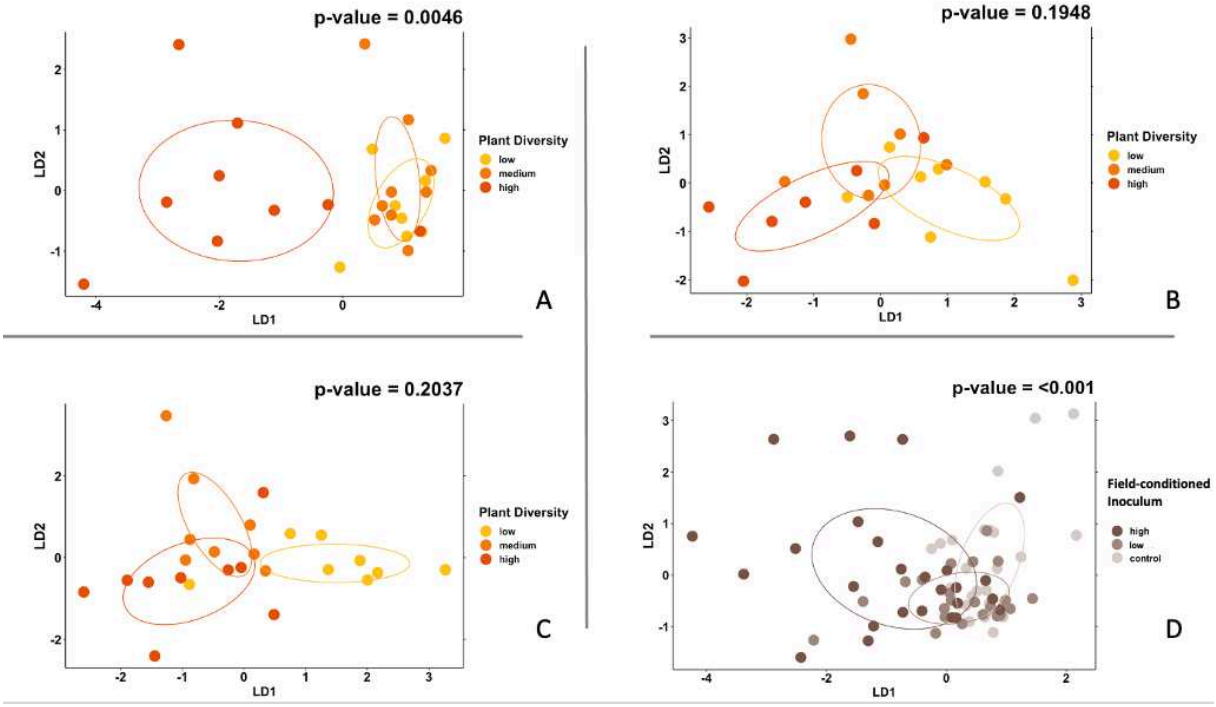


Figure 3.3. NMDS ordination representing the AM fungal community grouped by plant diversity within different soil conditioning treatments: (A) high field inoculum (B) low field inoculum (C) uninoculated control, and (D) AM fungal community grouped by field inoculum treatments. The different colors represent the greenhouse conditioned plant diversity treatments with yellow-orange representing low plant diversity (5 species), light-orange representing the medium plant diversity (15 species), and dark-orange representing high plant diversity (30 species).

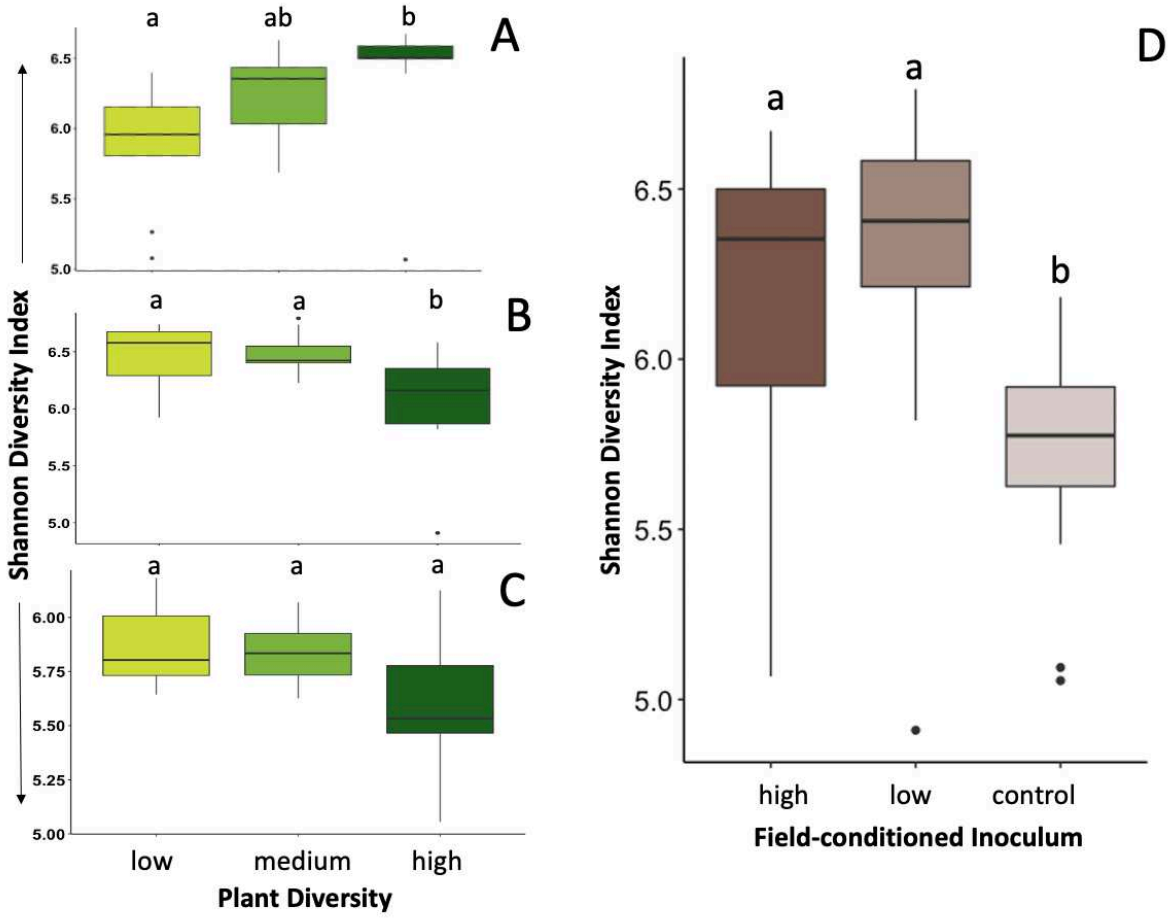


Figure 3.4. Boxplots representing the diversity of bacterial community composition using the Shannon diversity index. Each plot represents one of the three inoculum treatments: (A) high field inoculum, (B) low field inoculum, (C) uninoculated control, and (D) bacterial community grouped by inoculum treatments. Each color represents the greenhouse conditioned plant diversity treatments with yellow-green representing low plant diversity (5 species), light-green representing medium plant diversity (15 species), and dark-green representing high plant diversity (30 species).

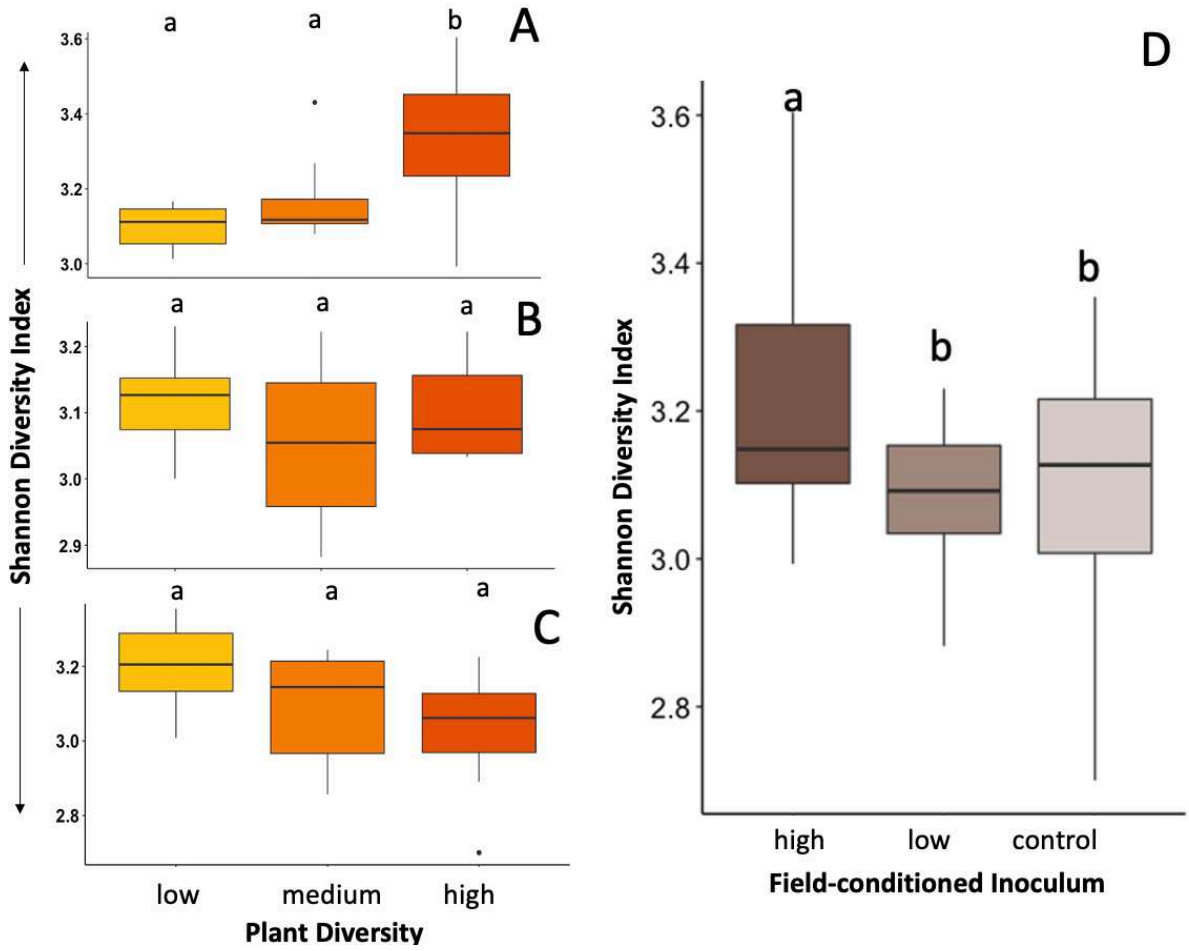
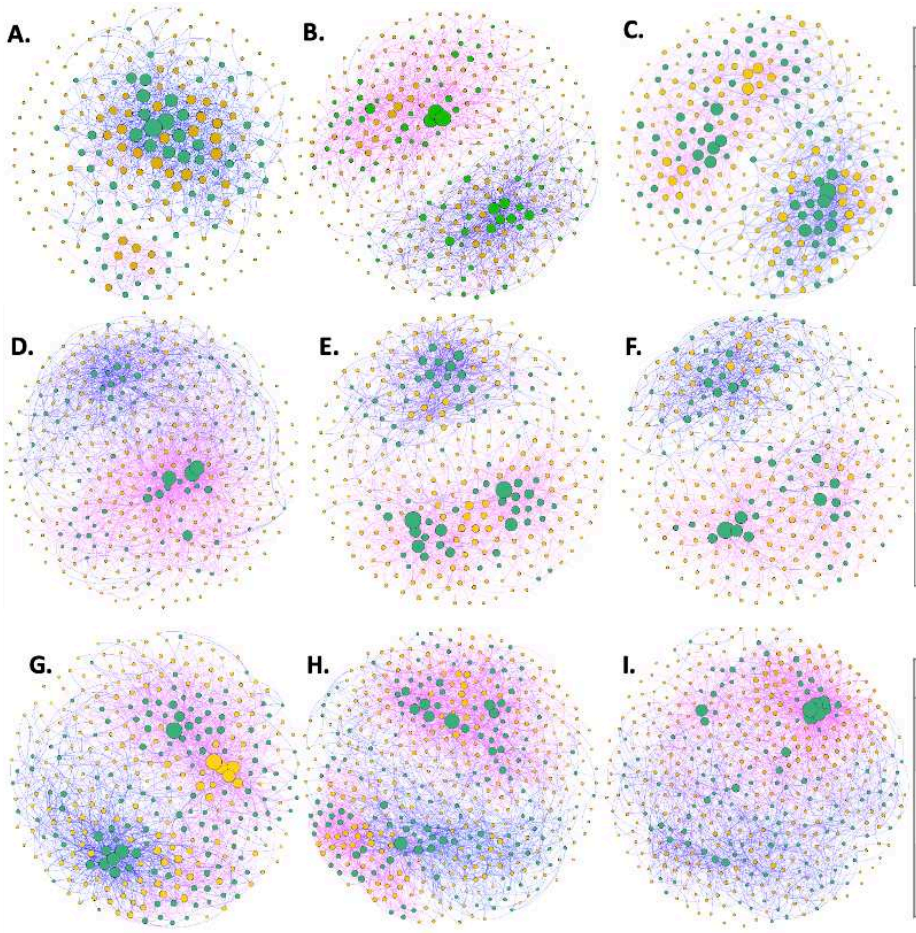


Figure 3.5. Boxplots representing the diversity of AM fungal community composition using the Shannon diversity index. Each plot represents one of the three inoculum treatments: (A) high field inoculum, (B) low field inoculum, (C) uninoculated control, and (D) AM fungal community grouped by inoculum treatments. Each color represents the greenhouse conditioned plant diversity treatments with yellow-orange representing low plant diversity (5 species), light-orange representing medium plant diversity (15 species), and dark-orange representing high plant diversity (30 species).



Attributes	High Plant Diversity	Medium Plant Diversity	Low Plant Diversity
Node	238	326	307
Edge	888	1430	1108
Positive	**84.68 %**	**46.92 %**	**46.21 %**
Negative	**15.32 %**	**53.08 %**	**53.79 %**
Bacteria	28.15 %	28.53 %	31.27 %
AM Fungi	71.85 %	71.47 %	68.73 %

Attributes	High Plant Diversity	Medium Plant Diversity	Low Plant Diversity
Node	376	312	304
Edge	1982	1402	1233
Positive	**36.88 %**	**34.95 %**	**44.04 %**
Negative	**63.12 %**	**65.05 %**	**55.96 %**
Bacteria	26. 86 %	25 %	27.96 %
AM Fungi	73.14 %	75 %	72.04 %

Attributes	High Plant Diversity	Medium Plant Diversity	Low Plant Diversity
Node	339	472	460
Edge	1866	2632	2188
Positive	**37.67 %**	**31.99 %**	**39.26 %**
Negative	**62.33 %**	**68.01 %**	**60.74 %**
Bacteria	40.12 %	33.9 %	30.43 %
AM Fungi	59.88 %	66.1 %	69.57 %

Figure 3.6. A graphical representation of co-occurrence networks between AM fungal and bacterial taxa. Yellow nodes represent AM fungal taxa. Green nodes represent bacterial taxa. The size of each node represents the degree associated with each node. Edges are represented as the lines between nodes. Edges colored in pink represent negative interactions (mutual exclusion) between nodes. Edges colored in blue represent positive interactions (copresence) between nodes. Network diagrams show samples that have been inoculated with different soil treatments: high field inoculum (A-C), low field inoculum (D-F), and uninoculated control (G-I) ; along with the greenhouse conditioned plant diversity treatments: high diversity (A, D, G), medium diversity (B, E, H), and low diversity (C, F, I). Medium diversity, G.-I. Low diversity). The tables associated with each soil conditioning treatment show network topological features that are represented in each co-occurrence network.

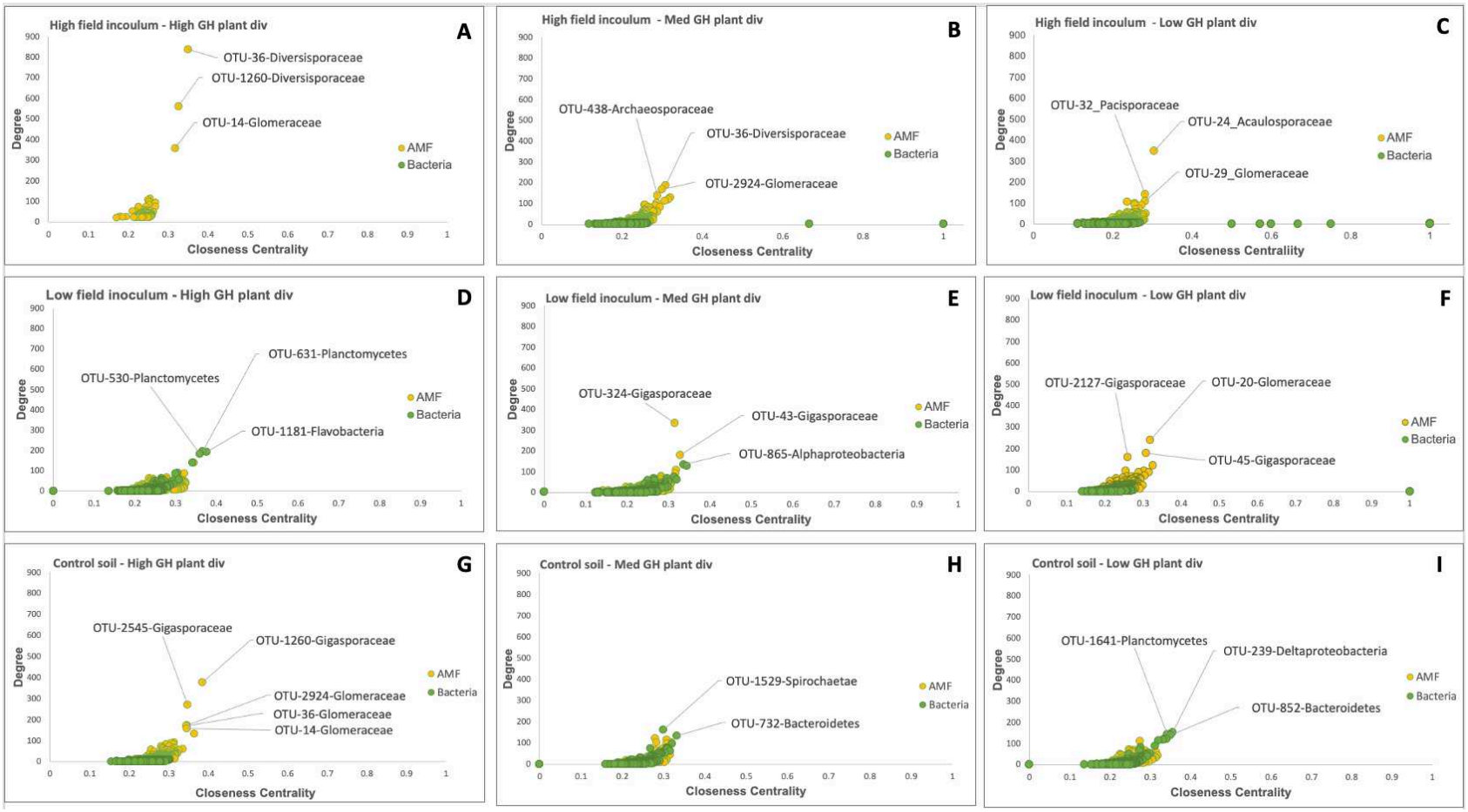


Figure 3.7. Scatterplots showing hub taxa that were determined by plotting network topological properties, closeness centrality and degree, of each node. Hub taxa were identified as those that exceeded a closeness centrality of 0.2 and a degree of 120. Network topological properties were obtained in conjunction with co-occurrence network formation. Fig 3.7A-C represent high soil conditioning treatments, Fig 3.7D-F represent monoculture soil conditioning treatments, and Fig 3.7G-I represent control soils.

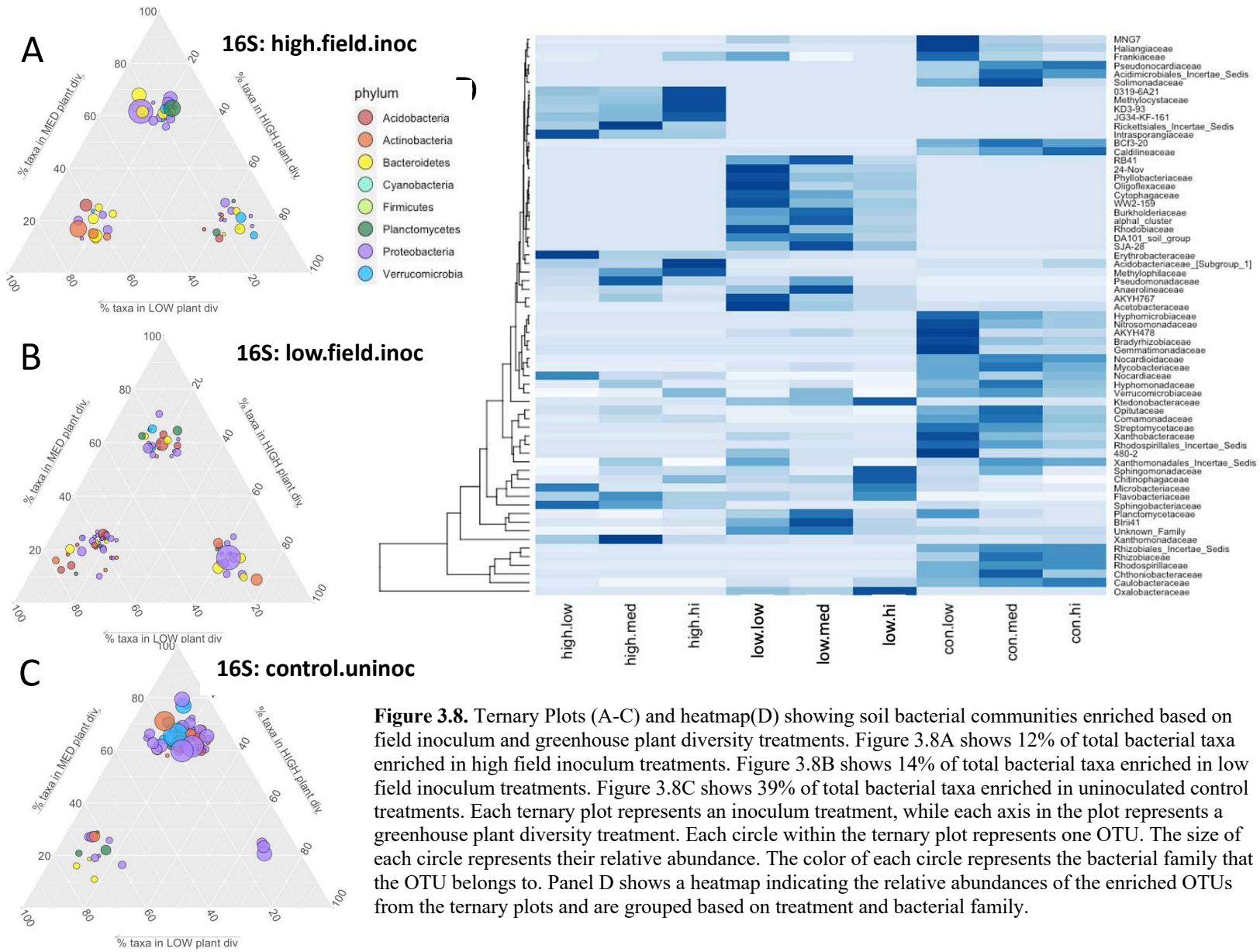


Figure 3.8. Ternary Plots (A-C) and heatmap(D) showing soil bacterial communities enriched based on field inoculum and greenhouse plant diversity treatments. Figure 3.8A shows 12% of total bacterial taxa enriched in high field inoculum treatments. Figure 3.8B shows 14% of total bacterial taxa enriched in low field inoculum treatments. Figure 3.8C shows 39% of total bacterial taxa enriched in uninoculated control treatments. Each ternary plot represents an inoculum treatment, while each axis in the plot represents a greenhouse plant diversity treatment. Each circle within the ternary plot represents one OTU. The size of each circle represents their relative abundance. The color of each circle represents the bacterial family that the OTU belongs to. Panel D shows a heatmap indicating the relative abundances of the enriched OTUs from the ternary plots and are grouped based on treatment and bacterial family.

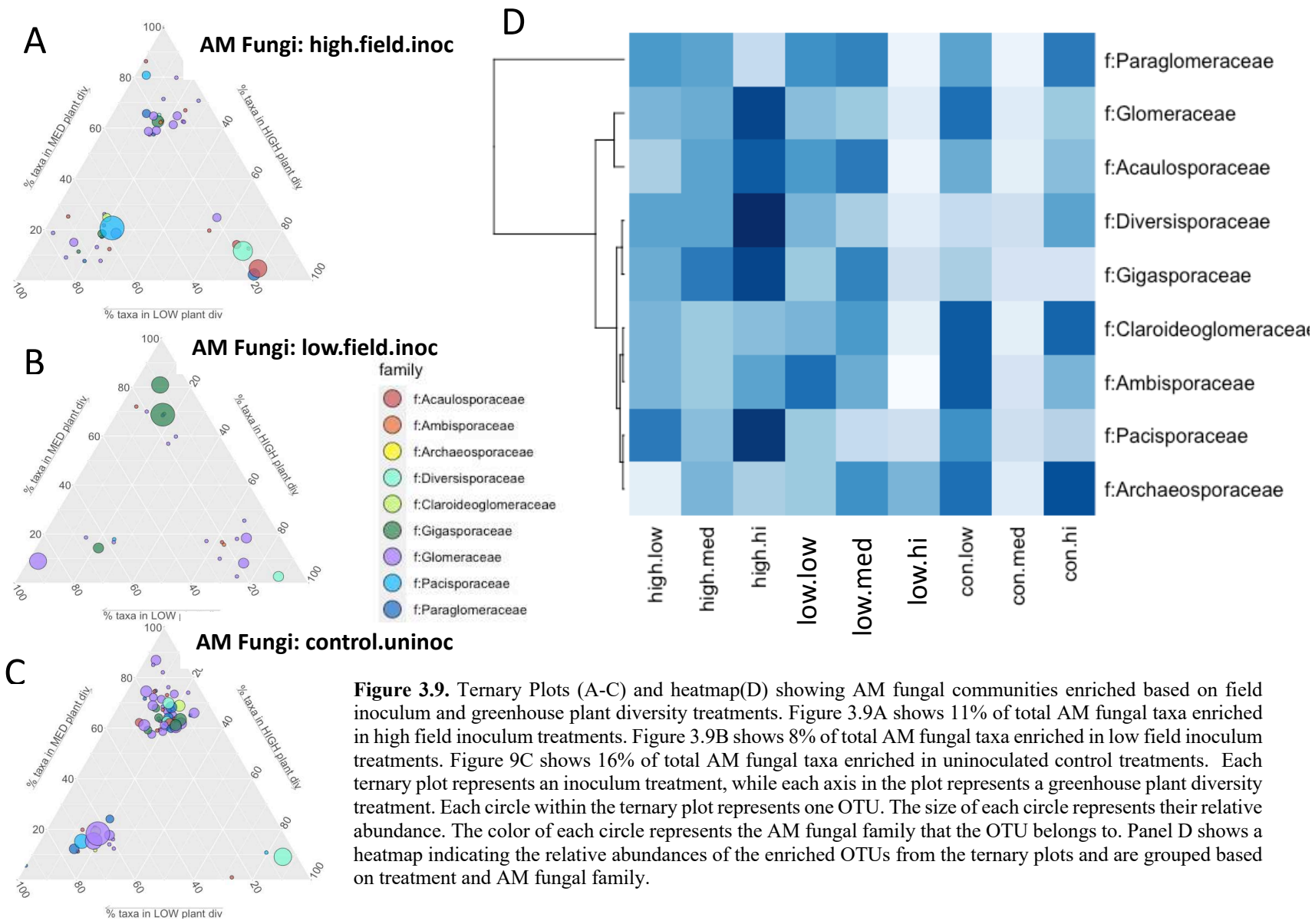


Figure 3.9. Ternary Plots (A-C) and heatmap(D) showing AM fungal communities enriched based on field inoculum and greenhouse plant diversity treatments. Figure 3.9A shows 11% of total AM fungal taxa enriched in high field inoculum treatments. Figure 3.9B shows 8% of total AM fungal taxa enriched in low field inoculum treatments. Figure 9C shows 16% of total AM fungal taxa enriched in uninoculated control treatments. Each ternary plot represents an inoculum treatment, while each axis in the plot represents a greenhouse plant diversity treatment. Each circle within the ternary plot represents one OTU. The size of each circle represents their relative abundance. The color of each circle represents the AM fungal family that the OTU belongs to. Panel D shows a heatmap indicating the relative abundances of the enriched OTUs from the ternary plots and are grouped based on treatment and AM fungal family.

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CHAPTER 4.

Influence of plant community diversity imparts stability of microbial network complexity in the face of disturbance

4.1. Introduction

Severe biodiversity loss and disruption of ecological assemblages is expected to increase over time in response to climate change (Bellard et al. 2012, Trisos et al. 2020). Plant diversity loss can influence ecosystem processes, like nutrient retention (Tilman et al. 1997) and soil carbon storage (Lange et al. 2015) through changes in litter quality, host plant exudates and carbon allocation that contribute to the general loss of soil microbial functional diversity (Jewell et al. 2017, Canals et al. 2019, Chen & Chen 2019). As the primary source of energy for many soil microorganisms, plant litter diversity alone has important implications for nitrogen release, microbial composition, and abundance (Santonja et al. 2017). Furthermore, aboveground stressors influencing plant productivity may contribute to feedbacks that shift litter quantity and diversity, which further influence rates of microbial decomposition and the availability of microbially-processed nutrients (Wardle et al. 2004).

In natural systems, aboveground stressors seldom occur independently, and multiple synergistic disturbances can pose major challenges to ecosystem restoration. Subtle environmental perturbations, like changes in carbon source, nutrient availability, and salinity levels (Kinsler et al. 2020), can influence the fitness of microbial taxa impacting network interactions and niche dynamics (Deng et al. 2019). Frequent, low intensity disturbances to aboveground communities can negatively impact belowground communities. Studies have shown that removal of aboveground biomass at high frequency decreases substrate quality resulting in decreased contributions from bacterial taxa to network dynamics, increased contributions from fungal taxa to network dynamics, and had a greater influence on fungal

community composition compared to bacterial composition (Zhang et al. 2020, Chen et al. 2021). These shifts in network dynamics influence microbial interactions, which can trigger cascading effects on plant diversity through plant-soil feedbacks (Meyer & Lenski 2020, Shaw et al. 2020). Soil microbes are sensitive to the loss of plant biomass due to altered plant contributions to its associated microbiota (Xue et al. 2016). To simplify the complexity of natural disturbances, some studies use clipping as a way of simulating disturbance-induced plant biomass loss to tease apart the effect it has on the dynamics within the rhizosphere microbiome. On the community level, removal of aboveground plant biomass has been known to favor AM fungal families with fast growth rates, like Glomeraceae, and greater carbon-use efficiency, like Acaulosporaceae (Dudinszky et al. 2019). Recent studies have proposed the use of stress-conditioned soil biota to accelerate plant community restoration efforts to achieve functional resilience within microbial communities (Hawkes et al. 2015, Valliere et al. 2020). Despite these propositions, soil microbial community tolerance to the removal of aboveground plant biomass has not been well studied.

Overall, microbial community response to stress is dependent on the community members and niche availability (Ofek-Lalzar et al. 2014). In AM fungal literature, the carbon-limitation hypothesis has been well supported, which predicts when plants lose biomass, AM fungal symbionts receive less carbon from their symbiotic plant partners (Wallace 1987, Gehrig and Whitham 1994, Klironomos et al. 2004, Saravesi et al. 2014). Immediately after the removal of aboveground biomass, plants shift their carbon allocation from shoots to roots where they are stored or released directly into soil through root exudates (Dyer et al. 1991, Holland et al. 1996). Symbiotrophic root mutualists, like AM fungi, do not access carbon through root exudates released into the soil, but directly from their plant hosts whose carbon allocation to roots dwindle

as stressors continue (Smith & Read 2008). Further investigation of the removal of plant biomass in different systems revealed that carbon allocation to roots differs based on host plant functional group (Barto and Rillig 2010). Therefore, carbon allocation from host plant to AM fungi is dependent on the plant types within that community. Changes in AM fungal community composition under stress will likely differ based on host plant carbon allocation under stress.

While changes in carbon allocation to AM fungal symbionts depend on plant type response to stress, plant root microbiomes assemble based on chemical shifts in root exudation (Rolfe et al. 2019). Increases in plant diversity also increase the amount of exudates released by plant roots, contributing to greater bacterial and fungal biomass (Eisenhaur et al. 2017); however, subtle perturbations, like clipping, decreases soil organic matter (SOM) mineralization and shifts microbial community composition, favoring gram negative bacteria (Shazad et al. 2012). It has been suggested that plant photsynthates, like carbon, is released via AM fungal hyphae before being exuded passively for bacterial communities downstream (Kaiser et al. 2014). Nonetheless, differences in niche occupation between AM fungi (carbon allocation from plant roots) and bacteria (root exudation into soil) indicate that these microbial communities will be differentially influenced by host plant stress, and both contribute to cooperative interactions in network dynamics, leading to increased stability due to higher modularity (Gao et al. 2018).

In this study, we investigate how AM fungal and bacterial interactions change when aboveground plants are removed under different greenhouse-established plant diversity levels. We set up different levels of plant diversities using experimental mesocosms inoculated with soils conditioned by different plant diversity levels in the field. After plants had been established for 6 months, we clipped aboveground biomass bimonthly over the course of 3 months. To evaluate the soil microbial community, we collected soil samples from each treatment and used

amplicon-based sequencing to identify AM fungal and bacterial community composition. Overall, we hypothesized that clipping aboveground communities would have a negative impact on fungal communities at low plant diversity. We assumed clipping reduced host plant carbon allocation to AM fungi within all plant communities. However, nutrients released from litter in high plant diversity communities likely contributed to the persistence of bacterial communities. We further hypothesized that after aboveground clipping, (1) bacterial communities would differ based on plant community diversity (due to changes in exudates released after clipping), (2) AM fungal communities would show little to no change based on plant community diversity (due to host plant reduced carbon allocation to AM fungi), (3) high plant diversity treatments would have greater modularity and show a greater proportion of positive interactions between AM fungi and bacteria compared to low plant diversity treatments, (4) microbial network dynamics would differ based on field inoculum source and plant diversity level. Overall, we aimed to provide a better understanding of how plant community disturbance and diversity influence soil microbial communities. Such information is critical for improving the disturbed plant communities where changes in plant diversity could lead to the degradation of soil microbial interactions and stability.

4.2. Methods

4.2.1. Greenhouse Study

Soil inoculum for our greenhouse study was collected from October 18-20, 2018 at experimental grassland diversity plots (located at UTM 14 E 534572.55, N 4510395.93) established in 2010 with two levels of plant diversity (low and high) (Gholizadeh et al. 2018). Twenty-five soil samples were collected in a grid-like pattern every 10-m (excluding a 10-m

buffer) in the high (4 plots) and low (4 plots) diversity plots. Soil samples were composited per plot replicate for a total of 8 soil inoculum samples. Soil inoculum was transported in coolers on ice and transferred to 4°C storage for preprocessing. Samples were air dried in a sterile laminar flow hood for 48 hours before being dry sieved (0.25mm sieve) and stored in -18°C until used as inoculum in the greenhouse study. Previous efforts to understand the microbial ecology of high and low field conditioned plant diversity treatments at this site showed that bacterial communities had significantly greater diversity and richness in high plant diversity plots compared to low plant diversity plots (*See Chapter2*). AM fungal communities did not show significant difference in diversity or richness between high plant diversity plots and low plant diversity plots (*See Chapter2*).

Throughout this paper, we used the term 'field-conditioned inoculum treatments' as soil inoculum that was collected from high and low plant diversity field plots (along with an uninoculated control treatment). We used these field inoculum treatments in a greenhouse experiment and planted various levels of plant diversity in replicated mesocosm pots. We refer to these mesocosm pots as greenhouse-established plant diversity treatments, which included a selection of 5, 15, and 30 native tallgrass prairie plant species chosen based on their presence in Nebraska and Kansas tallgrass prairies. To randomize the selection of plant species in each pot, we selected plant species based on a pool of 88 different native species. Seeds were purchased from regional seed suppliers. Seeds were stratified in a 75% sand 25% perlite mixture using plastic containers with drainage holes and germinated according to commercial seed provider instructions to ensure at least 70% germination. Stratified seeds were sprayed with water every 4-5 days to mimic outdoor moisture conditions.

All seed germination trays (30- x 60-cm) and were UV-radiated for 2 minutes on each side. Trays were filled with 2.5-cm of General-Purpose Premiere Pro-Mix BX potting-medium (without mycorrhizal treatment) that had been autoclaved 2 times (within 48 hours) for 1 hour at 375°C. In starting the seeds, a 2.5-cm layer of autoclaved potting mix was added to each tray and sprayed with water. Next, we added approximately 110-180 grams of seed to each tray. For some species, we cold-stratified seed in moist sand for 2-3 months prior to this step. One final layer of autoclaved potting-medium was added to each tray (amount varied based on seed size) and sprayed down with water. Each tray contained one plant species, and 2-3 trays per plant species were prepared for germination. Trays were watered every 48 hours and grew for 30 days in the greenhouse before emerged seedlings were transplanted into mesocosms.

Large pots (38 L) were used to prepare mesocosm communities. Pots were filled with 30 L of General-Purpose Premiere Pro-Mix BX potting-medium (without mycorrhizal treatment) and watered before transplanting. Inoculum was composited based on field-conditioned plant diversity, high and low, before being used as treatments. Pots that received inoculum treatments were topped off with a 1-cm layer of field soil (either low or high plant diversity field-conditioned soils) followed by a cap of 5 cm of Pro-Mix BX potting-medium and were watered prior to transplanting. Pots designated as control treatments did not contain inoculum from field conditioned soils but were prepared using only the Pro-Mix BX potting medium.

Each plant species used for the greenhouse conditioning treatment was given a designated number (using a random number generator) to select species to be included in each pot based on the diversity treatment: 5 species in low plant diversity pots, 15 species in medium plant diversity pots, 30 species in high plant diversity pots. Each pot contained a total of 60 individual plants. As a result, pots designated as low plant diversity contained a higher representation of

each plant species than others. For example, pots designated as low plat diversity had 5 plant species with 12 individuals transplanted per species. Pots designated as medium plant diversity had 15 plant species with 4 individuals per species. Pots designated as high plant diversity had 30 plant species with 2 individuals per species. Pots were watered 3-4 times a week and grew in the CSU greenhouse for 6 months. Seedlings that did not survive initial transplanting were replaced during the first 2 weeks of the study.

In total, our study included three greenhouse-established plant diversity treatments (low, medium, high diversity) and three inoculum treatments (control, low-conditioned field inoculum, high-conditioned field inoculum). With nine plant-soil treatment combinations and 10 replicates per treatment combination, we had a total of 90 mesocosm pots.

After 6 months of growth in the greenhouse, mesocosm plants were harvested by clipping all aboveground biomass. Therein, plants were clipped in all pots every two weeks for two months, with clipped plant litter being left on the soil surface of each mesocosm. After the two-month clipping period, there was one month of a recovery period for the microbial communities, that included a regular watering regimen. Soil samples were collected from each mesocosm after the one-month recovery period using a 5-cm wide by 10-cm deep soil corer. Three samples were collected at the soil surface of each pot approximately 10 cm from the center from the pot, with each collected soil core being 20 cm from the other two cores collected. The three cores from each pot were composited to yield one sample per pot, homogenized with large roots removed from the soil, stored in plastic bags, and transported on ice to the lab. At the laboratory, sample were air dried under a laminar-flow hood for 48 hours before being dry sieved (0.25mm sieve) and stored at -18°C prior to DNA extraction.

4.2.2. Molecular Methods and Bioinformatics

Using 0.2 g of air dried mesocosm soils, we extracted DNA from each of our samples using the DNeasy PowerSoil Kit. Due to the absorptive nature of the potting-medium soils, we altered the standard protocol using the following modifications: (1) Powerbead tubes consisted of 120µl of C1 solution and 600 µl of Powerbead solution, (2) after the first centrifuge, supernatant was pipetted into a new tube, (3) Powerbead tube was used again with an additional 200µl of Powerbead solution, vortexed briefly and centrifuged at 10,000 rpm for 1 minute. AM fungal libraries were prepped using a two-step PCR process. We cleaned the samples after the first PCR using Sera beads and cleaned again using a column cleaning procedure after pooling to equimolar concentration.

For our bacterial dataset, we amplified the V3 and V4 regions of the small sub-unit bacterial ribosomal RNA gene using primers universal prokaryotic primers 314F and 806R (Muyzer et al. 1993, Caporaso et al. 2011, Takahashi et al. 2014). The primers for the AM fungal dataset, WANDA and AML2, were selected based on its wide use in AM fungal community surveys, its SSU-based marker diversity, and its balanced amplification of AM fungal families (Egan et al. 2018, Vasar et al. 2021). Our bacterial dataset and our AM fungal dataset underwent two different methods of library preparation due to the difficult nature of amplifying AM fungal samples. A one-step PCR library preparation was implemented on our bacterial dataset using Earth Microbiome Project adapters. We used a two-step PCR process for our AM fungi samples, with a Serabead cleanup step between each PCR. Library preparation was performed using KAPA 3G plant PCR kit and polymerase. With a total reaction volume of 25µl, our first PCR reaction contained 12.5µl of buffer, 0.75µl of the forward primer, 0.75µl of the reverse primer, 0.2µl of Taq Polymerase, 1.5µl of $MgCl_2$, and 9.3µl of template DNA. The following conditions

were used to amplify the SSU region of AM fungal DNA: 95°C for 2 minutes, 30 cycles of 95°C for one minute, 64°C for 1 minute, and 72°C for 1 minute courses of 30 cycles. After the cycling period was over, each sample was amplified at 72°C for 1 minute and kept at 0°C until removed. All samples were then pooled at equimolar concentration and assessed using the PicoGreen assay. Pooled samples were then sequenced at the Colorado State University Sequencing Core with Illumina Miseq (paired-end 2x300). All reads were processed using USEARCH v10 software (Edgar 2010), using *cutadapt* (Martin 2011) and *UPARSE* (Edgar 2013) for demultiplexing and OTU construction. Reads were blasted against the SILVA_132 reference database (Quast et al. 2012) for 16S reads and the MaarjAM database for AM fungal reads (type sequence V.05/06/2019) (Öpik et al. 2010).

4.2.3. Statistical Analyses:

After completing our bioinformatic analyses, our bacterial dataset had 19, 971 OTUs across 86 samples and our AM fungal dataset had 3,996 OTUs across 87 samples . We took three steps into consideration when filtering our dataset. For our 16S and AM fungal datasets, the first step was to include OTUs that had at least 50 reads across our sample dataset. Next, we filtered to include exclude samples that had less than 5,000 reads total across all OTUs. Finally, we calculated occupancy by OTU to filter out OTUs that had less than 20% occupancy. This filtering step allows us to only include OTUs that are present in 20% of the samples to avoid making inference about taxa that occur in low numbers across all samples.

Each OTU table was rarified using the package *vegan* in Rstudio (Oksanen et al. 2007). CAPS analyses were performed for beta diversity ordinations using the Bray-Curtis dissimilarity metric within the *Biodiversity R* package (Kindt & Kindt 2019). Permanovas were run using the

adonis function in the *vegan* package (Oksanen et al. 2007). Alpha diversity was calculated with Shannon Diversity Index metrics using the *vegan* package (Oksanen et al. 2007). To determine statistical significance of alpha diversity plots, the *agricolae* package was used to calculate Tukey's Multiple Comparisons test (de Mendiburu & de Mendiburu 2019). All data wrangling and tidying was performed using the *tidyr* package and all figures were plotted using the *ggplot2* package (Wickham 2011, Wickham & Wickham 2017). In making the network analyses, we utilized the app CoNet (Faust & Raes 2016) within Cytoscape v3.7.2 (Shannon et al. 2003). Network visualization was performed using the platform Gephi 0.9.2 (Bastian et al. 2009). Nodes with a degree of less than 10 were eliminated to reduce noise in the network visualizations.

4.3. Results and Discussion

4.3.1. Beta Diversity

AM fungal and bacterial communities showed strong evidence that clipping differentially effects soil microbial community structure based on field inoculum treatments (Fig 4.1D, 4.2D). Inoculum and plant diversity treatments impacted beta diversities of bacterial communities, but only impacted some AM fungal communities. Clipping had a significant impact on bacterial community structure in all inoculum treatments; however, results were more significant for high plant diversity conditioned inoculum treatments ($p < 0.0001$) (Fig 4.1A-C). High field-conditioned plant diversity inoculum treatments showed the clearest separation between greenhouse-established high plant diversity and low/medium plant diversities (Fig 4.1A). Bacterial communities under greenhouse-established high plant diversity showed distinct clustering possibly due to diverse litter inputs and root exudation into the soils providing diverse

nutrients for bacteria. This may indicate bacterial community response to plant host clipping stress which differs based on plant diversity levels, as visualized by the stark clustering of greenhouse-established low and medium plant diversities from high plant diversity (Fig 4.1A). The low field-conditioned plant diversity inoculum treatments and uninoculated control also showed some evidence of different ordination patterns based on greenhouse-established plant diversity treatments (Fig4.1B-C). Since bacterial communities tend to be limited based on precipitation patterns (Martiny et al. 2017, Guo et al. 2018), degradation and utilization of plant litter by bacterial communities likely did not change. However, litter amounts accumulated over time with each clipping event, which may have contributed to positive feedbacks for soil bacteria.

AM fungal communities in high field-conditioned plant diversity inoculum and uninoculated control treatments showed similar ordinations, indicating community similarity across plant diversity treatments (Fig 4.2A, 4.2C). Nonetheless, it is likely that our uninoculated controls had become contaminated through air circulated from outside the greenhouse facilities. AM fungal communities showed weak evidence in clustering based on plant diversity, despite more diverse litter inputs. One study found that saprophytic fungal community diversity increases with plant litter diversity (Santonja et al. 2018, Habetewold et al. 2020); however, another study found that to not be the case with ectomycorrhizas that are host specific (Otsing et al. 2018). Host-specific fungi that obtain carbon from plant hosts likely rely on host plant carbon more than litter inputs (Otsing et al. 2018).

Our results indicate that clipping aboveground plant biomass in greenhouse-established plant communities shifted AM fungal communities from being different across greenhouse-established plant diversity treatments (*see Chapter 3*) to being similar across greenhouse-

established treatments. Changes in greenhouse-established plant diversity had little influence on the alpha diversity of AM fungal communities because all mesocosm pots maintained populations of 'carbon-resistant' AM fungal communities due to plant disturbance (Bachelot & Lee 2018). In one study, researchers found under stress, soil microbes allocate more resources to survival than growth (Malik et al. 2020), which may be the case for AM fungi in our study. Therefore, due to the stress on host plant communities, AM fungi likely allocated resources towards survival in response to low carbon allocation from host plants. Another study analyzing the effect of plant stressors on microbial communities found that meadow plant communities under stress strongly reduced carbon allocation to plant storage, which benefitted bacterial communities after leaf litter accumulation (Karlowsky et al. 2018). Therefore, evidence in our study indicates that bacterial communities were dependent on carbon from diverse leaf litter, whereas 'carbon-resistant' AM fungal communities persisted despite lower plant carbon allocation and changes to plant diversity.

Across AM fungal communities, low field-conditioned inoculum treatments showed evidence of clustering based on greenhouse-established plant diversity treatments, where high plant diversity clustered differentially from medium and low plant diversities (Fig 4.2B). This could have occurred because AM fungi associated with high greenhouse-established plant diversity had more specialized associations with their host plants. AM fungal communities associated with low and medium greenhouse-established plant diversities inoculated with low field-conditioned soils may have been dominated by generalist AM fungi, which were not supported under host plant stress.

4.3.2. Alpha Diversity

There was minimal difference in the majority (88%) of alpha diversities measured across the experiment, with the exception of bacterial communities in high field-conditioned inoculum treatments. Alpha diversity across field-conditioned inoculum treatments for both bacteria and AM fungi showed no significant differences (Fig 4.3D, 4.4D). Across greenhouse-established plant diversity treatments, AM fungal communities showed no significant differences for all field-conditioned inoculum treatments (high, low, and uninoculated control treatments) (Fig 4.4A-C). Bacterial community diversity for low field-conditioned inoculum and uninoculated control treatments did not show differences across greenhouse-established plant diversity treatments. However, bacterial alpha diversity differed between high and medium/low greenhouse-established plant diversities under high field-established inoculum treatments (Fig 4.3A). Bacterial communities in the high field-conditioned inoculum treatment under high plant diversity showed the greatest variation in diversity metrics (Fig 4.3A), which could have contributed to the significant differences observed between high and medium/low greenhouse-established plant communities.

Our first hypothesis suggested that, after aboveground clipping, bacterial communities would differ based on greenhouse-established plant diversity treatments. While we did not find consistent evidence across all inoculum treatments to support this hypothesis, we did find evidence in high field-conditioned plant diversity inoculum treatment that supports this hypothesis (Fig 4.3A). There was no evidence to support this trend under low field-conditioned plant diversity inoculum or uninoculated control treatments.

In our second hypothesis, we predicted that AM fungal communities would show little to no change based on field-conditioned inoculum and greenhouse-established plant diversity

treatments. Since AM fungal multiple comparisons showed no difference across treatments, there was no evidence to support that AM fungal alpha diversity was influenced in this study (Fig 4.4). Nonetheless, we did see some patterns in beta diversity that suggest that diverse plant communities stressed by clipping likely factored into different bacterial communities and AM fungal communities across inoculum treatments (Figs 4.1-4.2). Other studies have found that litter decomposition had little effect on alpha diversity of microbial communities, but increased plant exudation and fungal abundance (Che et al. 2020). Minimal differences in AM fungal communities based on greenhouse-conditioned plant diversity could likely indicate that stressed plants allocated less carbon to AM fungi, decreasing 'carbon-reliant' AM fungal communities and increasing 'carbon-resistant' AM fungal communities, which have likely adapted microbial trade-offs to support AM fungal survival instead of growth (Malik et al. 2020).

Our results suggest that there were few differences between microbial alpha diversity, but more evidence showing that bacterial and AM fungal beta diversity differed in response to plant diversity and plant communities stressed by clipping. This was likely the result of triggered root exudation due to clipping which caused shifts in bacterial communities along with changes to litter diversity. The few changes in AM fungal beta diversity that we observed could indicate decreased carbon allocation from plants, allowing 'carbon-resistant' AM fungal communities to persist despite changes in plant diversity.

4.3.3. Co-occurrence networks

Overall, network visualizations within the same greenhouse-established plant diversity treatments showed strong similarities (Figs 4.5A, D, G; 4.5B, E, H; 4.5C, F, I) as opposed to treatments within the same field inoculum treatments (Fig 4.5A-C, 4.5D-F, 4.5G-I). Across field

inoculum treatments, there were more nodes and edges in medium greenhouse-established plant diversity treatments compared to high and low greenhouse-established plant diversities while networks in the low greenhouse-established plant diversity treatment had the lowest number of nodes and edges (Fig 4.5C, F, I). Therefore, the medium greenhouse-established plant diversity treatment showed the most complexity due to the higher number of nodes and edges in this treatment. Our third hypothesis predicted that the high greenhouse-established plant diversity treatment would have greater modularity and positive interactions; however, our study showed that this was not the case. While modularity was greater in the high greenhouse-established plant diversity treatment, interactions were mostly negative (Table 4.1, Fig 4.5A, D, G). Since plant communities were stressed by clipping aboveground biomass, we expected that network properties would be more complex in the high greenhouse-established diversity treatment, as evident in the high plant diversity plus low field conditioned inoculum treatment prior to clipping (*see Results from Chapter 3*).

To explore network properties beyond node and edge count, we considered the positive and negative interactions that influenced network structure between all microbes. Overall, two-thirds of the treatment combinations showed greater negative edges than positive edges. More specifically, all high greenhouse-established plant diversity treatment showed negative correlations. This is likely because stressful environments are characterized by the processes driving negative associations, like competition and niche divergence (Hernandez et al. 2021). Therefore, clipping high greenhouse-established plant diversity likely increased network complexity and competitive interactions leading to more stable microbiomes. Across medium and low greenhouse-established plant diversities, there were higher proportion of positive edges compared to high greenhouse-established plant diversity treatments, which were only evident in

treatments where field-conditioned inoculum was utilized. Low field-conditioned inoculum treatments with medium greenhouse-established plant diversity exhibited the greatest proportion of positive edges (81.81%), which was approximately ¼ higher than the other two treatment combinations with high proportions of positive edges: the low field-conditioned inoculum plus low plant greenhouse-established diversity treatment and the high field-conditioned inoculum plus medium greenhouse-established plant diversity treatment. This finding is common in experiments using subtle perturbations, like clipping, which can trigger positive microbial feedbacks (Xue et al. 2016). Environmental stressors have been known to destabilize networks where positive associations between taxa tend to dominate (Hernandez et al. 2021). This is because species turnover is more common in microbial communities when negative correlations are less frequent (Coyte et al. 2015, Herron & McMahon 2017, Danczak et al. 2018).

Of the treatments with high positive edges, both medium greenhouse-established plant diversities combined with high and low field-conditioned inoculum treatments showed a greater contribution from bacteria to network interaction. This is likely the result of positive feedback mechanisms that favor bacterial communities through increased diversity of root exudates. Low field-conditioned inoculum combined with low greenhouse-established plant diversity showed a greater contribution from AM fungi to network interactions. This is likely because the few plants species contributing greenhouse-established plant diversity these mesocosms may have had a higher reliance on AM fungi, creating positive feedback for AM fungal communities. In grasslands, positive bacterial feedbacks have been linked to increased carbon exudation in roots caused by defoliation (Hamilton et al. 2008). Similar stressors, like clipping aboveground, in grasslands has been known to increase the abundance of bacterial genes responsible for labile and recalcitrant carbon degradation, nitrogen fixation, mineralization, denitrification, and

phosphorus utilization genes (Xue et al. 2016, Guo et al. 2018). Therefore, the higher percentages of positive interactions and bacterial contributions to network structure indicate that positive bacterial feedbacks and nutrient cycling were likely induced by plant exudation and sustained by litter accumulation due to clipping treatments.

In stressed environments where negative interactions dominate network structure, microbial groups are more stable due to the complexity of microbial interactions, leading to high modularity (Layeghifard et al. 2017). Clustering in soil microbial co-occurrence networks is represented through network modularity metrics, which have been shown to correlate with microbial functioning for nutrient cycling (Xue et al. 2022). One recent study showed networks with high modularity have a negative relationship with environmental stress but represent stable microbial communities (Hernandez et al. 2021). However, our study indicated that less plant diversity contributed to greater positive interactions (Fig 4.5) that encouraged the persistence of positive feedback loops, which rely on interactions that support the fitness of the microbes involved (Hernandez et al. 2021) and decreased the modularity of microbiome network structure (Table 4.1). In this study, positive feedbacks promoted microbial taxa that are highly interactive and are likely, collectively, correlated with microbiome function. Generally, modularity is reduced when taxa that are important to modular structure are not present (Agler et al. 2016). This is represented in our study where treatments with high bacterial contributions to network structure (Fig 4.5) also exhibit lower modularity, indicating that key microbial taxa are missing from microbial communities associated with low greenhouse-established plant diversity inoculated with high field-conditioned inoculum, medium greenhouse-established plant diversity low field-conditioned inoculum, and medium greenhouse-established plant diversity in uninoculated control treatments.

As predicted by our third hypothesis, we did find that high diversity plant treatments had more modularity, but more negative interactions indicating increased network complexity and competitive interactions that are often seen in stable microbiomes associated with stressed plants. Our results also showed that network dynamics differ across both field inoculum and plant diversity treatments. Overall, we found that networks were more stable in high plant diversity treatments and less stable in lower plant diversity treatments. Bacterial contributions to network structure were greater in lower plant diversity treatments. This is likely because bacterial communities perpetuated positive feedback loops triggered by plant clipping, which increased root exudation of particular plant species allowing for the bacteria that use those exudates as a food source to thrive. Interestingly, AM fungal communities dominated in contributions to network structure associated with high plant diversities. This likely contributed to competitive interactions that stabilized network dynamics between AM fungi and bacteria, impacting the functional plasticity of the high plant diversity microbiomes.

Since bacteria can utilize carbon directly from leaf litter and through root exudates triggered by clipping, individuals within the plant community likely have a decreased need for obligate symbiotic associations that rely on carbon allocation, such as those formed with AM fungi. Nonetheless, AM fungal taxa sustained interactions in the root microbiome, giving way to high modularity in high plant diversity treatments. This high modularity maintains the functional plasticity in the rhizosphere microbiome, allowing for greater stability of the microbial community (Vandenkoornhuyse et al. 2015). Although low field inoculum treatments lacked this plasticity, we see more positive interactions that are indicative of bacterial positive feedbacks that are potentially spearheaded by the exudation of particular plant species due to low plant

diversity. AM fungal contributions to stability in the rhizosphere microbiome should not be defined by alpha and beta diversity metrics, but instead be evaluated in its network interactions.

In support of our fourth hypothesis, our experiment provided evidence suggesting that network dynamics differ based on field-conditioned inoculum treatments and greenhouse-established plant diversity levels (Fig 4.5). Microbial communities in high greenhouse-established plant diversity had more negative interactions, leading to stability under stress to aboveground host plants. In medium and low greenhouse-established plant diversities, network interactions were mostly positive between microbial interactions, indicating potential negative plant-microbe feedback cycles. Interestingly, all field-conditioned inoculum treatments with high greenhouse-established plant diversities showed greater contributions from AM fungal taxa to network formation (Fig 4.5). Within disturbed habitats, assembly rules and patterns may be modified (Legras et al. 2019) because the pressure applied by stressors can be sufficient in displaying patterns of competition between taxa (Cornwell & Ackerly 2009). Biotic interactions have been shown to modify the effects of stress and develop new interactions due to stress (Bruder et al. 2019). Therefore, network interactions are particularly important when evaluating the effect of stress between taxonomic groups in the rhizosphere microbiome. Experimental validation of the functional influence of microbial interactions should be prioritized (Liu et al. 2020) to understand how they influence plant community restoration.

4.4. Conclusions

Overall, the results from this study suggest that clipping disturbances decrease the stability of cooccurrence microbial networks in low greenhouse-established plant diversity but maintains network stability in high greenhouse-established plant communities. Plant communities with high greenhouse-established diversity had greater contributions from AM

fungi to network structure, indicating that despite field-conditioned inoculum source. Through this experimental setup and soil microbial observations, we were able to detect few changes in bacterial and AM fungal alpha and beta diversity due to aboveground clipping across a gradient of plant community diversity. However, we were able to quantify distinct changes to soil microbial interactions and network stability after aboveground clipping which mostly differed due to changes in greenhouse-established plant diversity.

Overall, our findings indicate:

- Soil microbial diversity has limited implications for understanding how disturbances, like clipping, influence the stability of the soil microbiome.
- Disturbances that impact aboveground biomass have a greater effect on soil microbial interactions and stability in plant communities with low diversity compared to high diversity.
- Negative coocurrence network interactions between soil microbes in disturbed systems indicate competition that sustain stability between soil microbial communities.
- AM fungal interactions have high contributions to network stability in plant communities with high diversity when aboveground communities are disturbed.

The implications of this study extend to the restoration of plant community diversity by indicating that lower plant diversity systems can perpetuate plant-soil interactions that can only support low diversity communities. Furthermore, we observed AM fungi have greater contributions to network interactions in high plant diversity treatments, which shows that AM fungi play a large role in sustaining high diversity plant communities when under stress.

Table 4.1. Network modularity as calculated through network properties calculated in Cytoscape. This this table describes network modularity in terms of network structure indicating the presence of highly interconnected nodes that have few connections outside these highly connected groups (Zhou et al. 2011). Modularity indicates more connections within modules than would occur by random chance (Tian et al. 2018). Modularity less than 0.4 indicates low modularity and decreased network stability.

Network Modularity			
	high plant	**medium plant**	**low plant**
high inoculum	0.426	0.426	0.334
low inoculum	0.426	0.375	0.416
uninoculated control	0.506	0.396	0.62

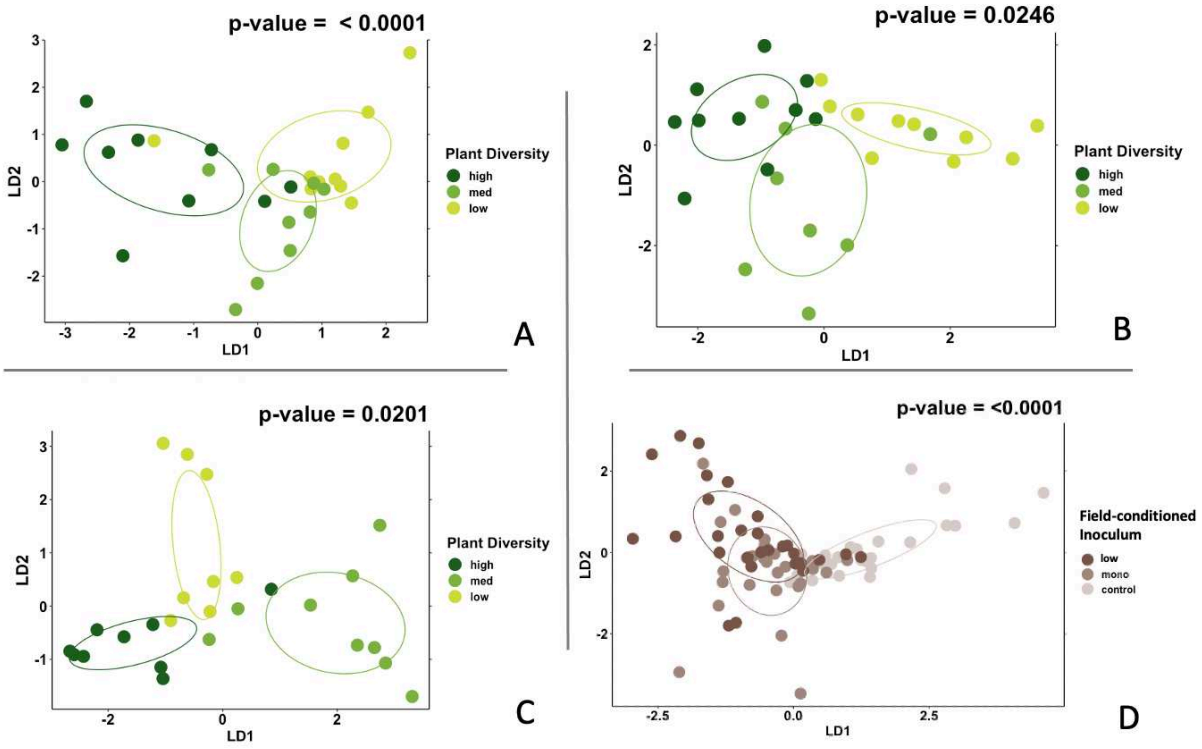


Figure 4.1. CAPS ordination representing the bacterial community within different inoculum treatments: (A) high field-conditioned inoculum (B) low field-conditioned inoculum (C) uninoculated control, and (D) bacterial community grouped by inoculum treatments. P-values for each graph was obtained through PERMANOVA analyses. The different colors represent the greenhouse-established plant diversity intensity with yellow-green representing low plant diversity (5 species), light-green representing the medium plant diversity (15 species), and dark-green representing high plant diversity (30 species).

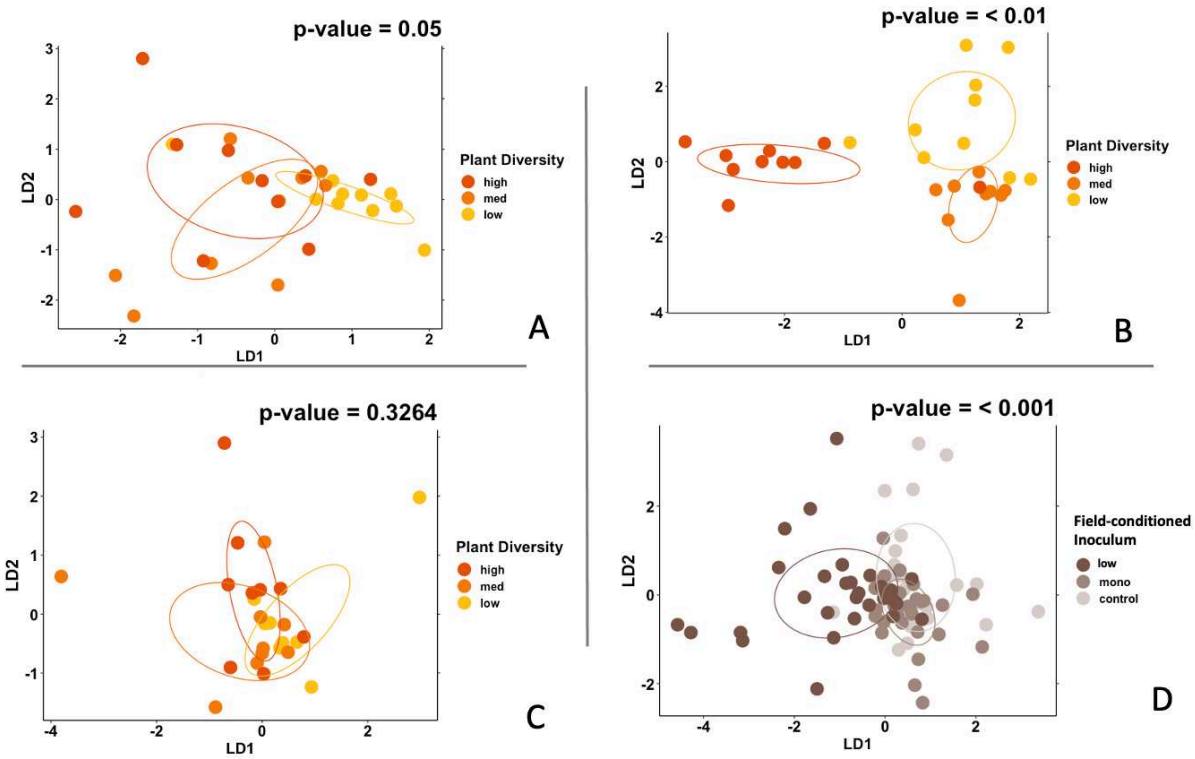


Figure 4.2. CAPS ordination representing the AM fungal community grouped by plant diversity within different inoculum treatments: (A) high field-conditioned plant diversity inoculum treatment (B) low field-conditioned plant diversity inoculum treatment (C) uninoculated control, and (D) AM fungal community grouped by inoculum treatments. P-values for each graph was obtained through PERMANOVA analyses. The different colors represent the greenhouse-established plant diversity intensity with yellow orange representing low plant diversity (5 species), light-orange representing the medium plant diversity (15 species), and dark-orange representing high plant diversity (30 species).

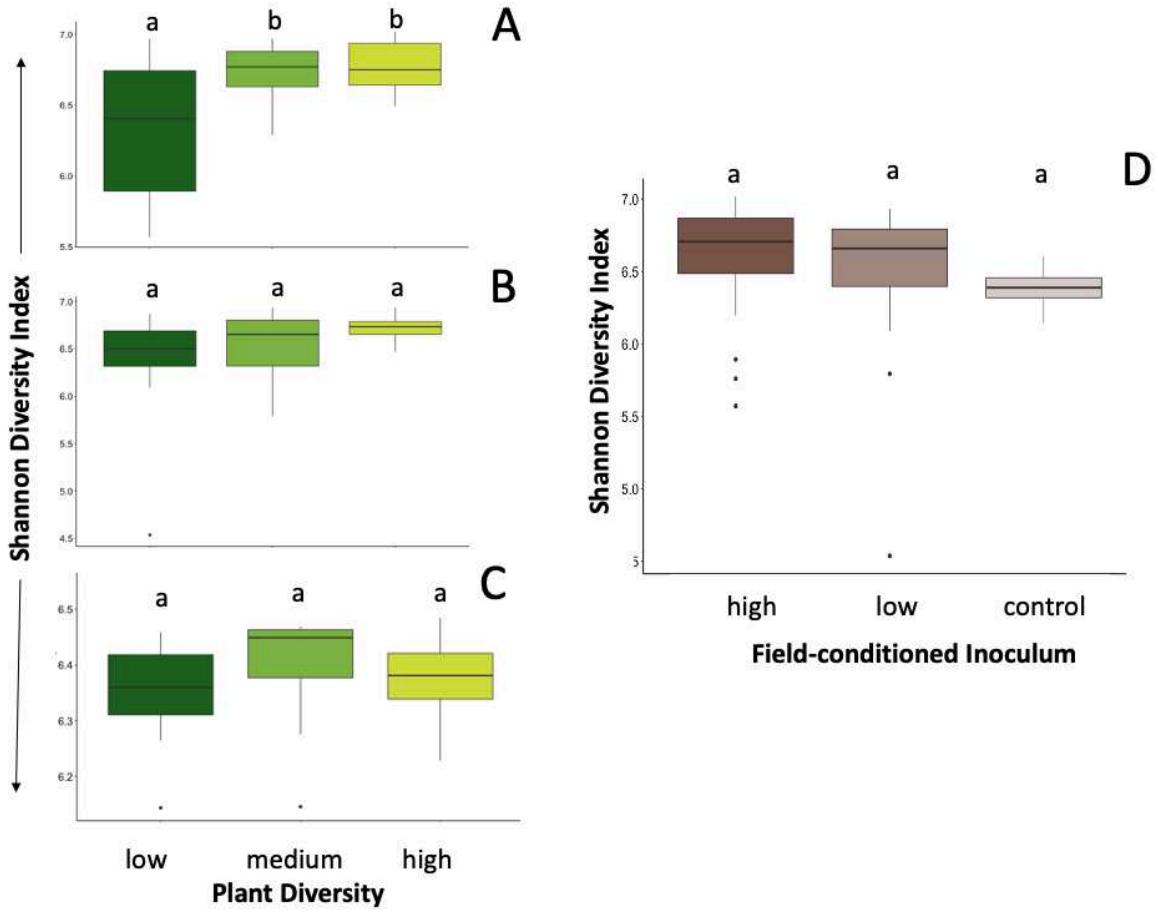


Figure 4.3. Boxplots representing the diversity of bacterial community composition using the Shannon diversity index. Each plot represents one of the three inoculum treatments: (A) high field-conditioned plant diversity inoculum treatment (B) low field-conditioned plant diversity inoculum treatment (C) uninoculated control, and (D) bacterial community grouped by inoculum treatments. Each color represents the greenhouse-established plant diversity intensity with yellow-green representing low plant diversity (5 species), light-green representing medium plant diversity (15 species), and dark-green representing high plant diversity (30 species). Lowercase letters represent significantly different microbial communities as obtained by Tukey's Multiple Comparisons test.

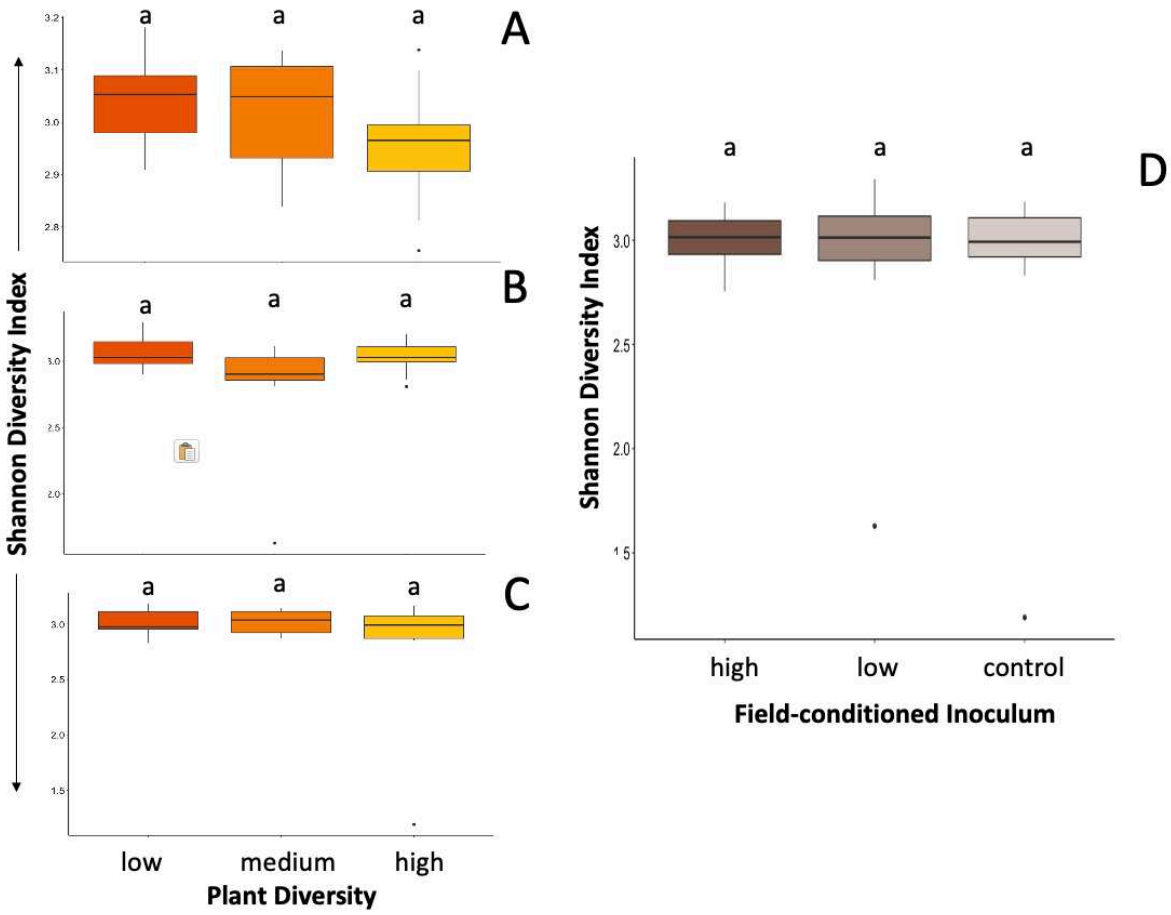
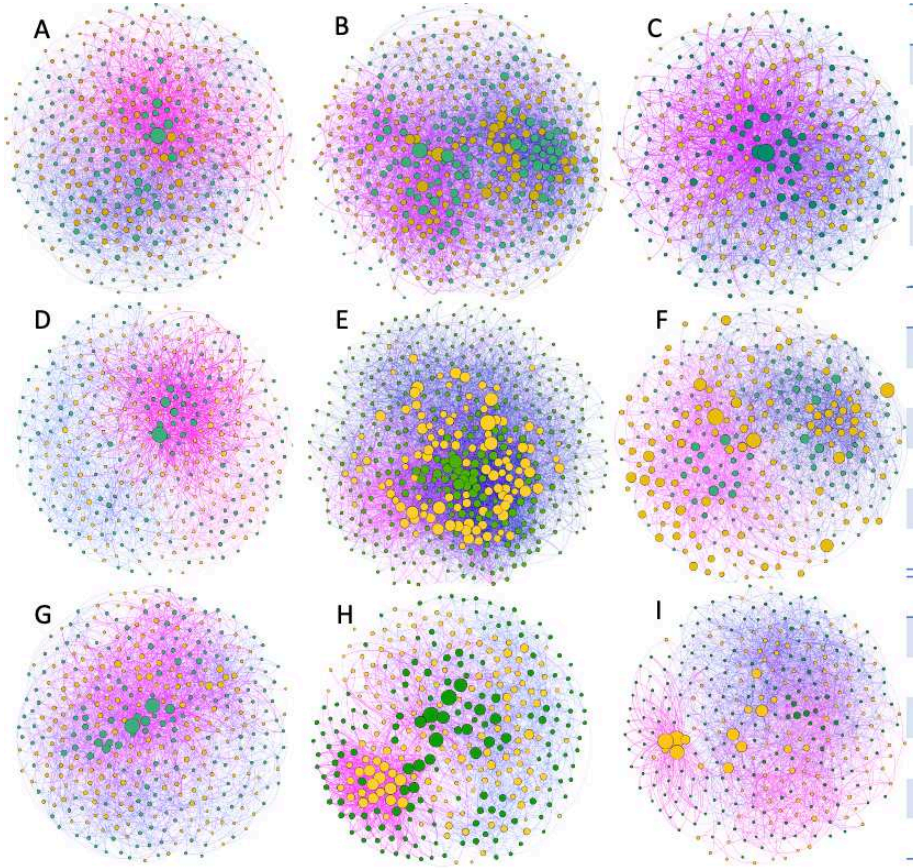


Figure 4.4. Boxplots representing the diversity of AM fungal community composition using the Shannon diversity index. Each plot represents one of the three inoculum treatments: (A) high field-conditioned plant diversity inoculum treatment (B) low field-conditioned plant diversity inoculum treatment (C) uninoculated control, and (D) AM fungal community grouped by inoculum treatments. Each color represents the greenhouse-established plant diversity intensity with yellow-orange representing low plant diversity (5 species), light-orange representing medium plant diversity (15 species), and dark-orange representing high plant diversity (30 species). Lowercase letters represent significantly different microbial communities as obtained by Tukey's Multiple Comparisons test.



Attributes	(A) High Plant	(B) Medium Plant	(C) Low Plant
Nodes	397	488	314
Edges	3492	4970	3329
Positive	45.93 %	59.28%	48%
Negative	54.07%	40.72%	52%
Bacteria	48.61%	55.33%	50.32%
AMF	51.39%	44.67%	49.68%

Attributes	(D) High Plant	(E) Medium Plant	(F) Low Plant
Nodes	373	473	313
Edges	2360	7008	2748
Positive	39.53%	81.81%	57.68%
Negative	60.47%	18.19%	42.32%
Bacteria	46.65%	75.26%	49.52%
AMF	53.35%	24.74%	50.48%

Attributes	(G) High Plant	(H) Medium Plant	(I) Low Plant
Nodes	380	334	323
Edges	3218	3066	2952
Positive	34.46%	44.78%	45.8%
Negative	65.54%	55.22%	54.2%
Bacteria	48.95%	50.9%	62.23%
AMF	51.05%	49.1%	37.77%

Figure 4.5. Co-occurrence networks containing both AM fungal and bacterial taxa. Yellow nodes represent AM fungal taxa. Green nodes represent bacterial taxa. The size of each node represents the degree associated with each node. Edges are represented as the lines between nodes. Edges colored in pink represent negative interactions (mutual exclusion) between nodes. Edges colored in blue represent positive interactions (copresence) between nodes. The tables associated with each inoculum treatment show network topological features that are represented in each co-occurrence. Red boxes within the table highlight treatments that have a higher proportion of negative edges along with the taxa contributing most to network formation. Green boxes highlight treatments that have a higher proportion of positive edges along with the taxa contributing most to network formation. Network diagrams show samples that were inoculated with field-conditioned soils from: high diversity plant community (A-C), low diversity plant community (D-F), and uninoculated control (G-I); along with greenhouse-established plant diversity treatments: high diversity (A, D, G), medium diversity (B, E, H), and low diversity (C, F, I).

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CHAPTER 5: SYNTHESIS

The future of soil restoration and the implementation of microbial amendments for restoring soil function greatly relies on the study of plant-soil feedbacks and microbe-microbe interactions. A common goal in ecological restoration is to establish, or maintain, high diversity amongst plant communities, which becomes a greater challenge in ecosystems that experience natural and human-induced disturbances (Farrell et al. 2020, Heneghan et al. 2008). Although diversity is one of the fundamental concepts in community ecology, its quantification has proven to be a limited strategy for understanding community patterns without providing context behind the mechanisms that contribute to diversity (Shade 2017). Network interactions can be used to make inferences about relationships between soil microbial communities and to further hypothesis generations that aid in the establishment and restoration of plant communities. Understanding how these interactions shift in the face of disturbance can help us reestablish complex relationships between soil microbiota to enhance the speed of restoration efforts (Moreno-Mateos et al. 2020).

My dissertation focused on changes in plant diversity and the use of long-term conditioning of soils by plant communities to evaluate its impact on cooccurrence network interactions between bacteria and AM fungi in the rhizosphere. I accomplished this by, first, observing how plant diversity influences microbial structure, composition, and network interactions in a field setting. Then, I experimentally manipulated plant diversity in greenhouse mesocosms paired with field-conditioned soil inoculum to understand how soils conditioned by particular plant communities differ in microbial cooccurrence interactions. Finally, we looked at how disturbance to the aboveground plant community impacts microbial cooccurrence patterns.

To address the how plant diversity influence microbes, I collected soil samples from long-term plant diversity plots in a mixed grass prairie ecosystem. Using diversity metrics and network analyses, I analyzed bacterial, overall fungal, and AM fungal communities. In evaluating my primary question, I found that plant diversity influences soil microbial communities differently. Bacterial diversity and species richness mirrored plant diversity, while AM fungal and overall fungal diversity and richness did not mirror plant diversity levels. In my main finding, I observed that 92% of microbial interactions associated with high diversity plant communities were positive interactions, while microbial interactions associated with low and monoculture plant diversity plots were mostly negative. Furthermore, network contributions from AM fungi and overall soil fungi were greatest in high plant diversity communities. These findings indicate that aboveground diversity is not always equivalent to belowground diversity but is dependent on the microbial groups observed. The higher percentage of positive interactions and fungal contributions to network dynamics reveal that the microbial communities associated with high plant diversity are more stable than those associated with lower plant diversities. This trend is suggested to be driven by fungal communities. Overall, plant communities with high diversity have an impact on bacterial diversity and microbial cooccurrence interactions, which contribute to the stability of the system.

To address how the microbial community responds to experimental changes in plant diversity, I created mesocosm communities using species of mixed grass prairie plant species and incorporated inoculum treatments using field soils (*from Chapter 2*). After 6 months of plant growth in the greenhouse, I used diversity metrics and network analyses to understand how microbial communities respond to changes in plant diversity. My main finding showed that AM fungal-bacterial cooccurrence networks that had more negative than positive interactions, which

can indicate feedback mechanisms typical of an stable networks under ideal conditions in a greenhouse setting (Coyte et al. 2015). It is evident that plant composition had a major impact on soil microbial diversity when using field-conditioned inoculum from high diversity plant communities based on bacterial and AM fungal diversity. Therefore, the high contribution of positive interactions to network dynamics evident in high field-conditioned inoculum and high greenhouse-established plant diversity treatments indicates network stability in this treatment combination. The results in this study also indicated that microbial communities respond differently to changes in plant community composition, which is mostly influenced my field inoculum source. Another major finding in this study indicated that most network hubs were dominated by AM fungal taxa, revealing quantitative evidence that AM fungi are key taxa in structuring of these networks.

To address the how microbial communities respond to disturbance, I used the mesocosm pots (*from Chapter 3*) and clipped aboveground biomass after the completion of the previous study to simulate disturbance of the aboveground community. My main finding showed that clipping created an aboveground disturbance, which resulted in more negative interactions than positive. This finding is supported by the general response of microbial communities to disturbance using network analyses (Cornwell & Ackerly 2009). In my study, microbial communities experienced a shift of interactions, from cooperative to competitive, after the aboveground community was clipped. Stressed plants have been known to cause changes to belowground carbon inputs that can shift soil microbial community composition and interactions. The shift from positive to negative interactions sustains competitive interactions that drive microbial diversity and prevents microbially-induced positive feedbacks from dominating interactions when host plants are stressed (Bruder et al. 2019). Modularity metrics provided

evidence that network stability was greater in high plant diversity treatments. Some microbial communities associated with low and medium plant diversities showed minimal changes in microbial community structure and lower network modularity. Low modularity could indicate a lack of stability in networks and could have contributed to negative plant-soil feedback cycles, that were quantified as positive microbe-microbe interactions in cooccurrence networks, due to the clipping disturbance (Gao et al. 2018).

This dissertation used a combination of observational field data and experimental greenhouse studies to understand how changes in plant diversity influence soil microbial interactions in the rhizosphere. By utilizing these methods, I have demonstrated that soil microbial dynamics are complex and are dependent on plant diversity and aboveground stressors. The stability of the interactions in the rhizosphere are dependent on the historic conditioning of soils, which is evident in the field inoculum treatments, and belowground inputs from diverse plant communities. This dissertation confirmed existing knowledge gaps and posed new questions by clarifying previous assumptions behind plant-soil relationships. Future studies should include the experimental manipulation of particular microbial taxa, specifically AM fungi due to its hub contributions to network dynamics, to evaluate the impact of different AM fungal families have on plant-soil feedbacks. This can be accomplished by using a variety of target plant species that have a strong evolutionary history with particular AM fungal taxa. Future research would have major implications for the field of restoration by identifying which plant species contribute to positive plant-soil feedbacks with AM fungal taxa. In restoration, identifying plant species that contribute to positive feedbacks can contribute to the reestablishment of native mycorrhizal communities, encourage stability in the soil microbiome, and aid in proliferating beneficial soil microbial communities that are more resilient to climate change and disturbances.

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APPENDIX

Appendix Table 1. ANOVA table for soil bacteria showing multiple comparisons (Tukey's) based on mean abundance in high and low plant diversity plots by bacterial genus.

BACTERIA (TAXA)								
Taxa (Genus)	**Phylum**	**P Diversity**	**FDR Diversity**	**P adjusted Diversity**	**F Value Diversity**	**P (Tukeys) low-high**	**high mean**	**low mean**
Bosea	Proteobacteria (A)	0.000016	0.00032	0.0028	14	0.000012	0.32	0.22
Bradyrhizobium	Proteobacteria (A)	0.000027	0.00042	0.0046	13	0.000023	1.55	1.11
Rhizobium	Proteobacteria (A)	0.00013	0.00084	0.02	11	0.00061	0.59	0.45
Burkholderia	Proteobacteria (B)	0.00003	0.00042	0.0051	13	0.000055	0.42	0.24
Nitrosospira	Proteobacteria (B)	0.0058	0.013	0.58	5.7	0.0066	0.15	0.098
Rhizomicrobium	Proteobacteria (A)	0.00027	0.0014	0.039	9.6	0.00016	0.5	0.37

Appendix Table 2. ANOVA table for soil bacteria showing multiple comparisons (Tukey's) based on mean abundance in high and low plant diversity plots by bacterial phylum.

BACTERIA (PHYLUM)								
Taxa (Genus)	**Phylum**	**P Diversity**	**FDR Diversity**	**P adjusted Diversity**	**F Value Diversity**	**P (Tukeys) low-high**	**high mean**	**low mean**
Acidothermus	Actinobacteria	0.00055	0.0021	0.074	8.6	0.0028	0.32	0.16
Actinocorallia	Actinobacteria	0.00022	0.0012	0.033	9.8	0.00013	0.33	0.22
Actinomycetospora	Actinobacteria	0.0035	0.0082	0.36	6.3	0.0062	0.16	0.11
Actinoplanes	Actinobacteria	0.00013	0.00084	0.02	11	0.000083	0.65	0.45
Aeromicrobium	Actinobacteria	0.0018	0.0047	0.2	7.1	0.0043	0.65	0.53
Agromyces	Actinobacteria	0.012	0.024	1	4.8	0.011	0.42	0.63
Amycolatopsis	Actinobacteria	0.0026	0.0062	0.28	6.6	0.0017	0.17	0.096
Angustibacter	Actinobacteria	0.000072	0.00063	0.012	11	0.000064	0.16	0.073
Arthrobacter	Actinobacteria	0.0056	0.013	0.57	5.7	0.0088	1.8	1.45
Cellulomonas	Actinobacteria	0.001	0.0031	0.12	7.8	0.00075	0.36	0.26
Conexibacter	Actinobacteria	0.000073	0.00063	0.012	11	0.00052	0.15	0.087
Cryptosporangium	Actinobacteria	0.000081	0.00063	0.013	11	0.000045	0.16	0.092
Dactylosporangium	Actinobacteria	0.00037	0.0016	0.051	9.1	0.0004	0.35	0.24
Gaiella	Actinobacteria	0.00032	0.0015	0.045	9.3	0.00029	0.6	0.48
Hamadaea	Actinobacteria	0.00048	0.0019	0.065	8.8	0.00049	0.29	0.13
Janibacter	Actinobacteria	0.00025	0.0013	0.037	9.6	0.00028	0.15	0.094
Kineosporia	Actinobacteria	0.00065	0.0022	0.084	8.4	0.0004	0.49	0.35
Kribbella	Actinobacteria	0.00025	0.0013	0.037	9.6	0.0014	0.49	0.38
Marmoricola	Actinobacteria	0.00013	0.00084	0.02	11	0.0008	0.44	0.31
Microbispora	Actinobacteria	0.0012	0.0034	0.14	7.6	0.00077	0.14	0.067
Modestobacter	Actinobacteria	0.00063	0.0022	0.084	8.4	0.0028	0.31	0.22
Mycobacterium	Actinobacteria	0.0029	0.0069	0.3	6.5	0.041	0.61	0.51
Nakamurella	Actinobacteria	0.017	0.033	1	4.4	0.028	0.36	0.27
Patulibacter	Actinobacteria	5.1E-06	0.00023	0.0009	15	0.0000053	0.44	0.24
Phycicoccus	Actinobacteria	0.0012	0.0034	0.14	7.6	0.0076	0.15	0.1
Phytohabitans	Actinobacteria	0.0087	0.019	0.84	5.2	0.01	0.28	0.2
Pseudonocardia	Actinobacteria	0.0013	0.0035	0.15	7.5	0.002	0.6	0.46
Rhodococcus	Actinobacteria	0.0012	0.0034	0.14	7.6	0.0066	0.91	0.4

Appendix Table 2. (continued) ANOVA table for soil bacteria showing multiple comparisons (Tukey's) based on mean abundance in high and low plant diversity plots by bacterial phylum.

BACTERIA (PHYLUM) (continued)								
Taxa (Genus)	Phylum	P Diversity	FDR Diversity	P adjusted Diversity	F Value Diversity	P (Tukeys) low-high	high mean	low mean
Solirubrobacter	Actinobacteria	0.000061	0.00058	0.0099	12	0.000093	0.9	0.7
Streptomyces	Actinobacteria	4.1E-09	7.4E-07	7.4E-07	28	0.000000018	0.58	0.4
Streptosporangium	Actinobacteria	0.0091	0.019	0.87	5.1	0.012	0.13	0.089
Terrabacter	Actinobacteria	0.00097	0.003	0.12	7.9	0.002	0.23	0.14
Virgisporangium	Actinobacteria	0.0023	0.0058	0.25	6.8	0.003	0.039	0.12
Chryseolinea	Bacteroidetes	0.00084	0.0027	0.11	8.1	0.0016	0.28	0.22
Mucilaginibacter	Bacteroidetes	0.025	0.044	1	3.9	0.031	0.59	0.41
Niastella	Bacteroidetes	0.0037	0.0085	0.38	6.2	0.0085	0.25	0.21
Segetibacter	Bacteroidetes	0.0025	0.0061	0.27	6.7	0.0018	0.29	0.23
Terrimonas	Bacteroidetes	3.9E-07	0.000035	0.00007	19	0.00000068	0.6	0.82
Burkholderia	Proteobacteria (B)	0.00003	0.00042	0.0051	13	0.000055	0.42	0.24
Nitrosospira	Proteobacteria (B)	0.0058	0.013	0.58	5.7	0.0066	0.15	0.098
Oligoflexus	Proteobacteria (B)	0.016	0.031	1	4.4	0.012	0.18	0.13
Polaromonas	Proteobacteria (B)	0.00063	0.0022	0.084	8.4	0.00056	0.17	0.27
Variovorax	Proteobacteria (B)	0.00034	0.0015	0.048	9.3	0.0006	0.18	0.36
Bacillus	Firmicutes	0.000044	0.00048	0.0073	12	0.000025	0.61	0.43
Clostridium_sensu_stricto_13	Firmicutes	0.0013	0.0035	0.15	7.5	0.0079	0.14	0.086
Paenibacillus	Firmicutes	7.4E-06	0.00026	0.0013	15	0.0000068	0.38	0.27
Sporosarcina	Firmicutes	0.000034	0.00044	0.0057	12	0.000083	0.44	0.24
Tumebacillus	Firmicutes	0.00095	0.003	0.12	7.9	0.00076	0.46	0.33

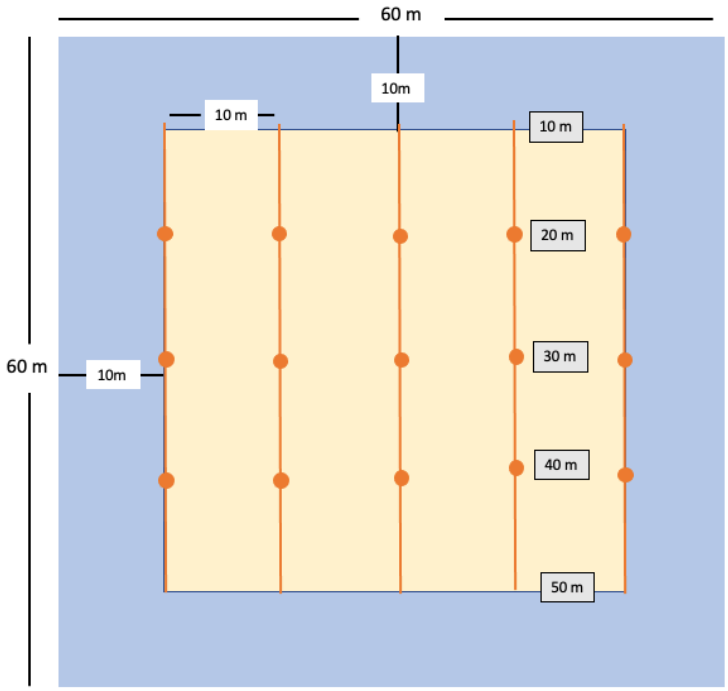
Appendix Table 3. ANOVA table for AM fungi showing multiple comparisons (Tukey's) based on mean abundance in high and low plant diversity plots by AM fungal genus.

AM FUNGI (TAXA)								
Taxa (Genus)	**Family**	**P Diversity**	**FDR Diversity**	**P adjusted Diversity**	**F Value Diversity**	**P (Tukeys) low-high**	**high mean**	**low mean**
Acaulospora	Acaulosporaceae	0.031	0.036	0.062	3.7	0.018	0.11	0.35
Archaeospora	Archaeosporaceae	0.012	0.021	0.048	4.8	0.017	3.72	1.69
Claroideoglomus	Claroideoglomeraceae	0.00065	0.0023	0.0039	8.4	0.00051	4.35	5.8
Diversispora	Diversisporaceae	0.017	0.024	0.051	4.4	0.043	3.18	2.33
Paraglomus	Paraglomeraceae	0.00012	0.00084	0.00084	11	0.000071	5.5	4.08
Scutellospora	Gigasporaceae	0.0067	0.016	0.034	5.5	0.0073	1.37	0.68

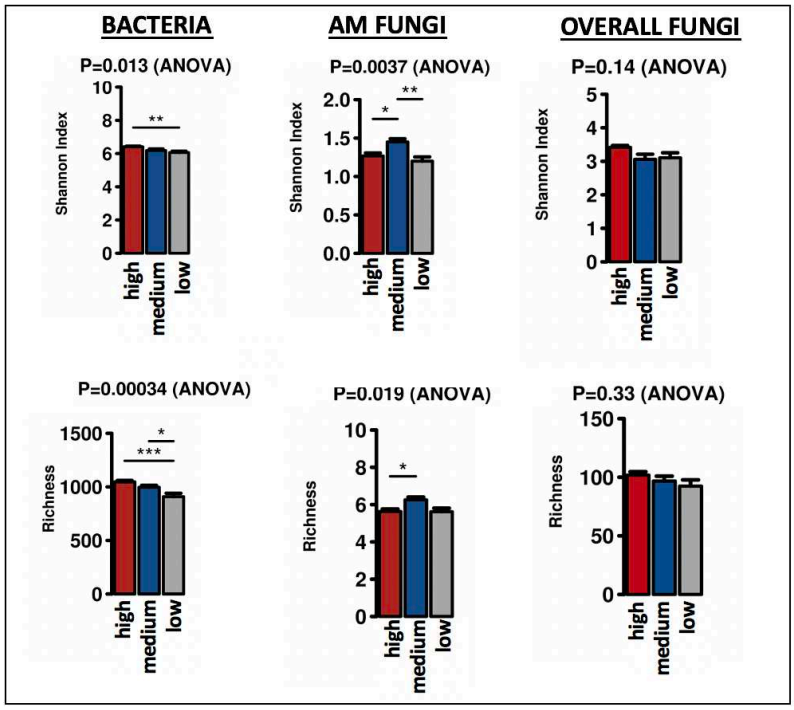
Appendix Table 4. List of plant species used in Chapters 3 and 4. Referred to as "greenhouse-established diversity" in text.

Greenhouse Experiment Plant Species List (69 species)		
Achillea millefolium	*Elymus virginicus*	*Ratibida columnifera*
Allium canadense	*Eragrostis trichodes*	*Rudbeckia hirta*
Andropogon gerardii	*Eupatorium altissimum*	*Rudbeckia subtomentosa*
Anemone canadensis	*Glycyrrhiza lepidota*	*Rudbeckia triloba*
Artemisia ludoviciana	*Helianthus annuus*	*Schizachyrium scoparium*
Asclepias sullivantii	*Helianthus maximillianii*	*Silphium laciniatum*
Asclepias syriaca	*Hesperostipa comata*	*Sisyrinchium campestre*
Asclepias tuberosa	*Heterotheca villosa*	*Solidago missouriensis*
Asclepias verticillata	*Lactuca ludoviciana*	*Solidago speciosa*
Astragalus canadensis	*Lespedeza capitata*	*Sorghastrum nutans*
Baptisia australis	*Liatris aspera*	*Sphenopholis obtusata*
Baptisia bracteata	*Liatris punctata*	*Sporobolus heterolepis*
Baptisia lactea	*Mirabilis nyctaginea*	*Symphyotrichum ericoides*
Bouteloua curtipendula	*Monarda fistulosa*	*Symphyotrichum laeve*
Calamagrostis canadensis	*Oenothera biennis*	*Symphyotrichum lanceolatum*
Callirhoe involucrata	*Oenothera rhombipetala*	*Symphyotrichum novae-angliae*
Dalea candida	*Oligoneuron rigidum*	*Symphyotrichum oblongifolium*
Dalea purpurea	*Panicum virgatum*	*Symphyotrichum sericeum*
Desmodium illinoense	*Pascopyrum smithii*	*Tridens flavus*
Echinacea angustifolia	*Penstemon digitalis*	*Triodanis perfoliata*

Appendix Figure 1. Map of Dahm's Diversity Plots at Platte River Prairies owned by The Nature Conservancy in Wood River, NE



Appendix Figure 2. Schematic of soil sampling design for each of the diversity plot within Dahm's Diversity Plots at Platte River Prairies owned by The Nature Conservancy in Wood River, NE.



Appendix Figure 3. Barplots showing alpha diversity levels of bacteria, overall soil fungi and AM fungi across high, medium, and low diversity plant communities.