Assessing the Contributions of Arbuscular Mycorrhizal Community Life-History Traits and Trait Diversity to Soil Carbon Cycling and Plant Nutrition

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Abstract

Arbuscular mycorrhizal (AM) fungi are soil mutualists that provide plants with nutrients in exchange for photosynthetic carbon (C). AM fungal life-history strategies can differ substantially among species, affecting their growth, life cycle, and reproduction. Variation in AM fungal life-history strategies have the potential to influence soil organic carbon (SOC) cycling and host plant nutrition. Soil contains almost three times more C than the atmosphere. Thus, knowing what factors encourage long-term soil C storage is important for climate change mitigation. Further, nutrition is central to plant health, and understanding how AM fungal traits influence nutrition could have implications in agriculture and ecosystem function. Despite the recognized benefits of AM fungi, the effect of their life-history strategies on SOC and plant nutrition are not well studied at the community level. To address these knowledge gaps, I grew Sudan grass (*Sorghum sudanense*) with AM fungal communities representing contrasting life-history traits and trait diversity: either five species from the *Gigasporaceae* family, representing a competitor life-history strategy, five species from the *Glomeraceae* family, representing a ruderal life-history strategy, or a combination of all ten species, representing wider life-history trait diversity. I used ^{13}C-CO_2 to investigate how AM fungal families affected SOC cycling and measured Sudan grass nutrient concentrations. All AM fungal communities decreased net total SOC relative to uncolonized plants, suggesting a SOC priming effect. Yet, despite the net SOC loss, all AM fungal communities contributed to SOC formation, but only *Glomeraceae* biomass contributed to the slower cycling SOC pool, making up 0.12% of the pool after harvest. The mixed-trait community increased plant phosphorus the most and the *Gigasporaceae* community the least, by 520% and 366% relative to the non-mycorrhizal control, respectively. Additionally, only the mixed-trait and *Glomeraceae* communities increased plant manganese concentrations.

The *Glomeraceae* community was the only one to decrease plant potassium and only *Gigasporaceae* species increased plant sodium. These results show functional variation at the family level with implications to SOC storage and plant nutrition.

Résumé

Les champignons mycorhiziens arbusculaires (AM) sont des mutualistes du sol qui fournissent aux plantes des nutriments en échange de carbone photosynthétique (C). Les stratégies d'histoire de vie des champignons AM peuvent différer considérablement d'une espèce à l'autre, affectant leur croissance, leur cycle de vie et leur reproduction. La variation des stratégies d'histoire de vie des champignons AM a le potentiel d'influencer le cycle du carbone organique du sol (COS) et la nutrition des plantes hôtes. Le sol contient presque trois fois plus de carbone que l'atmosphère. Ainsi, savoir quels facteurs favorisent le stockage à long terme du C dans le sol est important pour l'atténuation du changement climatique. De plus, la nutrition est au cœur de la santé des plantes, où comprendre comment les traits fongiques AM influencent la nutrition pourrait avoir des implications dans l'agriculture et la fonction de l'écosystème. Malgré les avantages reconnus des champignons AM, l'effet de leurs stratégies d'histoire de vie sur le COS et la nutrition des plantes n'est pas bien étudié au niveau communautaire. Pour combler ces lacunes dans les connaissances, j'ai cultivé de l'herbe du Soudan (Sorghum sudanense) avec des communautés fongiques AM représentant des traits d'histoire de vie et une diversité de traits contrastés : soit cinq espèces de la famille des *Gigasporaceae*, représentant une stratégie d'histoire de vie concurrente, cinq espèces de la famille des *Glomeraceae* , représentant une stratégie d'histoire de vie rudérale, ou une combinaison des dix espèces, représentant une plus grande diversité de traits d'histoire de vie. J'ai utilisé le ^{13}C-CO_2 pour étudier comment les familles de champignons AM affectaient le cycle du COS et mesuré les concentrations de nutriments de l'herbe du Soudan. Toutes les communautés fongiques AM ont diminué le COS total net par rapport aux plantes non colonisées, suggérant un effet d'amorçage du sol. Pourtant, malgré la perte nette de COS, toutes les communautés fongiques AM ont contribué à la

formation de COS, mais seule la biomasse de *Glomeraceae* a contribué au pool de COS à cycle plus lent, représentant 0.12 % de celui-ci après la récolte. La communauté à traits mixtes a le plus augmenté le phosphore des plantes et la communauté *Gigasporaceae* le moins, de 520 % et 366 % par rapport au témoin non mycorhizien, respectivement. De plus, seules les communautés à caractères mixtes et *Glomeraceae* ont augmenté les concentrations de manganèse dans les plantes. La communauté des *Glomeraceae* était la seule à diminuer le potassium des plantes et seules les espèces de *Gigasporaceae* ont augmenté le sodium des plantes. Ces résultats montrent une variation fonctionnelle au niveau de la famille avec des implications sur le stockage du COS et la nutrition des plantes.

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analyzing my soils for carbon (C) and ^{13}C content and the Stable Isotope Facility for Ecosystem Research, Natural Resources Analytical Laboratory (SIFER/NRAL) at the University of Alberta for tinning and analyzing my aboveground Sudan grass biomass samples for micro and macro nutrient, C, nitrogen (N), ^{13}C, and ^{15}N content.

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Contribution of Authors

This thesis is composed of an abstract, general introduction, three chapters, a general discussion, and conclusion. The general introduction briefly provides context for my research and presents this project's hypotheses and objectives. Chapter 1 is a literature review which describes the motivation for this thesis by overviewing previous scientific findings and knowledge gaps on arbuscular mycorrhizal (AM) fungal life-history traits and their effects on soil organic carbon (SOC) formation, stabilization, retention, and plant nutrition, and ends by restating the objectives in more depth. The results and findings of a greenhouse experiment are presented in Chapters 2 and 3 in manuscript format. Chapter 2 covers objectives 1 and 2, focusing on the effects of AM fungal community life-history strategies and diversity on SOC cycling, while Chapter 3 covers objectives 3 and 4 and focuses on the effects of AM fungal communities on plant nutrient uptake. The general discussion and conclusion summarize the project's key findings, their implications, and future directions.

The candidate was the lead author on all chapters, with co-advisors Dr. Cynthia Kallenbach and Dr. Pedro M. Antunes contributing overall conceptual and editorial guidance. The research, objectives, hypotheses, and experimental design were planned by the candidate with assistance from both co-advisors. The isotope chamber and methods used for isotopic labeling used in Chapter 2 were designed according to protocols available in Dr. Joann Whalen's lab and modified for this project by the candidate with assistance from co-advisors. The candidate conducted the greenhouse experiment, statistical analysis, and most of the laboratory work with occasional assistance. External laboratory assistance was provided by Jennifer Bridge (student in Dr. Pedro M. Antunes' laboratory) who quantified AM fungal colonization, the Ján Veizer Stable Isotope Laboratory at the University of Ottawa who analyzed soils for carbon and

isotopic concentrations, and the Stable Isotope Facility for Ecosystem Research, Natural Resources Analytical Laboratory (SIFER/NRAL) at the University of Alberta who analyzed plant chemistry. Findings were interpreted by the candidate with guidance from Dr. Pedro M. Antunes and Dr. Cynthia Kallenbach.

List of Abbreviations

Abbreviation	***Full Description***
AM	Arbuscular Mycorrhizal
AM MAOC	MAOC originating from AM sources
AM SOC	SOC originating from AM sources
B	Boron
BAS	Branched Absorbing Structures
C	Carbon
Ca	Calcium
CO_2	Carbon dioxide
Control	Community planted with Sudan Grass containing no AM fungi (a.k.a., the planted non-mycorrhizal control)
Cu	Copper
Fe	Iron
Giga	*Gigasporaceae*
Glom	*Glomeraceae*
IRMS	Isotope Ratio Mass Spectrometer
K	Potassium
MAOC	Mineral Associated Organic Carbon
Mg	Magnesium
Mix (Mixed)	The AM treatment containing all 10 *Gigasporaceae* and *Glomeraceae* Species
Mn	Manganese

N	Nitrogen
Na	Sodium
P	Phosphorus
S	Sulfur
SIFER/NRAL	Stable Isotope Facility for Ecosystem Research, Natural Resources Analytical Laboratory
SOC	Soil organic Carbon
Total MAOC	All MAOC found in the soil, including from fungal sources
Total SOC	All SOC found in the soil, including from fungal sources
Unplanted Control	Community containing AM fungal inoculant but no Sudan Grass
Zn	Zinc

General Introduction

Arbuscular mycorrhizal (AM) fungi are obligate symbionts forming relationships with 72% of vascular land plants. They are capable of altering landscape biogeochemical processes and ecosystem functions by transporting soil nutrients to their plant hosts in exchange for photosynthetic carbon (C) (Brundrett and Tedersoo 2018; Ricklefs 2010; Tedersoo and Bahram 2019). AM fungal functional traits are considered to vary primarily at the family level, especially for the two largest families of AM fungi, *Gigasporaceae* and *Glomeraceae* (Chagnon et al. 2013; Hart and Reader 2002). Species in the *Gigasporaceae* family are thought to utilize the competitor strategy as they prioritize acquiring growth-limiting resources such as phosphorus (P) and C to invest proportionally more in extraradical hyphae, at the cost of slower growth rates and delayed reproduction (Chagnon et al. 2013; Grime 1977; Hart and Reader 2002; Maherali and Klironomos 2007a; Staddon et al. 2003). The *Glomeraceae* family is thought to function as ruderals as they invest in faster growth rates and hyphal turnover and frequent reproduction at the cost of decreased allocation to extraradical hyphae. Data by Maherali and Klironomos (2007) supports these findings as inter-family AM communities were found to be more likely to co-exist than intra-family communities, indicating a degree of niche conservatism driven by differing life-history strategies. This thesis aims to address how variation in AM fungal community life-history strategies and trait diversity influence soil organic carbon (SOC) cycling and plant nutrition.

AM fungi can substantially alter SOC cycling by depositing photosynthetic C from their hosts directly into the soil. This AM fungal C could serve as a direct source of new inputs to the SOC pool, and has been seen to contribute up to 15% of new C in grassland and tropical ecosystems (Leake, Johnson, and Donnelly 2004; Miller and Kling 2000; Rillig et al. 2001).

Despite this direct contribution of AM fungal C inputs to SOC, little work has been done examining how AM fungal community trait variation impacts SOC accumulation and cycling. My first two objectives are to investigate how AM fungal community life-history strategies and trait diversity influence 1) the formation of SOC and 2) the slower cycling mineral associated organic carbon (MAOC) pool. The differences in life-history traits between *Glomeraceae* and *Gigasporaceae* families are likely to affect the rate and the amount of new AM fungal C inputs to soil, thus influencing SOC formation. On the one hand, *Gigasporaceae* may contribute more to SOC because of their relatively greater investment in extraradical hyphae and subsequently larger amount of fungal biomass inputs. On the other hand, *Glomeraceae* may contribute AM fungal C to soil at a faster rate than *Gigasporaceae* because of more rapid growth and hyphal turnover. Additionally, plants can vary in the amount of C they allocate to their AM associates, depending on the amount of soil P they receive in exchange (Kafle et al. 2019; Kiers et al. 2011). From a resource economy perspective, when P is limited, *Gigasporaceae* have been shown to have a higher capacity for P uptake in exchange for more photosynthetic C (Johnson et al. 2003). This may thus give *Gigasporaceae* more energy for investment into extraradical hyphae production (Johnson 2010). As such, I hypothesized that *Gigasporaceae* communities will contribute more to SOC than *Glomeraceae* due to greater extraradical hyphae biomass associated with better P acquisition capacity (Hart and Reader 2002). I further expect that communities with greater trait diversity will contribute the most to total SOC formation, where a mixed-trait community optimizes SOC formation through both rapid AM fungal input rates (*Glomeraceae*) and high biomass quantities (*Gigasporaceae*). Further, AM fungal C inputs have the potential to enter the slower cycling MAOC pool, and thereby increase the possibility of long term fungal C retention in soil (Lavallee, Soong, and Cotrufo 2020). As the majority of MAOC is composed of

microbially derived compounds and highly decomposed plant and fungal tissues, AM fungal C is more likely to accumulate in the MAOC pool if it is first processed by saprotrophic communities (Angst et al. 2021; Sokol, Sanderman, and Bradford 2019). As such, hyphal chemistry could be partly responsible for the journey of AM fungal C molecules through the soil and their probability of accumulating in the MAOC pool (Adamczyk et al. 2019; Frey 2019; Hättenschwiler, Sun, and Coq 2019a). For instance, some evidence shows that AM phenolic concentrations, associated with slower fungal decomposition, are correlated with slower hyphal growth rates (Fernandez and Kennedy 2018; Fernandez and Koide 2014). Alternatively, AM fungi that are relatively more enriched in nitrogen (N) and P would encourage faster decomposition, and similar to plants and some microbes, have faster hyphal growth rates (Fernandez and Koide 2014; Franklin et al. 2011; Manzella, Geiss, and Hall 2019; Siletti, Zeiner, and Bhatnagar 2017). Thus, I hypothesize that competitor AM fungi, like those in the *Gigasporaceae* family, with slower growth rates and slower extraradical hyphae turnover times may be slower to decompose but also less likely to enter the MAOC pool. As such, communities with greater AM fungal trait diversity may contain species with differing decomposition rates that enter the MAOC pool at different times and with varying C contributions.

AM fungi are primarily known for providing their hosts with soil nutrients. However, their relationship can range across a mutualism-parasitism spectrum (Johnson, Graham, and Smith 1997; Smith and Read 2008). Soil nutrient availability and the plant and fungal species involved are known to affect the symbiotic outcome. Yet, how AM fungal trait variation and diversity affect plant nutritional outcomes remains poorly understood (Neumann and Eckhard 2010; S. Smith and Read 2008). My second and third objectives are to determine how AM community life-history strategies and trait diversity differentially affect 1) plant nutrient

concentration and 2) growth. AM fungal colonization can increase plant P, N, and other micronutrients (e.g. copper and iron) and, has a result, host growth and fitness (Smith and Read 2008). For instance, AM fungal colonization is capable of increasing plant P by 152% compared to non-mycorrhizal controls and can contribute 100% of their host's P. It is thus possible that AM hyphae are the primary means of acquisition for some plant nutrients rather than direct root uptake (Hodge and Storer 2015; Oliveer et al. 1983; S. E. Smith, Smith, and Jakobsen 2004). This is partly due to the hyphae's ability to grow outside of roots' nutrient depletion zones to access nutrients (Smith et al. 2011). This is done using thick transport hyphae that extend away from their hosts to deploy fast cycling absorptive hyphae that extract nutrients from pores that are too small for roots (Neumann and Eckhard 2010; Parihar et al. 2020; Smith and Read 2008). Nutrients are then transported to their host and exchanged in intraradical structures known as arbuscules. The rate of intraradical root colonization has been found to correlate with increased AM fungal contributions to plant nutrition (Bruce, Smith, and Tester 1994). As absorptive hyphae have faster hyphal turnover times than transport hyphae, allocation to these two structures could be predicted by mean hyphal turnover times. Thus, the *Gigasporaceae* family may invest more in transportive hyphae than absorptive hyphae due to its relatively slower turnover. Since the *Gigasporaceae* family is often better at P uptake and predominates in some P-limited systems, I predict that *Gigasporaceae* communities are better at providing their hosts with nutrients than *Glomeraceae* due to greater investment in extraradical hyphae and transport hyphae, which could better enable them to search for nutrients in the heterogeneous soil environment. Additionally, Crossay *et al.* (2019) also found that co-inoculation by species in two AM fungal families improved plant growth and nutrient concentration when exposed to abiotic stressors, and Maherali and Klironomos (2007) found that increased AM community trait

diversity led to greater plant growth; potentially due to a diverse community's improved ability to extract nutrients from different niches. Therefore, I hypothesize that an AM fungal community with greater trait diversity would be the best at providing plants with soil nutrients.

While most vascular land plants associate with AM fungi, certain plants or ecosystems may favor symbiosis with *Gigasporaceae* while others may favor *Glomeraceae* (Chagnon et al. 2013; Jansa et al. 2002; Oehl et al. 2011). Thus, the impact of AM fungal traits and their diversity is key for our understanding of SOC storage and plant nutrition. This could influence climate change research, soil health, food security, and plant community ecology. Despite these implications, little research has been done on how varying AM fungal community life-history strategies and trait diversity differentially affect these factors. In summary, this thesis aims to address these knowledge gaps by focusing on the following specific objectives: 1) Determine how AM fungal community life-history strategies and trait diversity affect SOC accumulation; 2) Determine if AM community life-history strategies or trait diversity impact the amount of SOC accumulating in the slower cycling MAOC pool; 3) Evaluate how plant nutrition and 4) growth are affected by AM community life-history strategies or trait diversity.

Chapter 1: Literature Review

1.1 Mycorrhizal Symbioses and Classification

Mycorrhiza describes an obligate symbiotic relationship between some soil fungi and plant roots. The symbiosis is most often mutualistic but commensal and parasitic outcomes have been reported (Neumann and Eckhard 2010; Smith and Read 2008). Mycorrhizal fungi benefit from their plant hosts by receiving photosynthate carbon (C) in return for nutrients and water that may be out of reach of their hosts (Ricklefs 2010). Associating with mycorrhizal fungi can also increase plant biomass and fitness through improved nutrition and stress tolerance (Parke, Linderman, and Black 1983; Ricklefs 2010; Ruiz-Lozano, Azcon, and Gomez 1995).

Mycorrhizal symbioses can be found across most vascular plant taxa and it is now generally accepted that early land plants evolved alongside mycorrhizal fungi and may have used the symbiosis to access nutrients before the evolution of roots (Heckman *et al.*, 2001; Smith and Read, 2008). Mycorrhizal fungi can be found across three fungal phyla and can be categorized by their structural characteristics (Smith and Read, 2008). There are seven mycorrhizal classifications of which ectomycorrhiza (EcM) and arbuscular mycorrhizal (AM) fungi are the most common (Read, 1991; Smith and Read, 2008, pg 5). EcM evolved several times from the phyla *Basidiomycota*, *Ascomycota*, and *Glomeromycota*, whereas AM fungi are only formed by the *Glomeromycota* phylum (Schüßler, Schwarzott, and Walker 2001; Smith and Read 2008). There are eleven different families of AM fungi of which most described species are found in the families *Gigasporaceae* and *Glomeraceae* (Redecker et al. 2013; Schüßler, Schwarzott, and Walker 2001).

Approximately 72% of vascular terrestrial plant species worldwide are thought to associate with AM fungi, making the AM fungal condition the default for most plants, and non-

mycorrhizal species the exception (Brundrett 2017). In this review I focus on AM fungi, as they are the most ubiquitous group of mycorrhizae and they served as the basis for my research. AM fungi penetrate into root cells and form structures known as arbuscules where resource exchange primarily occurs (Fig 1.1) (Ricklefs 2010; Tedersoo and Bahram 2019). Around 80% of AM fungi also form resource storage structures within cell walls known as vesicles (Smith and Read 2008). Compared to EcM, AM fungi are the predominant mycorrhiza in temperate and tropical grasslands, agricultural soils, and shrublands, and soils with a relatively lower carbon (C) to nitrogen (N) ratio and higher pH (Read 1991). Unlike EcM, AM fungi are not capable of direct soil organic matter (SOM) decomposition.

AM fungi are not functionally homogeneous, as they can utilize different life-history strategies and possess a variety of functional traits. This diversity across AM fungal functional groups has the potential to affect integral aspects of plant health and ecosystem function (Sanders 2003). Despite this, most studies on trait variation have focused on differences between EcM and AM fungi, while less research has been done on variation among AM fungal families (Chagnon et al. 2013; Soudzilovskaia et al. 2019; Tedersoo and Bahram 2019).

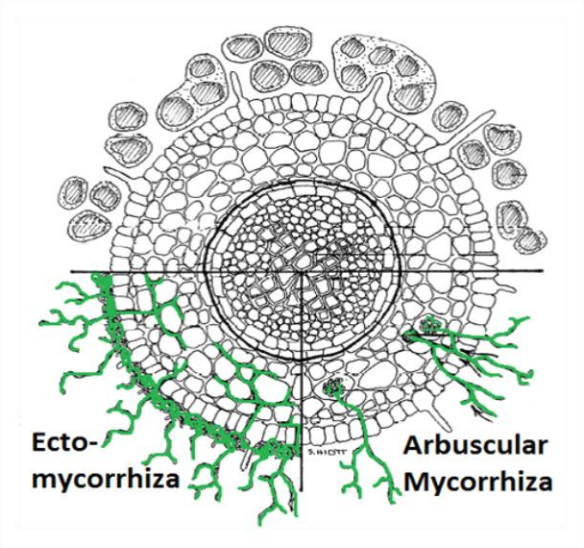

Figure 1.1 A cross-section of a root showing ectomycorrhiza (left) and arbuscular (right) mycorrhizal fungal colonization strategies. Mycorrhizal structures are colored green and plant tissues are shown in black. Adapted from: https://plantecology.files.wordpress.com/2012/05/mycorrhizaetypes.png

1.1.1 Life-History Models

Fungal species, including mycorrhizae, are frequently classified into functional groups according to a variety of different models to better predict, for example, how populations interact and how communities respond to environmental conditions (Treseder and Lennon 2015). The r- and K-selection strategies and Grime's C-S-R framework are two well-known models that have recently been applied to mycorrhizal fungi to comprehend strategies around resource conservation, reproduction, body size, and life cycle (Chagnon et al. 2013).

The r- and K-selection model considers two environmental contexts: 1) access to abundant resources and limited competition due to, for example, a sudden environmental change or introduction to a new habitat; and 2) limited resources, high competition, and a stable environment (Pianka 1970; Reznick, Bryant, and Bashey 2002). The first scenario would favor

the survival of r-strategists or populations that specialize in rapid resource acquisition with a smaller body size, shorter lifespan, faster rate of maturation, high fecundity, low investment in individual offspring, and investment in current over future reproduction (Mertz et al. 1966; Pianka 1970; Reznick, Bryant, and Bashey 2002). The second environment would favor the survival of K-strategists; those that specialize in high resource use efficiency, have larger body sizes, longer lifespans, delayed maturation, low fecundity, high investment in individual offspring, and long-term reproductive success. In reality, species exist on a spectrum between the two strategies, but examples of r-strategists such as rabbits (Leporidae family) and dandelions *(Taraxacum spp.)* or K-strategists such as oaks trees (*Quercus* spp.) and elephants (*Elephantidae* family) can still be provided.

The Grime's C-S-R framework was introduced by Grime (1977) and adds upon the r- and K-selection theory by focusing on functional traits adapted to three environmental conditions driven by stress and disturbance. Stress is defined as consistent disadvantageous conditions such as arctic, alpine, or desert biomes, while disturbance is considered a frequent dramatic event leading to major biomass loss due to, for example, grazing, wildfire, and frost (Chagnon et al. 2013; Grime 1977). These conditions create a two-axis framework with three potential life-history strategies: 1) competitors, 2) stress tolerators, and 3) ruderals (Grime 1974) (Figure 1.2). Competitors thrive in low disturbance and low-stress environments where their major obstacles come from competition for resources (Chagnon et al. 2013; Grime 1977). Competitors prioritize resource acquisition by delaying reproduction. In plants, competitors often form short-lived leaves, tall canopies, and dedicate a relatively small portion of their biomass to reproduction. Stress tolerators are adapted to high-stress, low disturbance environments and prioritize resource use efficiency, and functional biomass retention. In plants this is demonstrated by a slow growth

rate, small, needle-like, or leathery leaves, evergreen tendency, and a relatively small proportion of biomass devoted to reproduction. Finally, ruderals are adapted to high disturbance, low-stress environments by prioritizing high fecundity, rapid growth rates, and short reproductive cycles. In plants this is characterized by weedier species with large portions of their biomass allocated to reproduction. It is hypothesized that species capable of surviving in both high stress and disturbed environments do not exist (Chagnon et al. 2013; Grime 1977). Species are not confined to these three categories but rather exist on a spectrum. For instance, a species could evolve under conditions of moderate stress and moderate disturbance (Figure 1.2).

Models such as the r- and K- strategies and Grime's C-S-R framework help to understand species adaptations and to find patterns in functional life-history traits. Although, both r- and K-selection and Grime's C-S-R framework were primarily developed for their application with plants, they have also been applied to other kingdoms. When Grime (1977) first proposed his framework, he suggested how it could be applied to animals and fungi. Since then, Grime's C-S-R framework has been modified to fit numerous other organisms including corals, saprotrophic wood rot fungi, and soil microbes (Darling et al. 2012; Lustenhouwer et al. 2020; Malik et al. 2020). Categorizing AM fungi into functional groups with these models would be beneficial as it could help explain what evolutionary pressures drove their wide-ranging trait variation and predict how different AM traits could affect mutualisms and ecosystem function.

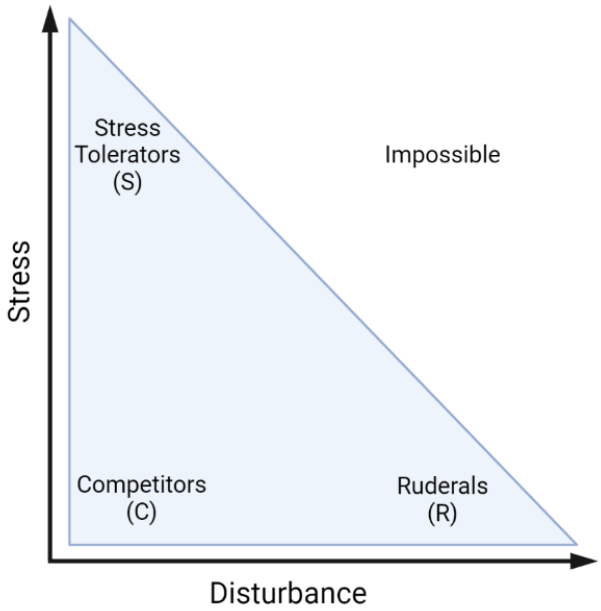

Figure 1.2 Grime's C-S-R triangle modified from Chagnon *et al.* (2013). The blue triangle represents the range of possible life-history strategies that species can occupy.

1.1.2 Proposed Mycorrhizal Life-History Strategies

AM fungal life-history strategies vary taxonomically, primarily at the family level (Hart and Reader 2002). Chagnon *et al.* (2013) suggested that the Grime's framework could apply to AM fungi at the family level. He proposed that the AM families, *Gigasporaceae*, *Acaulosporaceae*, *Glomeraceae*, could be classified as competitors, stress tolerators, and ruderals, respectively. Competitors excel at acquiring growth-limiting resources, but resources that are growth-limiting for a plant host may not be equally limiting for their fungal symbionts. It is thought that C is the most growth-limiting resource for most AM fungi and the amount of C a host plant provides to AM fungi is correlated with the amount of phosphorus (P) the AM fungi provides to its host in return (Kafle et al. 2019; Kiers et al. 2011; Olsson, Jakobsen, and Wallander 2002). Therefore, an AM family would be considered competitive if it possessed traits corresponding with high P acquisition such as heavy investment in extraradical hyphae and

decreased investment in reproduction. *Gigasporaceae* fit this description well, as they invest the majority of their biomass in relatively long-lasting extraradical hyphae, with delayed sporulation, and the production of fewer, larger spores (*Species Descriptions | Davis - INVAM | West Virginia University*; Hart and Reader, 2002; Staddon *et al.*, 2003; Oehl *et al.*, 2009; Chagnon *et al.*, 2013). *Gigasporaceae* species are often better at providing their hosts with soil P and have a high photosynthetic C sink compared to *Glomeraceae* (Johnson et al. 2003; Lerat et al. 2003; Maherali and Klironomos 2007b).

Ruderals can survive recurrent devastating biomass loss, which for AM fungi would mean damage to their hyphal networks. Species adapted to survive in such conditions are most likely to rapidly colonize and efficiently heal damaged hyphae through hyphal fusion – traits typically expressed by *Glomeraceae*. *Glomeraceae* have a fast growth rate, a high hyphal fusion and turnover rate, and a short life cycle resulting in frequent sporulation of many small spores (Chagnon et al. 2013; Hart and Reader 2002, 2005; De La Providencia et al. 2005; Oehl et al. 2009; Species Descriptions | Davis - INVAM | West Virginia University n.d.; Staddon et al. 2003). *Glomeraceae* are also the predominant mycorrhizal community in disturbed (e.g., tilled) agricultural soils (Jansa et al. 2002). Finally, stress tolerators can survive for extended periods in sub-optimal conditions. For AM fungi, a continuous shortage of C from their host would be a major stressor, and species best adapted to such conditions would most likely grow slowly and maintain their biomass due to the high cost of new biomass production. *Acaulosporaceae* fit this description as they grow slowly, produce less biomass than other families, and have been found in harsh, early successional sites in alpine ecosystems (Hart and Reader 2002; Oehl et al. 2011).

For the same reasons that the *Gigasporaceae* and *Glomeraceae* could be classified as competitors and ruderals, they also fit the descriptions of r- and K- strategists. *Gigasporaceae* is

slower growing, delays reproduction, invests in fewer but larger offspring, and grows larger long-lasting hyphal networks, as expected for a K-strategist. In contrast, *Glomeraceae* colonizes plants rapidly, reproduces frequently producing many small spores, and has higher hyphal turnover, matching the description of an r-strategist.

1.1.3 Potential Effect of Variation in AM Fungal Life-History Strategies

Mycorrhizal fungi can impact ecosystem functions and make up a large portion of total soil microbial biomass in some environments (Frey 2019; Sanders 2003). Thus, variation in AM fungal life-history strategies could impact landscape biogeochemical processes and plant nutrition (Tedersoo and Bahram 2019). AM fungi act as an interface between the soil and atmosphere. Plants photosynthesize C and allocate it to their mycorrhizal partners, essentially functioning as a pump bringing C below ground. Differing AM fungal life-history traits may influence how much C the plants provide the AM fungi, where that C will be placed within the fungi, and the chemical composition that C may take; all of which could influence soil organic carbon (SOC) cycling. Soils contain three times as much C than the atmosphere (Paustian et al. 2016). So, understanding factors that influence SOC accumulation and retention could aid in improving climate change modeling and mitigation strategies. Moreover, diversity in AM fungal functional traits may lead to variation in plant nutrition. Differing AM growth rates and strategies may affect fungal nutrient acquisition efficiency and consequently the nutrition and growth of its host. Understanding how AM life-history strategies influence plant nutrition could have implications to overcoming major challenges faced in agriculture and ecosystem restoration.

Diversity in AM fungal functional traits and niche conservatism could also influence AM fungal community assembly, further increasing the local impact of AM fungal traits. Maherali

and Klironomos (2007) inoculated plantain *(Plantago lanceolata)* with eight mycorrhizal species, either all from *Gigasporaceae*, *Glomeraceae*, or *Acaulosporaceae* families, combinations of two, or all three families. After one year of growth, communities that were inoculated with a more diverse community had greater realized AM fungal species richness than those inoculated with less diverse communities, indicating some degree of niche conservatism among the three families and reduced competition among AM fungi from different families (i.e., in phylogenetically over-dispersed communities). *Plantago lanceolata* grown with more taxonomically diverse communities also grew larger than those grown with single-family communities, possibly due to niche complementarity with the consequent greater abundance and array of resource acquisition.

1.2 Soil Organic Carbon

Soil organic carbon (SOC) ultimately originates from plants and much of it will inevitably be respired back into the atmosphere as CO_2. How an individual C molecule moves through the soil is governed by its chemical characteristics, the local decomposer community, and the physical soil environment (Lehmann and Kleber 2015; Waring et al. 2020). Proximity to a decomposer can accelerate a C molecule's journey through the soil, depending on whether the decomposer can break down the chemical components of the C molecule in question. Physicochemical processes such as occlusion in aggregates or association with soil minerals will slow down a C molecule's journey by stabilizing it in the soil matrix (Waring et al. 2020). The pore structure, temperature, and moisture of the soil environment can also influence a C molecule's life cycle through the soil. Due to how complex and variable these factors are, it is challenging to determine the ultimate fate of any one soil C molecule (Waring et al. 2020). However, SOC can be separated into functional pools associated with different turnover times,

formation mechanisms, and chemistries that may help predict soil C's fate related to disturbance responses and long-term persistence (Christensen 2001). A typical approach is to separate SOC into the operationally described pools: particulate organic matter (POM) and mineral associated organic carbon (MAOC) (Cambardella and Elliott 1992; Lavallee, Soong, and Cotrufo 2020). POM is mostly composed of light-weight, less decomposed organic material, often more similar chemically to plant tissue and varies in size from 53 to 2,000 μm (Cotrufo et al. 2019; Lavallee, Soong, and Cotrufo 2020). MAOC is formed mostly by low molecular weight compounds, from highly decomposed organic matter, microbial cellular and extra-cellular compounds, and root exudates. These compounds associate with soil minerals typically via strong mineral sorption mechanisms. MAOC can be operationally classified by its size, as SOC <53 μm and it is more nutrient dense than POM, has a lower C to N ratio, and requires a lower activation energy (Cotrufo et al. 2019; Lavallee, Soong, and Cotrufo 2020). POM can stay in the soil for years to decades if it is protected by occlusion in aggregates (Cotrufo et al. 2019; Lavallee, Soong, and Cotrufo 2020). MAOC, on the other hand, is protected from decomposition by mineral associations and can reside in the soil for decades to centuries, making it a relatively more persistent C pool and thus important for long-term C storage (Balesdent and Mariotti 1996).

MAOC can be formed directly from plant inputs through sorption of dissolved plant compounds to soil minerals or from microbial inputs and plant compounds first processed by microbes through the microbial turnover pathway (Sokol, Sanderman, and Bradford 2019). In most temperate and tropical ecosystems, the majority of MAOC is dominated by microbially derived compounds (Angst et al. 2021). As such, areas of high microbial density and metabolic activity, such as the rhizosphere will have a higher proportion of MAOC accumulating from microbially processed organic matter and direct microbial inputs (Sokol, Sanderman, and

Bradford 2019). Because of the importance of soil microbial communities in MAOC accumulation, connecting soil community trait variations and their impacts on MAOC accumulation is needed (Domeignoz-Horta et al. 2021). For example, several studies have indicated that the proportion of SOC increases with the higher fungal abundance (Angst et al. 2021; Frey 2019; Kallenbach, Frey, and Grandy 2016) but certain fungal traits may further determine the magnitude of this relationship and fate of fungal inputs to MAOC.

1.2.1 Arbuscular Mycorrhizal Fungi and Soil Organic Carbon

Mycorrhizal fungi can make up 20 to 30% of soil microbial biomass and constitute a unique boundary between soil C and atmosphere C (Frey 2019; Leake, Johnson, and Donnelly 2004). In grassland and tropical ecosystems AM fungi are estimated to contribute up to 15% of SOC (Leake, Johnson, and Donnelly 2004; Miller and Kling 2000; Rillig et al. 2001). AM fungi are capable of forming, stabilizing, and destabilizing SOC where their net impact on SOC concentrations is dependent on the balance between these three processes (Frey 2019).

AM fungi influence on SOC persistence (or longer soil residence time) has largely been associated with their ability to increase soil aggregation and thus physical protection of SOC. AM fungal hyphae can facilitate soil aggregation through enmeshment of soil particles and enhanced production of polysaccharides that act as glues to bind soil particles produced from bacteria that coexist on AM fungal hyphae (Parihar et al. 2020; Rillig 2004; Solaiman 2014; Tedersoo and Bahram 2019). AM fungi may also stabilize SOC through the production of insoluble, persistent, protein exudates known as glomalin (Wright and Upadhyaya 1998). Estimates have found that glomalin can constitute 20 times as much to SOC than microbial biomass (Parihar et al. 2020; Rillig et al. 2001) and it has been tied to soil aggregate formation

and stability (Parihar et al. 2020; Rillig 2004; Wright and Upadhyaya 1998). However, the origin and estimates of glomalin have recently come under question (Holátko et al. 2021).

Unlike EcM fungi, AM fungi are not thought to be capable of SOC decomposition, lacking the genes necessary for lignin decomposition, and most of the genes required for hemicellulose degrading enzymes (Tisserant *et al.*, 2013; Tang *et al.*, 2016; Frey, 2019). Thus, their ability to directly shift SOC mineralization rates is often considered negligible. However, AM fungi interact and influence the decomposer community as well as the soil environment. Depending on these community interactions, this could have consequences for both the stabilization and mineralization of SOC. First, soil microbial populations can travel through the soil matrix using AM hyphae as a pathway. Most soil microbes are sessile and motile microbes are often confined by heterogeneous water availability, but AM hyphae have moist surfaces and exude readily available C, creating "fungal highways" (Dechesne et al. 2010; Gorka et al. 2019; See et al. 2022). Bacterial "migrations" have been documented over these highways, providing a vector by which otherwise confined saprotrophs may be introduced to new SOC (See et al. 2022; Zhang et al. 2018). Further, AM fungi may be able to indirectly utilize these saprotrophic microbial communities to their advantage by "priming" the soil. In such a scenario, priming involves the addition of C to targeted soil areas in the form of hyphae or AM fungal exudates, to release inaccessible nutrients (Frey 2019; Kuzyakov 2002; Toljander et al. 2007; Zak et al. 2019). This newly deposited AM fungal C can increase decomposer activity and thus accelerate SOC mineralization.

Arbuscular mycorrhiza may influence SOC formation by changing how much photosynthetic C is fixed, where that C is allocated (e.g., AM fungal structures), and its chemistry. AM fungi are known to increase plant growth and net primary productivity, resulting

in more plant C entering the SOC pool. It is estimated that 30 to 65% of gross primary productivity is allocated below ground and in AM systems 2 to 20% of plant C is allocated to their mycorrhizal symbionts within 72 hours (Gill and Finzi 2016; Leake, Johnson, and Donnelly 2004).

In addition to the indirect effect of increasing plant C inputs, AM fungi contribute to SOC directly through their own biomass inputs. AM fungal C can take three forms: living AM fungal compounds, fungal exudates, and dead fungal biomass (i.e., necromass) (Frey 2019). Living hyphae can facilitate soil C formation by depositing AM fungal C near mineral surfaces, encouraging the formation of MAOC, and by enhancing aggregate formation (Frey 2019; See et al. 2022). The contribution of AM hyphae to MAOC through these means may be considerable as the MAOC pool is primarily derived from microbial C and living EcM hyphae can constitute over a third of total microbial biomass and up to half of fungal mycelia in some systems. As such, AM fungi may likewise provide considerable microbial and thus MAOC inputs (Angst et al. 2021; Bååth et al. 2004; Högberg and Högberg 2002). AM hyphae can also grow beyond the rhizosphere to form the "hyphosphere" or the area influenced by roots and soil fungi (Fig 1.3). The formation of the hyphosphere increases the area of active biological activity where C can be deposited (See et al. 2022). Further, fungal exudates (C-rich, low-molecular weight compounds) can reach mineral surfaces within micro pores inaccessible to microbes, promoting MAOC production (Frey 2019; Keiluweit et al. 2015). It has been estimated that, overall, plant exudates constitute 2 to 17% of plant net primary productivity and that AM exudates are the dominant source of new photosynthetic C to the soil (Frey 2019; Kaiser et al. 2015; Kuzyakov 2002; Nguyen 2003; Toljander et al. 2007). New hyphal necromass can either directly enter the SOC pool as free hyphae, similar to fine POM, or can bind to soil minerals to form MAOC through

the direct sorption pathway. The hyphal necromass that is initially within the fine POM fraction can be rapidly decomposed by saprotrophic microbes. This decomposition may move some of the free hyphae into the more persistent MAOC fraction, composed of smaller more microbial-processed compounds. Which pathway AM hyphae takes is likely partly influenced by hyphal chemistry which can vary across mycorrhizal species (Fernandez and Kennedy 2018; Frey 2019). The chemical composition of hyphae can affect both the rate of its decomposition and thus transition to MAOC and mineralization as well as its capacity to sorb to mineral surfaces.

Though AM fungal C likely provide inputs to SOC as described above, the absolute quantity and drivers of variation remain unknown. Since other classes of mycorrhizae (e.g., EcM) have far more hyphal biomass relative to AM fungi, AM fungal necromass inputs have largely been overlooked as a potentially important direct pathway of soil C inputs (Clemmensen et al. 2013). Instead, the impact of AM fungi on SOC has been more focused on the mechanisms associated with aggregation, glomalin, and priming.

The net impact of AM fungi on SOC cycling depends on the balance between its C formation, stabilization, and destabilization capacity. Since many factors influence SOC cycling, the balance between the three may be subject to change depending on environmental conditions, plant host identity, and AM life-history traits.

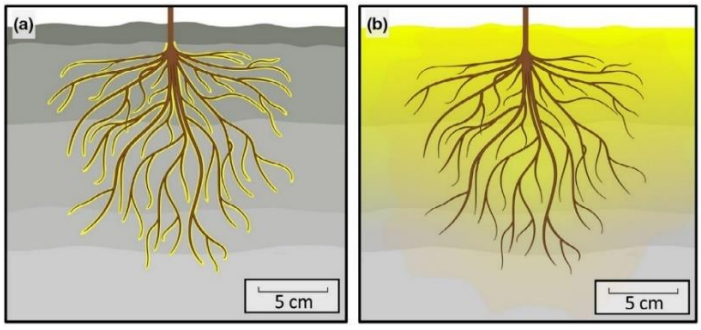

Figure 1.3 The soil volume under the influence of the soil rhizosphere (a) versus the hyphosphere (b), adapted from See *et al.* (2022).

1.2.2 Arbuscular Mycorrhizal fungal Chemistry and its Effects on Soil Organic Carbon

Microbial necromass can constitute over half of soluble SOC but its decomposition and inevitable path through the soil matrix is partly dependent on its chemical composition (Simpson et al. 2007). Mycelium C:N and C:P ratios have been found to vary drastically in saprotrophic fungi with the highest C:N and C:P ratios being over 31 and 74 times higher than their lowest values, respectively (Camenzind et al. 2021). These differences in stochiometric ratios would be expected to impact the rate of mycelium decomposition, with faster decay associated with great N and P enrichment. Stochiometric variability may occur in response to environmental stress, resource availability, and possibly across conserved fungal traits. For instance, similar to plants and other microbial species, high growth rates tend to support more N and P enrichment (Franklin et al. 2011; Manzella, Geiss, and Hall 2019; Siletti, Zeiner, and Bhatnagar 2017). Such variation in AM fungal hyphae nutrient concentrations is also highly plausible but methodological challenges limit isolating enough AM fungal hyphae mass for chemical analyses.

In addition to variable stoichiometry, fungal molecular compounds such as chitin and melanin, can affect hyphal decomposition with concentrations varying across AM fungal traits. A large portion of AM fungal C is found in chitin, a carbohydrate that is primarily present in fungal cell walls (Kögel-Knabner 2002; Solaiman 2014). Chitin is an accessible source of C and N for microbes and thus decomposes relatively rapidly (Tedersoo and Bahram 2019; Zeglin and Myrold 2013). Despite chitin's relative chemical lability, it can last in the soil for decades, likely because its protein group preferentially bonds with phenolic compounds and clay minerals (Adamczyk et al. 2019; Gleixner et al. 2002; Hättenschwiler, Sun, and Coq 2019b). Melanins are also found in some fungal cell walls and protect the fungi against microbial attack, UV radiation, and water stress (Deveautour et al. 2020; Kögel-Knabner 2002). These compounds are large

complex nonhydrolyzable polymers formed of phenolic or indolic monomers (Fernandez and Koide 2014; Frey 2019). High-cost oxidative enzymes are needed to break down melanin, making it difficult for microbes to decay (Frey 2019). Fernandez and Koide (2014) found that the melanin concentrations of EcM necromass were negatively correlated with biomass decomposition and that fungal necromass decomposed faster when melanin synthesis was inhibited. Similarly, Siletti, Zeiner and Bhatnagar (2017) found that SOC correlated with soil melanin concentrations in coniferous forests, indicating that melanin is resistant to decomposition and may drive necromass accumulation (Fernandez and Koide 2014; Frey 2019). Melanin, like other phenolic-based compounds, can suppress decomposition when it interacts with nitrogen (N) in a similar way to plant lignin-N complex (Fernandez and Koide 2014). At the same time, high N concentrations in hyphae and the soil environment can accelerate decomposition rates if the microbial community is relieved of its N-limitations.

The chemical composition of AM fungal necromass can also influence its bioavailability (susceptibility to decomposition) and thus its journey through the soil matrix. While melanin and chitin are specific compounds that may delay decomposition, N-rich hyphae and other energy C-rich compounds such as some short-chain lipids and polysaccharides within fungal hyphae may be more susceptible to initial decomposition. The ease of breaking down chemical compounds found in hyphal necromass can influence how likely the necromass is to be decomposed by soil microbes and the metabolic efficiency of these compounds to contribute to new biomass growth. These factors influence the residence time of hyphal necromass C, as its likelihood of entering the more persistent MAOC pool is thought to increase following greater microbial decomposition (J. Lehmann and Kleber 2015; Olayemi, Kallenbach, and Wallenstein 2022). Given the potential importance of nutrients, chitin, and melanin on hyphal decomposition, it

would be important to know if their concentrations vary with fungal life-history traits. While it has yet to be shown for AM fungi, EcM fungal melanin concentrations are often negatively correlated with growth rate and biomass production and it is unclear if chitin concentrations also vary with growth strategies (Siletti, Zeiner, and Bhatnagar 2017).

1.2.3 Effect of Arbuscular Mycorrhizal Fungal Diversity on Soil Organic Carbon

Variation in AM fungal life-history traits at the family level could directly affect SOC by influencing its formation, stabilization, and decomposition; the net effect of which could alter the size of SOC pools. The *Gigasporaceae* and *Glomeraceae* families utilize different life-history strategies and whether this confers differences in SOC pools is unknown. *Gigasporaceae* are classified as competitors or k-strategists and are better at acquiring growth limiting resources, potentially by investing more in extraradical hyphae (Chagnon et al. 2013; Maherali and Klironomos 2007a). In contrast, *Glomeraceae*, classified as ruderals or r-strategists, invest more of their biomass on intra-radical than extra-radical. These main differences in life-history traits could all affect their relationship with SOC.

Mycorrhizal life-history traits that may increase SOC formation are also those that would lead to an increase in the amount of C entering the soil. Host plants increase the amount of C they provide their AM associates in accordance with the amount of P they receive in exchange. Whether or not they may do the same for other limiting nutrients is unknown (Kafle et al. 2019; Kiers et al. 2011). Species in the *Gigasporaceae* family dominate under N-rich, P-limited field conditions, and are often, but not always, more adept at P uptake, and thereby may receive more C more consistently than *Glomeraceae* species (Johnson et al. 2003). For instance, a long-term greenhouse experiment found the *Gigasporaceae* family to be best at increasing English plantain's *(Plantago lancelota)* P concentrations, (Maherali and Klironomos 2007b). At the same

time, *Gigasporaceae* species may also be stronger C sinks than *Glomeraceae*. Lerat *et al* (2003) found that the *Gigasporaceae* species *Gigaspora rosea* had a stronger photosynthetic C sink strength in a split-root system compared to an uncolonized control when grown with both barley *(Hordeum vulgare)* and sugar maple *(Acer saccharum)*, while the *Glomeraceae* species' results were less consistent. The *Gigasporaceae* family's C sink strength may be due to its greater investment in extraradical hyphae which may better enable them to search the heterogeneous soil environment for nutrient-rich pockets, while simultaneously depositing photosynthetic C across a larger volume of soil. However, *Glomeraceae* species are generally faster growing and have a faster turnover time (Chagnon et al. 2013). As such, they may be better at rapidly absorbing available soil nutrients and thus be able to obtain more C than *Gigasporaceae*.

AM fungal traits may also be associated with divergent hyphal chemistries, influencing whether AM suppress decomposition or contribute to MAOC formation (Frey 2019). AM hyphal chemical composition can influence the probability of hyphal C remaining as POM or being consumed by soil microbes, and thus its likelihood of entering the MAOC pool (Cotrufo et al. 2013). In plants there is often a negative correlation between growth rates and the concentration of defense metabolites (Donaldson, Kruger, and Lindroth 2006). Thus, faster growing *Glomeraceae* may have a lower melanin concentration than *Gigasporaceae* (Chagnon *et al.*, 2013). Melanin is not universally produced across AM fungal species, but biomass N often demonstrates a similar relationship to growth rates, influencing decomposition potential. Faster growing *Glomeraceae* would be expected to have higher hyphal N content relative to slower growing *Gigasporaceae.* As such, *Gigasporaceae* necromass may be more resistant to microbial decay, but simultaneously less likely to end up in the slower cycling MAOC pool. At the same time, *Gigasporaceae's* larger investment in extraradical hyphae may suggest that they could

stimulate saprotrophic metabolic activity over a larger volume of soil. If this is the case, it is possible that *Gigasporaceae* could facilitate faster SOC mineralization compared to *Glomeraceae*.

In nature, these AM fungal families can coexist in the same environments and colonize some of the same plant hosts. Although they may compete for similar nutrients, they could coexist by occupying different niches. Maherali and Klironomos (2007) found that, species competition was relatively smaller in taxonomically diverse AM fungal communities and both Maherali and Klironomos (2007) and Crossay *et al.* (2019) found that AM taxonomic diversity improved plant nutrition. If this is the case, one would expect diversity-productivity effects in terms of total AM fungal biomass.

Although some research has been done on AM fungal species C sink strength, little is known about how AM community functional traits and trait diversity differentially affect SOC cycling (Lerat et al. 2003). Greater investment in extraradical hyphae, associated with better P acquisition capacity, may be a key trait for SOC formation if it leads to increased C deposition across a larger volume of soil. In which case, a *Gigasporaceae* community would be expected to contribute more to SOC than a *Glomeraceae* community (Hart and Reader 2002). Yet, a community dominated by traits associated with slow growing hyphae may also limit the rate of new fungal C inputs due to slower colonization rates and hyphae turnover. Therefore, it may be that communities with greater trait diversity will contribute the most to total SOC formation. For instance, co-inoculation of *Gigasporaceae* and *Glomeraceae* has been seen to increase plant growth and P compared to single inoculants, explained by the presence of a diversity of nutrient acquisition strategies (Crossay et al. 2019). As plants provide AM fungi C proportional to the P they provide in return, a mixed AM community may attain more plant derived C. Increased AM

fungal diversity may also lead to increased hyphal growth, where a mixed-trait community optimizes SOC formation through both rapid AM fungal input rates (*Glomeraceae*) and high biomass quantities (*Gigasporaceae*). Further, as the *Glomeraceae* family is faster growing than the *Gigasporaceae* family, its necromass C may be more likely to enter the MAOC pool due to more microbially accessible compounds and thus faster decomposition. However, an AM fungal community with greater trait diversity would, by default, consist of AM fungal species with different SOC input and cycling rates, that could result in a more constant C supply. Over time, this could lead to more diverse microbial communities, leading to greater variation in SOC chemistries and more total SOC accumulation (Kallenbach, Frey, and Grandy 2016).

1.3 Arbuscular Mycorrhiza Fungi and Plant Nutrition

EcM are dominant in ecosystems with high soil C to N ratios, low pH, and rich organic matter, whereas AM fungi dominate in P-limited environments, with fast N cycling, high pH, and lower C to N ratios (Hobbie and Högberg 2012; Read 1991; Tedersoo and Bahram 2019). Unlike some EcM, AM fungi lack the capacity to degrade lignin, cellulose, and proteins. (Frey 2019; Hobbie and Högberg 2012; Tang et al. 2016; Tisserant et al. 2013). As such, AM fungi cannot directly decompose organic material and are less efficient at providing their hosts with N and P (Tedersoo and Bahram 2019). AM fungi are still able to absorb nutrients from organic and inorganic sources and they can prime the soil by exudating photosynthetic C to stimulate saprotrophic microbial activity in areas with inaccessible nutrients (Frey 2019; Hodge and Storer 2015; Khanday et al. 2016; Kuzyakov 2002; Zak et al. 2019).

AM fungi absorb soil nutrients through extraradical hyphae, which can take two dominant forms: transport and absorptive hyphae (Friese and Allen 1991). Absorptive hyphae have thin cell walls and an average turnover time of only 5-7 days, and are responsible for

absorbing nutrients from the soil (Neumann and Eckhard 2010). They are usually smaller than 4 µm in diameter and, as such, are able to enter soil pores that are too fine for roots, enabling the hyphae to increase nutrient accessibility (Neumann and Eckhard 2010; Smith and Read 2008). Absorptive hyphae can form highly branched absorbing structures (BAS), that resemble intracellular arbuscules, that may exploit nutrient-rich areas in heterogenous soil (Bago et al. 1998; Neumann and Eckhard 2010; Souza et al. 2005). Absorptive hyphae grow out of thick transport hyphae, also known as runner-hyphae, which carry nutrients and photosynthetic C. Runner-hyphae have thicker cell walls and wider diameters, varying between 5-20 µm on average (Neumann and Eckhard 2010; S. E. Smith and Smith 2011). They can grow up to 25 cm away from their host's roots at a rate of 1-2 mm a day. These hyphae can grow outside the nutrient-depleted zones surrounding roots and in doing so may be able to expand the area of active microbial activity from the rhizosphere into the larger hyphrosphere (Gupta, Aggarwal, and Asha 2018; Neumann and Eckhard 2010; See et al. 2022). This extension enables plants to access nutrients from areas otherwise beyond their reach.

Though AM fungi are capable of increasing plant access to nutrients, the mechanisms involved, and the effects of increased plant nutrition are complex and rarely consistent. Such variability in the AM symbiosis often depends on the plant and fungal species, and their physical environment (Smith and Read 2008). AM plants can take up nutrients in two ways 1) through their own roots and 2) through the AM hyphae (Fig. 1.4) (Smith and Smith 2011; Smith and Read 2008). AM colonization has been shown to increase plant phosphorous (P), nitrogen (N), iron (Fe), calcium (Ca), copper (Cu), potassium (K), magnesium (Mg), manganese (Mn), sulfur (S), and zinc (Zn) concentrations, but only the direct uptake of P, N, Fe, S, and Zn through AM hyphae has been confirmed (Neumann and Eckhard 2010). The increase seen in Ca, Cu, K, Mg,

and Mn could be due to direct hyphal uptake that has yet to be observed or through indirect chemical mobilization of the nutrients by AM fungi either by reducing soil pH or by stimulating the microbial community with exudates (Frey 2019; Hodge and Storer 2015; Neumann and Eckhard 2010; Smith and Read 2008).

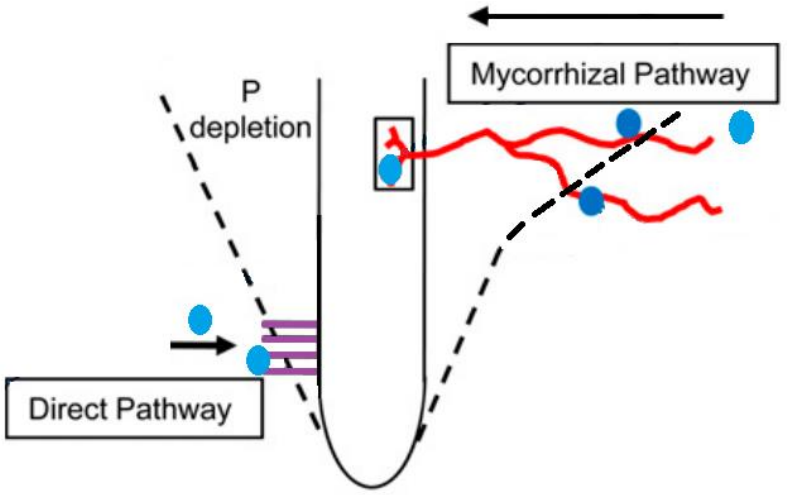

Figure 1.4 Plant nutrient uptake via the direct root hair absorption versus through AM fungal hyphae, modified from Smith *et al.* (2011). The dotted line indicates the zone of phosphorus (P) depletion around the root, root hairs are drawn in purple, AM fungal hyphae are drawn in red, and P molecules are depicted in blue.

1.3.1 The Contribution of Arbuscular Mycorrhizal Fungi to Plant Nutrient Uptake

One of the most difficult nutrients for plants to obtain is P. Phosphorus is key for plant growth and nutrition but is poorly mobile in soils, as it is rapidly fixed by soil minerals and cations or into microbial biomass (Schachtman, Reid, and Ayling 1998; Smith and Read 2008). Roots can form zones of P depletion around them that can make it difficult for direct P uptake, but AM hyphae are capable of growing outside the P depleted zone, increasing their host's access to P (Fig 1.4) (S. E. Smith et al. 2011). For this reason, P uptake may be one of the

greatest drivers of the AM fungal symbiosis, with AM fungal colonization increasing plant growth twice as much under low P conditions than low N (Hoeksema et al. 2010). As such, the effect of AM fungal colonization can vary depending on soil P availability. Oliveer *et al.* (1983) found that AM fungal colonization increased subterranean clover *(Trifolium subterraneum)* growth and P concentration under conditions of low P availability by 45 and 152%, respectively. However, the benefit of AM fungal colonization decreased with increasing P availability until ~ 0.4 mmol P $kg^{-,}$ above which AM fungi began to decrease plant growth and nutrition. This same trend can be seen in AM fungal colonization, where P availability is negatively correlated with the percentage of roots colonized by AM fungi (Smith et al. 2011). Even with this variability in mind, AM fungal associations are thought to be the dominant source of P for AM plants, over direct root uptake (Smith et al. 2011). Smith, Smith and Jakobsen (2004) found that AM colonization could be a plant's exclusive means of P acquisition, contributing 100% of P uptake but without increasing plant growth or P content compared to an uncolonized plant. This would indicate that, regardless of plant response, AM colonization can contribute to P uptake more than roots (S. Smith and Read 2008).

It was previously thought that AM fungi did not provide N to their hosts, as most AM dominant ecosystems have relatively high N availability (Read and Perez-Moreno 2003). Yet, AM hyphae are now known to take up both organic and inorganic N from soil and transfer it to their host (Hodge and Storer 2015). Plants require around ten times as much N as they do P, so mycorrhizal associations may be able to provide AM plants with a competitive advantage over non-mycorrhizal plant species (Hodge and Storer 2015; S. Smith and Read 2008). Research with ^{15}N isotopic labeling has shown that AM hyphae can take up both ammonium (NH_4^+) and nitrate (NO_3^-) (Govindarajulu et al. 2005; Hawkins, Johansen, and George 2000; S. Smith and Read

2008; Tedersoo and Bahram 2019). Ammonium requires less energy for anabolism and so is the preferred form of N for AM fungi (Hodge and Storer 2015; Nygren et al. 2012). Yet, NO_3^- is the dominant form of N acquired by AM fungi, most likely due to its higher availability in soils (Hodge and Storer 2015). AM associations can provide plants with a substantial amount of their total biomass N, and have been seen to contribute up to 42% of their host plant's N uptake (Mäder et al. 2000; Wang et al. 2020; Xie et al. 2022). AM fungi can also transfer N through common mycelial networks (CMN), an interconnection of plants through AM mycelium of several AM fungal species (Hodge and Storer 2015). AM fungal N transfer from N_2-fixing to non-N_2-fixing AM plants has been seen through CMN, with one-way N contributions ranging from 0.1 to 80% (He et al. 2009). Although AM fungi are now known to take up and transfer N, the contributions of AM fungi to plant N are still variable and poorly understood. Further work is required to understand how different plant and AM fungal species differ in their N uptake capacity and how the soil environment can affect this relationship (Hodge and Storer 2015).

Although P and N appear to be the major drivers of AM fungal associations, how much the symbiosis contributes to the uptake of other nutrients can still be considerable and should not be overlooked (Neumann and Eckhard 2010). There is no direct evidence of hyphal uptake of Ca, Cu, K, Mg, and Mn; yet AM fungal colonization has been seen to alter plant uptake of these nutrients (Neumann and Eckhard 2010). For instance, Li, Marschner and George (1991) found that AM fungal colonization contributed up to 62% of total Cu in white clover *(Trifolium repens)*, irrespective of soil P levels. Likewise, a meta-analysis conducted by Lehmann and Rillig (2015) found that AM fungal colonization had a substantial positive effect on plant Cu concentrations ranging from 26 to 41%. However, AM fungal colonization does not always have a positive effect on plant nutrition, and has usually been found to decrease plant K and Mn

concentrations (Lehmann and Rillig 2015; S. Smith and Read 2008). This could be due to competition between the fungi and plant or rapid plant growth stimulated by AM fungi that could cause other nutrients to become limiting (Neumann and Eckhard 2010; Smith and Read 2008). These results show that AM fungal colonization does affect plant nutrient availability but that this effect differs greatly depending on the nutrients.

AM fungal research has progressed exponentially over the last few decades, revealing how vital AM fungal colonization is to plant nutrient uptake, often being the default method of nutrient acquisition in most plants. Yet, it is still unclear how hyphae absorb some nutrients and what factors drive the variation in plant nutrition seen between different plant and fungal partners. Further work examining AM fungal species as individual organisms with their own evolutionary pressures and functional diversity is necessary (Hodge and Storer 2015).

1.3.2 Arbuscular Mycorrhizal Fungal Trait Effects on Plant Nutrient Concentration

The functional reasons driving the range of ecological responses to AM fungal symbiosis are poorly understood as outcomes can vary depending on soil nutrient availability, the physical environment, and both the plant and fungal species (Neumann and Eckhard 2010; Smith and Read 2008). Understanding what AM physiological factors are responsible for enhanced plant nutrition could improve our understanding of plant health and plant community assembly. The AM families *Gigasporaceae* and *Glomeraceae* have differing physiology and life-history strategies that could alter their nutrient uptake capabilities and host nutrition.

Gigasporaceae prioritize acquiring growth limiting C by exchanging P with their plant hosts, and as such have often been seen to increase plant P concentrations more than *Glomeraceae* species (Gosling, Jones, and Bending 2016; Johnson et al. 2003; Kiers et al. 2011; Maherali and Klironomos 2007). *Gigasporaceae*'s P acquisition strategy may be driven by their

relatively higher investment in extraradical hyphae (Chagnon et al. 2013; Maherali and Klironomos 2007). *Gigasporaceae*'s longer lasting extraradical hyphae may indicate more transport hyphae that enable greater capacity to exploit heterogeneous nutrient availability at longer distances (Fig 1.3 and 1.4). However, if P and other nutrient depleted zones have yet to form around roots, greater investment in distance-covering transport hyphae may not be advantageous. Further, greater investment in extraradical hyphae does not necessarily mean a species will be better at acquiring nutrients, they may instead be searching for other host roots (Smith and Read 2008).

In contrast to *Gigasporaceae*, the *Glomeraceae* family's fast turnover times may make it better at acquiring nutrients near the roots. Their faster extraradical hyphae turnover times may point to greater investment in faster cycling absorptive hyphae, which may enable them to absorb available nutrients in a shorter time period (Bago et al. 1998; Neumann and Eckhard 2010). Additionally, *Glomeraceae* species are able to colonize roots more rapidly than *Gigasporaceae* species and invest more of their biomass in intraradical hyphae, increasing their total root colonization, which is associated with increased AM nutrient contribution (Bruce, Smith, and Tester 1994; Chagnon et al. 2013; Hart and Reader 2002). Thus, it would be reasonable to predict that *Glomeraceae's* faster turnover, higher colonization rates, and proximity to roots may give their hosts a greater advantage in nutrient acquisition.

A mixed AM fungal community of *Gigasporaceae* and *Glomeraceae* species may have the greatest capacity to improve its host nutrition, as it is more likely to be able to exploit nutrients from different niches and through different acquisition mechanisms (Cornwallis et al. 2021; Maherali and Klironomos 2007). In support of this, Maherali and Klironomos (2007) and Crossay *et al.* (2019) both found that plants grown with AM species from both families grew

larger than those grown with only one family. If *Gigasporaceae* and *Glomeraceae* families use different nutrient acquisition strategies, then a community that is capable of efficiently accessing nutrients through both strategies may be ideal in a heterogeneous environment.

Plants with certain strategies may benefit most from associating with AM fungal communities with some functional traits more than others, as different AM life-history traits may provide their hosts with different primary benefits. Yet, little is known about how or even if AM fungal life-history traits and trait diversity influence their nutrient uptake capabilities (Neumann and Eckhard 2010). AM fungal traits conserved at the family level are likely selected for under specific environmental conditions or plant communities that favor one family over another. For instance, as *Glomeraceae* species may be best at acquiring nutrients quickly after establishment they could dominate in annual plant communities, while *Gigasporaceae,* given their slower colonization rate and longevity, may dominate in perennial plant communities. If certain plant and AM fungal strategies are best suited to improve each other's fitness, understanding how both partner's identities drive plant-soil interactions could have implications on agricultural practices, plant-soil community ecology, and ecosystem function (Neumann and Eckhard 2010).

1.4 Importance of Trait Variation across Arbuscular Mycorrhizal Families

The AM families *Gigasporaceae* and *Glomeraceae* utilize different life-history strategies that influence their growth, reproduction, and most likely biomass chemistry. The effects of trait variation across AM families and trait diversity have not been well studied, and few experiments have looked at variation between AM families at the community level. These differences in functional traits can influence SOC accumulation, retention, destabilization, and plant nutrient uptake.

A better understanding of how distinct AM fungal communities and their associated traits impact C and plant nutrient uptake is valuable for agriculture and conservation as it could enable farmers to manage AM fungi for improved plant nutrition and may explain plant community composition in different ecosystems (Chagnon, Bradley, and Klironomos 2015). Moreover, a better understanding of the variable effects of AM fungi on C cycling could improve climate change models and allow us to better predict which AM community-host interactions are more or less likely to facilitate SOC accumulation.

For my thesis research, I conducted a greenhouse experiment where I grew Sudan Grass (*Sorghum sudanense*) with either a community of five competitor *Gigasporaceae* species, five ruderal *Glomeraceae* species, or a combination of all ten species for 89 days. I assessed the effect of AM fungal community composition on SOC dynamics, plant shoot nutrition, and plant biomass. This experiment aimed to address the following gaps in the literature:

1. Do AM fungal communities that differ in their life-history traits or trait diversity affect SOC accumulation?
2. Do AM fungal communities that differ in their life-history traits or trait diversity impact the amount of SOC stabilized in the MAOC pool?
3. How is plant nutrition affected by AM fungal communities representing distinct life-history traits and trait diversity?
4. How are plant biomass and growth affected by AM fungal communities representing distinct life-history traits and trait diversity?

Chapter 2:

The Role of Arbuscular Mycorrhizal Trait-Based Communities in Soil Carbon Cycling

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2.1 Abstract

Soil is the largest terrestrial carbon (C) pool, containing nearly three times more C than the atmosphere. Thus, understanding what factors encourage long-term soil organic carbon (SOC) storage is important for climate change mitigation. Fungi can be a considerable input to SOC and a large portion of fungal biomass accumulates in the more persistent mineral associated organic carbon (MAOC) pool. Yet, little is known about the contributions of arbuscular mycorrhizal (AM) fungi, in particular the role of AM community traits and trait diversity, to SOC accumulation. To determine if AM fungal communities representing different traits and trait diversity impact hyphal contributions to SOC and MAOC following decomposition, I grew Sudan grass (*Sorghum sudanense*) in a greenhouse with communities composed of: 1) five species in the AM family *Gigasporaceae* representing Grime's C-S-R framework's competitor life-history strategy due to its proportionally high investment in extraradical hyphae and slow growth rates, 2) five species in the fast-growing *Glomeraceae* family representing the ruderal strategy, or 3) a mixed community combining all ten AM species representing functional traits diversity. Sudan grass was pulse labeled with ^{13}C-CO_2 weekly for 12.5 weeks to assess new AM fungal biomass, then root-free hyphal compartment soils were incubated for a month. I quantified AM hyphal contributions to total SOC and MAOC in both pre and post incubation soils. Before the incubation, *Glomeraceae* and *Gigasporaceae* communities decreased total pre-incubation SOC by 15% and 14% compared to the planted non-mycorrhizal control, respectively ($p < 0.05$). Net SOC losses were likely caused by AM fungal-mediated SOC priming. Despite these net SOC losses, hyphal C from all three AM fungal communities contributed an average 2.5 mg of fungal C to new SOC formation. Notably, before incubation only the *Glomeraceae* community contributed to MAOC (0.12% of total MAOC), but after one month of incubation,

both the *Glomeraceae* and the mixed-trait communities ($p = 0.51$) contributed inputs to MAOC, potentially due to divergent hyphal chemistry influencing decomposition rates. Given that AM fungal family abundances are subject to environmental selection pressures (e.g., disturbance, plant communities, nutrient availability), this study helps inform how selection for community trait dominance or trait diversity at the family-level may impact SOC accumulation and loss.

2.2 Introduction

Soil contains three times more carbon (C) than the atmosphere. As such, even a relatively small change in the soil C flux has the potential to exacerbate or mitigate climate change (Lehmann and Kleber 2015; Paustian et al. 2016). Microbial community composition and function can influence soil C cycles, shaping soil C accumulation and residence-time (Domeignoz-Horta et al. 2021). Arbuscular mycorrhizal (AM) fungi are a known source of soil organic carbon (SOC), exchanging soil resources for plant photosynthetic C (Frey 2019; Ricklefs 2010). AM fungi can alter SOC concentrations by stimulating saprotrophic community decomposition, and by protecting SOC from mineralization through aggregate formation (Frey 2019; Waring et al. 2020; Yakov Kuzyakov 2002). Direct C inputs from AM fungal spores, hyphal exudates, and extraradical dead hyphae (i.e., necromass) can directly enter the soil and may also be effective at driving SOC accumulation (Frey 2019). AM fungal traits that affect biomass turnover and allocation to extraradical hyphae could thereby impact new SOC formation. For AM fungi, these traits vary primarily at the family level; thus certain AM fungal traits or community assemblages may encourage SOC accumulation more than others (Hart and Reader 2002; Powell et al. 2009).

Several microbial trait-based frameworks suggest that functional and life-history traits can be effective predictors of SOC accumulation and SOC response to disturbances (Cotrufo et

al. 2013; Hicks et al. 2022; Malik et al. 2020; Wallenstein and Hall 2012). Chagnon *et al.* (2013), explicitly considered non-saprotrophic AM fungi and proposed that two to of the largest AM families, *Gigasporaceae* and *Glomeraceae,* use distinct life-history strategies described in Grime's C-S-R framework (Grime 1977). Species in the *Gigasporaceae* family act as competitors as they invest heavily in growth-limiting resource acquisition by allocating proportionally more of their biomass to extraradical hyphae at the cost of delayed sporulation, and often exhibit slower growth rates (Chagnon et al. 2013; Hart and Reader 2002, 2005; Maherali and Klironomos 2007a; Staddon et al. 2003). Conversely, species in the *Glomeraceae* family function as ruderals, prioritizing fast growth rates and frequent sporulation, leading to higher hyphal turnover and decreased investment in extraradical hyphae.

These different strategies may impact how communities dominated by different AM fungal families contribute to SOC accumulation. For instance, AM fungi receive C from their hosts in accordance with the amount of phosphorus (P) they provide (Kafle et al. 2019; Kiers et al. 2011). *Gigasporaceae* species are thought to be better P scavengers than *Glomeraceae*, due to more extensive extraradical hyphae. In turn, this may lead to greater C acquisition compared to *Glomeraceae* species (Johnson *et al.*, 2003; Maherali and Klironomos, 2007). Alternatively, *Glomeraceae* species' faster growth and turnover rates may promote more rapid nutrient absorption and thus faster photosynthetic C deposition.

In addition to effects on C deposition, AM life-history traits may also determine the proportion of fungal C that accumulates in the slower cycling mineral-associated organic C (MAOC), an important pool for longer-term C storage (Balesdent and Mariotti 1996; Torn et al. 1997). MAOC typically has a high abundance of compounds originating from microbial inputs or highly decomposed plant and fungal tissues (Angst et al. 2021; Gleixner et al. 2002; Grandy

and Neff 2008). The close interaction and sorption of these microbial and decomposed plant compounds with mineral surfaces, helps protect them from decay (Oades 1988). See et al., (2021) suggested that variations in hyphal growth rate, length, and chemistry across different fungal groups (ectomycorrhizae, arbuscular, and saprotorophs) impact the formation of MAOC in different ways.

AM fungal inputs to MAOC may result from the direct contact between hyphae and mineral surfaces, or after decomposition to low molecular weight compounds, increasing their mineral sorption potential (See et al, 2022). AM fungal chemical composition, which can vary across species, likely affects fungal C decomposition and thus its transition to MAOC (Fernandez and Kennedy 2018; Frey 2019; Waring et al. 2020). For plants, ectomycorrhizae, and some bacteria, variation in chemical composition can be associated with life-history traits such as growth rate (Donaldson, Kruger and Lindroth, 2006; Franklin *et al.*, 2011; Fernandez and Koide, 2014; Siletti, Zeiner and Bhatnagar, 2017; Manzella, Geiss and Hall, 2019). For example, phenolics and N concentrations are often respectively negatively and positively correlated with growth rate, (Franklin et al. 2011; Siletti, Zeiner, and Bhatnagar 2017). While a community dominated by extensive, yet slow growing, extraradical hyphae (e.g., *Gigasporaceae*) may obtain more C inputs, MAOC accumulation could be limited by a relatively slow microbial decay of hyphal necromass. Conversely, fungal C from a community exhibiting traits for less extensive, yet fast-growing, and short-lived extraradical hyphae (e.g., *Glomeraceae*) may be relatively more microbially processed and result in a higher proportion of C entering MAOC, but accumulation would also be limited by lower initial C inputs.

The connections between distinct AM fungal family-level traits and contributions to SOC must also consider that no one set of traits may be optimal under varying temporal scales and soil

heterogeneity. AM fungal communities with greater trait diversity may deposit C at different times and with different chemistries, potentially causing diverse communities to have a larger impact on fungal SOC accumulation. A community with a diversity of traits may have different "waves" of MAOC formation as AM fungal C from different life-history strategies is produced and decomposed at different times. While *Glomeraceae* could initially contribute relatively more fungal C to MAOC due to faster turnover rates, C from *Gigasporaceae* should also accumulate in MAOC, just at a slower rate. In addition, total photosynthetic C deposition may also increase with increasing trait diversity. For instance, AM fungal trait diversity, contributes to greater plant growth and P concentrations, thereby potentially increasing AM fungal C acquisition and deposition (Crossay et al. 2019; Maherali and Klironomos 2007a). As such, AM fungal trait diversity, not individual traits themselves, may be the best predictor of SOC accumulation and retention.

Despite their widespread occurrence, the impact of AM fungal community traits and diversity on SOC formation and potential for accumulating as MAOC has not been well documented. This study addresses this knowledge gap by examining if and how AM fungal community traits and trait diversity impact AM fungal inputs to SOC and MAOC. I predicted that 1) AM colonization would increase total SOC, especially in the MAOC pool; 2) *Gigasporaceae* species would contribute more to new total SOC than *Glomeraceae* due more extensive extraradical hyphae, 3) that *Glomeraceae* species would contribute relatively more to MAOC due to faster hyphal decomposition; and 4) a diverse mixed-trait community, composed of species across the *Gigasporaceae* and *Glomeraceae* families, may contribute most to both SOC and MAOC formation, due to greater total hyphal biomass and variation in hyphal decomposition rates.

2.3 Materials and Methods

2.3.1 Experimental design

A greenhouse experiment was conducted with Sudan grass grown with four completely randomized AM fungal treatments: 1) five *Gigasporaceae* species (Giga); 2) five *Glomeraceae* species (Glom); 3) a mixture of all ten mycorrhizal species (Mixed); or 4) sterilized inoculum (Control). AM treatments were replicated nine times, for a total of 36 pots. Sudan grass in all treatments were exposed to ^{13}C-CO_2 to isotopically label AM fungal C inputs into the soil.

Sudan grass was chosen as the host plant because the species is highly mycotrophic, establishing associations with all AM species kept in culture collections (Morton, Bentivenga, and Wheeler 1993). Experimental pots had distinct rhizosphere and hyphal compartments in order to separate hyphal C from root C. To achieve this, a 30 μm mesh bag, was placed in the center of each pot to prevent roots but not hyphae from passing through to the surrounding soil (Fig. 2.1).

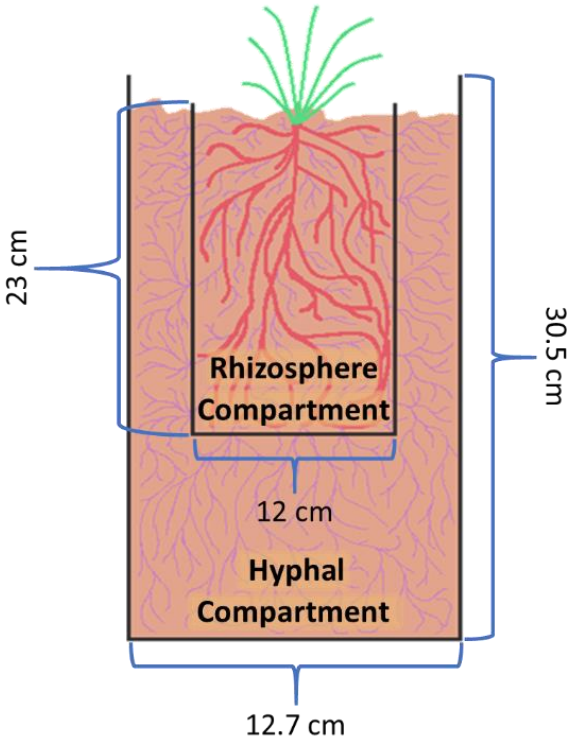


Figure 2.1 Pot layout with rhizosphere and hyphal compartments. Tree-pots were 12.7 cm wide, 12.7 cm long, and 30.5 cm tall, and held 5 L of soil. The hyphal compartment was made of a 24 x 23 cm piece of mesh folded in half and glued together, resulting in an approximately 23 tall x 12 cm wide bag. The top edge of the mesh bag was placed approximately 1 cm above the soil surface. Sudan grass roots could not grow through the mesh bag which created a root-free hyphal compartment.

2.3.2 Arbuscular Mycorrhizal Fungal Inoculum

All AM fungal isolates, each representing one species, were obtained from the International Cultural Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM; West Virginia). *Gigasporaceae* isolates were chosen for their relatively low fecundity and large spore size, while *Glomeraceae* isolates were chosen for their high fecundity and relatively small spores (Table 2.1) (Species Descriptions | Davis - INVAM | West Virginia University n.d.).

To increase the total amount of AM fungal inoculum, isolates were bulked up in pure cultures with Sudan grass for at least 4 months before spore containing soil was collected, dried, and stored at 4 °C. The resulting 10 isolate inocula were then used to prepare the two inoculum blends representing the *Gigasporaceae* and *Glomeraceae* families. Each mixture was made by combining 460 g from each of their five respective isolates, plus an additional 200 g of fresh INVAM inoculum from the same isolates. The mixed treatment inoculum, comprised of 10 isolates, contained equal parts of the *Gigasporaceae* and *Glomeraceae* mixtures. The control treatment's inoculum was prepared by autoclaving the mixed treatment inoculum.

Table 2.1 Arbuscular mycorrhizal fungal isolates used in the greenhouse experiment and organized by family.

Family	Isolates
Gigasporaceae	*Cetraspora pellucida*
	Dentiscutata heterogama
	Gigaspora margarita
	Racocetra fulgida
	Scutellospora calospora
Glomeraceae	*Claroideoglomus etunicatum*
	Funneliformis mosseae
	Rhizophagus clarus
	Rhizophagus intraradices
	Septoglomus deserticola

2.3.3 Planting

Sudan grass seeds were surface sterilized with a 50% ethanol solution for one minute and germinated in seedling trays containing an autoclaved 1:1 soil-sand substrate. Three seedlings were transplanted to 5 L tree-pots containing an autoclaved 1:1 v/v sand-soil mixture. Soil was collected from the McGill Morgan Arboretum in Canada (45° 25' 45.4" N; 73° 56' 32.5" W), mixed with all-purpose sand. Soil is a clay loam Gleysol in the Sainte Rosalie series, with a pH

of 6.31, a mean soil C content of 1.36%, and a mean ^{13}C isotopic signature of -26.4 $\delta^{13}C$. Soil was sieved to 2 mm. During transplant, pots were inoculated with 97.2 g ± 0.2 g of their respective dry AM inoculum prepared as described above. In addition, I included three pots without seedlings to use as controls for $\delta^{13}C$ in the absence of plant photosynthesis.

After six days, seedlings were thinned to one plant per pot. The Sudan grass was grown in the greenhouse for 89 days. Greenhouse temperatures were kept at 16 °C for 16 hours during the day and 19 °C at night. Plants received natural sunlight with an average photoperiod of 11 hours and 20 minutes.

2.3.4. Microbial Inoculum

A microbial community inoculum, free of any AM fungi, was added to each AM fungal treatment to establish a saprotrophic microbial community to facilitate AM fungal hyphae decomposition, as AM fungi are generally not considered saprotrophic. This microbial inoculum also allowed me to standardize for any potential variability between the different families' AM fungal inoculum's non-mycorrhizal microbial communities. The microbial inoculum was made by mixing 600 g of the sieved field soil and 150 g of each of the *Gigasporaceae and Glomeraceae* inoculum in 2 L of deionized water and passing the resulting slurry sequentially through a 500 µm, 38 µm, and 20 µm sieve twice. The 20 µm sieve does not allow AM fungal spores or hyphae to pass through but is large enough for bacteria, viruses, and some saprotrophic fungal spores. Each pot received 20 mL of the resulting slurry, which was added at the base of the plant, 14 days after transplant.

2.3.5. ^{13}C Isotope Labeling

To determine the amount of new AM fungal C in each treatment, I exposed Sudan grass to ^{13}C labeled CO_2 using a pulse label method similar to Bromand, et al. (2001). Pots were sealed

in an air-tight chamber and exposed to ~33 atom percent ^{13}C-CO_2 for a 4–6-hour period weekly for twelve weeks. A 4–6-hour labeling period was considered appropriate to provide AM fungi with a targeted ^{13}C enrichment of ~5 atom percent. The chamber (121 cm wide, 121 cm long, by 60 cm tall) was constructed from clear plexiglass, and contained two fans to circulate CO_2. Carbon dioxide was added by reacting sulfuric acid with 33 atom% ^{13}C sodium carbonate to produce ^{13}C-labeled CO_2. Chamber CO_2 concentrations were monitored with a LI-COR (LI-830, LI-COR, Lincoln, Nebraska, USA). For the first four weeks, CO_2 concentrations were kept between 370 – 430 ppm. As the Sudan grass grew and photosynthesis rates increased, target CO_2 concentrations in the chamber were expanded to 340 – 460 ppm for the remaining eight weeks. When photosynthesis caused CO_2 concentrations in the chamber to fall below the pre-established target concentrations (i.e., 340 or 370 ppm), ^{13}C-CO_2 was added to the chamber to raise CO_2 concentrations back to their target high concentrations (i.e., 430 or 460 ppm).

2.3.6 Sample Collection

I determined sampling time based on when spores were first detected in the hyphal compartment of all AM fungal treatments, indicating that the isolates had established and matured outside of the rhizosphere compartment. On November 18th, 2020, 84 days after transplanting, spores were detected in all three mycorrhizal treatments. Spores were extracted from one pot in each AM fungal treatment, prepared for destructive sampling, using a 60% sucrose solution and quantified under a microscope (M. Brundrett et al. 1994).

On November 23rd, 2020 above ground biomass was cut one centimeter above the soil and oven-dried at 60 °C to determine dry weight. Roots were rinsed, padded dry, weighed, and subdivided for quantification of mycorrhizal colonization and root dry weight. For analysis of mycorrhizal root colonization, part of each root system was placed in two tissue cassettes and

stored in 50% ethanol. The remaining roots were weighed again, and oven-dried at 60 °C to determine total root dry weight. Soil from the hyphal compartment was homogenized for thirty seconds and then subsampled for C analyses and for the subsequent one-month incubation described below. Soil samples for the C analyses were oven-dried at 105 °C.

2.3.7 Soil Incubation

To allow for hyphal decomposition, I incubated homogenized soil collected from each hyphal compartment, nine days after Sudan grass biomass harvest. For the incubation, 100 g of soil was placed in a 1-quart mason jar and covered with parafilm. Soils were incubated in the dark at 20 °C at 50% water holding capacity for 34 days. At the end of the incubation, all soils were oven-dried at 105 °C for C analyses.

2.3.8 Soil Carbon and ^{13}C Analyses

I determined the total soil mineral associated organic matter (MAOM) amount and MAOC concentrations from the oven-dried hyphal compartment soil 2-4 days after Sudan grass harvest and after one month of incubation. To isolate the MAOM fraction, I used the particle size fraction method (Bradford, Fierer, and Reynolds 2008). Briefly, 20 g of dry soil was added to 80 mL of a 0.008 M sodium hexametaphosphate solution and mixed on an end-to-end shaker for ~18 hours to disperse aggregates. The resulting slurry was washed through a 53 µm sieve to collect the clay and fine silt fraction, which were oven-dried at 105 °C and then weighed.

Incubated MAOM and pre-incubation MAOM and bulk soil from the hyphal compartments were homogenized with mortar and pestle and two replicate samples from each pot were analyzed for total C and ^{13}C at the Ján Veizer Stable Isotope Laboratory, University of Ottawa, Canada. Soil C and ^{13}C concentrations were determined on a flash combustion elemental analyzer (VarioEL Cube; Elementar ©, Langenselbold, Germany) paired with an isotope ratio

mass spectrometer (IRMS) (DeltaPlus Advantage; Thermo Electron Corporation ©, Massachusetts, USA) via a coupling interface (ConFlo III Universal Interface, Thermo Electron Corporation ©, Massachusetts, USA). The measurements from the two replicates from each pot were averaged and used for analysis.

Above ground Sudan grass biomass ^{13}C was analyzed at the Stable Isotope Facility for Ecosystem Research, Natural Resources Analytical Laboratory (SIFER/NRAL) at the University of Alberta using a flash-combustion organic elemental analyzer (Thermo FLASH HT Plus 2000; Thermo Fisher Scientific Inc., Milan, Italy), joined with an IRMS (Thermo Delta V Advantage; Thermo Scientific Inc. Bremen, Germany) via a coupling interface (ConFlo IV, Thermo Scientific Inc. Bremen, Germany).

2.3.9 Isotopic Calculations

An isotopic mixing model was used to determine the fraction of C in soil samples that originated from fungal hyphae (Eq. 2.1).

$$F\ fungal\ Derived\ C\ in\ soil = \frac{(\text{atom\% } soil\ fraction - \text{atom\% unplanted } control)}{(\text{atom\% } plant - \text{atom\% unplanted } control)}$$

Equation 2.1

Where F is the fraction of C from fungal hyphae in the soil fraction of interest (e.g., incubation MAOC and pre-incubated bulk soil and MAOC from the hyphal compartment); atom% soil fraction is the ^{13}C atom% for the soil fraction of interest; atom% unplanted control is the average ^{13}C atom% soil not seeded with Sudan grass; and atom% plant is the ^{13}C atom% of the sample's respective plant above ground tissue. For example, to determine F ^{13}C for the *Glomeraceae* treatment, I used the average ^{13}C atom% of the three unplanted controls that were inoculated with *Glomeraceae* but were not planted with Sudan grass for atom% control (Table

S7.1). This allowed me to account for any potential variation in host or soil autotrophic ^{13}C enrichment (e.g., cyanobacteria). Each C pool of interest was multiplied by its respective F ^{13}C value to determine the fraction of that pool that originated from the AM hyphae (Eq. 2.2).

$$\text{Pool AM C} = \text{F } ^{13}\text{C} * \text{Pool C}$$

Equation 2.2

Where Pool AM C is the amount of C in the pool of interest that originated from AM fungi, F ^{13}C is the fraction of ^{13}C from AM fungi in the soil fraction of interest, calculated in Equation 2.1, and Pool C is the amount of C found in the pool of interest.

Since there is potential for some ^{13}C contribution in the hyphae compartment from root exudates, I included a planted non-mycorrhizal control that allowed me to account for ^{13}C contributions not associated with AM fungal C. I then used this value to subtract out potential non-AM C (e.g., root exudates) from AM fungal treatments. An AM fungal community's contribution to any given C pool, was calculated as the average Pool AM C value of the AM community of interest minus the average Pool AM C value of the non-mycorrhizal planted control which would contain only non-AM C inputs (e.g., root exudates) (Eq. 2.3).

$$\text{AM C Community Contribution} = \text{AM Community Average} - \text{Control Average}$$

Equation 2.3

Where, AM C Community Contribution is the contribution of an AM fungal community of interest to a C pool of interest, AM Community Average is the average Pool AM C for the AM community of interest in the C pool of interest, and Control Average is the average Pool AM

C value for the planted uncolonized control in the respective C pool of interest. For example, to calculate the contribution of the *Glomeraceae* community to the MAOC pool, I took the average mixing model value for *Glomeraceae* in MAOC and subtracted it by the average mixing model value of the MAOC in the uncolonized planted control.

2.3.10 Root Colonization

Roots were stained using the ink-vinegar method by Vierheilig *et al.* (1998) and colonization was analyzed using the methods by McGonigle *et al.* (1990). Percent colonization was calculated as the number of intersections where AM fungal colonization was visible divided by the total number of intersections, multiplied by 100. Percent vesicles and percent arbuscules were calculated the same way.

2.3.11 Statistics

R version 4.1.0 was used to conduct one-way ANOVA's to determine AM fungal treatment effects on mycorrhizal colonization, soil C pools, and plant biomass before and after the incubation. Replicates were treated as a random effect in the ANOVAs. The sterilized, AM fungal-free control, planted with Sudan grass was used to compare the effect of AM fungal community. I used the Shapiro-Wilk test for normalcy and Levene's test for homogeneity of variance to assure the ANOVA assumptions were met. Rosner's Test was used to identify potential outliers, which I observed with histograms, box and whisker plots, and by plotting studentized residuals. If outliers were causing the data to not meet the assumptions of the model and there was either a methodological reason for a point to be considered an outlier or if a data point consistently appeared as an outlier, it was removed. I removed no more than one outlier per ANOVA. If the assumptions of the model were unable to be met through transformations, I used a non-parametric Kruskal-Wallis. Tukey tests were used to make pairwise comparisons if

ANOVA results were significant. Dunn's tests were used if Kruskal-Wallis tests were significant. Treatments were considered significant at $\alpha = 0.05$.

2.4 Results

2.4.1 Mycorrhizal Root Colonization

Colonization data showed that the experimental setup was successful. All AM treatments were colonized and there was no evidence of contamination in the control (Fig 2.2 and Table S7.2). The non-mycorrhizal treatment's colonization rates were near zero, where any minimal AM observations were attributed to human error. All AM communities' root colonization rates were significantly different from each other, with the mixed community being the most colonized, followed by *Glomeraceae*, and the *Gigasporaceae* community the least colonized. There was potential evidence of cross-contamination between AM communities, as the *Gigasporaceae* community contained some vesicles, contradicting its vesicle-free strategy observed in the literature (Souza et al. 2005). Yet, the mixed and *Glomeraceae* communities respectfully contained 2.1 and 1.8 times more vesicles than the *Gigasporaceae* community on average (Table S7.2). Thus, the treatments appeared to be properly colonized in accordance with the experimental design, except for some possible *Glomeraceae* species contamination in the *Gigasporaceae* community.

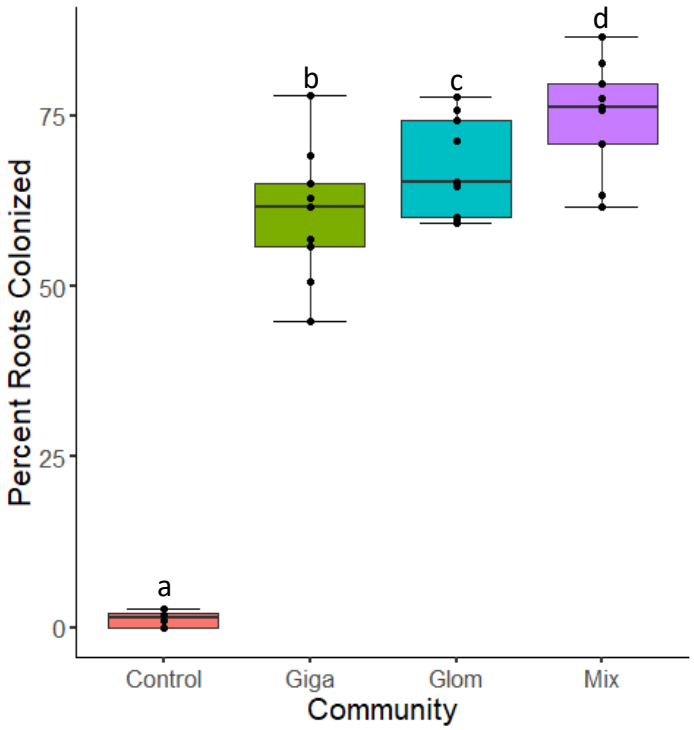


Figure 2.2 Percent of arbuscular mycorrhizal colonization observed on Sudan grass roots. Gig (*Gigasporaceae*); Glom (*Glomeraceae*); Mix (*Gigasporaceae* plus *Glomeraceae*i); Control (planted with sterile soil). Letters indicate pairwise differences among communities ($p < 0.05$). Df = 3, chi-squared = 23.031, n = 9.

2.4.2 Pre-Incubation Total SOC and MAOC

AM fungi significantly affected pre-incubation total SOC, with *Glomeraceae* and *Gigasporaceae* communities significantly lowering total SOC with an average decrease of 15% and 14% compared to the control, respectively (Fig. 2.3 A; Table S7.3). However, there was only weak evidence the mixed community decreased total SOC ($p = 0.057$) compared to the control (Fig. 2.3). Despite total SOC losses, AM fungi neither affected the total MAOC content in the soil nor the proportion of total MAOC within total SOC (Fig. 2.3 B; Table S7.3).

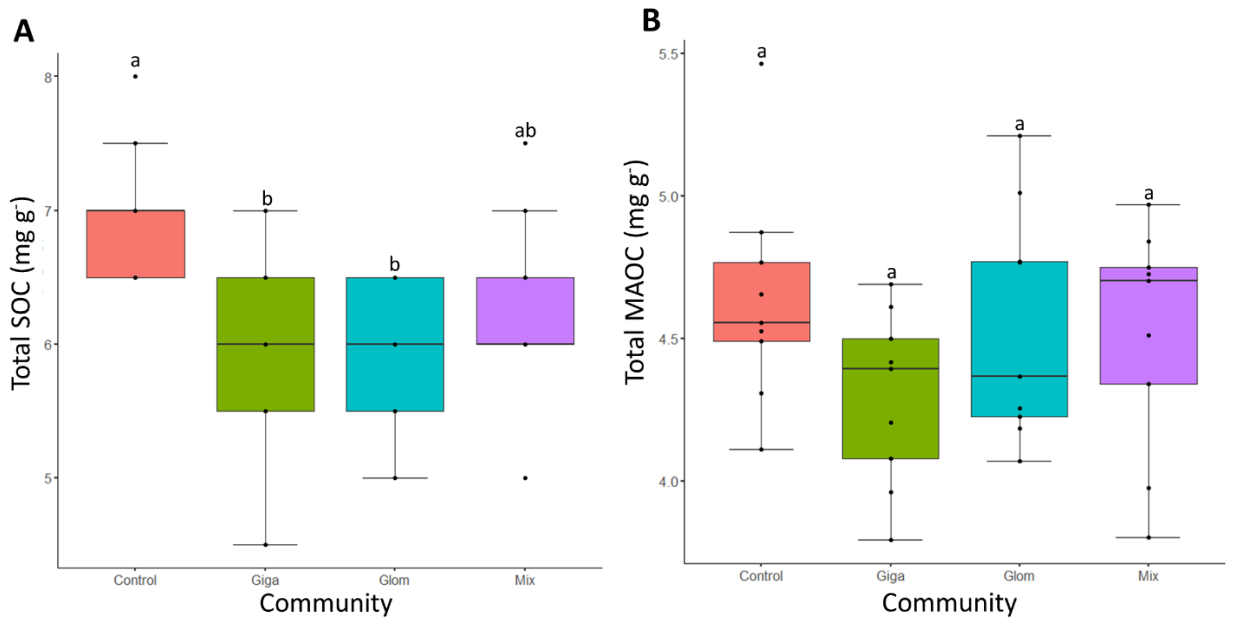


Figure 2.3 Effect of arbuscular mycorrhizal fungal treatments on pre-incubation soil carbon. Pre-incubation total soil organic carbon (SOC) in hyphal compartment soil by community (mg total SOC g^- of total soil) (A). Pre-incubation total mineral associated organic carbon (MAOC) in hyphal compartment soil by community (mg total MAOC g^- of total soil) (B). Gig (*Gigasporaceae*); Glom (*Glomeraceae*); Mix (*Gigasporaceae* plus *Glomeraceae*i); Control (planted with sterile soil). Letters indicate pairwise differences among communities ($p < 0.05$). Df = 3, F-value = 4.875, n = 9.

2.4.3 Pre-Incubation Arbuscular Mycorrhizal fungal SOC

Immediately following Sudan grass harvest, I detected strong evidence for ^{13}C enrichment in the hyphae only soil, across all AM fungal communities, compared to the planted non-mycorrhizal control soil. The mean ^{13}C enrichment was -21.0 δ ^{13}C with the AM fungal communities and -25.1 δ ^{13}C in the control. The control was slightly more enriched compared to the starting soil enrichment level (-25.8 δ ^{13}C). This was most likely due to inputs from ^{13}C enriched root exudates. While all communities had evidence of ^{13}C enrichment, their effect on

fungal SOC contributions to soil varied (Table S7.3), where only the *Glomeraceae* and mixed communities increased the amount of fungal SOC to soil compared to the planted non-mycorrhizal control.

Unlike with whole soil, all AM fungal communities significantly increased AM fungal SOC concentrations in total SOC, collectively contributing an average of 2.5 mg g^- of fungal C to SOC (0.25% of SOC) (Fig. 2.4; Table S7.3) ($p < 0.05$). There was no evidence for differences within the AM fungal communities' SOC contributions, but a trend emerged where *Glomeraceae* contributed the most fungal C to total SOC and *Gigasporaceae* the least, contributing 4.8 and 3.0 times more C respectively compared to the planted non-mycorrhizal control. As total SOC concentrations declined with AM colonization, detectable C inputs from AM fungi suggest this total SOC loss is associated with non-AM fungal C (Fig. 2.4).

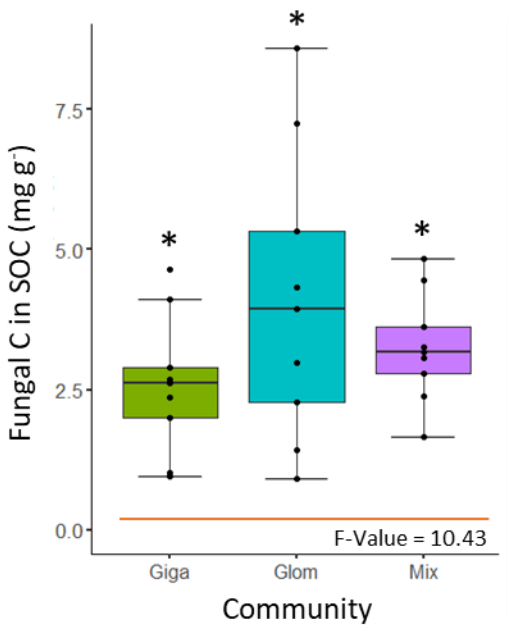


Figure 2.4 The pre-incubation soil organic carbon (SOC) concentrations derived from arbuscular mycorrhizal fungi by community (mg fungal C g^{-} of soil carbon). The red line is the planted non-mycorrhizal control's median value, representing likely background enrichment from root exudates. Gig (*Gigasporaceae*); Glom (*Glomeraceae*); Mix (*Gigasporaceae* plus *Glomeraceae*); asterisks indicate a significant difference between a community's carbon (C) and the planted non-mycorrhizal control ($p < 0.05$). Df = 3, n = 9.

2.4.4 Arbuscular Mycorrhizal fungal MAOC

I examined the amount of fungal C that accumulated as MAOC both following the plant harvest and after one month of incubation. Pre-incubation as with fungal accumulation in SOC, there was an overall significant increase in fungal MAOC in soils with colonization ($p < 0.05$). However, this effect was driven by the *Glomeraceae* community which was the only AM fungal community to significantly increase fungal MAOC in soil compared to the planted non-mycorrhizal control. The *Glomeraceae* community increased both pre and post incubation soil fungal MAOC by an average of 150% and 190% (Table S7.3). The amount of fungal MAOC in

soil did not significantly change following incubation, where fungal MAOC in the soil was similar between pre and post incubation soils and across AM communities.

I also compared fungal MAOC in total SOC to understand its relative contribution to observed changes in total SOC stocks. Similar to total soil, pre-incubation, only the *Glomeraceae* community significantly contributed to the proportion of fungal MAOC in SOC relative to the planted non-mycorrhizal control, by an average of 0.99 mg fungal MAOC g^- SOC. This corresponds to 36% of the *Glomeraceae* community's fungal SOC being composed of fungal MAOC (Fig. 2.5 A; Table S7.3). Although, pairwise comparisons between AM fungal communities were not significant, *Glomeraceae* contributed the most to fungal MAOC in SOC and *Gigasporaceae* the least (Fig 2.4 and 2.5 A).

After one month of incubation, the same trend in fungal MAOC contributions to total SOC was observed. The mean fungal community MAOC contribution was 2.9 times higher compared to the planted control after the incubation (Fig. 2.5 B; Table S7.3). *Glomeraceae* was still the only community that significantly differed from the planted control, with fungal MAOC in SOC 3.5 times higher. For the *Glomeraceae* community, approximately 0.1% (1.0 mg g^-) of total SOC was composed of fungal MAOC. There was some weak support that the incubation also increased the mixed-trait community's fungal MAOC contribution to total SOC ($p = 0.051$). Though, pre-incubation, the mixed community showed no evidence of fungal MAOC inputs to SOC, after one-month of incubation the mixed community MAOC was higher than the planted non-mycorrhizal control ($p = 0.051$). Fungal MAOC in SOC was 2.8 times higher in the mixed community on average compared to the planted non-mycorrhizal control and contributed approximately 0.75 mg g^- of fungal MAOC to SOC.

In addition to examining fungal MAOC in total SOC, I also compared the contributions of fungal MAOC to the total MAOC fraction, as total SOC was decreased by AM communities. While the fungal MAOC inputs to total MAOC show the same pattern as fungal MAOC inputs to total SOC, the relative contribution of fungal MAOC is higher within total MAOC compared to within total SOC. At pre-incubation, across all AM communities, the average amount of fungal MAOC in total MAOC was 0.86 mg g^{-}, whereas the average fungal MAOC in SOC was 0.71 mg g^{-}.

Across all AM communities, in both pre and post incubation soils, mean fungal MAOC in total MAOC was higher by 110% and 140% respectively compared to the planted non-mycorrhizal control (Figure 2.6 A and B; Table S3.2). *Glomeraceae* was primarily responsible for the AM fungal effect of increased fungal MAOC in total MAOC, as it was the only community significantly different from the planted control in both pre and post incubation soils. *Glomeraceae* contributed approximately 1.2 mg g^{-} before incubation and 1.0 mg g^{-} of fungal MAOC to total MAOC following incubation. Both pre- and post-incubation, though pairwise comparisons were not significant, the *Glomeraceae* community contributed the most to fungal MAOC in total MAOC and *Gigasporaceae* the least.

The incubation had no effect on the amount of fungal MAOC within total SOC or total MAOC (Fig. 2.5 C and 2.6 C; Table S3.2). Yet, the incubation formed opposing trends between the two comparisons, with fungal MAOC in total MAOC trending towards a decline after one month while fungal MAOC in total SOC trended towards an increase.

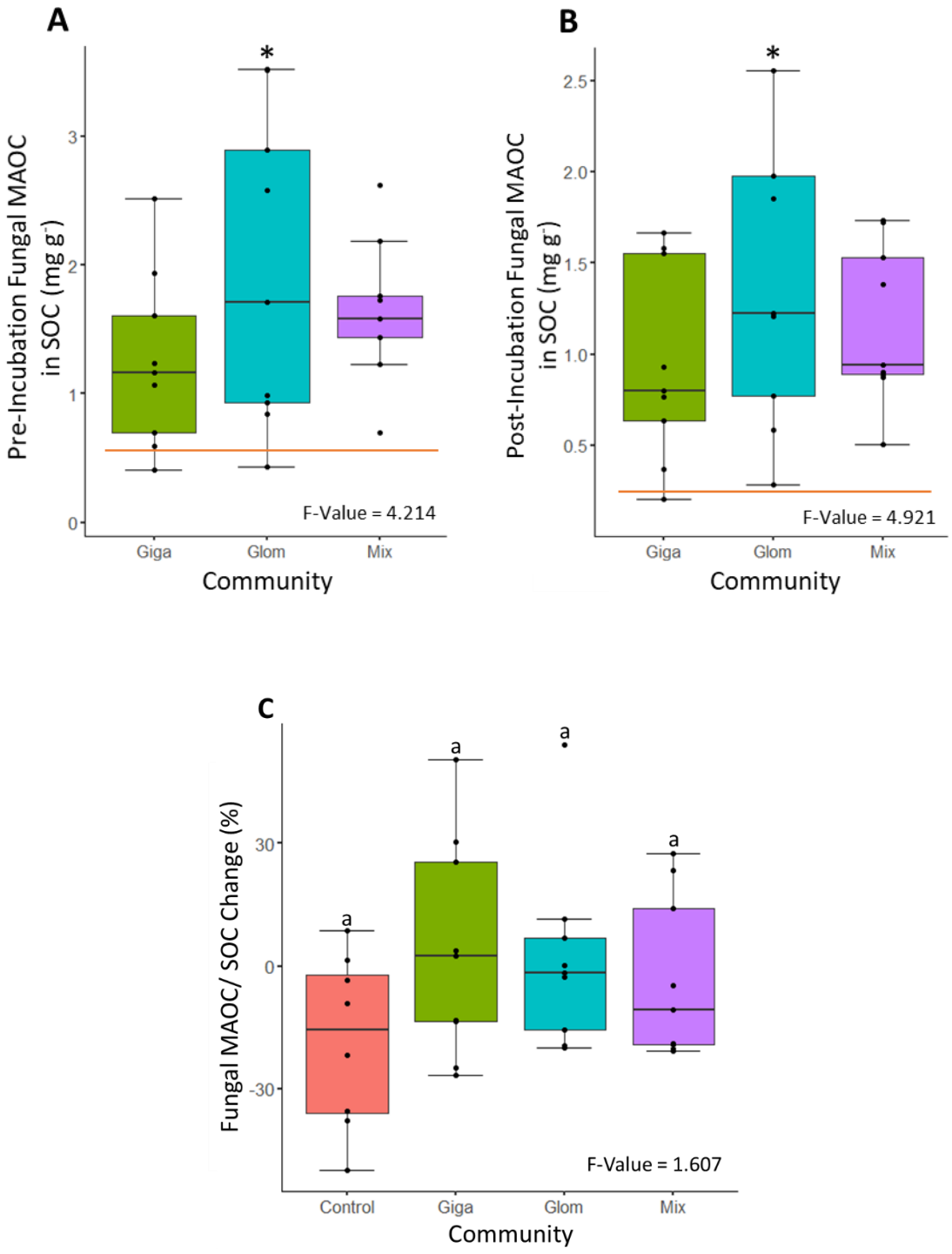
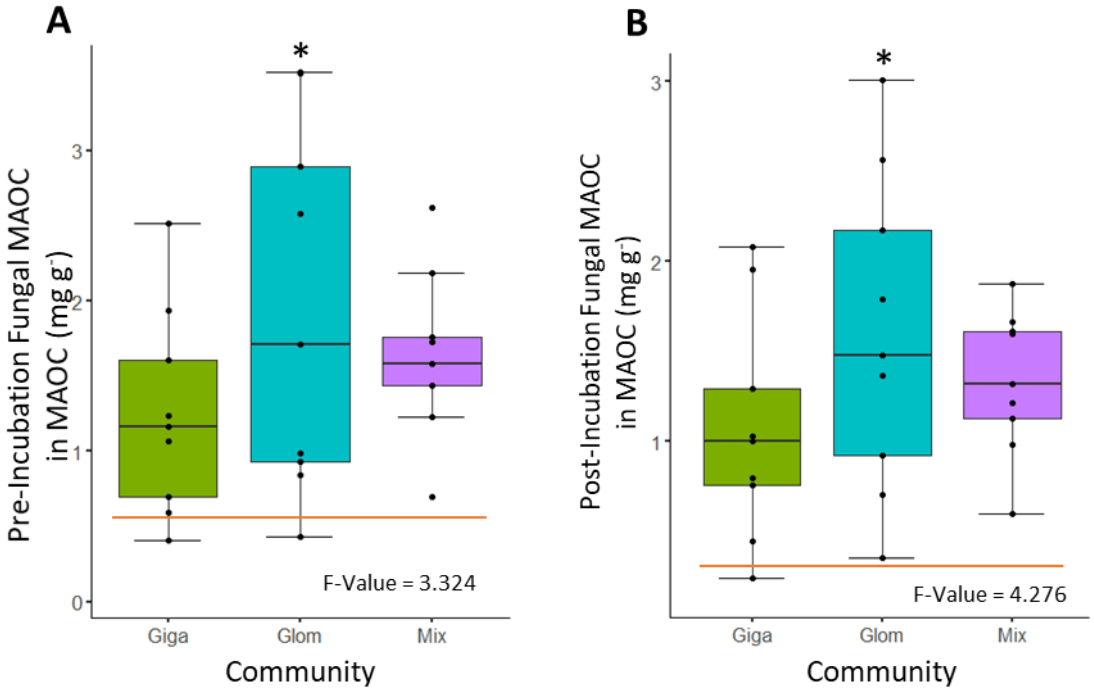


Figure 2.5 The proportion of arbuscular mycorrhizal (AM) fungal mineral associated organic carbon (MAOC) in soil organic carbon (SOC) by community for pre-incubated soil (mg fungal MAOC g^{-} SOC) (A). The proportion of fungal MAOC in SOC by community after one month of

incubation (mg fungal MAOC g^{-} SOC) (B). Proportional change of AM MAOC in SOC over the one-month incubation (mg g^{-}) (C). The red line in figures A and B is the planted non-mycorrhizal control's median value, representing likely background enrichment from root exudates. Gig (*Gigasporaceae*); Glom (*Glomeraceae*); Mix (*Gigasporaceae* plus *Glomeraceae*); Control (planted with sterile soil). Asterisks indicate a significant difference between a community's carbon and the planted non-mycorrhizal control and letters indicate pairwise differences among communities ($p < 0.05$). Df = 3, n = 9.



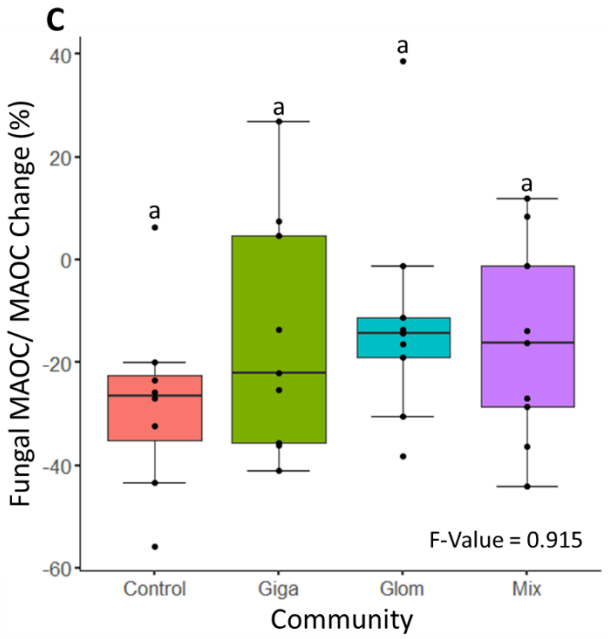


Figure 2.6 The proportion of arbuscular mycorrhizal fungal mineral associated organic carbon (MAOC) in total MAOC by community for pre-incubated soil (mg fungal MAOC g^{-} MAOC) (A). The proportion of fungal MAOC in total MAOC by community for post-incubated soil (mg fungal MAOC g^{-} MAOC) (B). Proportion of fungal MAOC in MAOC lost during the incubation (mg g^{-}) (C). The red line in figures A and B is the planted non-mycorrhizal control's median value, representing likely background enrichment from root exudates. Gig (*Gigasporaceae*); Glom (*Glomeraceae*); Mix (*Gigasporaceae* plus *Glomeraceae*i); Control (planted with sterile soil). Asterisks indicate a significant difference between a community's carbon composition and the planted non-mycorrhizal control and letters indicate pairwise differences among communities ($p < 0.05$). Df = 3, n = 9, except where outliers were removed n > 8.

2.5 Discussion

This experiment tested whether AM fungal communities with divergent life-history strategies and levels of trait diversity differentially affected SOC cycling. Specifically, I tested

whether AM fungal communities composed of competitors *(Gigasporaceae)*, ruderals *(Glomeraceae)*, or diverse traits (mixed) varied in their effect on SOC and MAOC accumulation and retention before and after a one-month incubation. All communities contributed to new SOC accumulation, but nonetheless total SOC concentrations generally decreased compared to the planted non-mycorrhizal control. Initially, only C from the *Glomeraceae* community accumulated in the MAOC pool, yet after the soil incubation, both the *Glomeraceae* and the mixed communities contributed to MAOC accumulation.

2.5.1 AM Fungal Priming may be Responsible for Decreased Total SOC

AM fungal priming may have been responsible for the observed total SOC loss (average decrease of -14%) (Fig 2.3 A and 2.7; Table S7.3). While I initially expected that AM fungal C inputs would increase net total SOC, I instead found that the *Gigasporaceae* and *Glomeraceae* communities decreased pre-incubation total SOC and there was weak evidence that the mixed community did the same. Although it is believed that AM fungi are incapable of direct decomposition, as they lack genes used in lignin and hemicellulose decomposition, AM fungi can indirectly accelerate SOC mineralization (Frey 2019; Tang et al. 2016; Tisserant et al. 2013). This occurs when AM fungi deposit C, in the form of hyphae or exudates, to targeted nutrient-rich sites to stimulate saprotrophic activity (Frey 2019; Toljander et al. 2007; Yakov Kuzyakov 2002; Zak et al. 2019). Notably, this decrease in SOC with AM fungi was observed immediately following plant harvest, indicating that some SOC loss occurs while AM fungi are active and in association with their host. Active AM fungi may benefit from increased saprotrophic organic matter mineralization that increases AM fungal access to available nutrients. Further, AM fungal hyphae provide a network of moist surfaces through the soil matrix that can increase saprotrophic microbial mobility (Gorka et al. 2019; See et al. 2022). Thus improving

saprotrophic bacteria's access to SOC, resulting in higher SOC mineralization (Dechesne et al. 2010).

Unlike total SOC, neither the total MAOC fraction nor the proportion of total MAOC in total SOC was affected by AM fungi, prior to the incubation (Fig 2.3 B; Table S7.3), However, an increase in the proportion of total MOAC to total SOC should be expected since total SOC decreased but MAOC did not. It may be that the total MAOC pool did marginally decrease but it was not detectable when compared to total soil (Fig 2.7). A small but not detectable decrease in total MAOC with a relatively larger total SOC decrease would indicate preferential but not exclusive decomposition of faster cycling C pools. This would support the literature as it would indicate that that MAOC is mineralized at a slower rate than faster cycling C pools (Balesdent and Mariotti 1996; Lavallee, Soong, and Cotrufo 2020).

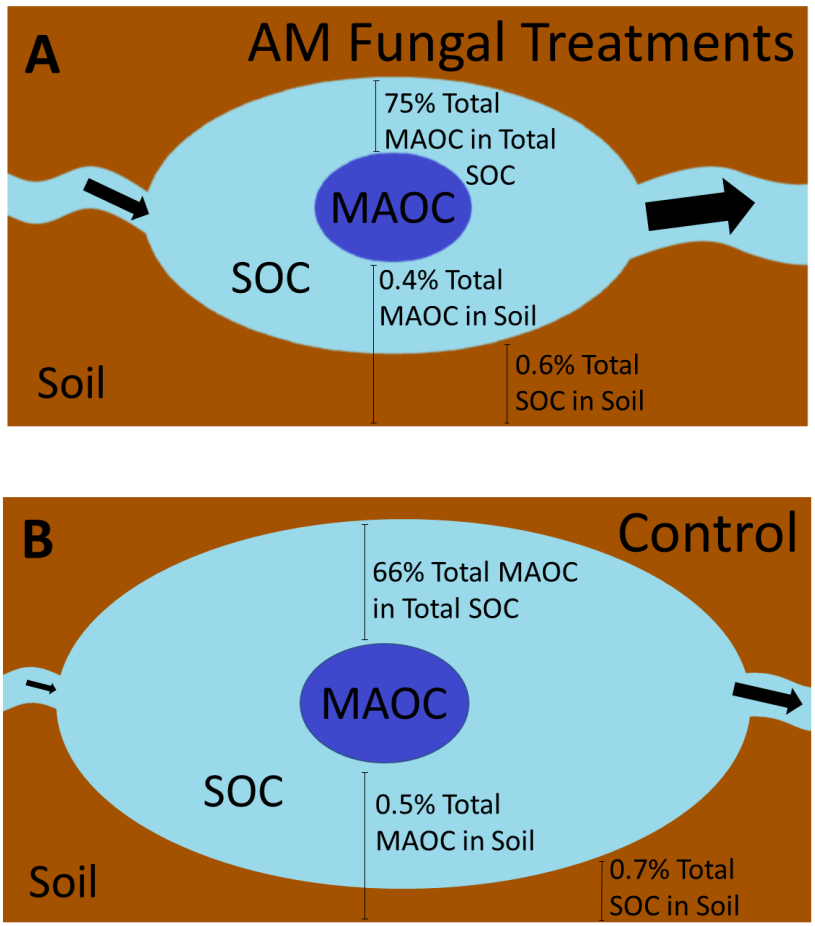


Figure 2.7 Conceptual figure demonstrating the potential shift in the SOC and MAOC pools in the AM fungal treatments (A) compared to the planted non-mycorrhizal control (B). The total SOC pool, represented by the light blue pools, decreased in the presence of AM fungi (B), likely due to increased SOC mineralization caused by increased saprotrophic microbial activity and mobility. This is despite additional carbon (C) inputs from hyphal C deposition, represented by the larger inflow arrow. Conversely, neither total MAOC nor the proportion of total MAOC in total SOC, represented by the deeper blue pools, was affected by AM fungi despite the net decrease in total SOC, suggesting that non-detectable decreases in MAOC may have occurred. Values indicate proportional averages across AM fungal (A) and control (B) treatments.

2.5.2 AM Hyphal C Universally Contributed to SOC Formation

I anticipated that the mixed-trait community would contribute the largest amount of new fungal C input to SOC, followed by the *Gigasporaceae* community. However, all AM fungal communities contributed to fungal SOC formation similarly. This indicates that regardless of the dominant trait or trait diversity of a community, AM fungal hyphae may be notable contributors to SOC. Over the short 12.5-week greenhouse experiment, AM fungal hyphae contributed 0.25% of total SOC on average after accounting for potential root exudate inputs (Fig 2.4; Table S7.3). Although this may be relatively small amount, the number of times hyphal C turned over, depositing new C, was likely low, as AM hyphae usually take around 12 days to turn over (Allen 2007). Thus, over time, the proportion of fungal SOC is likely to increase as hyphae continue to grow and turnover and as isolates mature.

It is also possible that community differences in fungal SOC inputs could emerge with more time for *Gigasporaceae* species to grow. *Glomeraceae* spores usually colonize roots within 1-4 weeks of planting while *Gigasporaceae* spores can take up to 8 weeks (Hart and Reader 2002). Hart and Reader (2005) found that 7 weeks after planting, *Glomeraceae* species had around four times more runner hyphae and 3.5 times more absorptive hyphae than *Gigasporaceae* species, demonstrating *Glomeraceae* specie's faster growth and colonization rates. On a longer time-scale than this study, the greater investment in extraradical hyphae associated with slower-growing *Gigasporaceae* could eventually lead to more total hyphal necromass production. However, it is unknown if at the same time, the *Glomeraceae* community's continued and more frequent hyphal inputs would outpace the inputs from *Gigasporaceae*. Indeed, while it was not significant, I observed that the fast-growing *Glomeraceae* community did contribute more to SOC production than *Gigasporaceae*. Although

we found no evidence of differences within AM fungal communities to SOC, this study can conclude that AM fungal hyphae are important contributors to SOC formation, and with time may constitute a notable portion of SOC.

2.5.3 Only the *Glomeraceae* Community Carbon Accumulated in the MAOC Pool

I had expected that the *Gigasporaceae* community would contribute more to fungal MAOC accumulation than the *Glomeraceae* community, and that the mixed community would contribute the most. I found that only communities containing *Glomeraceae* species contributed to fungal MAOC accumulation (Fig 2.5 A and B and 2.6 A and B; Table S7.3). In many cases, the majority of MAOC is composed of compounds previously assimilated by microbes; thus, more microbially available hyphal compounds may be more likely to enter the MAOC pool (Angst et al. 2021). Hyphal phenolics and N concentrations are known regulators of decomposition in fungi and other organisms, with phenolics, such as melanin, being negatively correlated with biomass decomposition, and N concentrations positively correlated with decomposition rates (Fernandez and Koide 2014; Franklin et al. 2011; Frey 2019; Manzella, Geiss, and Hall 2019). Like with plants and some other microbial species, slower-growing and longer-lived species often have lower nutrient concentrations and invest more in defensive compounds like phenolics (Donaldson, Kruger, and Lindroth 2006; Siletti, Zeiner, and Bhatnagar 2017). As *Glomeraceae* species are faster growing than *Gigasporaceae* species, it is possible that the *Glomeraceae* community also had hyphal compound chemistry supportive of faster decomposition and thus more rapid transformation into the MAOC pool.

It is also possible that the *Glomeraceae* community's greater MAOC production was partly due to its faster growth rates and turnover times increasing the amount of new fungal C entering the system and thus the probability of new fungal SOC associating with minerals. Faster

growth rates and turnover times associated with *Glomeraceae* species could regularly produce more total fungal necromass than *Gigasporaceae* species, increasing the chances of *Glomeraceae* necromass C entering the MAOC pool. Yet, this could have shifted if the experiment had run longer, allowing the *Gigasporaceae* community to fully mature. With time, not only would the *Gigasporaceae* community's total biomass increase, as discussed above, but the plant may also change how it allocates its C. Plants provide AM fungi with photosynthetic C in proportion to the amount of P AM fungi provide in return. It could be expected that P-depleted zone around the roots could grow over time, creating a more competitive environment that *Gigasporaceae* species are better adapted to. With a fully developed *Gigasporaceae* community, their extra-radical hyphae may be better adapted at reaching outside of larger P-depleted zones than *Glomeraceae*. As such, plant C allocation to *Glomeraceae* may decrease if they are less capable of supplying P, inadvertently slowing *Glomeraceae* species' growth. Yet, further research would be needed to examine if community growth rates and C inputs change over time. Under shorter timeframes, *Glomeraceae* species should have faster growth rates, likely increasing their C inputs and thereby probability of fungal MAOC production.

Notably, after the one month of decomposition, there was weak evidence that the more diverse mixed trait community also contributed to fungal MAOC production compared to total SOC, but not when compared to total soil or total MAOC (Fig 2.5 A and B and 2.6 A and B; Table S7.3). Although there was no evidence of a change between pre and post incubation soils, these results indicate that further decomposition during the incubation lead to movement of pre-incubation fungal C into MAOC (Fig. 2.5 C and 2.6 C). As the mixed trait community is composed of AM fungal species from both the *Glomeraceae* and *Gigasporaceae* families, and there was no evidence that the *Gigasporaceae* community contributed to MAOC formation

either pre or post incubation, it is likely that the mixed community's component *Glomeraceae* species were largely responsible for most of the MAOC formation. This further supports that, within the incubation period, only *Glomeraceae* species (whether as part of the *Glomeraceae* or mixed communities) contributed to MAOC formation; however, the specific mechanisms driving *Glomeraceae* species' MAOC formation are still unknown.

2.6 Conclusion

In conclusion, these results demonstrate trait-based AM communities' differential ability to alter SOC cycling. Priming most likely drove initial losses in AM fungal communities total SOC stocks, while hyphal C simultaneously contributed to fungal SOC formation. Although there was no evidence of an AM community composition effect on fungal SOC formation, only the ruderal *Glomeraceae* community contributed to the MAOC pool in pre-incubation soils. The characteristic faster growth and turnover rates associated with *Glomeraceae* species may have led to more rapid MAOC accumulation, and the additional decomposition period during the incubation may have led the mixed trait community to also contribute to MAOC formation. This variability in AM fungal community contributions to soil MAOC, suggests that AM community life-history strategy and trait diversity can differentially alter SOC cycling and retention. This could have implications for climate change modeling and mitigation practices. A time-sensitive multi-year experiment could provide deeper insight on the varying effects of AM community assembly on SOC cycling over time, and future work examining AM fungal chemistry could inform how additional controls outside of growth traits regulate AM fungal community MAOC formation.

2.7 References

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Chapter 3:

How do Arbuscular Mycorrhizal Community Life-History Traits and Community Trait Diversity Influence Host Nutrient Concentration?

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3.1 Abstract

Approximately 72% of terrestrial plant species associate with arbuscular mycorrhizal (AM) fungi, making AM symbiosis a key nutrient uptake pathway for most plant species. Life-history strategies differ substantially among AM fungal families, potentially effecting hyphal nutrient acquisition efficiency, host nutrition, and thereby plant health and ecosystem function. Despite these implications, the role of AM fungal community life-history strategies and trait diversity on host nutrient uptake is poorly understood. To address this knowledge gap, I grew Sudan grass (*Sorghum sudanense*) with communities representing contrasting life-history strategies and trait diversity: either 1) five species in the AM family *Gigasporaceae* representing competitor traits, 2) five species in the family *Glomeraceae* representing ruderal traits, or 3) a mixed community combining all ten AM species representing functional trait diversity. After 12.5 weeks, I measured above and below ground plant biomass and the concentration of 12 nutrients in aboveground biomass. The *Glomeraceae* community increased plant P concentrations 1.1 times more than the *Gigasporaceae* community. The community with diverse life-history traits generally showed either the highest plant nutrient concentrations or nutrient concentrations between those of its component life-history strategies. Importantly, trait diversity increased plant P 1.2 and 1.3 times more than *Glomeraceae* and *Gigasporaceae* communities. However, this did not translate to having the greatest plant biomass. These findings demonstrate that AM fungal community trait composition affects plant nutrient uptake, but no community is uniformly best at improving host nutrition.

3.2 Introduction

It is estimated that 72% of vascular terrestrial plant species associate with arbuscular mycorrhizal (AM) fungi, and this symbiosis is one of the primary means of terrestrial plant nutrient uptake (Brundrett and Tedersoo 2018; Smith et al. 2011). Although AM fungi are usually mutualists, the relationship varies across a spectrum from mutualism to parasitism (N. C. Johnson, Graham, and Smith 1997; S. Smith and Read 2008). Soil nutrient availability, the physical soil environment, and both the plant and fungal symbiont identity are factors influencing the symbiosis' outcomes (Neumann and Eckhard 2010; S. Smith and Read 2008). As these factors typically covary, predicting the effect of AM fungal species on plant health is a challenge, particularly considering AM fungi's taxonomic diversity. As environmental factors and plant hosts likely filter AM fungi based on a set of traits and not taxonomy, a trait-based community approach may prove more promising for ecologists to develop predictable patterns in the AM symbiosis (Aguilar-Trigueros et al. 2015; Crowther et al. 2014). For instance, from an AM fungal trait perspective, it may be more tractable to identify trait trade-offs in resource use than to consider host plant response to varying taxonomic assemblies (Powell and Rillig 2018). While recognition of the importance of trait-based fungal ecology is growing, the AM functional traits responsible for a successful mutualism remain poorly understood (Neumann and Eckhard 2010; Smith and Read 2008). Determining which AM fungal traits enhance AM host nutrient uptake, and thus have the strongest benefit to plant nutrition could improve our understanding of plant health and ecosystem function (Neumann and Eckhard 2010).

AM fungi exhibit diverse functional traits that are conserved primarily at the family level (Hart and Reader 2002). Chagnon *et al.* (2013) suggested that two of the largest AM fungal families, *Gigasporaceae* and *Glomeraceae*, utilize distinct life-history strategies that could be

categorized by Grime's C-S-R framework (Grime 1977; Schüßler, Schwarzott, and Walker 2001). They proposed that species in the *Gigasporaceae* family may follow a competitor strategy, thriving in conditions of low stress and low disturbance by growing relatively slowly, delaying sporulation, and prioritizing biomass allocation to extraradical hyphae to acquire growth limiting-resources (Chagnon et al. 2013; Hart and Reader 2002, 2005; Maherali and Klironomos 2007a; Staddon et al. 2003). Species in the *Glomeraceae* family, on the other hand, use a ruderal strategy, adapted to conditions of high disturbance and low stress by prioritizing fast growth rates with high hyphal turnover and frequent reproduction at the cost of low biomass allocation to extraradical hyphae. Due to these trait differences, *Glomeraceae* species may be best at rapid nutrient uptake when resources are plentiful, while *Gigasporaceae* species may be better at providing their hosts with resources in nutrient-poor environments.

While *Gigasporaceae* are often more dominant in phosphorus (P)-limited soils, they might not always increase plant nutrient uptake. In a one-year greenhouse experiment with English plantain *(Plantago lancelota)*, *Gigasporaceae* provided its host with more P than *Glomeraceae* but in a 15 week greenhouse experiment with onion *(Allium cepa L) Glomeraceae* species were seen to improve plant nutrition more than *Gigasporaceae* species (Gosling, Jones, and Bending 2016; Nancy Collins Johnson et al. 2003; Maherali and Klironomos 2007b). These opposing responses indicate that resource acquisition via AM fungi is dependent on both life-history strategies, growing conditions and host identity, including host traits such as lifespan. Further, the *Gigasporaceae* family's slower root colonization and slower turnover rates could potentially indicate a greater investment in transport hyphae over absorptive hyphae (Souza et al. 2005). Transport hyphae, can grow 1-2 mm a day, up to 25 cm away from their host's roots, enabling hyphae to obtain nutrients out of reach to roots (Neumann and Eckhard 2010; S. E.

Smith and Smith 2011; S. Smith and Read 2008). This may better enable *Gigasporaceae* to reach beyond P-depleted zones or search for scarce resources, particularly important in nutrient-limited conditions (Fig. 3.1). The *Glomeraceae* family's fast colonization rates and high hyphal turnover time could indicate greater investment in absorptive hyphae that, compared to transport hyphae, have a relatively fast turnover time and narrower diameter, allowing them to extract nutrients from smaller soil pores (Bago et al. 1998; Neumann and Eckhard 2010; S. Smith and Read 2008). Investment in absorptive hyphae would more likely be beneficial in high resource environments and give *Glomeraceae* a competitive advantage when resources are abundant (Hart and Reader 2002; Maherali and Klironomos 2007a). Thus, these contrasting AM families have traits that may confer different benefits for plant nutrient acquisition (Neumann and Eckhard 2010; S. Smith and Read 2008).

As environmental conditions are not always stable or uniform, plants may derive the greatest benefit by associating with communities that have diverse AM life-history traits. Such trait diversity could increase AM fungal resistance and resilience to stress and disturbances. Maherali and Klironomos, (2007) and Crossay *et al.* (2019) found that co-inoculation by AM fungi with differing life-history strategies improved plant growth and nutrient concentration. Maherali and Klironomos, (2007) also found that AM fungal communities with greater functional trait diversity experienced less competitive exclusion. Taken together, AM communities with increased trait diversity, not just species diversity, may best promote plant nutrition and growth.

Different AM traits may benefit certain plant species more than others based on both partners' combined life-history strategies. For instance, the *Gigasporaceae* family's slow growing strategy may be better at acquiring plant nutrients in perennial systems, whereas annual

plants, and plants with fine root architecture and rapid root turnover may benefit most from fast adapting associates like *Glomeraceae*. Thus, understanding the plant's responses to AM functional traits could help explain plant community assembly, and better inform management practices such as applying AM fungal inoculants to agricultural fields. Yet, little work has been done comparing plant responses to different AM fungal families with varying functional traits and diversity. This study examines whether 1) AM fungal communities that differ in their set of life-history strategies cause different responses in their host plant's nutrient concentration and biomass and; 2) if greater trait diversity promotes better plant nutrient concentration and growth. To test these questions, I grew Sudan grass (*Sorghum sudanense*) with either a community of competitors (five *Gigasporaceae* species), a community of ruderals (five *Glomeraceae* species), or a mixture of both (all ten AM species) for 12.5 weeks, and measured above and below ground biomass and the concentration of macro- and micro-nutrients in above ground biomass. I expected both plant nutrient concentration, especially plant P, and plant biomass to vary by trait-community. However, given that the *Gigasporaceae* community typically invests more in extraradical hyphae and potential transport hyphae, they may be better at acquiring limiting nutrients compared to the *Glomeraceae* community. I also expected the mixed-trait community to be best at providing plants with soil nutrients and increasing biomass compared to the *Gigasporaceae* or *Glomeraceae* communities due to niche complementarity.

3.3 Materials and Methods

3.3.1 Experiment Design Overview

I used the same greenhouse experiment as in Chapter 2, with the four treatments grown with Sudan grass to compare the influences of contrasting AM fungal community life-history strategies and trait diversity on plant nutrient uptake and biomass. Briefly, the experiment used

four AM fungal communities which were inoculated with: 1) five *Gigasporaceae* species; 2) five *Glomeraceae* species; 3) all ten mycorrhizal species (Mix); or 4) sterilized inoculum (Control) (Table 2.1). Each treatment was replicated 9 times and grown in a greenhouse for 89 days. Experimental pots had separate rhizosphere and hyphal compartments in order to create a root-free zone from which only AM fungi could acquire nutrients. This was done by growing the Sudan grass inside of a 23 cm tall by 12 cm wide mesh bag with a 30 μm pore size that prevented roots but not hyphae from passing through to the surrounding soil (Fig. 2.1).

3.3.2 Mycorrhizal Inoculum

I obtained the mycorrhizal fungal isolates from INVAM and chose them based on their reproductive traits (http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html). The five *Gigasporaceae* species selected were: *Cetraspora pellucida, Dentiscutata heterogama, Gigaspora margarita, Racocetra fulida*, and *Scutellospora calospora.* The five *Glomeraceae* species were *Claroideoglomus etunicatum, Funneliformis mosseae, Rhizophagus clarus, Rhizophagus intraradices, and Septoglomus deserticola.* The inoculum was mixed by dry weight so that each treatment contained equal proportions of each individual isolates' inoculum. The control treatment was made by autoclaving the inoculum mixture containing all ten isolates.

3.3.3 Planting and Microbial Inoculum

Before planting, Sudan grass seeds were surface sterilized with 50% ethanol for one minute and then germinated in seedling trays. Three seedlings were transplanted to 30 μm mesh bags, approximately 23 x 12 cm, placed at the center of 5 L tree-pots. The mesh pore size prevented roots from passing but permitted hyphae to access surrounding soil, creating distinct rhizosphere and hyphal compartments (Fig. 2.1). The soil I used was an autoclaved 1:1 mixture

of all-purpose sand and a clay-loam gleysol in the Sainte Rosalie series collected from a grassland at the McGill Morgan Arboretum Québec Canada. Mycorrhizal inoculum was added to each pot during transplant. I selected Sudan grass as a host plant because the species is highly mycorrhizal. Pots were transferred to the greenhouse on August 27th, 2020, and seedlings were thinned 5 days later. Twenty mL of a microbial wash was added to each plant 14 days after transplant to: 1) return an AM fungi-free natural soil saprotrophic community and 2) to normalize for potential variation in the non-AM microbial community associated with each inoculum. To make the inoculum, 600 g of the original field soil and 150 g of the mixed *Gigasporaceae and Glomeraceae* inoculums were added to 2 L of deionized water and sieved twice through a 20 µm sieve. Mycorrhizal spores are too large to pass through the sieve, but bacteria and other saprotrophs are small enough to enter the resulting slurry.

3.3.4 Sample Collection

I ran the experiment until spores could be found in the root-free compartment of all mycorrhizal treatments, demonstrating that all AM fungal communities matured. Spores were detected 84 days after transplanting. Five days later, I collected above and below ground biomass. Above ground biomass was collected by cutting the plant at the base of the stems. For root biomass, soil within the root compartment was gently removed and roots were cleaned, padded dry, and weighed before being divided into samples for mycorrhizal colonization and root dry weight. Roots designated for colonization analysis were stored in a 50% ethanol solution. Roots designated for dry weight analysis were weighed again and oven-dried at 60 °C with the above ground biomass.

3.3.5 Above Ground Biomass Nutrient Analyses

Above ground biomass was subsampled for nutrient concentrations by collecting a ~4.5 cm fragment from the base of the stem, along with 2-4 similarly sized stem fragments from further up the stem, and portions of the base, middle, and ends of several leaves. I sent subsamples to the SIFER/NRAL at the University of Alberta, Canada, for carbon (C), macro and micro nutrient analysis. Macronutrients were nitrogen (N), P, potassium (K), calcium (Ca), sulfur (S), and magnesium (Mg). Micronutrients were boron (B), copper (Cu), iron (Fe), manganese (Mn), sodium (Na), and zinc (Zn). Biomass C and N were analyzed via flash combustion on an elemental analyzer (Thermo FLASH 2000, Thermo Fisher Scientific Inc., Bremen, Germany). All other nutrients were analyzed using an inductively coupled plasma-optical emission spectrometer (Thermo iCAP6300 Duo (N. America), Thermo Fisher Corp., Cambridge, United Kingdom).

The University of Alberta's SIFER/NRAL also analyzed the above ground biomass for ^{13}C and ^{15}N content with an elemental analyzer (Thermo FLASH HT Plus 2000; Thermo Fisher Scientific Inc., Milan, Italy) connected via a coupling interface (ConFlo IV, Thermo Scientific Inc. Bremen, Germany) to an isotope ratio mass spectrometer (Thermo Delta V Advantage; Thermo Scientific Inc. Bremen, Germany).

3.3.6 Root Colonization

The methods from Vierheilig *et al.* (1998) were used to stain the roots with ink and vinegar and I determined colonization using the method by McGonigle *et al.* (1990). I calculated the percent root colonization as the number of intersections with AM fungi divided by the total number of intersections, multiplied by 100.

3.3.7 Statistical Analysis

I used R version 4.1.0 to conduct one-way ANOVA's for differences in AM colonization, Sudan grass nutrient content, isotopic signatures, and plant biomass between AM communities and the sterilized planted control. I used Shapiro-Wilk's test for normalcy and Levene's test for homogeneity of variance to assure the assumptions of the model were met. Rosner's Test was used to flag potential outliers, which were then observed with histograms, box and whisker plots, and by plotting the studentized residuals. If an outlier was either consistent across measured variables or if there was a methodological reason (e.g., damaged plant) for a datapoint to be an outlier, it was removed. No more than three outliers were ever removed per ANOVA and no more than two outliers were ever removed from the same treatment. Removing an outlier changed the results of the ANOVA for plant Na, shifting the result from insignificant to just below a p-value of 0.05. If the assumptions of the model were unable to be met through transformations, I used a non-parametric Kruskal-Wallis test instead of an ANOVA. If ANOVA results were significant, Tukey tests were performed to observe pairwise comparisons between AM fungal communities. Likewise, if the results of a Kruskal-Wallis tests were significant, a Dunn's test was used to examine pairwise comparisons between AM fungal communities. AM community effects on nutrients and biomass were considered significant at $\alpha = 0.05$.

3.4 Results

3.4.1 Colonization

The experimental inoculation and AM fungal root colonization was a success according to the high rates of colonization observed in all three AM fungal treatments (Table S7.2). The non-mycorrhizal control showed minor AM fungal contamination, but any observations of colonization were likely due to human error (Fig. 2.2; Table S7.2). The mixed community had

the highest average root colonization rate, 57 times higher than the control, and *Gigasporaceae* had the lowest, with a colonization rate 46 times higher than the control, matching the life-history strategies observed in the literature (Table S7.2) (Souza et al. 2005). However, some vesicles were found in the *Gigasporaceae* community, potentially indicating some *Glomeraceae* species contamination, as *Gigasporaceae* are not thought to produce vesicles (Table S7.2) (Souza et al. 2005). Thus, despite the potential for *Glomeraceae* species contamination in the *Gigasporaceae* community, the treatments appear to be properly colonized in accordance with the experimental design.

3.4.2 AM Fungal Effects on Aboveground Host Nutrient Concentration

AM fungal communities affected most macro and micronutrient concentrations in above ground Sudan grass biomass relative to the non-mycorrhizal control. Of the 12 measured nutrients, only N, Fe, B, and Zn showed no response to AM colonization (Figs. 3.1 and 3.3 B; Table S7.4). Plant Ca and P had the strongest response to AM colonization, with a respective overall mean increase of 2.8 and 5.3 larger than the control. On average, across all AM fungal communities, colonization also increased above ground biomass Ca, Cu, Mg, Mn, Na, P, and S relative to the control, except for K which had a mean decrease of 31.6%. Host plant Ca, Cu, Mg, P, and S were the only nutrients in which a significant increase was observed when comparing each of the three AM communities to the control (Fig. 3.1; Table S7.4).

Plant tissue K, Mn, and Na concentrations were only significantly affected by some AM communities compared to the planted non-mycorrhizal control. *Glomeraceae* was the only AM fungal community that affected K, decreasing K concentration by 41.2% compared to the control (Fig. 3.1 and 3.2 A; Table S7.4). Conversely, only the *Gigasporaceae* community significantly increased Na concentration, increasing it on average 3.4 times higher from the control (Fig. 3.1

and 3.2 C; Table S7.4). Both the *Gigasporaceae* and mixed communities positively affected Mn concentrations increasing average Mn by 81.5% and 82.0% from the control, respectively (Fig. 3.1 and 3.2 B; Table S7.4).

Plant P was the only nutrient to show differences among AM fungal communities. Among AM fungal communities, the mixed community was associated with the largest increase in P concentration whereas *Gigasporaceae* had the lowest P increase compared to the control, with 520% and 366% higher P concentrations, respectively (Fig. 3.1 and 3.2 D; Table S7.4). The mixed and *Gigasporaceae* communities had the largest difference in P concentrations between each other, with the mixed community containing 1.3 times more P than *Gigasporaceae*. The same pattern was found in plant C:P ratios, where colonization generally lowered plant tissue C:P from the control by 84.1% and differed among all AM fungal communities. The mixed community had the lowest plant C:P (178), 86.6% lower than the control and 24.5% lower than that of the *Gigasporaceae* family. *Gigasporaceae* had the highest C:P (236) within AM communities, with an average decrease of 82.2% from the control (Table S7.4).

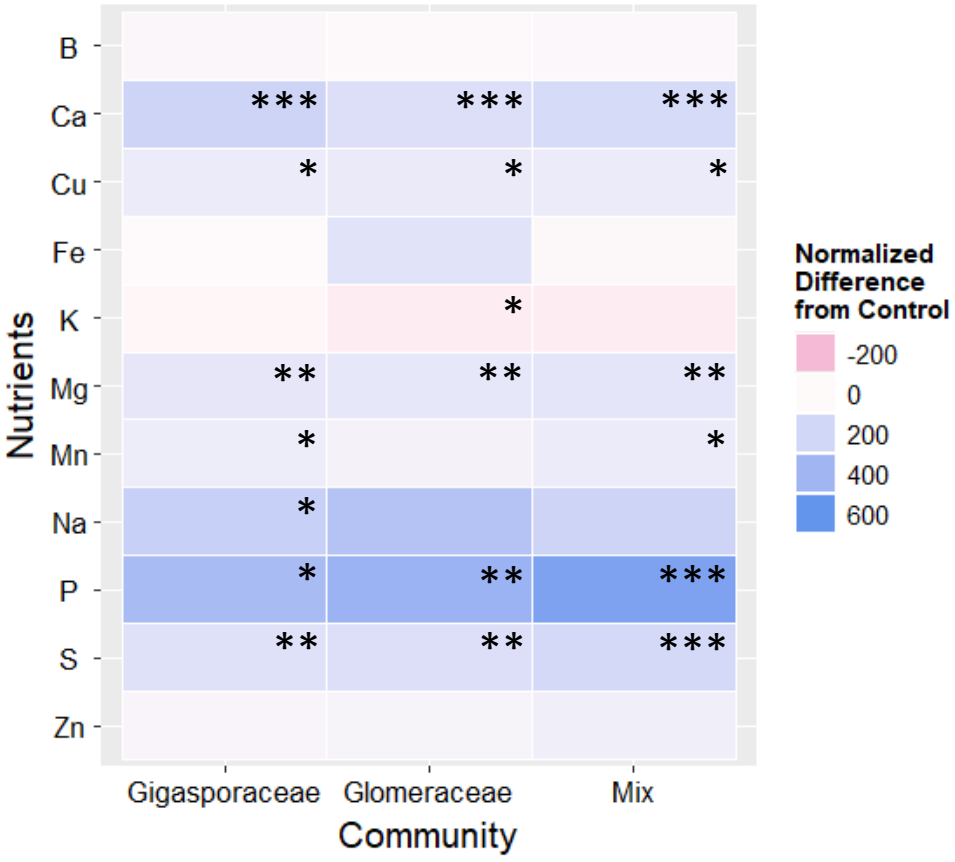

Figure 3.1 Effect of AM fungal community on Sudan grass' aboveground nutrient concentrations (mg kg^{-}). The colors indicate the percent change relative to the control where blue colors are an increase and red colors are a decrease from the uncolonized control. Asterisks indicate significant differences from the control where (*) indicates a p-value < 0.05, (**) a p-value < 0.01, and (***) a p-value <0.0001. Df = 3, n = 9, except where outliers were removed n > 7.

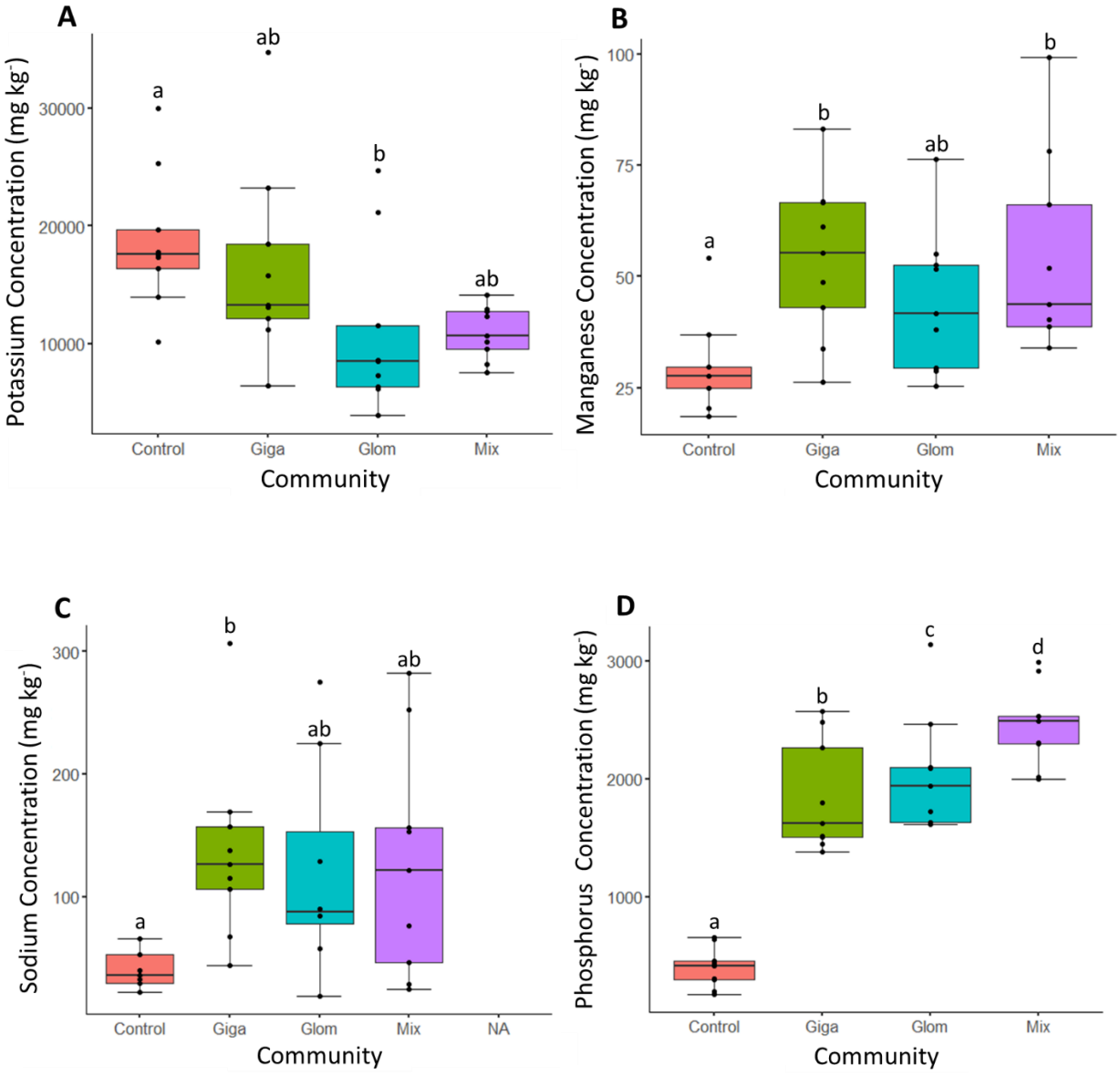

Figure 3.2 Effect of arbuscular mycorrhizal fungal community on plant nutrient concentration. Potassium (A); Manganese (B); Sodium (C); and Phosphorous (D) concentration in above ground Sudan grass biomass (mg kg^{-} dry biomass) by AM community. Gig (*Gigasporaceae*); Glom (*Glomeraceae*); Mix (*Gigasporaceae* plus *Glomeraceae*); letters indicate pairwise differences between communities ($p < 0.05$). Df = 3, n = 9, except where outliers were removed n > 8, ANOVA F-Values = 4.5, 3.9, and 4.2 respectively for A-C, Chi-Squared = 24.0 for D.

3.4.3 AM effects on Aboveground Plant tissue ^{15}Nitrogen and Total Nitrogen

AM colonization increased the δ ^{15}N of Sudan grass above ground biomass, compared to the uncolonized Sudan grass. The mean δ ^{15}N across all colonized treatments was 12.8 δ ^{15}N, whereas in the control it was 8.89 δ ^{15}N. Between the colonized treatments, there were no significant differences in δ ^{15}N (Fig. 3.3 A). AM colonization had a marginally significant ($p < 0.1$) effect on aboveground biomass total N concentrations, with colonized treatments decreasing N concentrations by an average of 15% compared to the control (Fig. 3.3 B; Table S7.4). The C:N ratio of Sudan grass above ground biomass was not affected by AM fungal colonization (Table S7.4).

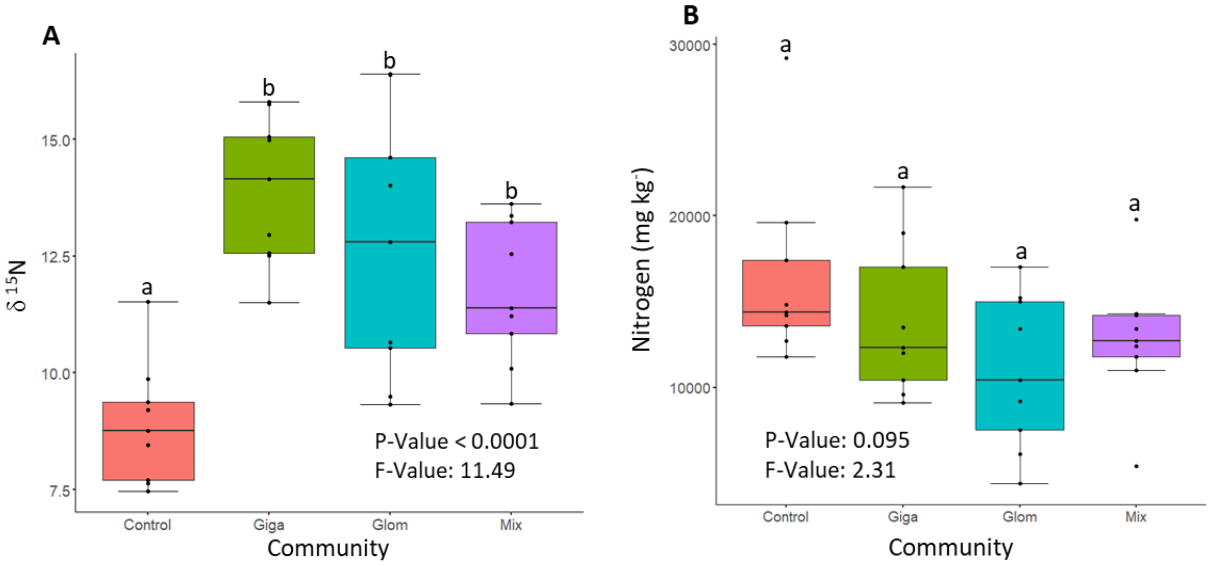

Figure 3.3 Sudan grass above ground biomass δ ^{15}N by AM community (A). Sudan grass above ground biomass total nitrogen concentration (mg kg^{-} dry biomass) by AM community (B). Gig (*Gigasporaceae*); Glom (*Glomeraceae*); Mix (*Gigasporaceae* plus *Glomeraceae*); letters indicate pairwise differences between communities ($p < 0.05$). Df = 3, n = 9, except where outliers were removed n > 8.

3.4.4 Aboveground Biomass

AM colonization had a significant positive impact on both root and shoot biomass compared to the control, but the effect varied by community. Both the *Glomeraceae* and mixed communities significantly increased the shoot biomass, with an average biomass increase of 1.56 and 1.44 times higher than the control, respectively. Shoot biomass was also significantly impacted by the pot placement within the greenhouse (p-value = 0.047). Only the *Glomeraceae* community significantly increased root biomass compared to the control, with root biomass 1.96 times higher on average in *Glomeraceae* communities (Table 3.1). Despite these increases in root and shoot biomass with colonization, the root:shoot ratio was not significantly changed by AM colonization (Table 3.1).

Table 3.1 Mean Sudan grass biomass and standard deviations by mycorrhizal treatment for above ground C concentration, root biomass, above ground biomass, and root:shoot ratio. Statistical significance for the model is a p-value < 0.05. Letters indicate pairwise differences between communities ($p < 0.05$). Df = 3, n = 9, except where outliers were removed n > 7.

Variable	Control	*Gigasporaceae*	*Glomeraceae*	Mixture	ANOVA p-Value
C Concentration (w/w%)	43.15 ± 1.3	40.69 ± 4.5	42.79 ± 1.6	42.84 ± 0.9	0.906
Root Dry Weight (g)	2.09 ± 0.99^{a}	2.96 ± 1.0^{ab}	4.09 ± 1.9^{b}	3.09 ± 0.75^{ab}	0.049
Shoot Dry Weight(g)	4.31 ± 0.90^{a}	5.21 ± 1.6^{ab}	6.73 ± 1.6^{b}	6.23 ± 0.82^{b}	0.003
Root:Shoot	0.42 ± 0.14	0.58 ± 0.16	0.54 ± 0.13	0.49 ± 0.08	0.120

3.5 Discussion

This study examined if AM fungal community life-history strategies or trait diversity differentially affect host plant nutrition and growth. Specifically, I determined whether AM communities composed of competitor species *(Gigasporaceae)*, or ruderal species

(Glomeraceae), varied in how they affected their host plant's nutrient concentrations and biomass. I also tested if AM fungal communities with greater life-history trait diversity improved plant nutrition and growth more than AM fungal communities with only one life-history strategy. In comparing the two communities with divergent life-history traits I found that the ruderal community, *Glomeraceae*, provided more P to its host than *Gigasporaceae*, potentially due to faster growth and colonization rates. I also found that, depending on the element, the diverse mixed-trait community's plant nutrient concentrations were usually either greater or in-between those of its component communities. The differing responses may be related to soil nutrient availability and nutrient uptake strategies. AM fungal-induced increases in plant biomass was not always associated with increased nutrient concentrations, likely due to a dilution effect of higher biomass.

3.5.1 AM Fungal Communities Affected the Magnitude, not Direction, of Plant Nutrients

Similar to several studies, AM fungal colonization increased biomass P, Ca, Cu, Mg, Mn, Na, and S concentrations, while plant K concentrations were decreased (Fig. 3.1; Table S7.4) (Giri, Kapoor, and Mukerji 2007; Smith and Read 2008). In all cases, the direction of the effect of AM communities on nutrient concentration was similar across the three communities. For example, all three AM communities increased plant Ca and S concentrations compared to the planted non-mycorrhizal control (Fig. 3.2 and 3.3; Table S7.4). There was no situation where one community increased their host's concentration of a specific nutrient while another community decreased the concentration of that same nutrient. This suggests that, under the same environmental conditions and host plant, AM fungal communities with different life-history strategies and levels of trait diversity will all either increase or decrease the concentrations of their host's nutrients compared to an uncolonized plant.

While the direction of the nutrient response was similar across AM fungal communities, I observed community dependent variations for some of the nutrients (P, K, Mn, and Na), indicating an influence of life-history traits. AM fungal community differences emerged in 1) the magnitude of the response and 2) which nutrients were most affected by each community. This community-dependent effect was strongest for P, where the mixed trait community increased P the most and the *Gigasporaceae* community the least (Fig. 3.2 D; Table S7.4). As such, I found that no AM fungal community was best at increasing all nutrient concentrations, instead varying AM community life-history strategies and levels of trait diversity primarily differed by which plant nutrient they affected the most.

3.5.2 The Ruderal *Glomeraceae* Community, Increased Plant P More than the Competitive *Gigasporaceae* Community

The *Glomeraceaceae* community increased plant P concentrations more than the *Gigasporaceae* community, potentially due to *Glomeraceae*'*s* faster growth rates and greater investment in absorptive hyphae (Fig. 3.2 D; Table S7.4). AM hyphal P uptake is thought to drive AM symbiosis because soil P is generally immobile despite being a primary nutrient needed for plant growth (Hoeksema et al. 2010; Smith et al. 2011). Thus, AM hyphal absorption is thought to be the primary pathway of P acquisition in AM plants (sometimes contributing up to 100% of plant P uptake) over direct root uptake due to their ability to grow beyond the root P-depleted zones (Fig. 3.4) (Smith et al. 2011; S. E. Smith, Smith, and Jakobsen 2004). I initially expected that the *Gigasporaceae* community would increase plant P more due to its greater investment in extraradical hyphae and potentially higher amount of transport hyphae. Such investment strategies would enable them to more effectively reach beyond the P-depleted zones and search the heterogenous soil environment for nutrient-rich pockets. However, this was not

supported by my results for plant P (though it was for other trace nutrients). It may be possible that the experiment's 12.5-week period was not long enough for P-depleted zones to form around roots or for the slow-growing competitive *Gigasporaceae* community to fully develop. Instead, the experiment's relatively short period, and setup that resembled a more disturbed environment, could have combined to favor the ruderal *Glomeraceae* community.

Ruderals are adept at surviving in disturbed environments, thus *Glomeraceae* species can establish quickly, and should be able to rapidly take advantage of available resources (Grime 1977; Pianka 1970; Reznick, Bryant, and Bashey 2002). As such, *Glomeraceae* species may be better at P uptake in annual or rapidly changing environments such as agroecosystems. The conditions of this experiment may be similar to an agricultural system as AM fungi developed from spores and hyphal fragments instead of common mycorrhizal networks (CMN). The AM fungal inoculum had a relatively short period of time to colonize the host and explore the soil environment. Compared to *Gigasporaceae* species, *Glomeraceae's* fast growth rate, higher root colonization, and potentially greater investment in absorptive hyphae may have better enabled them to rapidly absorb available soil nutrients found near their host's roots (Fig. 3.4) (Bruce, Smith, and Tester 1994). Further, the soil used in the experiment was autoclaved before planting which is known to cause a spike in plant-available P, similar to fertilizing (Serrasolsas and Khanna 1995; Serrasolses, Romanyà, and Khanna 2008). It is possible that the *Glomeraceae* community's fast-paced strategy was better at taking advantage of the early P spike than the slower *Gigasporaceae* community. Similar results were seen by Gosling, Jones and Bending (2016), who found that *Glomeraceae* species improved plant P more than *Gigasporaceae* after 15 weeks in a greenhouse experiment with sterilized soil, reinforcing that *Glomeraceae* species P acquisition strategies are most adept at rapidly colonizing newly disturbed systems.

As competitors, *Gigasporaceae,* thrive in low stress and low disturbance environments, where their greatest environmental pressure comes from competition for available resources (Chagnon et al. 2013; Grime 1977). Consequently, *Gigasporaceae* species may dominate in perennial grasslands or temperate forests, where AM fungi could survive alongside their hosts for multiple years, and where P-depleted zones could be more extreme. In such ecosystems, greater investment in long-term survival and reproduction may be more advantageous than fast life cycles. Thus, AM fungi in such ecosystems may live multiple years, primarily colonize new plants through pre-existing CMN, and allowing AM spores to establish over longer periods. Additionally, high-cost extraradical hyphae and transport hyphae may be advantageous in perennial systems that are often relatively more nutrient-limited, enabling AM fungi to grow further into P available regions and search for nutrient-rich pockets (Fig. 3.4). Supporting this, Maherali and Klironomos (2007) found that a *Gigasporaceae* community was best at improving plant P uptake after 1 year of growth in a greenhouse. Thus, my experiment may have needed to run longer for conditions favoring competitors to form and *Gigasporaceae* P acquisition strategies to be advantageous.

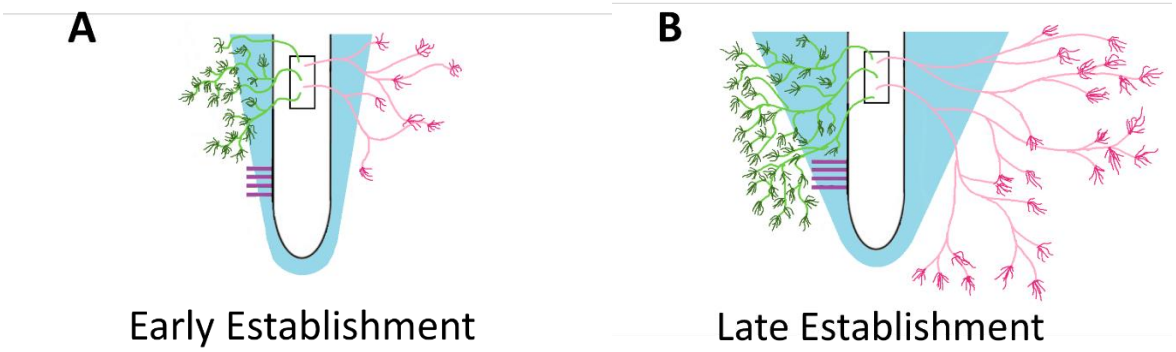

Figure 3.4 Hypothetical depiction of *Gigasporaceae* and *Glomeraceae* nutrient uptake strategies in a recently disturbed system (A) and in a stable, low-disturbance, and low-stress system (B), modified from Smith *et al.* (2011). Absorptive hyphae are the branched structures extending off the transport hyphae. *Glomeraceae* hyphae are depicted in green *Gigasporaceae* hyphae are depicted in pink. The root is depicted in white, the zone of P depletion is depicted in light blue, and root hairs are drawn in purple. In a recently disturbed system, where P-depleted zones around roots may still be small, the *Glomeraceae* community's life-history strategy may be best at rapidly absorbing abundant resources due to its faster growth rate, higher root colonization, faster turnover times, and potentially greater investment in absorptive hyphae (A). In older and stable environments, where *Gigasporaceae* and P-depleted zones have had time to grow, the *Gigasporaceae* community's competitor life-history strategy may be best at acquiring resources, due to its greater investment in extraradical hyphae, and potentially transport hyphae. These may enable growth beyond P-depleted zones and a more efficiently search for nutrient-rich pockets in the heterogenous soil environment (B).

3.5.3 Limiting Resources May drive Synergetic effects in Mixed Community

Nutrient responses to AM fungal trait diversity (mixed community) tended to be either synergetic of or in between those of the *Gigasporaceae* and *Glomeraceae* communities. The

mixed community increased plant P the most, supporting my hypothesis that mixed communities would be best at improving host plant nutrition; yet this effect was not universal across all nutrients (Fig. 3.2 D; Table S7.4). No other nutrient had significant differences between AM fungal communities, but community trends were still present. Plant Mg and S concentrations showed weak evidence that they increased the most in response to the mixed community (Table S7.4). The mixed community plant Ca, K, Mn, and Na concentrations fell in-between that of the *Gigasporaceae* and *Glomeraceae* communities', and the mixed community's plant Cu concentrations were the lowest (Fig. 3.2 A, B, and C; Table S7.4). Despite these variable outcomes, it is important to recognize that I observed the largest positive effect of trait diversity for plant P, which is generally considered the dominant nutrient driving AM symbiosis.

Nutrient limitations could explain why the mixed-trait community's plant nutrient concentrations were either synergetic or in between that of its component communities'. Plants may 'reward' their AM symbionts with a larger amount of photosynthetic C in return for the provision of limiting nutrients, such as P, compared to more readily available plant nutrients (Hoeksema et al. 2010; Smith et al. 2011). Thus, plant-limited high-reward soil nutrients may motivate hyphal exploration, while AM fungi may acquire less in-demand nutrients more passively. For the mixed trait community, this may cause synergistic responses for high-demand limiting nutrients like P, with niche complementarity resulting from both component communities allocating resources to acquire limiting nutrients. This may explain why this study and others found higher plant P within a diversity of AM families of different traits (Maherali and Klironomos, 2007; Crossay *et al.*, 2019). On the other hand, middling mixed trait nutrient responses may be due to more passive nutrient acquisition for a nutrient that is in less demand by the host (e.g., Ca, Na), where the benefit from a diversity of nutrient acquisition traits would not

necessarily be manifested. In such cases, passive nutrient uptake may be more random and result in the diverse mixed-trait community having nutrient concentrations between that of its component communities. As such, my observations where the mixed trait community's synergetic nutrient effects emerge for plant P but not necessarily other nutrients may be a function of how niche complementarity does not uniformly apply across all nutrients. Further experimentation manipulating soil nutrient concentration and time would be required to investigate this more thoroughly.

3.5.4 Plant Biomass Did Not Correlate with Increased Nutrient Concentration

There was no clear association between plant nutrient concentrations and growth. This may be explained by increased plant biomass diluting some nutrient concentrations, due to AM fungi-mediated increases in nutrient uptake (Smith, Smith, and Jakobsen 2004). Although the mixed community increased plant P the most, the *Glomeraceae* community was the only one to increase both above and below ground plant biomass (Table 3.1). It is difficult to establish a relationship between plant growth and nutrient concentrations because the most limiting nutrient will both drive and prevent biomass growth (i.e., Liebig's Law of the Minimum) (De Baar 1994). Thus, increased plant nutrient uptake from AM hyphae does not inevitably lead to increased plant growth or nutrient concentration. For instance, while AM fungi can contribute 100% of their host's P uptake, their host's biomass and plant tissue P concentration will not necessarily increase in response. Colonization by AM fungi can, in some circumstances, lead to decreased plant nutrient concentrations, as is often the case for K, like it was in this study (Fig 3.2 A; Table S7.4) (Lehmann and Rillig 2015; Smith and Read 2008). Decreased plant nutrient concentrations may be due to competition with AM fungi for nutrients or AM uptake of limiting resources driving plant growth, while simultaneously decreasing the concentration of non-limiting

nutrients (Neumann and Eckhard 2010; S. Smith and Read 2008). Consequently, increased plant biomass, driven by AM fungal uptake of limiting plant nutrients, may inadvertently decreased or have no effect on other plant nutrient concentrations.

The ^{15}N data provided evidence that AM hyphae did increase plant N uptake, but their contribution did not lead to increased plant N concentration. Ammonium is relatively enriched in ^{15}N compared to nitrate and is usually the preferred form of N for both plants and AM fungi, but less enriched ^{15}N nitrate usually contributes more to plant N due to its greater mobility (and sometimes abundance) in soil (Hodge and Storer 2015; Nygren et al. 2012). Plant ^{15}N concentrations were higher in all AM communities than in the non-mycorrhizal control, suggesting that AM fungi did contribute to plant N uptake, primarily through ammonia acquisition (Fig 3.3 A) (Hobbie and Högberg 2012; Tatsumi et al. 2021). However, AM communities did not increase plant N concentration compared to the planted non-mycorrhizal control (Fig 3.3 B). As such, AM fungal contributions to plant N uptake may have increased plant growth rates, instead of nutrient concentrations (Smith, Smith, and Jakobsen 2004). This demonstrates how there is no easily identifiable connection between plant nutrient concentration and biomass. Instead, AM community strategies that maximize the uptake of the most limiting nutrients could be the main drivers of plant growth.

3.6 Conclusion

In summary, I found that AM fungal communities differentially affect plant nutrient concentration and growth; yet responses to AM fungal communities were not uniform, indicating that no AM community life-history strategy or level of trait diversity is best at improving plant nutrition. The *Glomeraceae* community was found to increase plant P concentrations and total biomass more than *Gigasporaceae* potentially due faster growth rates and possibly more

investment in absorptive hyphae. Niche complementarity seems to be important as plant P increased most in response increased AM fungal community trait diversity. These findings provide further insight on how different AM fungal community traits and diversity affect host nutrition, improving our understanding of plant health and ecosystem function (Neumann and Eckhard 2010).

3.7 References

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General Discussion

While most vascular land plants associate with AM fungi, certain symbiotic context can either favor symbiosis with taxa in the *Gigasporaceae* or *Glomeraceae* families, which have contrasting life-history strategies (Brundrett and Tedersoo 2018; Jansa et al. 2002; Oehl et al. 2011). While one can expect that AM fungal traits and their diversity are key for understanding important factors such as SOC storage and plant nutrition, little research has examined cause and effect relationships in this context (Frey 2019; Ricklefs 2010). To address this knowledge gap, I tested if AM fungal communities composed of competitor *(Gigasporaceae)* species, ruderal *(Glomeraceae)* species, or communities with greater trait diversity (*Gigasporaceae* and *Glomeraceae* species) differentially affected SOC (Chapter 2) and host plant nutrition and growth (Chapter 3). I found that the effects of AM fungi on SOC cycling and plant nutrient uptake were associated with community life-history strategies and diversity. Although all AM fungal families contributed to fungal SOC formation, C only accumulated in the slower cycling pool in the presence of the *Glomeraceae* community, possibly due to faster necromass decomposition rates. Although there was AM fungal community-dependent variation in host nutrient concentration, no community was consistently the best at improving plant nutrient uptake. Yet, the more diverse mixed community, including both *Glomeraceae* and *Gigasporaceae* species, increased plant P the most, followed by the *Glomeraceae* community, potentially due to niche-complementarity and *Glomeraceae* species' faster growth rates. These results indicate that AM fungal life-history strategy and trait diversity differentially effect both SOC cycling and plant nutrient uptake. Below, I discuss the influence of temporal variability, future directions, and broader implications of my data.

Temporal Dynamics

The fungal communities I examined were chosen in part for their distinct growth traits, with *Glomeraceae* species having relatively faster growth, colonization, and turnover rates (Hart and Reader 2002, 2005; Staddon et al. 2003). Consequently, temporal dynamics must be considered when interpreting my findings. First, the 12.5-week period of the experiment may not have been long enough for some community effects to become significant. For instance, the contribution of AM fungal communities to total SOC and MAOC formation may appear to be relatively small after the short experimental period. However, it is key to emphasize that over time, fungal SOC contributions would continue to accumulate. In addition, the non-significant trend where the *Glomeraceae* community constantly contributed the most to total SOC and MAOC and *Gigasporaceae* the least may have grown with time as *Glomeraceae* species continued to grow faster. Similar results could be possible with plant nutrition, where differing nutrient uptake strategies may lead plant nutrient concentrations to further differentiate with time. What remains uncertain is if my observations on the rate of new AM fungal SOC accumulation can be projected linearly for longer time periods or if, instead, AM fungal SOC deposition rates accelerate or decline as communities change where they are in their life cycle. For instance, the short experimental period, likely initially favored the fast-growing ruderal *Glomeraceae* species (Hart and Reader 2002, 2005; Klironomos and Hart 2002). However, with more time, the *Gigasporaceae* species may have contributed more to total SOC formation and plant P uptake. *Gigasporaceae* species are thought to grow further away from their host's roots and invest more in extraradical hyphae compared to *Glomeraceae*. As such, given an optimal time to establish an extensive hyphal network, *Gigasporaceae* hyphae might have occupied a greater soil volume, interacted more so with soil minerals and nutrient pools, and produced

relatively more total hyphae, all of which could contribute to more P uptake and more hyphae entering the MAOC pool. Thus, while in my experiment the *Glomeraceae* community contributed the most to the slower cycling MAOC pool, it is important to consider potential limitations associated with the duration of the experiment.

Future Directions

Future work could expand upon this thesis by conducting a long-term nutrient manipulation study and examining hyphal chemistry. As described above, a long-term destructive sampling experiment would allow for monitoring changes in AM community SOC cycling or host nutrient concentration that this study may not have captured, potentially revealing more of the subtle differences between communities or time-dependent shifts. Further, experimental nutrient manipulations may help explain the mechanisms behind differing AM fungal community responses. For instance, when I consider my combined findings from Chapters 2 and 3, there appears to be a relationship between P uptake and SOC that I was unable to directly test. It is possible that soil P uptake may drive fungal SOC deposition via the symbiosis, thereby elucidating the role of AM fungal community diversity on SOC cycling. Further, the mixed-trait communities synergetic and middling nutrient effects may be driven by active verses passive nutrient scavenging. As such, a study manipulating the spatial distribution of soil P relative to host roots or altering limiting nutrient concentrations could provide insight into the relationship between AM fungal communities' nutrient uptake strategies and C inputs.

Additionally, it would be beneficial to examine AM community hyphal chemistry and biomass to determine if hyphal compounds or nutrient concentrations vary with functional traits, and if so, if they influence the fate of new AM fungal C inputs. Similar to faster growing plants, I hypothesize that *Glomeraceae* species' hyphal biomass would have relatively more microbially

available compounds, which would encourage faster decomposition rates and thus C accumulation in the MAOC pool (Donaldson, Kruger, and Lindroth 2006). If true, this could explain why the *Glomeraceae* community was the only community to contribute to MAOC formation in my study. A comparison of community hyphal molecular chemistry or phenolic:N ratios may be a helpful next step to determine if hyphal chemistry varies with AM life-history strategies and can partly explain the differences I observed in fungal MAOC production.

Broader Implications

Considering that most terrestrial plants associate with AM fungi, the findings of this study that show community-dependent effects on both SOC and plant nutrition are relevant for supporting and understanding above and below ground ecosystem services and ecology (Brundrett and Tedersoo 2018). As AM fungal communities are abundant across different environments, knowledge of community-dependent variation could be used to explain and predict patterns of SOC formation and retention (Jansa et al. 2002; Oehl et al. 2011). This could be valuable information for climate change mitigation as soils are large C reservoirs and soil fungal communities are an important source of this C (J. Lehmann and Kleber 2015; Paustian et al. 2016). Additionally, understanding how differing AM community life-history strategies and diversity affect plant nutrient uptake could lead to improved practices in agriculture and restoration ecology. Agricultural producers are increasingly using AM fungal inoculants to improve plant health, so knowing which fungal species are most conducive to improving crop growth could help the development of inoculants for increased yields. Moreover, as host specificity may be a factor in the AM fungal symbiosis, the strengths and weaknesses of different AM fungal life-history strategies could function as predictors of plant community assembly (Chagnon et al. 2013). Understanding, variation in fungal and plant community assembly could

also be valuable in landscape restoration by informing what AM fungal communities would be best at promoting the re-establishment of native plants.

General Conclusion

AM fungal life-history strategies are conserved at the family level but how they influence soil C cycling and plant nutrient concentration remains uncertain. My thesis contributed to filling this knowledge gap by showing that both plant nutrient acquisition and soil C formation are associated with AM fungal community functional trait richness and composition. More specifically, I found that only communities containing ruderal *Glomeraceae* species contributed to slower cycling MAOC accumulation, with the *Glomeraceae* community's fungal MAOC contributing 0.12% and 0.10% of total MAOC, both pre-and post-incubation, respectively. *Glomeraceae* species' faster hyphal growth rates and turnover times may be responsible for increased MAOC formation, by increasing the amount and regularity of AM fungal necromass C deposition available for saprotrophic microbial decomposition. Further, compared to *Gigasporaceae*, the *Glomeraceae* community also resulted in more aboveground host P concentrations. The *Glomeraceae* community's faster growth rates may have enabled it to acquire soil P faster than the slow-growing *Gigasporaceae* community. Importantly, the diverse mixed-trait community improved aboveground host P concentrations the most, suggesting niche complementarity. Yet, the mixed community's higher host P concentrations did not equate to the greatest plant growth, where only the *Glomeraceae* community increased both above and belowground biomass compared to the control. When comparing across all nutrients, no community was consistently best at improving Sudan grass nutrient concentrations. As 72% of terrestrial plants establish associations with AM fungi, differential impacts of AM fungal community traits could have global ramifications in agriculture, conservation, and C storage (Brundrett and Tedersoo 2018). Knowing how AM fungal communities affect plant nutrient uptake could explain variation in plant community composition across dynamic environments

and help agricultural producers manage AM fungal inoculants to improve crop health (Chagnon, Bradley, and Klironomos 2015). Further, a better understanding of AM fungal SOC formation and storage could help to understand an ecosystem's capacity to store SOC, improve climate change modeling, and potentially increase SOC retention through crop inoculation. Future research should investigate variation in AM fungal hyphal chemistry and examine whether AM fungal community effects on SOC and plant nutrition change over time or with differing soil nutrient availability.

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Supplementary Material

Table S7.1 Mean, standard deviations, and ANOVA p-values for δ ^{13}C vpdb values for plant biomass and the inoculated un-planted control soils used in the mixing model. Where "Pre" stands for pre-incubation, "post" stands for post-incubation, "SOC" stands for soil organic C, and "MAOC" stands for mineral associated organic C. Statistical significance for the model is a p-value < 0.05. Letters indicate pairwise differences between treatments ($p < 0.05$). Df = 3, n = 9 for biomass, n = 3 for control soils.

	Control	*Gigasporaceae*	*Glomeraceae*	Mixed	ANOVA p-value
		δ ^{13}C vpdb			
Pre-Incubation Total SOC	-26.0 ± 0.09	-26.1 ± 0.23	-25.8 ± 0.54	-26.0 ± 0.13	0.692
Pre-Incubation Total MAOC	-26.3 ± 0.09[a]	-26.0 ± 0.16[ab]	-25.6 ± 0.27[b]	-26.0 ± 0.11[ab]	0.0243
Post-Incubation Total MAOC	-26.7 ± 0.09	-26.5 ± 0.17	-26.5 ± 0.14	-26.5 ± 0.10	0.392
Above Ground Plant Biomass	1350 ± 210	1420 ± 240	1510 ± 250	1450 ± 280	0.591

Table S7.2 Mean percent root colonization, standard deviations, and ANOVA p-values. Statistical significance for the model is a p-value < 0.05. Letters indicate pairwise differences between treatments ($p < 0.05$). Df = 3, n = 9.

Colonization	Control	*Gigasporaceae*	*Glomeraceae*	Mixed	ANOVA p-value
Total	1.3 ± 1.1^{a}	61 ± 9.3^{b}	68 ± 6.9^{c}	75 ± 7.8^{d}	<0.0001
Arbuscules	0.59 ± 0.66^{a}	56 ± 8.6^{b}	61 ± 9.6^{bc}	70 ± 8.6^{c}	<0.0001
Vesicles	0 ± 0^{a}	10 ± 10^{b}	18 ± 8.0^{c}	22 ± 14^{d}	<0.0001

Table S7.3 Mean, standard deviations, and ANOVA p-values for AM soil C contribution to different C pools approximated by Pool AM C averages. Where "Pre" stands for pre-incubation, "post" stands for post-incubation, "SOC" stands for soil organic C, and "MAOC" stands for mineral associated organic C. Statistical significance for the model is a p-value < 0.05. Letters indicate pairwise differences between treatments ($p < 0.05$). Df = 3, n = 9, except where outliers were removed n > 7.

AM C Contributions to C Pools	Control	*Gigasporaceae*	*Glomeraceae*	Mixed	ANOVA p-value
SOC / Soil (%)	0.70 ± 0.05^{a}	0.60 ± 0.07^{b}	0.59 ± 0.05^{b}	0.62 ± 0.08^{ab}	0.007
MAOC / SOC (%)	66 ± 5.6	73 ± 11	77 ± 6.6	74 ± 10	0.106
MAOC / Soil (%)	0.46 ± 0.04	0.43 ± 0.03	0.45 ± 0.04	0.45 ± 0.04	0.278
AM MAOC / AM SOC (%)	250 ± 210	36 ± 11	37 ± 9.0	37 ± 7.1	0.142
mg AM SOC / g Total SOC	0.86 ± 1.7^{a}	2.6 ± 1.2^{b}	4.1 ± 2.44^{b}	3.2 ± 0.92^{b}	<0.0001
Pre-Incubation: mg AM MAOC / g SOC	0.49 ± 0.49^{a}	0.92 ± 0.49^{ab}	1.5 ± 0.85^{b}	1.2 ± 0.41^{ab}	0.013
Post-Incubation: mg AM MAOC / g SOC	0.41 ± 0.44^{a}	0.95 ± 0.51^{ab}	1.4 ± 0.79^{b}	1.2 ± 0.41^{ab}	0.006
AM MAOC / SOC Change (%)	3.4 ± 65	3.8 ± 25	1.4 ± 21	-3.3 ± 19	0.208
Pre-Incubation: mg AM MAOC / g MAOC	0.75 ± 0.75^{a}	1.2 ± 0.64^{ab}	1.9 ± 1.1^{b}	1.6 ± 0.52^{ab}	0.032
Post-Incubation: mg AM MAOC / g MAOC	0.55 ± 0.57^{a}	1.1 ± 0.59^{ab}	1.6 ± 0.83^{b}	1.3 ± 0.38^{ab}	0.012
AM MAOC / MAOC Change (%)	-8.7 ± 56	-15 ± 22	-8.4 ± 19	-16 ± 19	0.445
µg AM SOC C / g Soil	6.0 ± 12^{a}	16 ± 8.0^{ab}	24 ± 13^{b}	20 ± 5.1^{b}	0.004
Pre-Incubation: µg AM MAOC / g Soil	3.4 ± 3.4^{a}	5.4 ± 2.8^{ab}	8.5 ± 4.5^{b}	7.3 ± 1.9^{ab}	0.024
Post-Incubation: µg AM MAOC / g Soil	2.9 ± 3.1^{a}	5.6 ± 2.9^{ab}	8.3 ± 4.2^{b}	7 ± 2.0^{ab}	0.0111

Table S7.4 Mean nutrient concentrations (mg kg^{-} of dry tissue), standard deviations, and ANOVA p-values. Statistical significance for the model is a p-value < 0.05. Letters indicate pairwise differences between treatments ($p < 0.05$). Df = 3, n = 9, except where outliers were removed n > 7.

Nutrients	Control	*Gigasporaceae*	*Glomeraceae*	Mixed	ANOVA p-value
B	2.64 ± 0.79	3.23 ± 0.67	2.91 ± 0.77	3.07 ± 0.90	0.482
Ca	3330 ± 1210^{a}	10500 ± 3390^{b}	8440 ± 2700^{b}	9260 ± 2150^{b}	<0.0001
Cu	5.67 ± 1.66^{a}	10.6 ± 2.53^{b}	10.7 ± 4.49^{b}	10.5 ± 3.04^{b}	0.006
Fe	42.1 ± 38.5	58.4 ± 14.2	51.7 ± 26.5	47.8 ± 20.9	0.219
K	18700 ± 5550^{a}	16500 ± 7830^{ab}	10900 ± 6770^{b}	10900 ± 2120^{ab}	0.010
Mg	3150 ± 1460^{a}	6670 ± 1640^{b}	6580 ± 1820^{b}	6870 ± 1130^{b}	<0.0001
Mn	29.7 ± 10.1^{a}	53.8 ± 16.8^{b}	44.3 ± 15.4^{ab}	54 ± 21.4^{b}	0.018
N	14800 ± 2380	13800 ± 4170	10900 ± 4200	12800 ± 3540	0.214
C:N	28.3 ± 6.78	31.9 ± 9.54	37.0 ± 12.0	32.0 ± 4.53	0.323
Na	40.1 ± 13.4^{a}	137 ± 70.9^{b}	121 ± 81.1^{ab}	127 ± 88.6^{ab}	0.013
P	395 ± 162^{a}	1840 ± 442^{b}	2040 ± 475^{c}	2450 ± 325^{d}	<0.0001
C:P	1330 ± 617^{a}	236 ± 64.8^{b}	221 ± 45.8^{c}	178 ± 24.0^{d}	<0.0001
S	916 ± 277^{a}	2240 ± 479^{b}	2310 ± 715^{b}	2620 ± 830^{b}	<0.0001
Zn	11.4 ± 3.02	15.1 ± 4.51	13.2 ± 3.42	15.8 ± 4.95	0.159