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Effects of AM Fungi from Conventional and No-till Michigan Crop Fields on Plant and Soil Health

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Abstract

Centuries of conventional till (CT) management in agriculture has depleted soil organic matter (SOM) by over 50%. While only comprising 5% in most soils, SOM provides soil with fertility and productivity. To compensate for SOM depletion, producers have been forced to increase their reliance on fertilizer and irrigation to maintain yields. In the coming decades, climate change is expected to challenge food production and threaten an already fragile system. With no remaining land left to cultivate, conservation management strategies such as no-till (NT) look to restore SOM and increase the resilience of food production for an ever growing, increasingly food insecure global population. An ecological outcome of NT management is the fostering of arbuscular mycorrhizal fungi (AMF). AMF are obligate biotrophs that form symbiotic associations with most terrestrial plants. Their plant host supplies organic carbon, and in return, AMF primarily enhance the acquisition of limiting nutrients and reduce osmotic stress. AMF also contribute to SOM through a protein glomalin, found in their cell walls which is deposited into soils during hyphal turnover. We performed a controlled greenhouse experiment with populations of AMF from CT and NT soils to isolate their influence glomalin yield and on corn and soybean growth under well-watered (WW) and drought stress (DS) conditions. NT soils used for AMF inoculum contained over 4X ($p=0.01$) the AMF spores as CT soils with a distinct community. Colonization rates ranged from 71-97% in corn, 69-95% in soybean and were generally higher in NT over CT and DS over WW. For both plants, AMF had the strongest influence on shoot growth particularly under WW conditions. Corn shoot biomass was reduced by both AMF communities under WW conditions ($p=0.004$), while soybean shoot biomass was increased by NT AMF ($p=0.03$). NT inoculum produced the most glomalin under WW condition for both plants. Much

more work is required to understand the plant-fungal dynamics in agricultural ecosystems. Identifying and fostering beneficial relations between plants and AMF may be a strategy to reduce inputs while maintaining yields, increasing agricultural sustainability.

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Chapter I: An overview of the challenges in modern agriculture and the role AMF may play in mitigating these challenges.

Soil Organic Matter

"A nation that destroys its soil, destroys itself" (Pres. Franklin Roosevelt, 1937). Quality agricultural soils are fertile and productive primarily due to soil organic matter (SOM), whose primary component is soil organic carbon. SOM only comprises ~5% of most soils but is critical in forming healthy soil structure, storing nutrients required for plant growth, fostering beneficial microorganisms, and retaining water (Osman, 2012). The intensification of agricultural systems and widespread use of conventional tillage has led to a global decline in soil quality (Lal, 2009). Arable land has decreased from 0.44 ha per capita in 1960 to 0.18 ha in 2005 and is likely to decrease to 0.1 ha by 2050 from soil degradation coupled with rising population levels (FAO, 2013). Not only are we degrading the quality of our agricultural lands, but we are also actively removing agriculture from these fields. For example, between 1992 and 2012, 31 million acres of farmland were lost to urbanization in the United States (Freedgood et al., 2020). While farmland is continually degraded and replaced, the remaining land that could be cultivated is either ecologically sensitive or unsuitable for agriculture with inherently poor soil quality (Lal, 2009). Once SOM is reduced in agricultural fields from pre-cultivated levels, it takes generations to rebuild. A single inch of topsoil, that is high in organic matter where plant roots grow, can take anywhere from 100-500 years to create depending on numerous factors such as the input material, climate and organisms present in the system (NRCS: Soil Formation; Lal, 1984;

Montgomery, 2007). Globally, agricultural systems must focus on retaining the remaining SOM and employ efforts to rebuild SOM, our soils most valuable resource.

Conventional Till

For centuries, conventional tillage (CT) management practices that rely on intensive soil disturbance to prepare fields for planting have been widely practiced and are the root cause of SOM depletion in agricultural soils (Balesdent et al., 2000). CT practices turn over soils to bury crop residue and manure at average depths of 15-30 cm which in most soils is nearly the entire A-horizon (topsoil) where the majority of organic matter and microorganisms reside. CT practices are also traditionally performed to prepare soil for planting and to remove weeds that compete for nutrients and water (Morugán-Coronado et al., 2020). As of 2017, 177 million acres (63%) in the United States continue to employ tillage as part of their overall field management (USDA NASS, 2017 Census of Agriculture). SOM depletion has already surpassed 60% in the temperate regions and 75% in the tropical regions from tillage operations in agriculture (Lal, 2004). When soil is mechanically tilled, often multiple times a year, the overall structure is altered and organic carbon within soil aggregates is exposed to the environment. This soil organic carbon is then oxidized by aerobic microorganisms and CO_2 is lost to the atmosphere at rates much greater than in undisturbed soils (Al-Kaisi & Yin., 2005). CT management also contributes to overall poor soil structure which further reduces crop productivity (Baker & Saxton, 2007). It results in increased soil compaction, restricting root growth which reduces the volume of soil that plants have access to for nutrients and moisture. This compaction further increases the necessity for fertilizer and irrigation in soils with low organic matter. CT managed soils also have less aeration

and lower water infiltration rates that results in increased runoff during rain events, removing topsoil and nutrients (Baker & Saxton, 2007; Zhang et al., 2007). Continued depletion of SOM and degradation of soil structure from CT management practices in our agricultural ecosystems has forced producers to increase their reliance on artificial inputs into these systems to maintain yields and has greatly reduced the sustainability of agriculture.

Fertilizer & Irrigation

Nitrogen is the most important and often a limiting nutrient in plant growth. Nitrogen cycles through the atmosphere and can be added to soils naturally via biological fixation or artificially from the Haber-Bosch process and is considered a renewable resource for agricultural production (Alewell et al., 2020) although production requires fossil fuels. Present-day agricultural yields are only possible in continually degraded soils through the over reliance on artificial inputs, primarily fertilizer and irrigation. This practice started during the first Green Revolution of the 1960s and 1970s and the use of inputs continues to increase (Lal, 2009). For example, while corn yields in the United States have increased by only 5 times since 1940, the application of increasingly expensive nitrogen fertilizer has increased by over 12 times during the same period (Cao et al., 2018). Globally, fertilizer use has increased from 30 million tons (Mt) in 1960 to 140 Mt in 2000, while yield increases have not kept pace (Lal, 2009). Fertilizer usage is often over-reliant on nitrogen while other essential nutrients are often overlooked (Lal, 2009). Although nitrogen is the most important nutrient for plant growth (Osman, 2012) limiting other essential nutrients can result in a specific soil nutrient that limits plant growth even with excess

nitrogen. In addition, most fertilizers contain trace amounts of heavy metals and salts that are accumulating in agriculture fields, impacting crops and further degrading soil quality.

The second most important nutrient for plant growth is phosphorus (Osman, 2012; Alewell et al., 2020). Phosphorus is naturally added to soils via the slow process of rock weathering. Phosphorus fertilizer mined from mineral deposits is considered a non-renewable resource and deposits are expected to last for the next few centuries at current rates of consumption and loss via erosion. While we are currently depleting a non-renewable resource required for food production, the costs associated with applying phosphorus is also unsustainable. From 1961 to 2015, phosphorus fertilizer increased in price by 875% which is higher than background inflation rates and more than twice the increase in the value of corn over the same time period (Alewell et al., 2020). Furthermore, a significant amount of the artificial phosphorus used in agriculture goes from fields to aquatic environments where it has been linked to many negative environmental impacts including eutrophication and toxic algae blooms (Alewell et al., 2020). To ensure long term food system stability it is essential that we improve the efficiency of how we use phosphorus chemical fertilizers in agricultural systems since phosphorus is 1 of the 3 main components of chemical fertilizer and required for plant growth and yield (Osman, 2012). To increase the sustainability of food production, future systems must develop ways to increase their fertilizer use efficiency.

In order to maintain yields in agricultural soils with low organic matter and poor soil structure from CT management supplemental irrigation is required. Globally, irrigated land has increased from 100 million hectares (Mha) in 1950 to 275 Mha in 2000 (Lal, 2009). In 2017 over 58 million acres of cropland were irrigated across the United States with over 2 million acres in

Michigan alone which accounts for ~80% of groundwater usage (USDA NASS, 2017 Census of Agriculture; USDA ERS, Irrigation & Water Use). Although freshwater is a renewable resource, in 2018 it was reported that in Ottawa County, MI, USA ground water reserve consumption is occurring faster than it can be replenished (Curtis et al., 2018). Supplemental irrigation cannot be relied upon to make up for deficiencies in soil quality and is showcased by Zhang et al. (2007). This experiment compared various parameters related to water use efficiency such as water infiltration, runoff and sediment loss between soils managed by CT and more sustainable conservation till. They found that CT soils had over 10X more runoff, 49X more sediment loss and less than half the infiltration rate of soils managed under conservation practices. These differences were attributed primarily to the field under conservation practices having nearly double the soil carbon and show that changes must be made in management to improve water use efficiency. Not only is relying on supplemental irrigation unsustainable, it also can be prohibitively expensive for producers to establish and maintain. For a standard 160-acre center pivot irrigation system that covers 132 acres, the instillation cost in Michigan is approximately $78,000 with an annual depreciation of $3,300. Annual operating costs for an annual supply of 6” of supplemental irrigation costs over $15,000 or approximately $114 per acre (USDA NRCS, Irrigation). Agricultural production must not rely on more inputs into the system to make up for deficiencies caused by management practices. These inputs only treat the symptoms while we continue to perpetuate the causes. For long term food system stability, there must be a shift in the mindset of production from input focused to management focused.

Combining poor soil quality from CT management with excessive use of fertilizer and irrigation to make up for depleted soils results in serious health, economic and environmental

consequences (Withers et al., 2014). For example, nearly 50% of the nitrogen fertilizer applied in agriculture is not used for plant growth and is lost due to leaching which eventually enters aquatic ecosystems (Olivares et al., 2013). Besides the numerous ecological consequences from eutrophication from agricultural fertilizer, there is a significant economic impact as well. The United States spends approximately 2.2 billion dollars annually to correct issues from eutrophication such as the treatment of drinking water and protection of threatened species (Dodds et al., 2008). Both producers and the surrounding environment will benefit from improving the quality of agricultural soils thus allowing for reduced inputs and improved production efficiency. The looming climate crisis further increases the urgency for improving the quality of agricultural soils.

Climate Change

"The close link between soil C sequestration and world food security on the one hand and climate change on the other can neither be over emphasized nor ignored" (Lal, 2004). Climate change is expected to heavily influence crop productivity and food production. Early studies suggested that rising CO_2 levels would increase crop production however, these studies largely ignored the yield reducing factors that increase due to climatic change (Fuhrer, 2003; Goudriaan & Zadoks., 1995). For example, an increase in temperature is expected to decrease grain yield and even with a doubling of CO_2, a warming of only 1.6-4.0^{o}C will have a negative effect on yield (Amthor, 2001). CO_2 and temperature increase from anthropogenic climate change also influences a plants water use efficiency and a fields water availability (Fuhrer, 2003). Lockwood (1999) modeled an evaporation increase of 2-3% for each 1^{o}C rise in temperature.

Elevated evaporation, combined with increased frequency of extreme weather events (including droughts), means areas currently dealing with inadequate soil moisture will find maintaining productivity even more challenging in the near future. Besides increased temperature reducing yields and increased soil moisture evaporation, anthropogenic climate change is expected to increase the abundance of insect pests, alter plant disease cycles and expand the range of problematic weeds (Fuhrer, 2003). Agriculture will not only be influenced by climate change but can also be used to help mitigate its effects. As of 2018, ~39% of land globally was devoted to annual agriculture (FAOSTAT, 2020). While atmospheric carbon pools are estimated at 760 gigatons (Gt), global soil carbon is 3.3 times that at 2500 Gt (Lal, 2004). By reducing our reliance on CT practices that release CO_2 into the atmosphere and changing to practices that help accumulate soil carbon, we can both help slow the effects of CO_2 on the atmosphere and improve soil quality. Both of which are required to feed an increasing global population that already struggles with securing enough healthy food.

Hunger & Food Insecurity

The number of people affected by food insecurity globally has been on the rise since 2014 and global disruptions such as the COVID-19 pandemic worsen the issue. Current estimates are that nearly 690 million people (8.9% of world population) are hungry, and 2 billion people are food insecure which means they do not have regular access to safe, nutritious and enough food (FAO, 2020). While issues surrounding access to food disproportionately effect developing nations (FAO, 2020), in 2018 an estimated 11.5% (over 11 million) children in the United States were food insecure with over twenty-three thousand in Michigan's Ottawa county alone

(Feedingamerica.org). Unfortunately, global population trends will worsen the issue. Current population models predict a global population addition of 1 to 3 billion in the next 50 years (Bongaarts, 2009; Lutz et al. 2001) and a two-fold increase in food demand over the same time period (Tilman et al. 2002). Globally, agriculture must shift to more conservation minded practices that build SOM, reducing our reliance on artificial inputs and contributing to a more stable food system to meet the challenges of climate change and rising population levels.

No-till

The second Green Revolution must be based on the sustainable management of soil and water resources to feed a projected 9.8 billion people by 2050. This second revolution must focus on the implementation of conservation practices such as no-till (NT) to enhance SOM levels and not the continued reliance on costly and environmentally damaging artificial inputs (Lal, 2009). The primary benefit of NT is in slowing the rate of carbon oxidation, retaining higher levels of soil organic matter for crop productivity and reducing additional inputs of CO_2 into the atmosphere. Reducing oxidation and maximizing plant residue remaining after harvest results in carbon sequestration and the accumulation of SOM (Baker & Saxton, 2007). While estimates state that soils within the United States have lost 30-50% of the organic matter they contained prior to cultivation (Kucharik et al., 2001), Kimble et al (1998) estimates most can be restored in a 50-year period at a rate of 24-40 Mt C $year^{-1}$ using NT practices (Lal et al., 2003). In 2018, agriculture contributed 10% (667.7 million metric tons) of the greenhouse gas emissions in the United States (EPA, Greenhouse Gas Emissions) The widespread adoption of NT practices and subsequent carbon sequestration is one of the best ways to mitigate further climate change.

Besides SOM accumulation, NT systems have demonstrated numerous other ecological and economic benefits compared to CT systems as reviewed by Baker & Saxton (2007). An 80% reduction in fuel usage is achieved when converting from CT to NT because of less time in the field and the ability to use a smaller horsepower tractor that is more fuel efficient. While CT operations require 5-10 trips over a field within a season, NT operations only require 1-3 which saves time, money and increases the time interval between maintenance. NT practices encourage microbial and earthworm populations that help to improve soil structure, cycle nutrients and decompose plant material which adds to SOM. NT practices also improve overall soil structure, increasing both water and nutrient relations. Even after five decades of promotion, only ~6% of cropland area worldwide employs NT practices (Lal, 2009). As of 2017 in the United States, nearly 105 million acres (37%) of cultivated fields were operated under no or reduced tillage which is a 2% increase from 2012 (USDA NASS, 2017 Census of Agriculture). Unfortunately, however, only 25% of Michigan farms implement NT which is down from 2012 (USDA NASS, 2017 Census of Agriculture).

Arbuscular Mycorrhizal Fungi

One of the ecological benefits of NT is the cultivation of symbiotic arbuscular mycorrhizal fungi associations. Arbuscular mycorrhizal fungi (AMF) are an ecologically and economically important fungal group (Morton & Benny., 1990) that are present in almost all terrestrial ecosystems (Read, 1991). AMF are in in the phylum Glomeromycota, an asexual group of fungi that have been forming associations with land plants for over 400 million years (Redecker et al., 2000). Ecologically, AMF are obligate biotrophs that form a symbiotic relationship with an

estimated 74% of all plant species and obtain all their organic carbon from their host plant (Smith & Read, 2010). In return, AMF primarily enhance the acquisition of limiting nutrients (nitrogen and phosphorus) and reduce osmotic stress (Smith & Read, 2010). AMF associations also impact pollination (Cahill et al., 2008), salt tolerance (Al-Karaki et al., 2001), pest resistance (Vannette & Hunter, 2009), drought resistance (Ji et al., 2019), and reduce the toxicity of heavy metals (Ma et al., 2016). AMF also improve soil quality by contributing to SOM, which improves productivity and thus further reduces the requirement for inputs to maintain yields (Wright & Nichols, 2002). Of the various agricultural benefits associated with AMF, we are most interested in further investigating the interplay between the impact of NT and CT on AMF communities and their impact on growth, yield, nutrient acquisition and accumulation (N and P), contributions to SOM and water relations.

Nutrient Absorption

Nitrogen (N) is required by all living systems and is frequently the most limiting macronutrient in plant growth (Osman, 2012). AMF are proposed to influence the N-cycle in both spatial and temporal aspects that relate to agricultural yield and overall efficiency. First, the outcome of AMF colonization is often an increase in overall growth and root system volume (Ji et al., 2019). With larger root systems, plants are able to forage through larger volumes of soil and thus gain access to more N and other limiting nutrients. Also, when AMF colonize roots, they effectively act as an extension of the root system and translocate nutrients to plant root cells (Schachtman et al., 1998; Osman, 2012). Second, AMF can modify the physical properties of the environment by mediating soil aggregation which increases aeration and thus allows more N_2 to

enter the soil, increasing N deposition via fixation (Tisdall & Oades, 1982). Third, AMF have demonstrated the ability to modify the microbial community within the rhizosphere (Rillig et al., 2006). The assisted proliferation of N-fixing microbes combined with a decrease in competition within the root zone, may increase plant available N and subsequently biomass (Vazquez et al., 2000). A 10-week greenhouse experiment demonstrated that AMF presence was required to maintain N-fixing bacterial populations (Andrade et al., 1998). Nitrogen fixation normally occurs within the root nodules of legumes (e.g., soybean and peas), and inoculation of legumes with AMF increases their N-fixing ability (Toro et al., 1998). Finally, AMF may limit leaching and subsequent N loss in intensively managed agricultural ecosystems. Bender & van der Heijden (2015) showed that both the composition and total amount of leachate was positively influenced by AMF in a greenhouse experiment. Clearly AMF influence the ability for plants to acquire nitrogen and must be considered an important component when considering plant-N interactions.

Phosphorus is one of the most difficult nutrients for plants to obtain and second most limiting macronutrient for plant growth. Making up about 0.2% dry weight of plants, phosphorus is often abundant in soils although in forms unavailable to plants (Schachtman et al., 1998). Roots colonized by AMF can have an influx of phosphorus 3 to ten times greater than non-colonized roots (Schnepf et al., 2008; Smith & Read, 2010). AMF hyphae have high-affinity P transporters that are similar in structure and function to ones found in plants which allows AMF to uptake P and transfer P along their hyphae into plant root cells (Schachtman et al., 1998). It is estimated that in agricultural field conditions a reduction of 80% of the recommended phosphate fertilizer could be replaced by inoculation of AMF (Jakobsen, 1995). Therefore, AMF

should be considered a critical aspect of sustainable agriculture in helping to maximize fertilizer (N & P) use efficiency, allowing for reduced reliance on these environmentally damaging and costly inputs.

Osmotic Relations

AMF influence a plants osmotic regulation, and a plants ability to resist and recover from droughts. For crop plant productivity, drought is considered the most detrimental environmental stress (Lambers et al., 2008) and is predicted to increase in both severity and frequency due to global climate change, especially in areas already subject to drought (IPCC, 2019). Osmotic stress limits plant growth and negatively impacts both yield quantity and quality (Zhu et al., 1997; Osman, 2012). AMF symbiosis can decrease plant osmotic stress by increasing water use efficiency; thus reducing a producer's reliance on irrigation (Augé, 2001). AMF hyphae are able to penetrate soil pores and transport water to the plant that root hairs alone cannot access effectively increasing a plants root zone (Ruiz-Lozano & Azcon, 1995). AMF colonization also alters the osmoregulation of the plant through the interaction of inorganic ions and uncharged organic compounds (Goicoechea et al., 1998; Kubikova et al., 2001). This indirect interaction is responsible for postponing declines in leaf water content after the onset of drought conditions (Dixon et al., 1994) and also helping leaf water content return to pre-drought levels quicker than non-mycorrhizal plants (Subramanian et al., 1997). Although not as thoroughly investigated as leaf water content, root water content appears to be positively influenced by AMF colonization also (Ruiz-Lozano & Azcón, 1995; Goicoechea et al., 1996).Therefore, AMF can play a vital role in decreasing the dependency on irrigation in agricultural ecosystems while soils are regenerated

by NT practices. AMF can also help during periods of drought and high temperatures predicted to increase in severity from global climate change in the coming decades.

Glomalin

In agricultural systems, AMF also indirectly contribute to SOM and soil structure via the protein glomalin found in their hyphal cell walls (Tisdall & Oades, 1982; Wu et al., 2014). Glomalin is a glycoprotein (Wright & Upadhyaya, 1996) that is released into the soil during hyphal turnover, after the death of AMF (Driver et al., 2005). Glomalin is a water insoluble, heat resistant molecule with homologous sequences to plant heat shock protein 60 (Gadkar & Rillig., 2006). Glomalin is a stable compound with a glue-like nature and likely hydrophobic in its native state (Wright et al., 1996, 1999; Wright & Upadhyaya, 1998). Glomalin is also a ubiquitous compound that is 2-3X more common in the A horizon (topsoil) of the soil profile, although it can be found at depths of 140cm (Rillig et al., 2003). Do to glomalin's ubiquitous and stable nature, it has been proposed that glomalin comprises a large part of soil organic carbon and is a major component of SOM (Wright & Nichols, 2002). Like other sources of soil organic matter, tillage is detrimental to glomalin levels. Tilled fields have lower levels of glomalin and total carbon (Wright et al., 2007), but glomalin concentrations can double in only three years after switching from CT to NT management (Wright et al., 1999) suggesting a rapid improvement in soil quality from glomalin accumulation when switching to conservation management. Therefore, it is clear that AMF play a crucial role in retaining and adding to soil organic matter and should be considered a major component of carbon sequestration in agricultural systems managed with NT practices.

AMF Unknowns

Although AMF colonization is often viewed as a benefit to the plant (Smith & Read, 2010) the plant-AMF interaction is context dependent and is heavily influenced by numerous biotic and abiotic variables (Hoeksema et al., 2010) especially in agricultural ecosystems. Recent advancements in the molecular identification of fungal species (Raja et al., 2017) has demonstrated increased host specificity in AMF (Öpik et al., 2009) and greater variation in functional traits between AMF species (Gamper et al., 2010) than previously thought. While biodiversity may act as a buffer against stresses (Hector & Bagchi., 2007) and AMF species may complement one another within the same root system (Koide, 2000; Maherali & Klironomos., 2007) much more work needs to be done to uncover this interplay in agricultural systems. Some studies have shown AMF diversity to increase parameters of plant growth (van der Heijden et al., 1998; Gustafson & Casper., 2006; Jansa et al., 2008; Hoeksema et al., 2010). While others have demonstrated that plant biomass was greater when inoculated with a single AMF species, rather than a mixture of species (Mickelson & Kaeppler., 2005; Jansa et al., 2008). Furthermore, artificial selection pressures in agriculture heavily influence AMF species composition (Smith & Read, 2010). Tillage decreases AMF species diversity (Jansa et al., 2002; Menendez et al., 2001) while high levels of fertilizer have resulted in decreased AMF root colonization and caused AMF to store nutrients instead of transferring them to the plant (Mäder et al., 2000; Nijjer et al., 2010). Understanding how farming practices affect AMF populations and how subsequent AMF communities affect soil and plant health is essential in establishing and managing future sustainable agricultural systems.

Chapter II:

Introduction

Managing agricultural soils with conventional tillage (CT) has degraded global soil quality which threatens food system security (Lal, 2009). CT practices that physically disturb topsoil in an effort to reduce weed pressure and bury plant residue, also causes soil carbon oxidation at rates much higher than in undisturbed soils (Morugán-Coronado et al., 2020). Soil carbon is the major component of soil organic matter (SOM) and once oxidized, it is lost to the atmosphere as CO_2 (Al-Kaisi & Yin, 2005). Although SOM only makes up a small percentage of most soils, it is this vulnerable component that provides soil fertility and productivity (Osman, 2012). In the relatively short period of time since Europeans first cultivate soils in North America for agricultural production, up to half the SOM has already been depleted (Kucharik et al., 2001). Since CT practices deplete our soils most valuable resource, producers rely more and more on increasingly expensive and environmentally damaging artificial inputs such as fertilizer and irrigation to maintain yields in SOM depleted soils (Lal, 2009). Our current fragile food system is going to be increasingly threatened by climate change in the coming decades. Increased global temperature is expected to reduce the production of grain crops (i.e., corn, rice, and wheat), increase evaporation of soil moisture and increase the frequency and intensity of spring floods and summer droughts (Lockwood, 1999; Amthor, 2001; IPCC, 2019). While climate change is going to challenge food system stability, population levels are going to rise while the number of people food insecure is also expected to rise (FAOSTAT, 2020). There is no more land left to cultivate (Lal, 2009), we must improve the quality of the land already under cultivation to meet

the challenges of a changing climate and to feed an ever growing, increasingly hungry population.

Fortunately, conservation management practices that look to build soil quality and improve agricultural sustainability exist. In particular, no-till (NT) practices that reduce soil disturbance and subsequent oxidation of soil carbon are shown to build SOM. NT practices also improve soil structure which improves water and nutrient relations and retains plant residue which benefits soil microbes that further help in building organic matter (Baker & Saxton, 2007). While there are several decades of research into the benefits associated with NT practices, only ~6% of the cropland globally is managed in this way (Derphsch, 2007). In 2017, only 25% of Michigan cropland was managed by NT practices which was down from 2012 and 12% lower than the national average (USDA NASS, 2017 Census of Agriculture). In speaking with local soil conservationists in Ottawa county Michigan, encouraging producers to adopt NT practices is among their top priorities (Varboncoeur, 2019). In our study, we investigated how populations of arbuscular mycorrhizal fungi respond to NT management and then how these unique communities influence plant growth and soil health.

Arbuscular mycorrhizal fungi (AMF) are present throughout all terrestrial ecosystems (Read, 1991), pre-date the evolution of land plants (Redecker et al., 2000) and form associations with the roots of most plants (Smith & Read, 2010). They obtain their carbon from plants and in return, primarily enhance the acquisition of limiting nutrients (N & P) and improve water relations (Smith & Read, 2010). AMF also directly contribute to SOM via glomalin, a cell wall protein that is added to soils during hyphal turnover (Tisdall & Oades., 1982; Driver et al., 2005). Glomalin is a stable glycoprotein that is estimated to make up ~33% of soil organic carbon, the

main component of soil organic matter (Wright & Nichols, 2002). Besides contributing to a large portion of SOM, glomalin also improves soil structure and correlates strongly with soil parameters related to positive water and nutrient relations such the abundance of water stable aggregates (Wright et al., 2007; Zhang et al., 2007). AMF communities in our agricultural soils have the potential to help improve agricultural efficiency by reducing the reliance on fertilizer and irrigation while also improving soil quality via glomalin accumulation.

While AMF generally have a positive influence on plant growth, the relationship is highly context dependent in agricultural ecosystems (Hoeksema et al., 2010). Tillage (Jansa et al., 2002) and fertilizer (Mäder et al., 2000; Nijjer et al., 2010) significantly influence AMF population dynamics in terms of relative species abundance and presence. CT practices generally result in a reduction of AMF diversity and species density as compared to NT practices (Castillo et al., 2006; Verzeaux et al., 2017). Not only do management practices influence AMF communities, but crop cultivars also display significant variation in growth patterns when colonized by different AMF communities (Kabir et al., 1994). This is primarily because these modern high yielding cultivars were developed without considering the plant-fungal interaction (Lehman et al., 2012) and therefore some do well with AMF and others are negatively impacted (Sajedi et al., 2010; Verbruggen et al., 2012). Recent studies that have shown more functional diversity between AMF species (Gamper et al., 2010) and more plant-fungal specificity (Öpik et al., 2009) than previously recognized. Therefore, how agriculture practices like NT and CT impact AMF populations and how those manipulated AMF populations influence crop growth is still relatively unknown.

To uncover how AMF communities are influenced by CT and NT management practices, and how these subsequent populations influence crop growth, we performed a controlled greenhouse experiment to isolate the influence of AMF. We chose to investigate AMF's influence on both corn (*Zea mays L.*) and soybean (*Glycine max L.*) as these plants are the two most widely grown and economically important crops in the United States. As of 2017, there were ~180 million acres of corn and soybean grown throughout the country and 90% of that land was annually rotated between the two crops (Figure S1). The third most widely grown crop, wheat, only accounted for ~38 million acres (USDA NASS, 2017 Census of Agriculture). The implementation of crop rotations increases yield, reduces pest and disease pressure, and improves the efficiency of nutrient uptake (Selim, 2019). We also chose to investigate the relationship under well-watered and drought stress conditions. We followed a standard watering regime (Marulanda et al., 2003; Verbruggen et al., 2012) where all plants receive equal and adequate water for a period of time before half of the plants are subjected to drought stress. These conditions mimic the typical conditions found in Michigan, USA with wet springs followed by dry summers that are expected to increase in severity due to climate change in the coming decades (IPCC, 2019). Since these two crops dominate agriculture and are grown in the same soils, it is critical to investigate both when considering plant-fungal dynamics as a result of management practice.

We expected; (1): The NT soil would contain a higher density and diversity of AMF species, (2): The AMF community from our NT soil would improve plant biomass measures and nutrient uptake in our greenhouse experiment over the AMF community from CT soils

particularly under drought-stress conditions, (3): Glomalin production would be greater for pots with NT than CT inoculum and both would be higher than control pots.

Materials & Methods

Field Spore Inoculum

We sampled adjacent CT and NT fields on June 16th and 17th located in Zeeland, Michigan (42.8125N, 86.0187W). Both farms were managed under a corn-soybean rotation for the previous 15 years, neither site received supplemental irrigation, and both contained sandy loam soils of similar texture (Table S1). To account for differences in field size, we sampled 3-acre subjects within each field (Figure S2). We collected soil samples from 18 locations across each field following composite random sampling (Soil Survey Manual, 2014). Briefly, this technique attempts to spread sample locations evenly throughout a site and focus on the uniform topography. Areas within the field that do not represent the majority of the field are avoided or sampled separately. We collected soil cores to a depth of 20-cm, using a 5-cm diameter PVC pipe. Immediately after taking each core, we transferred the soil to zip-lock bags and then placed the bags on ice within a cooler slow microbial metabolism and thus preventing spore degradation (Soil Survey Manual, 2014). After collection, we air dried soil samples at room temperature (20°C) for 2 weeks (Verbruggen et al., 2012). We pooled the air-dried soil cores within fields which were then used as AMF inoculum in our greenhouse experiment and to determine spore abundance.

West Virginia Universities' INVAM Laboratory was referenced for the following spore extraction procedure (INVAM: https://invam.wvu.edu/methods/spores/spore-extraction). We first added 200 g of dried pooled soil to the top of a mesh sieve stack (top to bottom: 4000, 2000, 500, 250, 125 & 63 µm) with a capture tray on the bottom. We then added the mesh sieve stack to an electric sieve shaker and agitated the soil for 15 minutes, separating soil particles and

AMF spores based on size. After shaking, we collected the remaining material from the bottom catch and 3 sieves (250, 125 & 63 µm), then mixed them together. To separate spores from soil, we used 20 mL (10 mL each) of a 20%/60% table sugar concentration gradient added to 50 mL centrifuge tubes taking care to keep the solutions separated once poured. Then we added 15 mL of sieved mixed soil to each 50 mL tube and centrifuged (3 min, 960g). We then passed the supernatant containing AMF spores over a 125 µm sieve which was placed over a 20 µm sieve. We then collected 125-500 µm spores and material from the 125 µm sieve and 20-125 µm spores and material from the 20 µm sieve, forming two size classes that were analyzed separately. The collected spores and small particles from the 2 sieves were added to separate petri dishes with deionized water and kept at 4^{o}C until analysis.

To count spores, we used a gridded paper with 3.175 mm squares laid under each Petri dish. Spores within a total of 12 randomly chosen squares per dish were counted under 8x magnification. This resulted in 120.97 mm^2 of area observed from each dish. Knowing the overall area of each Petri dish (5672 mm^2), we then multiplied our count by 46.9 (5672 ÷ 120.97) to estimate the number of spores within 200g of soil. Examples of AMF spores are shown in Figure S3.

Experimental Design

Our greenhouse experiment consisted of randomized block design comprised of three inoculation treatments (control, CT and NT) and two irrigation treatments (well-watered and drought) forming six treatment groups. Six replicates of each treatment were performed for both corn and soybean, totaling 72 pots (36 corn and 36 soybean) with one plant per pot (Figure 1).

Greenhouse Growth

We started by sterilizing via autoclaving at 121°C for 30 minutes both our potting soil (BFG Grower Select M.1) and quartz-sand separately (Marulanda et al., 2003). We then scrubbed and cleaned our pots (4.7L) with water, followed by 70% ethanol to remove any residue from the manufacturing process. After cleaning, we added river rocks to the bottom of each pot to prevent our soil-sand mixture from escaping through the drainage holes while still allowing for water drainage. Equal amounts by weight (0.750 kg) of sterilized potting soil and quartz-sand were then mixed and added to each pot (1.5 kg total). The resulting soil-sand mixture physical and nutrient parameters are summarized in Table S1.

Three seeds of either corn (Blue River, Brand: 26B78, Variety: 1719858) or soybean (Zeeland Farm Service 1821) were sown to their respective pots separated by 5 cm at depths of ~5 cm for corn and ~3 cm for soybean using a market dowel (Thelen, 2012; Staton, 2016). Our chosen cultivars are grown throughout West Michigan under both CT and NT operations as discussed with a Ben Glass, a representative of Zeeland Farm Services, whose company distributes crop seeds throughout West Michigan (B. Glass, personal communication, June 18, 2019). After sowing, we added 37.5 g (2.5% by weight) of AMF soil inoculum over the top of the seeds to ensure physical contact (Marulanda et al., 2003). For the control groups we added 37.5 g of the sterilized soil-sand mixture instead of AMF inoculum. Once ready, we placed each labeled pot into its assigned location within the randomized block. Corn and soybean plants were in separate 3x12 grids.

We measured soil moisture with a meter (Vegetronix VG-200 Meter), which measures volumetric soil water content. Volumetric soil water content (VSWC) is the ratio between the volume of water within a sample and total volume of the soil sample, expressed as a percentage (Marulanda et al., 2003). For example, dry soil would be near 0% while waterlogged soil would be closer to 100%. We took measurements near the center of the pots at a depth of 9 cm for proximity to the root zone and to avoid higher fluctuations at the soils surface.

The greenhouse experiment started on June 24th. Soil moisture was below 3% for all pots prior to watering. To encourage germination, we initially soaked all pots for 10 seconds for three consecutive rounds separated by 30 minutes. This resulted in all pots having a VWSC above 30%. The watering regime we followed was similar to previous studies (Marulanda et al., 2003 and Verbruggen et al., 2012), in which all pots receive equal watering for a period of time before drought conditions are induced for half of the plants. This also reflects a wet and cool spring followed by warm and dry summer that is typical of Michigan which is expected to increase in severity in the coming decades due to climate change (EPA: What Climate Change Means for Michigan, 2016: https://19january2017snapshot.epa.gov/sites/production/files/2016-09/documents/climate-change-mi.pdf).

For 30 days after seeding, all pots received equal water on 11 occasions spread evenly across the period (Marulanda et al., 2003 and Verbruggen et al., 2012). Moisture measurements however were only recorded on 8 of those days due to self-quarantine actions taken by our research team. During this period, Grand Valley State University's greenhouse manager Christina Hipshier graciously maintained the experiment. We recorded soil moisture measurements and watered plants between 10a.m. and noon for the entirety of the experiment. We recorded soil

moisture levels each day prior to watering, which gave the most amount of time for the soil moisture levels to stabilize.

Returning from self-isolation 14 days after seeding, both corn and soybean displayed over 99% emergence. Only one corn (#34) and one soybean (#43) failed to emerge. All soybean plants were in growth stage V2, while corn plants were in late V3 or early V4. Typically thinning is performed within days of emergence while plants are young and root systems can be removed. Attempting to remove our more developed plants would have resulted in damage to the root system of the remaining plant. Instead, plants were cut at the soil surface with shears and root systems were left in place. Although not ideal, no regrowth was recorded and upon harvest the small root systems were nearly decomposed. Thinning resulted in a final spacing for both corn and soybean plants of ~35 mm between rows and ~23 mm within rows. After 30 days of growth, where all plants received adequate and equal moisture, we induced drought conditions for half of the plants and these conditions remained until harvest.

To confirm soil moisture levels we performed an analysis of variance (ANOVA) and found pre-drought soil moisture levels were not different between soybean groups ($F=1.64$, $p=0.149$) but were between corn groups ($F=4.891$, $p=0.000262$). A post hoc TukeyHSD test showed that there was a difference between corn groups C&B ($p=0.0019$) and D&B ($p=0.0023$). Pre-drought soil moisture levels are shown in Figure S4. This unfortunate difference is recognized as a potential source of error within the experiment although the variation is minimal.

Moisture levels after inducing drought conditions were ideal. Moisture levels were different between water treatment groups for both corn ($F=219.5$, $p<2e-16$) and soybean

(F=82.36, p,2e-16). TukeyHSD post hoc tests confirmed no differences within water treatment groups and significant difference between for both corn and soybean plants (Fig S5).

We harvested soybean plants 54 days after seeding and corn plants 57 days after seeding. Corn and soybean were harvested on different days due to time constrains and workload. Corn plants received one more day of watering than soybean on day 54. The above ground biomass from each plant was removed 2.5 cm above the soils surface with shears and air dried for at least 2 weeks at 25^{o}C. Root systems were carefully removed from pots and soil was separated by agitating the roots in a water bath. After removing the loose soil, the remaining soil particles were removed with tweezers and the nodules on soybean roots were collected and dried. While harvesting and preparing roots to dry, we collected a small section (~0.5 g wet weight) of lateral root fragments from each root system and placed them into 50 mL tubes containing 70% ethanol where they were stored at room temperature. We used these root fragments to determine AMF root colonization. After measuring various growth and development parameters, we pooled dried leaf tissue within groups and sent it to A&L Great Lakes Laboratories for nutrient analysis. Finally, we collected 50 g of soil from each pot, pooled the soil within groups and air dried for one week at 20^{o}C in aluminum baking pans. The air-dried soil was used to determine glomalin concentrations.

To determine root colonization, we followed (Penn State: https://plantscience.psu.edu/research/labs/roots/methods/methods-info/staining-of-mycorrhizal-fungi). We first cleared roots by removing them from the 70% ethanol, placing them into a 10% KOH solution and autoclaving for 15 minutes or until the roots were transparent and white. Larger diameter root fragments required a second round of autoclaving to remove a

yellow tint. After clearing, we washed them with tap water 3 times to remove residual KOH and then placed them in 5% HCl for 1 minute. After one minute, we poured out the HCl and added trypan blue stain. Root fragments remained in the stain for 24 hours to absorb the stain fully. To quantify colonization, we used the gridline intersect method (McGonigle et al., 1990). Similar to spore enumeration, we placed stained roots into Petri dishes above 3.175 mm (1/16th inch) grid paper where they were observed under a microscope. We counted the first 25 root segments that crossed a grid line below and noted whether or not there was also fungal hyphae that originated from the section of the root above the line. For example, if 20 of the 25 counts also contained fungal hyphae, the root colonization percentage would be 80%. Examples of roots colonized with AMF are shown in Figure S6.

To extract and quantify glomalin from soils we followed the prevailing protocol (Wright & Upadhyaya., 1996; Rosier et al., 2006; Janos et al., 2008) of autoclaving 1 g of soil in 8 mL of 20 mM sodium citrate in 50 mL centrifuge tubes for 30 minutes. Immediately after autoclaving, we centrifuged the tubes at 3320g for 20 minutes and the supernatant was immediately poured into separate tubes and stored at 4^{o}C until analysis.

To determine glomalin concentrations, we mixed 0.05 mL of supernatant with 1.5 mL of room temperature Coomassie Plus Reagent^{TM} (ThermoFisher Scientific) and placed the mixture into a spectrophotometer set to 595 nm. Between samples, we zeroed the machine by reading a cuvette filled with distilled water. We recorded absorbance values for each sample and protein concentration was determined by comparing against a curve of diluted albumin (BSA) standards of known concentrations.

Statistical Analysis

Despite efforts to transform field spore data, they remained heteroscedastic; thus, non-parametric tests (Wilcoxon Sum Rank Test) were used. All remaining greenhouse related data were subjected to either one or two factor analysis of variance (ANOVA) followed by TukeyHSD post hoc testing. One-factor ANOVAs were used to compare between inoculum treatments (control, CT and NT) within one of the water treatments (well-watered or drought). Two-factor ANOVAs were used when comparing between both water and inoculum treatments. Statistical significance was determined at the 5% probability level. Normality was assessed via the "shapiro_test" function and heteroscedasticity was assessed via the "levene_test" function in RStudio. Outliers remain in figures and did not change significance of results when removed. All analyses were conducted in RStudio v1.2.5033 (RStudio Team, 2020).

Results

Inoculum Spore Count

18 soil cores taken from each the CT and NT fields were pooled and used for spore extraction and enumeration. Overall, spore density was over four times higher in NT soils than CT soils (Wilcoxon Sum Rank test, W=80.5, p=0.01032). Specifically, NT soils contained 155% more spores 125-500 µm size class and 488% more spores in the 20-125 µm size class than CT soils (Fig 2). Issues with heteroscedasticity were not corrected via data transformations, thus non-parametric tests were used.

Corn Biomass

AMF CT and NT inoculum treatments resulted in a significant reduction in both shoot and total biomass for corn plants under well-watered conditions as compared to the control (Fig 3.) Shoot biomass under well-watered conditions was 30.3% and 26.4% less than control plants for NT (p=0.005) and CT (p=0.01) inoculum treatments respectively. Total biomass under well-watered conditions was reduced by 28.7% and 25.4% as compared to control plants for NT (p=0.005) and CT (p=0.01) inoculum treatments respectively. Overall, corn biomass was reduced by AMF inoculum treatments for both water conditions and resulted in a reduction in shoot to root ratio. No corn biomass parameters measured were significantly different between our CT and NT AMF inoculum treatments.

Soybean Biomass

Soybean shoot, root and total biomass was increased by AMF inoculum under well-watered conditions and reduced for plants subjected to drought (fig 4). Shoot biomass under well-watered conditions was significantly increased by 29.0% from the NT ($p=0.03$) inoculum treatment as compared to the control. Under drought stress conditions, both root ($p=0.0004$) and total ($p=0.003$) biomass were significantly lower for NT inoculated plants compared to control. As compared to the control group, NT inoculum resulted in a 40.2% reduction in root biomass and 25.0% reduction in total biomass. Under both water conditions, AMF inoculum caused an increase in the shoot to root ratio of soybean plants. No soybean biomass parameters measured were significantly different between our CT and NT AMF inoculum treatments.

Soybean Root Nodules & Bean Pods

Under well-watered conditions, the production of root nodules (42.8%, $p=0.01$) and bean pods (113.3%, $p=0.01$) was significantly increased by CT inoculum over non-mycorrhizal control plants (Fig 5). Under drought stress conditions however, there was no significant difference in either root nodule or bean pod production.

Root Colonization

Under well-watered conditions, root colonization was not significantly different between CT and NT inoculum for either corn or soybean plants. Under drought stress conditions however, NT inoculum resulted in significantly higher colonization rates than CT inoculum for both corn ($p=0.0001$) and soybean ($p=0.0003$) plants. For corn plants subjected to drought stress, NT

inoculum resulted in 24.6% more colonization than CT inoculum. For soybean plants under drought stress, the difference was marginally higher at 28.0% (Table 1). Control group root colonization was less than 30%.

Plant Nutrients

Plant nutrients are shown in table 2. Under well-watered conditions, corn plants within the control group contained more nitrogen and phosphorus than plants within the CT and NT groups. Under drought conditions, corn plants in the control group contained the most nitrogen while plants within the CT group contained the most phosphorus.

Under well-watered conditions, soybean plants within the NT inoculum group contained more nitrogen and phosphorus than plants within either the control or CT groups. Under drought stress conditions, soybean plants in the CT group contained the most nitrogen while plants within the NT group contained the most phosphorus.

Glomalin Production

Glomalin yield for corn and soybean plants grown for 57 and 54 days respectively, was increased by both AMF inoculum treatments as compared to control (Fig 6). Under well-watered conditions, NT inoculum resulted in a significant glomalin yield of 16.3% for corn ($p=0.01$) plants and 22.2% for soybean ($p=0.0007$) plants as compared to the control group. For corn plants subjected to drought conditions, glomalin yield was increased by 7.5% and 16.3% as compared to the control groups for NT ($p=0.03$) and CT ($p=0.01$) inoculum treatment groups respectively. While glomalin yield was increased by both AMF inoculum treatments for soybean plants

subjected to drought conditions they were not significantly different than the non-mycorrhizal control group.

Discussion

Inoculant Spore Count

In comparing CT and NT soils from adjacent sites in Ottawa Michigan, NT soils contained over 4X the spore density as CT soils. This result is consistent with previous studies that have demonstrated a higher spore density in NT versus CT fields (Castillo et al., 2006; Verzeaux et al., 2017) often attributed to the fact that CT operations physically disturb soil communities and result in a decrease in overall abundance of AMF (Jansa et al., 2002; Menendez et al., 2001). The 4X increase is higher than the 2X overall increases reported by Verzeaux et al. (2017) in a six-year field experiment in Northern France, and as reported by Castillo et al., (2006) in a 2-year study in Quebec Canada. Furthermore, the large difference in CT and NT spore density is noteworthy because while AMF spore density is generally higher in NT soils, many investigations have found no significant difference between NT and CT soils (Schalamuk et al., 2006; Curaqueo et al., 2011; Hu et al., 2015). In our study system, there are 2 possible explanations for the 4X difference in spore density between NT and CT sites. First, both fields were under continuous management for at least the previous 15 years which is longer than the studies mentioned above. The longer the period of time under continuous management the further apart these communities can be driven apart. Second, both sites receive fertilizer which has been shown to increase spore density in NT systems and cause a decrease in CT systems (Verzeaux et al., 2017).

Not only did NT and CT soils vary in spore density but also in the composition of spores found in the soil. NT soils contained 488% more spores from the smaller size class (20-125 µm) while there was no significant difference in the abundance of spores in the larger size class (125-500 µm) (Fig 2). Spore diameter is a morphological marker for species identification (INVAM:

https://invam.wvu.edu/methods/spores/enumeration-of-spores). For example, spores from the groups Acaulospora (<120 µm), Archaeospora (120-250 µm) and Gigaspora (>250 µm) do not overlap in size and thus spore size can help distinguish between species. Not only do studies find higher AMF species richness in NT sites, but they also find significant differences in the relative abundance of individual AMF species (Franke-Synder et al., 2001; Menéndez et al., 2001). In comparing adjacent CT and NT sites, Menéndez et al. (2001) not only found that AMF species relative abundance was significantly different between sites, but some species were missing from the CT site that were found in the NT site and vice versa. Therefore, although species level identification was not conducted, it is reasonable to conclude based on the size distribution of spores that the NT and CT AMF communities used for inoculation most likely varied in both species' composition and relative abundance of species between sites.

Corn Biomass

AMF inoculum from our CT and NT soils caused an overall reduction in corn biomass under both water treatments as compared to the non-mycorrhizal control groups and had a stronger, more negative influence on shoot than root growth (Fig 3). Similar to our finding, Verbruggen et al. (2012) demonstrated that inoculum containing AMF from organic and conventional agricultural fields significantly decreased corn biomass compared to control non-mycorrhizal plants. However, Sajedi et al. (2010) found that AMF had little influence on corn growth, yield and nutrient accumulation compared to the non-mycorrhizal groups when grown under field conditions. Furthermore, Jeong & Lee. (2006) demonstrated that some AMF species improve inoculated corn growth, and others were no different than the non-mycorrhizal control.

In our study there are three potential reasons for the general reduction in growth from AMF symbiosis. First, the soil nitrogen concentration in our greenhouse media was approximately half that found in both the CT and NT fields. AMF have a high nitrogen demand and can compete with the plant at low nitrogen soil levels and decrease plant growth (Hodge & Fitter., 2010). However, even in soils with high nutrient concentrations, such as those often found in agricultural systems, AMF can also detract from plant growth (Johnson et al., 2015). Second, the outcome of AMF colonization on growth depends on the specific corn cultivar and the specific AMF community (Kabir et al., 1994; Liu et al., 2000). This is further complicated because modern high yielding crop cultivars were developed to grow under high soil nutrient conditions in conventional agriculture (Lehman et al., 2012). Third, due to the arrangement of the greenhouse, corn plants did not receive direct sunlight until between 10:00am and 11:00am during their 57 days of growth, most likely decreasing their "normal" photosynthetic output. The AMF draw on photosynthetic carbon is estimated to range between 5 and 20% (Douds et al., 2000) which may have been enough in this context to reduce growth as compared to the non-mycorrhizal plants. Therefore, it is impossible to rule out whether soil and environmental variables or a potential mismatch between our cultivar and AMF explain the decrease in biomass.

In comparing the influence that our CT and NT AMF communities had on corn growth there was no significant difference in shoot, root or total biomass under both water treatments (Fig 3). Similar to our results, the two other studies comparing whole AMF communities derived from soils under different management practices report only marginal differences in corn growth (Bender et al., 2019; Saia et al., 2020). However, a recent meta-analysis by Zhang et al., (2019)

found that AMF had an overall positive influence on corn yield (13% increase). There is abundant data that suggests the results of AMF colonization are dependent on numerous biotic and abiotic factors (Hoeksema et al., 2010). For example, Pinos et al. (2019) found that a single AMF species varied in its influence on the growth pattern of a particular corn cultivar when plants were grown in different soil types. Haro et al. (2020) found that whether corn biomass was improved by AMF depended on the specific AMF species. Furthermore, in a greenhouse experiment Liu et al. (2000) found that 1 of the 3 corn varieties had a significant increase in the shoot to root ratio when inoculated from soil under conservation management. Moreover, the increase in the ratio was driven by a significant increase in shoot biomass (15.3%) which was opposite to our result of a decrease in shoot biomass. Further studies are required to more fully understand corn-AMF interactions in agricultural ecosystems if the ultimate goal is to use this symbiotic relationship to further improve agricultural sustainability. In particular, breeding programs should look to develop varieties that form positive AMF associations while still being high yielding. Positive AMF associations in the form of enhanced nutrient uptake and improved water relations (Smith & Read, 2010) would allow producers to reduce their reliance on fertilizer and irrigation while maintaining yields by taking advantage of these ubiquitous soil fungi.

Soybean Biomass

Our AMF communities had opposite effects on soybean biomass when grown under well-watered verse drought stress conditions. Under well-watered conditions, both AMF communities caused an overall increase in biomass as compared to the non-mycorrhizal control group. This increase was driven by a significant increase in shoot biomass while root biomass was only

marginally different. Previous studies have also found that soybean growth was improved by AMF as compared to non-mycorrhizal plants under varying moisture and phosphorus levels (Wang et al., 2011 and Adeyemi et al., 2020). However, Grümberg et al. (2015) found that AMF caused shoot and root biomass decreases in soybean under well-watered conditions. As with corn, the differences in studies could be due to both different AMF communities and soybean cultivars. Nevertheless, the increase in shoot biomass is a positive to farmers who want to maximize output while minimizing input. The fact that roots did not increase suggests the fungi are really preforming the function of roots and providing a major benefit to the plant.

Under drought stress conditions both AMF communities caused an overall decrease in total biomass, driven by a significant reduction in root biomass while shoot biomass was only marginally different (Fig 4). Grümberg et al. (2015) found there was no significant difference in plant biomass for soybean plants subjected to drought stress. Again, the reduction in root biomass suggests fungi providing a real benefit while allowing plants to allocate nutrients where the farmers want it. So, while more research needs to be conducted, it is clear that for soybeans, AMF benefit farmers even under drought conditions.

In comparing the influence that our CT and NT AMF communities had on soybean growth the outcome was dependent on soil moisture. Under well-watered conditions, total biomass was nearly equal (1.1% difference) while NT plants had a higher shoot to root ratio (13.8% difference), driven by an increase in shoot biomass (4.5% difference) and reduction in root biomass (12.5% difference). For a producer, an increase in shoot to root ratio generally results in a greater yield since more biomass is allocated above ground (Wang et al., 2020). This suggests that under well-watered conditions, the AMF community derived from our NT soils were able to

supplement the root system, allowing the plants to allocate energy to above ground growth. Under drought stress conditions however, plants inoculated with our NT AMF communities performed worse than CT plants in terms of total biomass and both shoot and root biomass. This shows that our NT AMF community had a stronger influence on soybean growth and whether the association is beneficial was heavily dependent on adequate soil moisture. These results further emphasize the importance of understanding the interaction between AMF communities, crop cultivars and environmental conditions. Like corn, breeding programs must take AMF into account when developing high yielding cultivars and further research is required to improve our understanding of the plant-AMF interaction in agricultural ecosystems in an effort to improve food system sustainability.

Soybean Root Nodules & Bean Pods

Both root nodule and bean pod production were highest in CT and not NT inoculated soybean plants under well-watered conditions (Fig 5). These results suggest variation in functional traits between our CT and NT microbial communities because while the NT inoculum increased overall biomass, the CT inoculum increased parameters related to N-fixation (root nodules) and yield (bean pods). Under drought conditions however, the differences between CT and NT treatment groups were not significant. This suggests that under ideal conditions the species assemblage of AMF may matter more in terms of yield than under stressed conditions.

Using whole soil cores for AMF inoculum, incorporates other microbial populations such as rhizobium bacteria that form root nodules. Root nodules are where biological nitrogen fixation takes place and are often viewed as a proxy for total nitrogen fixation (Wang et al.,

2011). Like AMF, rhizobium bacteria are a ubiquitous indicator of soil quality and their populations respond rapidly to soil changes (Torabian et al., 2019). In agricultural systems, rhizobium bacteria populations are strongly influenced by management practices and are different between tillage systems (Kaschuk et al., 2006). In a recent review article investigating the influence CT and NT practices on nitrogen fixation in legumes, Torabian et al., (2019) found that while NT practices generally improve root nodulation and nitrogen fixation, the results also depend on the legume in question. Contrary to our results, in a field study looking at soybean cultivars grown under NT and CT management, Okoth et al. (2014) found that overall, NT management resulted in more root nodules, nitrogen fixation and greater yield (bean pods) although there was also significant variation between cultivars. The degree of nodulation and colonization also depends on the specific AMF species and rhizobium strain(s) in question (Wang et al., 2011). These results may have also been influenced by the difference in soil structure between fields and less so by the variation in rhizobium communities as NT practices generally result in greater soil aeration (Zhang et al., 2007). Increased aeration boosts the availability of atmospheric nitrogen available for fixation. Our results in the greenhouse suggest that different AMF and rhizobium communities are present in our CT and NT inoculant soils which potentially accounts for the variation in nodulation.

In the United States approximately 90% of corn fields are annually rotated with soybean which is known to boost yields, reduce pest and disease pressure, and more evenly use soil nutrients (USDA NASS, 2017 Census of Agriculture; Selim, M. 2019). If legumes (primarily soybean) are grown in rotation, how rhizobium communities respond to management practices and how their subsequent populations interact with the plant-fungal relationship is yet another

important consideration when trying to increase the sustainability of our agricultural ecosystems. Like corn, matching soybean cultivars to the microbial community to form more positive associations could result in reduced inputs while maintaining yields.

Root Colonization

Plants inoculated with AMF from NT soils resulted in the highest root colonization across all treatments. Under drought stress conditions, root colonization was significantly higher in NT inoculated plants then those that received CT inoculum (Table 1). An increase in colonization by NT inoculum is partially due to the increased density of AMF spores, increased AMF diversity often found in NT soils (Jansa et al., 2002; Menendez et al., 2001; Castillo et al., 2006; Verzeaux et al., 2017) and the hypothesis that diverse communities of AMF offer functional complementarity to their plant host (Koide, 2000). However, it is also possible the community of AMF in NT soils are more likely to form associations than those in CT soils. Regardless of plant performance, increasing AMF colonization in agricultural soils is potentially beneficial to long-term soil regeneration when coupled with NT practices. A more abundant AMF community can build SOM through deposition of glomalin and improve soil structure, both of which are required to reduce the reliance placed on fertilizer and irrigation to maintain yields in degraded soils.

The colonization rates in all of our treatments were relatively high. After 2 months of growth in a greenhouse experiment, corn inoculated with AMF from soils under conservation management resulted in colonization rates from 46 to 66% (Liu et al., 2000). Similarly, when investigating the response of soybean plants to a mixed species AMF inoculum, colonization rates ranged from 56-72% (Adeyemi et al., 2020). Colonization is an intricate interaction

controlled by plant root exudates in response to various environmental stimuli, primarily limiting soil resources (Ma et al., 2016) and partner specificity depends on both plant and AMF species (Öpik et al., 2009). In general, phosphate availability in soils is the major determining factor in AMF colonization (Smith & Read, 2010). When present in high concentrations AMF colonization is inhibited (Breuillin et al., 2010) and stimulated when at low concentrations (Gutjahr & Parniske., 2013). When plants are grown under low nutrient conditions (NPK) they secrete more root exudates which are the signal molecules that initiate AMF colonization (Ma et al., 2016). The growth media used in our greenhouse experiment contained 29 ppm of phosphorus and 94 ppm of potassium, both of which are considered 'Below Optimum' in regard to field recommendations (Michigan State University: Soil and Plant Nutrient Laboratory) and are among the 3 most important nutrients for plant growth. This is potentially why colonization rates were above 66.7% for all treatments and as high as 97.3% which is higher than often found.

Although colonization rates were similar between our corn and soybean plants which is often used as a proxy for beneficial associations (Liu et al., 2000), it is clear that in this experiment, colonization by AMF resulted in very different responses between our corn and soybean cultivars and that the nature of the relationship for soybean plants was dependent on soil moisture. We suggest that colonization alone should not be relied upon as a proxy for beneficial associations when trialing cultivars in a field setting. While colonization rates may be similar between species or cultivars, the impacts of those associated AMF communities may benefit or detract from plant growth.

Plants within our control, non-mycorrhizal groups were colonized with AMF in our greenhouse experiment. The highest colonization found in any control plant was less than 30%.

The most likely cause is cross contamination of spores from adject, inoculated pots that were transferred while watering. AMF spores are light enough to travel via wind through the atmosphere (Egen et al., 2014) therefore it is extremely likely they were able to move between pots within water droplets or small soil particles. We recognize this as a source of potential error, however, if cross contamination by AMF spores was severe, we would expect no significant differences in biomass, colonization or glomalin yield. We suggest that future studies use micro-drip irrigation and cover the surface of pots to prevent cross contamination of AMF spores.

Tissue Nutrients

Corn and soybean shoot tissue nutrient levels are summarized in Table 1. Although based on pooled tissues within treatments (n=1), total nutrient levels generally followed shoot growth trends suggesting both larger plants of higher nutrient density. The only group to go against this trend was the soybean plants subjected to drought stress. While shoot biomass was reduced by both AMF treatments, nutrient levels were higher, suggesting increased nutrient density of the smaller overall plants.

Glomalin Production

AMF inoculation resulted in increased glomalin yields for both plants under both water treatments (Fig 6). Under well-watered conditions, glomalin yield was greater than under drought stress conditions, most likely due to improved overall growth in a non-limiting environment for both the plant and subsequently the AMF community. Glomalin increases SOM and directly contributes to improved soil structure which increases water infiltration rates,

reduces runoff, increases water holding capacity, increases soil retention of nutrients, and boosts plant productivity (Baker & Saxton, 2007; Zhang et al., 2007; Osman, 2012). Building SOM via glomalin accumulation may reduce the level of required fertilizer (Osman, 2012) and allow producers to reduce their reliance on supplemental irrigation and better resist drought conditions (Zhang et al., 2007). So much is still unknown about glomalin, but it is clear that both AMF and glomalin deposition must be taken into account when considering the revival of degraded lands and long-term agricultural sustainability.

Under well-watered conditions in soybean plants, the amount of glomalin in NT plants was significantly higher than in CT inoculated plant. There is significant variation between AMF species in terms of glomalin production (Wright & Upadhyaya., 1999) and AMF species composition is different based on management practices (Franke-Synder et al., 2001; Menendez et al., 2001). In agricultural soils with diverse AMF communities, tillage is the most influential factor in glomalin dynamics. Tillage results in a decrease in glomalin levels (Wright et al., 1999, 2007) from elevated carbon oxidation rates (Al-Kaisi & Yin., 2005) and both a simplification and reduction of the AMF community (Jansa et al., 2002; Menendez et al., 2001). Our results suggest that our NT AMF community would produce more glomalin, and improve soil quality faster.

In transitioning from CT to NT management, producers start the process of accumulating and keeping glomalin in soils (Wright et al., 1999) which contributes to SOM (Wright et al., 2007) and results in improved soil quality and structure (Baker & Saxton, 2007). Glomalin concentrations are expected to increase from both a shift in the AMF community, and through the reduction in carbon oxidation and subsequent loss to the atmosphere via CO2 (Al-Kaisi & Yin., 2005). By improving soil quality through NT practices that increase glomalin concentrations,

producers can hopefully reduce their reliance on environmentally harmful and economically costly artificial inputs while maintaining yields.

Conclusion

This experiment showcases the complexity of plant-fungal interactions in agricultural ecosystems and how the outcome is heavily context dependent. Corn, a C_4 grass, was hindered by AMF from both communities in terms of biomass while soybean, a legume, was assisted by AMF only under well-watered conditions. While there was no significant influence on corn growth between our CT and NT AMF communities under both water treatments, soybean growth was dependent on both the AMF community and soil moisture. In terms of agricultural production, corn and soybean are annually rotated on approximately 180 million acres across the United States and are the two most economically important crops (2017 Census of Agriculture). Improving our current high yielding crop cultivars to take advantage of AMF symbiosis and then matching these cultivars to field specific AMF communities is an essential aspect of sustainable food production.

Although there was variation in how our corn and soybean cultivars responded to AMF symbiosis from our CT and NT soils, there was significant glomalin deposition after less than 2 months of growth. We know that from previous studies, glomalin concentration can double in only 3 years after converting from CT to NT. The top priority must remain the widespread adoption of NT practices for the proven ability to rebuild SOM, soil structure and overall soil quality. As soils are restored, continued work into understanding the plant-AMF and plant-microbial interactions is necessary for increasing the environmental, economic, and societal sustainability of agricultural production.

List of Figures

Figure 1. Experimental design for greenhouse corn and soybean plants.

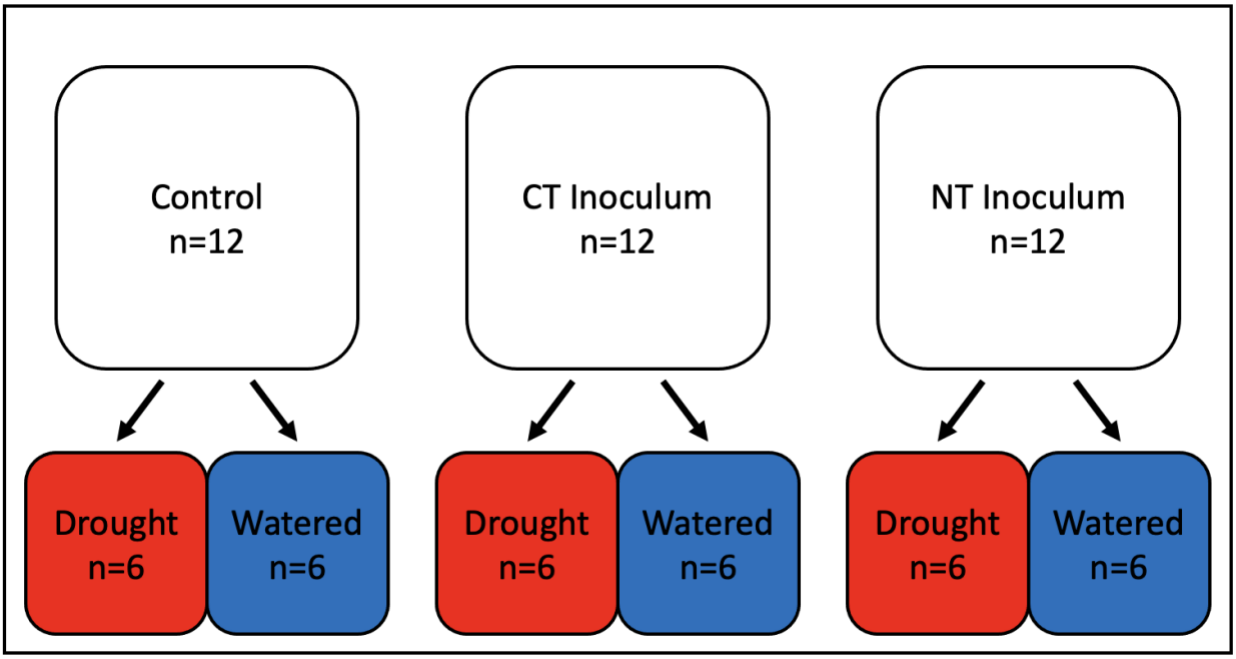


Figure 2. Spore counts from 200 g of field collected soil used as AMF inoculum. Different letters above the boxes indicate significant difference ($P < 0.05$) as determined by Wilcoxon Sum Rank tests. (a) Spore count comparison between fields (n=18). (b) Spore count comparison between fields separated by spore size classes (n=9).

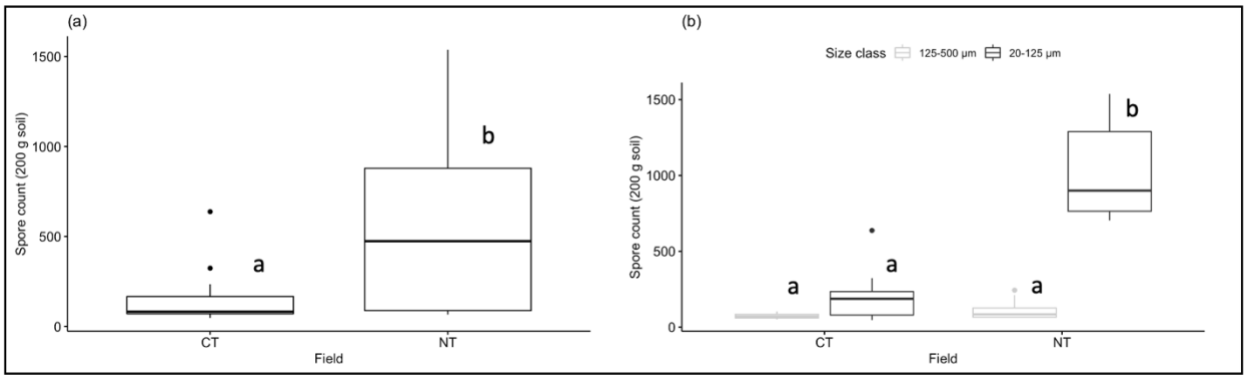


Figure 3. Corn plant biomass parameters. Values are means of six replicates. Different letters above the boxes indicate significant difference ($P < 0.05$) as determined by ANOVA and TukeyHSD post hoc tests.

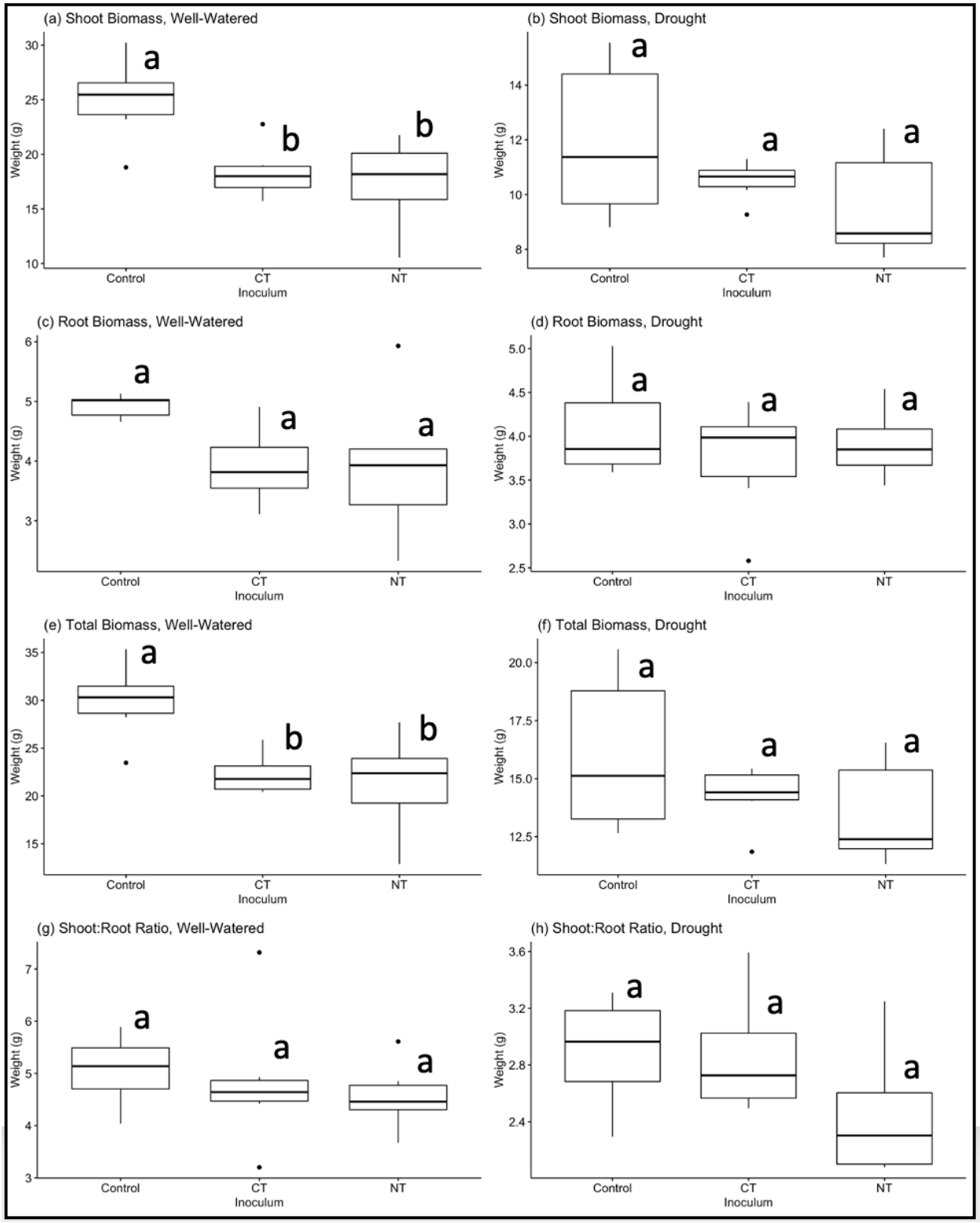


Figure 4. Soybean plant biomass parameters. Values are means of six replicates. Different letters above the boxes indicate significant difference ($P < 0.05$) as determined by ANOVA and TukeyHSD post hoc tests.

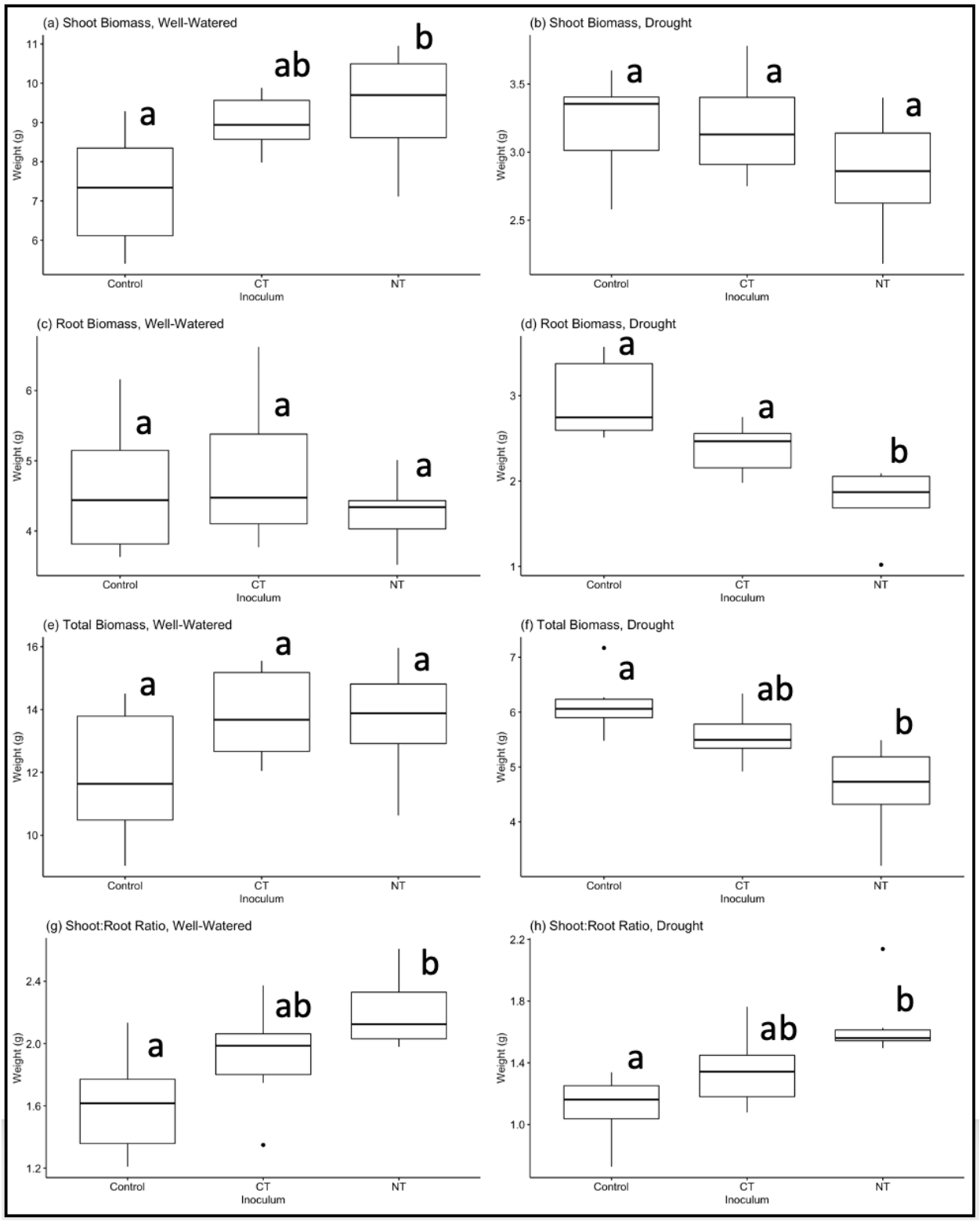
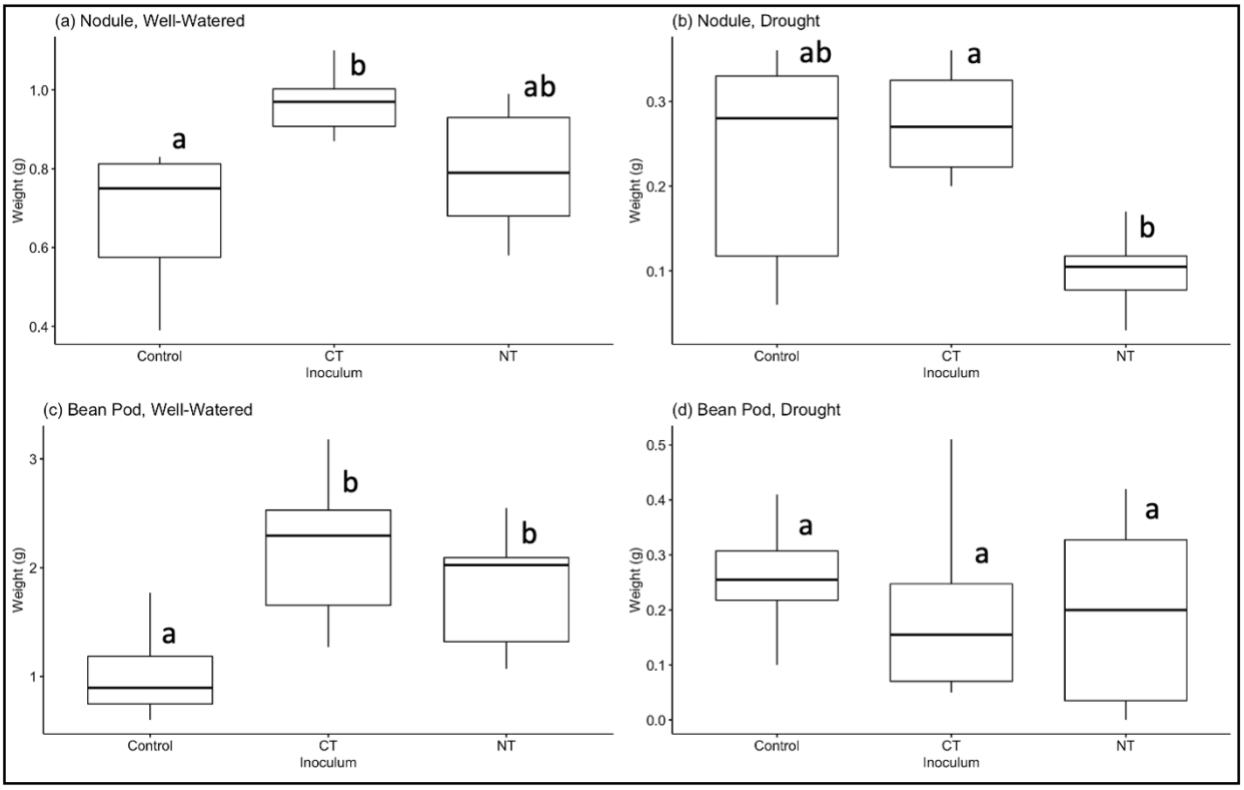


Figure 5. Soybean root nodule and bean pod production. Values are means of six replicates. Different letters above the boxes indicate significant difference ($P < 0.05$) as determined by ANOVA and TukeyHSD post hoc tests.



List of Tables

Table 1. Root colonization (%) for corn and soybean plants. Values are means of six replicates. Significant difference ($P < 0.05$) within water treatments indicated by an asterisk, as determined by an ANOVA.

Corn				Soybean			
Well-Watered		Drought*		Well-Watered		Drought*	
CT	NT	CT	NT	CT	NT	CT	NT
70.7	80.7	72.7	97.3	69.3	83.3	66.7	94.7

Table 2. Plant nutrient data for corn (top) and soybean (bottom). **Bold** values represent the highest value within water treatment groups. Values are based on pooled plant tissue samples within each group (n=1).

	Corn					
	Well-Watered			Drought		
Nutrient (mg / plant)	Control	CT	NT	Control	CT	NT
Nitrogen	**117.4**	91.9	97.6	**70.4**	63.0	62.2
Phosphorus	**67.4**	58.8	57.5	32.2	**34.7**	29.7
	Soybean					
	Well-Watered			Drought		
Nutrient (mg / plant)	Control	CT	NT	Control	CT	NT
Nitrogen	234.1	369.6	**375.5**	73.0	**111.2**	97.4
Phosphorus	35.0	36.0	**41.4**	15.7	15.3	**16.2**

Supplementary Material

Figure S1. Acres of corn (a) and soybean (b) harvested in 2017. 1 blue dot equals 10,000 acres.

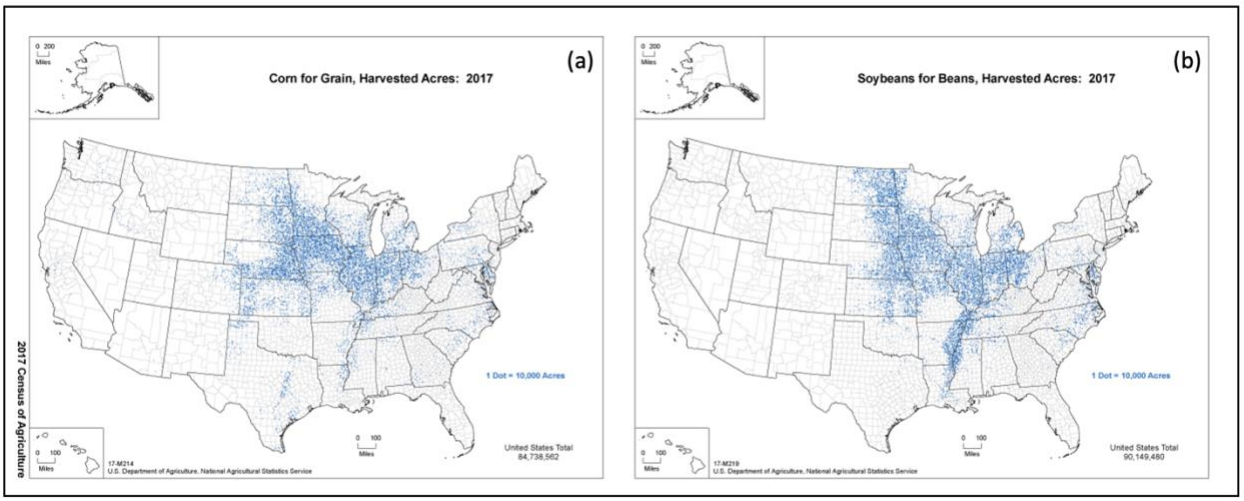


Figure S2. Field map of our sampled CT (right) and NT (left) field in Zeeland, MI.

Figure S3. AMF spores under 6X magnification. Examples are denoted with arrows.

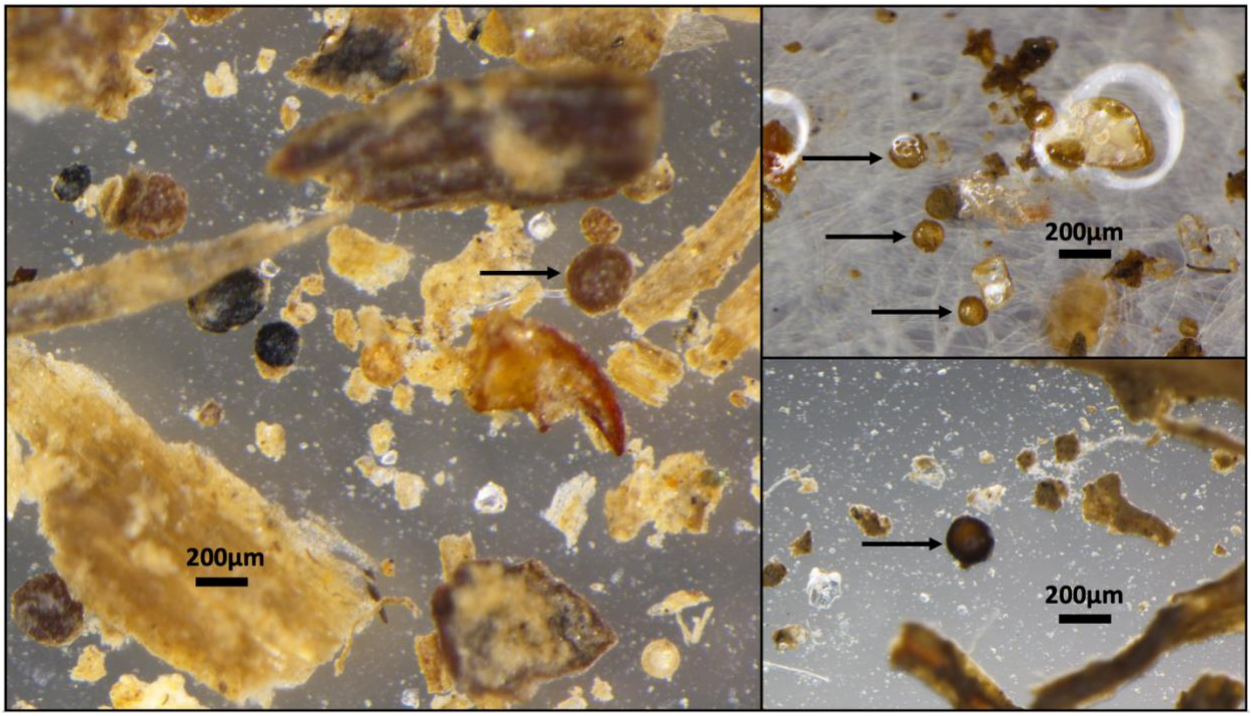


Figure S4. Soil moisture before inducing drought conditions. Different letters above the boxes indicate significant difference ($P < 0.05$) as determined by ANOVA and TukeyHSD post hoc tests. (a) Well-watered (A, C, E) and drought (B, D, F) corn treatment groups (n=48, 6 plants, 8 measurements each). (b) Well-watered (G, I, K) and drought (H, J, L) soybean treatment groups (n=48, 6 plants, 8 measurements each).

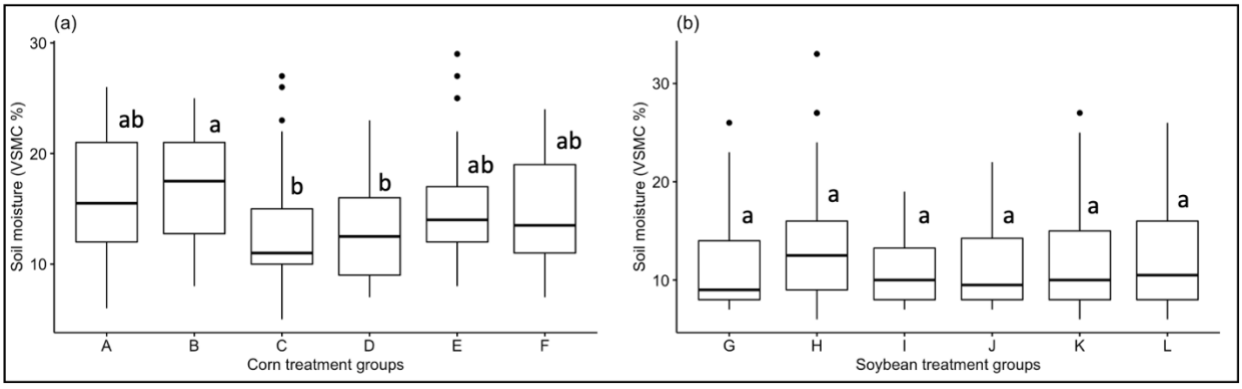


Figure S5. Soil moisture after inducing drought stress conditions. Different letters above the boxes indicate significant difference ($P < 0.05$) as determined by ANOVA and TukeyHSD post hoc tests. (a) Well-watered (A, C, E) and drought (B, D, F) corn treatment groups (n=72, 6 plants, 12 measurements each). (b) Well-watered (G, I, K) and drought (H, J, L) soybean treatment groups (n=66, 6 plants, 11 measurements each).

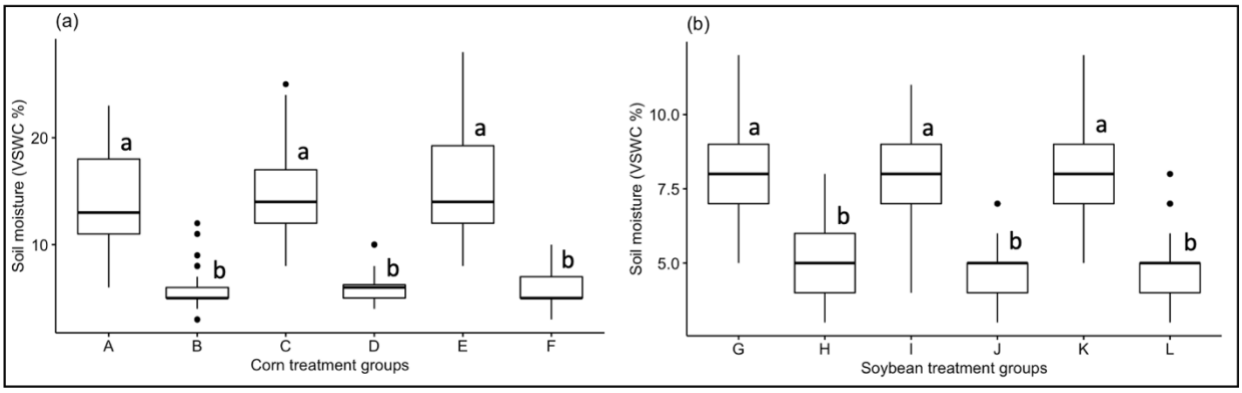


Figure S6. Example of stained soybean roots from NT inoculum treatment.

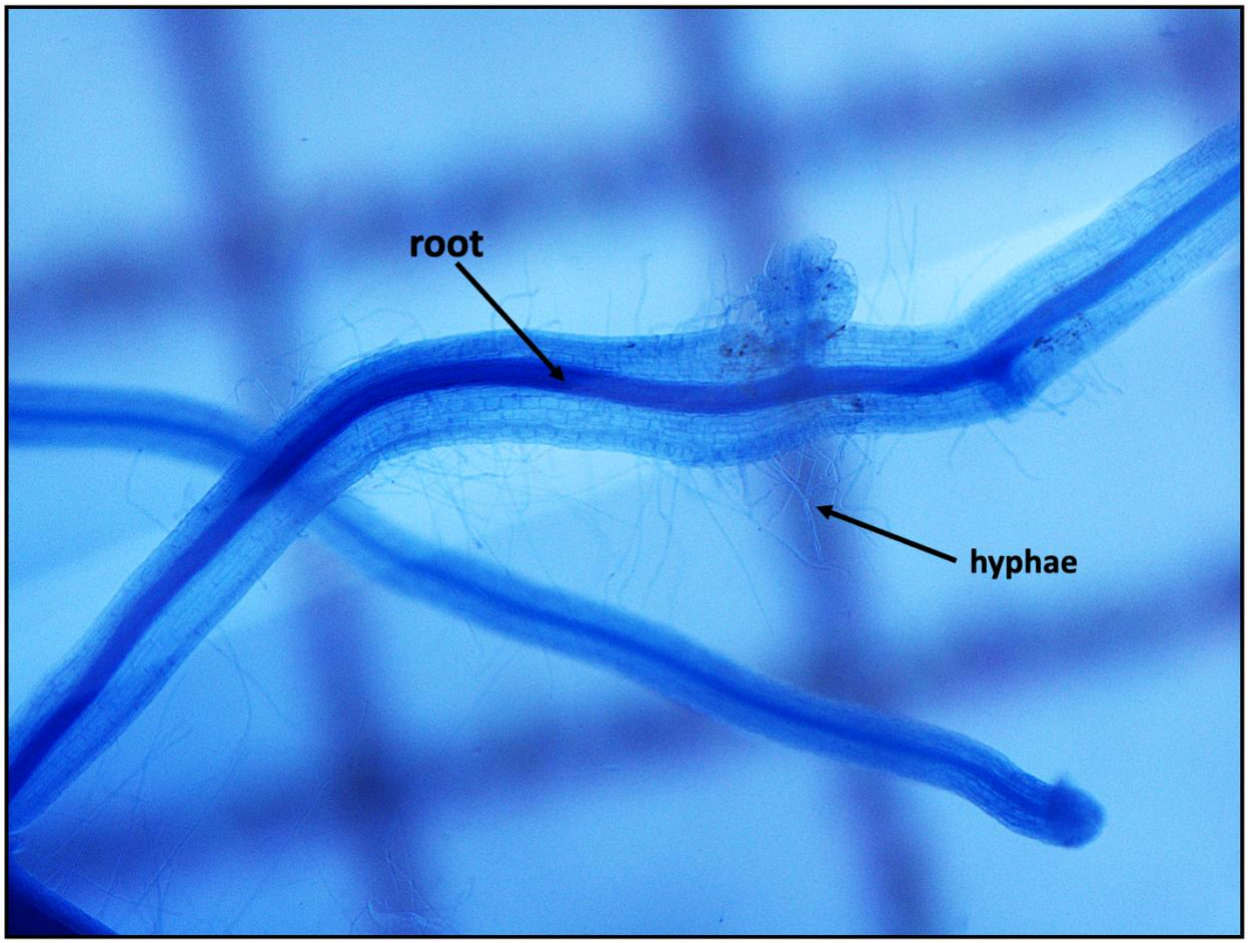


Table S1. Chemical and physical parameters for CT and NT soils and greenhouse growth media.

Soil Parameter	CT	NT	Greenhouse
pH	6.5	6.5	6.0
Nitrogen (%)	0.13	0.13	0.07
Phosphorus (ppm)	96	184	29
Potassium (ppm)	186	112	94
Organic Matter (%)	2.8	2.8	10.3
Sand (%)	73.9	73.4	*
Silt (%)	11.8	13.3	*
Clay (%)	14.3	13.3	*

*Unable to determine soil texture of greenhouse.

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