EFFECT OF ORGANIC SOIL AMENDMENTS AND CONTINUOUS TOMATO MONOCULTURE ON ARBUSCULAR MYCORRHIZAL COMMUNITIES

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
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To my parents for their undying support

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As interest in crop management alternatives to conventional high input farming increases, understanding the impacts of organic soil amendments and crop rotations on microbial components of the agroecosystem is desirable, especially for arbuscular mycorrhizal (AM) fungi. Soil amendments are thought to be more conducive for the activity of AM fungi, depending on nutrient supply. The effects of broiler litter and urban plant debris on infectivity and community structure of AM fungi were evaluated in a tomato (*Solanum lycopersicum*) microplot study. Tomato plants were cultivated under conventional methods for two years, and then transitioned for three years with alternative organic soil amendment treatments and rotation with cover crops (Sunn hemp (*Crotolaria juncea*) and Japanese millet (*Echinochloa crusgallii*). Mycorrhizal inoculum potential (MIP) of rhizosphere soil was assayed using maize (*Zea mays L*.) to assess infectivity of AM fungi. Soil DNA was assayed using real-time quantitative PCR for the presence of three key rDNA phylotypes in an attempt to track changes in the AM fungal community over time at different stages of management transition. Despite high levels of soil disturbance, and high supply of phosphorus and nitrogen that potentially

caused plant nutrient sufficiency, AM fungal colonization was not suppressed. A significant effect of tomato on the incidence of one of the phylotypes suggests a host mediated community shift, and evidence for differing fungal biological characteristics. High AM fungal infectivity and incidence supports previous findings of AM fungal resilience despite repeated disturbance and plant nutrient inputs. These conditions may favor AM fungi that colonize or sporulate rapidly, impacting the community structure and functioning in a transitioning or organic agroecosystem.

CHAPTER 1
INTRODUCTION

Less than half the Earth's land is arable, of which 15% is affected by soil degradation caused by erosion, nutrient mining, salinization, water logging, urbanization, and most prominently, unsustainable agricultural practices (Halberg et al., 2006). Half of the world's population is engaged in agriculture, mostly in the tropics and subtropics (Thurston, 1997) where high rainfall and temperatures pose major challenges to sustainable land use in many areas where there is a predominance of weathered soils, lower crop productivity, deforestation, and poor farming practices. To accommodate increases in human population and resource demand, an understanding of the effects of alternative soil management strategies is necessary for the development of sustainable agricultural systems. A major impact of intensive, conventional agriculture is soil erosion, which is one of the primary causes of soil degradation worldwide (Sanders, 1992), with topsoil losses in the continental U.S. estimated to be roughly 1 billion Mg per year (Lal, 1994). This results not only in loss of soil volume for plant roots, but also losses in soil nutrients, organic matter, and microbial biodiversity. These losses may lead to eutrophication of lakes, nutrient loading in water supplies, lower crop yields, and depletion of micronutrients after repeated harvests (Halberg et al., 2006).

Conventional farming practices typically involve high inputs of synthetic fertilizer and pesticides to maintain high crop yields with lower cost and labor. While this intensive management strategy has led to increased yields in recent decades, the environmental detriments have become more apparent in issues such as excessive use of synthetic fertilizer that accelerates soil degradation by increasing the rate of organic

matter decomposition, soil acidification, and depletion of soil nutrients not included in the fertilizer, a process known as nutrient mining (Bruun et al., 2006). Cultivation has been found to enhance the mineralization of soil organic matter by exposing it to microorganisms, air, and heat as a result of tillage and other practices, which reduces soil aggregates and structural stability (Harris et al., 1966; Tisdall and Oades, 1982), soil fertility, and crop growth, and in some cases yields over time (Thompson, 1991, Gavito and Miller, 1998b; Kabir et al., 1998).

The soil's integral role in ecosystem functioning highlights its fundamental role in organic farming. Since synthetic fertilizers and pesticides are not used and are prohibited in organic production systems, a farmer must rely on, maintain, and ideally build the soil's ability to provide nutrients (i.e. organic fertility sources and soil amendments such as manures, composts, incorporation of green manure crops, and biological N-fixation), and the ecosystem's ability to ward off pests. Alternative methods of supplementing nutrients and pest control are being implemented as a means of reducing environmental impacts and creating a more sustainable agricultural ecosystem as an alternative to . Examples of alternative or low input practices include: organic sources of fertilizer such as compost and chicken litter; pest control by soil solarization under plastic mulch; conservation (minimal) tillage to preserve soil structure; and adding organic matter and utilizing crop rotation to promote microbial activity and diversity. Sustainable practices can also involve improved water management, incorporation of crop residues, intercropping, leguminous cover crops, and crop rotation regimes (Bruun et al., 2006). These management strategies can decrease or eliminate the need for synthetic fertilizer, minimize losses, prevent erosion, minimize the impact of pests and

diseases, and can increase yields in some instances (Bruun et al., 2006). In conjunction with alternative practices, soil biota can provide ecosystem services such as decomposition, nutrient cycling, enhanced soil stability and water and nutrient retention capacity, for agriculture when conditions are manipulated to foster their utility. Practices that promote diverse and beneficial soil microbial communities can potentially reduce or eliminate the need for certain conventional inputs.

An integral aspect of determining a soil's quality and health is the monitoring of biological indicators, including plant health and soil components such as microbial biomass, activity, and diversity (Chellemi and Porter, 2001; van Bruggen and Semenov, 2000). By monitoring responses of these soil indicators to different stress factors, the ecosystem's health, stability, and resilience can be better quantified and predicted (van Bruggen and Semenov, 2000). As many farms transition to lower input systems due to an increasing market demand for organic produce, it is important to identify the most responsive, measurable and interpretable indices so that farmers can optimize their management practices (Stockdale and Watson 2009).

An understanding of the functioning and diversity of rhizosphere microorganisms is necessary before alternative soil amendments can confidently be applied or recommended to produce predictable outcomes (Bolton et al. 1992). Biodiversity of soil organisms in particular has been linked with enhanced plant productivity and ecosystem sustainability (Brussaard et al., 2007; van der Heijden et al., 2008). Soil microorganisms play a vital role in the biogeochemical cycling of nutrients and in the maintenance of soil quality in terrestrial ecosystems. While bacteria are the most diverse and abundant microorganisms in soil (Gans et al., 2005), fungi dominate

belowground biomass and metabolic activity due to their relatively large size (Brady and Weil, 2002). Many soil microorganisms are endophytes, which invade and colonize the root cortex, with effects on plant health and productivity ranging from beneficial to detrimental (Kennedy et al., 1997). All plants form intimate associations with at least one taxon of fungal endophyte, and are often indiscriminate in their associations (Sieber, 2002).

The most ubiquitous and ancient fungal root endophyte, arbuscular-mycorrhizal (AM) fungi, forms symbiotic relationships with over 80% of plant species (Jeffries et al., 2003). Probably the most abundant fungi in agricultural soils, AM fungi account for up to 50% of the soil microbial biomass (Olsson et al., 1999). Those in the phylum Glomeromycota are the most important in agroecosystems (Schussler et al., 2001). They improve the plant's ability to acquire limiting nutrients such as Phosphorus (P), (and to a lesser degree, Nitrogen (N)), as well as other trace elements and water by transporting nutrients via hyphae. The most important benefit that AM fungi provide to their host plant is the improved uptake of P (and sometimes P use efficiency). By extending up to 15cm from the root, hyphae bridge the gap between the zone of nutrient depletion and bulk soil (Barea and Jeffries, 1995; Smith and Read, 1997), drastically improving the plant's ability to obtain nutrients especially in times of nutrient stress. In exchange, the plant allocates up to 20% of its photosynthesized Carbon (C) for AM fungi (Jakobsen and Rosendahl, 1990). Host P status is a primary regulator of C exchange in the plant–AM fungal relationship (Graham and Abbott, 2000)

AM fungi interact with and affect many biotic and abiotic components of an ecosystem. They increase soil aggregation, and thus aeration and drainage, by physical

aggregation with enmeshing hyphae (Tisdall, 1991; Tisdall et al., 1997), as well as the adhesive glycoprotein exudate, glomalin (Hooker and Black, 1995). Glomalin, as well as hyphal turnover, provide C to other soil microorganisms (Jastrow et al., 1998), and can also sequester large amounts of C in the soil, thereby regulating C flow (Rygiewicz and Andersen, 1994) and acting as a major component in climate change dynamics (Fitter et al., 2000). AM fungal root exudates have been widely studied, and have been shown to cause changes in the rhizosphere microbial community (Dar et al., 1997), the host root architecture (Vigo et al., 2000) and changes to root biochemistry connected with plant defense mechanisms (Azcón-Aguilar and Barea, 1996; Gianinazzi- Pearson et al., 1996). Exudates may also include phosphatase (Tarafdar and Marschner, 1994; Koide and Kabir, 2000) and citric acid (Tawaraya et al., 2006), which solubilize organic and inorganic sources of P. AM fungi also directly and indirectly affect plant community composition (Bever, 2001), increase resistance to foliar-feeding insects (Gange and West, 1994), improve drought resistance (Augé et al., 1994), increase resistance to soil pathogens (particularly fungal) (Newsham et al., 1995; Pozo et al., 2002) and increase tolerance of salinity, extreme pH, and heavy metals (Shetty et al., 1995; Diaz et al., 1996; Al-Karaki et al., 2001; Feng et al., 2002; Mohammad et al., 2003; Jeffries et al., 2003). Considering the ubiquity and plant dependency on AM fungi, they can be considered a keystone mutualism in agroecosystems, and therefore a better understanding of their activity is desirable in order to implement the best management practices in a given agro-ecosystem type (Rasmann et al., 2009). In general, conventional agricultural practices have been found to have a negative impact on the

mycorrhizal association; agricultural soils have fewer species of AM fungi (Helgason et al., 1998; Menéndez et al., 2001).

The size and diversity of microbial communities has been found to be sensitive to agricultural practices (Marx et al., 2001), and they have the potential to act as indicators of the effects of such practices on the status of soil health and quality, more so than biochemical parameters, although a variety of indices should be assessed (Bending et al., 2004). Manipulation or disturbance of the plant-mycorrhizal relationship can have substantial negative consequences in terms of plant community degradation, health or productivity. Loss of propagules of AM fungi may result in a decreased capacity of crops to take up nutrients, thus lowering crop productivity (Jeffries et al., 2003). Understanding the various ways that different crop management practices impact AM fungal diversity, resilience, and colonization of the host crop is especially relevant when nutrient levels, soil fertility, and intensity of management are of concern, and in order to predict fungal behavior and crop response under different conditions.

Florida is the largest producer of fresh tomatoes in the U.S., accounting for about 50% of all fresh tomatoes produced domestically, and one third of the total value of all fresh vegetables produced in Florida each year (Florida Agricultural Statistical Service). Approximately 32,000 acres in Florida are under cultivation for the fresh tomato market; however, very little of this production is organic or low-input. In Florida, much of the tomato crop is grown in the Florida Everglades Watershed (FEW), an ecologically sensitive 25,000km^2 region consisting of multiple land use types and levels of development (Chellemi et al., 2011). Most agricultural soils in this watershed are Alfisols and Spodosols. They are poorly-drained, situated over perched water tables, very low in

organic matter, and comprised mostly of fine sand soil types (Obreza and Collins, 2002). To maintain profitable yields, Florida tomato producers must balance environmental concerns and sustainability with escalating production costs for weed management and control of soilborne pests and pathogens (Chellemi et al., 2011), and other prevailing economic challenges in farming systems such as rising energy and fertilizer costs.

The main goal of this research was to measure the effects of different organically-based land management strategies on AM fungal colonization and community structure in a subtropical vegetable production system. This research was associated with a broader multidisciplinary effort to examine the biological impacts on soil microbial communities and soilborne pests of tomato during transition from conventional production practices to organically based production practices. This study focused on land management and crop production practices that are known to affect AM fungi and to be representative of certain types of alternative options that can improve the soil health of an agro-ecosystem. A field site with a uniform soil type was selected for the construction of microplots to realistically simulate conditions common to vegetable production systems in Florida, and minimize the interaction of soil type with organic amendments. The microplots had previously been used for a study to evaluate effect of soil fumigants on root-knot nematodes.

A specific goal of this project was to identify how the transition to an organic system and crop host rotation affected two AM fungal parameters: level of infectivity and community structure. DNA-based primers were developed to detect AM fungal phylotypes that were known to be prevalent in FEW agro-ecosystems under either

conventional or organic management (E. J. Johnson, unpublished data). Although much literature exists describing the impacts of various conventional and organic agricultural practices on AM fungi, there is little information regarding the ability and extent to which populations recover from detrimental conditions once management changes (Rasmann et al., 2009). Regarding the long-term repercussions of conventional farming on AM fungi and how they are affected by major changes in agricultural practices, little is known about AM fungal infectivity or community structure beyond simple diversity. The quantification of the AM fungal parameters over time is important because it can impart better information on soil microbial responses and the system's soil health as a whole during and after substantial ecosystem changes.

The infection potential of field soil under treatments of varying fertilizer levels was assayed in a greenhouse maize experiment, and AM fungal community structure was described in a qPCR analysis of three phylotypes in extracted field soil DNA using three selected primers. The above work was accomplished to examine the impact of divergent and transitioning agricultural practices, as perpetuated in nine treatments, on the infectivity and diversity of AM fungi.

Literature Review

A great quantity of literature has described the presence, diversity, and functioning of AM fungi in a variety of soil types and host crops. AM fungal diversity has been shown to vary greatly among the ecosystems studied. For example, a review of spore diversity studies by Douds and Millner (1999) reported the number of morphotypes to vary from 3 to 46 per site. The effects of fertilization, tillage, and crop rotation were found to have the strongest effects on AM fungi, yet can vary greatly depending on crop nutrition needs and soil type. A meta-analysis by Treseder (2004) found that

mycorrhizal abundance was reduced by 15% due to N fertilization and 32% by P fertilization, which indicates that there are direct adverse impacts of fertilization. Therefore, soil fertility is an important factor to study, specifically what happens to the mycorrhizal symbiosis when this factor changes over time. An excellent review by Gosling et al. (2006) examines the existing research on how farm management practices influence the AM association, and how alternative management options can be employed to benefit it. In the following sections, the literature on the major agricultural influences on AM fungi, divergent management strategies, and different common approaches to studying various parameters of the mycorrhizal symbiosis will be reviewed. Organic agriculture may include various combinations of alternative practices, some of which are low-input, lower cost, lower labor, or low/no-till, but not necessarily.

Only farms that meet the criteria and standards set by the USDA for legal certification. Individual alternative practices that are common in organic farms will be discussed further, and a general comparison between organic and conventional systems will be made with the understanding that organic farms vary widely in their constituent set of management practices. The experimental microplot system in this study is neither certified organic, nor is it low-input or low-till, but it serves to portray several common alternative practices that are likely to affect soil biota. Therefore, the term ‘organic’ when used generally in this discussion refers to the collection of alternative practices that are likely or potentially present in certified organic farms, or used in non-certified alternative farms. A transitioning system is one that has begun to

incorporate alternative practices, but is not yet certified organic, or has ended the typical conventional practices such as using synthetic fertilizer, fumigants, and/or monoculture.

1. Farming Practices and AM Fungi

1.1 Fertilizers

Effects of AM fungi on crop nutrition. AM fungi increase volume of exploitable soil for the crop plant due to the very large surface area of its hyphae, and increased distance of exploration from the root surface (Miller and Jastrow, 1990; Spurr, 1998). Improved uptake of P is generally regarded as the most important benefit that AM fungi provide to their host plant, and plant P status is often the main controlling factor in the plant-fungal relationship (Thompson, 1987; Smith and Read, 1997; Graham and Abbott, 2000). In addition to increased P uptake, AM fungi may also facilitate P use efficiency, and can increase crop growth and yield (Koide et al., 2000a, 2000b). AM fungi also assist in the uptake of other plant essential nutrients, such as Zn, Mg, Cu, Fe, N, K, and Ca; sometimes the availability of these trace nutrients controls the initiation of the symbiosis (Ryan and Angus, 2003). AM fungi produce extracellular enzymes that aid OM decomposition and produce organic acids which release plant nutrients from soil minerals, thereby increasing the solubility of the inorganic P (Joner et al., 2000; Tawaraya et al., 2006). Direct decomposition of organic matter has been documented for orchidaceous and ectomycorrhizae root symbionts, but not for AM fungi (Barea and Jeffries, 1995). The concentration of various soil nutrients may mediate differential uptake of nutrients (Liu et al., 2000), causing an increased uptake of one nutrient, but a decreased uptake in another (Kothari et al., 1990). It is important to remember that AM fungi do not substitute for fertilizer inputs and that nutrient mining as a consequence of

continuous cropping will result in depletion and availability of nutrients both inorganic and organic, for AM fungi to deliver to the crop (Ryan and Tibbett, 2008).

Effects of fertilizers on AM fungi. The amount of plant available P has been shown to predict the levels of AM fungal colonization in some studies. When a high amount of plant-available P exists in the soil, such as in the case of synthetic fertilizers, plants may more easily take up P and reach a state of plant sufficiency (Bethlenfalvay 1983; Hetrick et al., 1996; Thingstrup et al., 1998; Sorensen et al., 2005). The plant, having obtained the necessary P, suppresses AM fungal colonization by decreasing allocation of photosynthetic C to the fungi, or fails to respond to colonization by native AM fungi (Ryan et al., 2002). Decreases in soil populations of AM fungi due to elevated soil P concentrations from intensive agriculture are well documented (Smith and Read, 1997). However, it has also been found that in some cases of very high P levels, percent colonization is not drastically reduced, and that hyphal continuity is more important in determining colonization levels and persistence (Miller et al., 1995). In cases where colonization persists despite very high levels of plant-available P in the soil, crop yield may decrease (Gavito and Varela, 1995; Kahiluoto et al., 2001) due to the plant-fungal relationship shifting toward parasitism, when the delivered mineral benefits do not outweigh the C costs (Ryan and Graham, 2002; Johnson, et al., 1997; Koide, 1991; Bethlenfalvay, et al., 1983). However, decrease in yield is not always the case (Galvan 2009). High fertilization rates not only reduce colonization and propagule numbers, but may also select for AM fungal species that provide less of a benefit to the host plant (Johnson, 1993), thereby altering fungal community composition.

In contrast to synthetic fertilizers, organic sources may stimulate rather than suppress AM fungi (Harinikumar and Bagyaraj, 1989; Ryan et al., 1994; Douds et al., 1997; Kabir et al., 1998; Miller and Jackson, 1998; Joner, 2000; Alloush and Clark, 2001). Sources of organic fertilizer include natural materials high in organic matter (OM) such as animal manure and plant compost. OM provides benefits to the agroecosystem through improvement of microbial biomass and activity, soil structure, porosity, cation exchange capacity, water retention, nutrient storage, water use efficiency, and decreased runoff and erosion (Thurston, 1997). According to Glover et al. (2000), OM produces a 'more is better' effect; soil and water quality is improved with increasing soil OM content. Nutrient and water holding capacity are especially improved in sandy soils such as those in Florida, which are notoriously low in nutrient exchange capacity and OM content, lacking proper balance of cations that plants need, and are prone to rapid nutrient losses due to leaching.

The nutrient composition of soil amendments containing animal manures varies according to type of animal, feed composition, type of bedding material, storage conditions, and the various components of the "litter" (feces, urine, bedding, spilled feed, feathers, soil, etc) (Dewes and Hunsche 1998; Shepherd et al. 1999). Sixty-eight percent of the manure used in the US is poultry litter, which is considered the best source of animal manure for use as a crop fertilizer due to its low water content (30-50%) and high content of nutrients and trace elements (Hatfield and Stewart, 1998). Animal manure amendments stimulate assimilation of nutrients by soil microbes, initially causing nutrient immobilization and then release of macro- and micronutrients in plant available forms (Brady and Weil, 2002). Manure also increases microbial diversity,

enhances soil structure, water retention, and drainage; the use of manure can be less expensive in some instances compared to synthetic fertilizers (Hatfield and Stewart, 1998).

P can easily become immobilized in the soil and made unavailable to plants due to several processes, but AM fungi can “short-circuit” that process. Dead vegetation usually becomes mineralized, creating orthophosphate, which is where P is bound up and immobilized in soil. If mycorrhizae are present and active, they can directly assimilate P via hyphal colonization of decaying organic matter (Joner and Jakobsen, 1994; Tarafdar and Marschner, 1994b; George et al., 1995). In addition, organic matter can form chelates with Fe, Al and Mn which in turn prevents them from reacting with P. As microbes mineralize organic matter, P is slowly released and available over a greater span of time at a lower risk of being lost due to leaching.

Indeed, a major concern with the use of animal manure on organic farms is the potential for excessive nutrient loading and pollution of water sources. For instance, nitrates can leach into groundwater, runoff can carry bacteria, carbon, metals, and P into rivers, salinity damage to vegetation can be exacerbated due to buildup of N and K salts, and ammonia volatilization can contribute to acid rain (Hatfield and Stewart, 1998). These environmental problems may be mitigated by employing best management practices such as proper timing and placement of applications, incorporating manure into the topsoil to reduce N losses, optimizing composting stage to stimulate microbial activity, and determining the best application rates for specific crops and regions. With regards to soil bacterial communities, the C:N ratio of the compost will affect N mineralization rates, decomposition rates, and nutrient

immobilization. Selecting compost with an ideal C:N ratio can help prevent N leaching between crops by supplying N in amounts corresponding to crop demand (Watson et al., 2002). AM fungi can increase root branching (Berta et al., 1995), thereby improving exploration of the soil and altering patterns of fungal exudation (Hooker and Black, 1995) which in turn alters rhizosphere bacterial communities (Meyer and Linderman, 1986; Linderman, 1991). This may be especially important in organic systems since nutrients are coming from organic sources, the decomposition of which depends on soil bacterial communities and their potential stimulation by AM fungi. Thus, AM fungi can act synergistically with OM in fertilizers if conditions are optimal, thereby enhancing plant uptake (Hodge et al., 2001), and improving soil structure and nutrient retention.

1.2 Tillage

Tillage is the practice that has been shown to be the most detrimental to AM fungi due to the severe physical disruption of the fungal mycelium (hyphal network), thus reducing the viability of the hyphal network and its benefits to the plant (Miller et al., 1995). The network functions not only as the means for nutrient and water transport to the plant, but also serves as the primary method for colonizing seedling root systems in natural ecosystems (Read et al., 1976). Tillage severely disrupts this activity and may delay or reduce colonization and reduce the volume of soil that is exploited by the AM fungi (Jasper et al., 1989a,b, 1991; Evans and Miller, 1990). Thus, in some cases nutrient uptake, crop growth and yield, may be reduced (Evans and Miller, 1988; McGonigle et al., 1990; Kabir et al., 1999; Gavito and Miller, 1998b; Kabir et al., 1998). The negative effects of tillage is highly dependent on soil type (Mulligan et al., 1985; Kabir et al., 1998), level of dependency of crop on the AM symbiosis (Mozafar et al. 2000), and may not have long-term detrimental effects (McGonigle and Miller, 1993).

In addition to affecting colonization levels, tillage can cause a shift in the AM fungal community structure (Jansa et al., 2002, 2003; E. J. Johnson, unpublished) and may favor those fungi that are primarily propagating from spores rather than root fragments, hyphal fragments, or the mycelial network. Johnson et al. (1992) noted that the prolific spore-forming AM fungi may not be the most beneficial for the host, as they divert a larger proportion of their resources (and plant C allocation) to spore production instead of delivering nutrients to the host plant. No-till and reduced-tillage systems provide an alternative method of preserving the integrity of the hyphal network, especially from previous crops, which is a critical factor in the greater, earlier P absorption observed in undisturbed systems (Miller, et al., 1995). This has frequently been shown to increase subsequent colonization levels, spore counts, nutrient levels in plant tissues, and nutrient uptake (Galvez et al. 2001). However, tillage plays an important role in weed suppression in organic farming systems, a difficult task in the absence of synthetic herbicides. Limiting tillage may reduce damage to the mycelial network, and allowance for some weeds in the agroecosystem can provide plants that act as continuous AM fungal hosts between cropping periods (Gosling et al., 2006).

1.3 Crop rotations

Conventional agricultural systems are characterized by crop monoculture and possible uncultivated seasons, which may decrease soil microbial diversity due to low host diversity (An et al., 1993; Burrows and Pfleger, 2002; Oehl et al., 2003). Johnson et al. (1992) hypothesized that monocropping selects AM fungal species which grow and sporulate rapidly, and that due to such an increased allocation of resources towards their own growth and reproduction, these species will offer the less benefit to the plant.

This may result in decreased benefits of AM fungi to the host over time as monocropping continues.

Crop rotation, however, is a way to provide a constant host for the fungi and return nutrients and organic matter to the soil between crop cycles instead of bare fallow. Cover crops, especially leguminous plants, can benefit the biological, chemical, and physical structure of soil and the overall agroecosystem in many ways. The plants decrease sediment loss as a result of erosion caused by wind and rain, provide habitat for other (often beneficial) organisms, increase biodiversity, increase organic matter content of the soil if residues are retained, reduce nutrient loss from leaching and runoff (Clement and Williams, 1967; Grace et al., 1995). Rotations can also be designed to minimize the spread of weeds, pests, and diseases (Altieri, 1995). Cover crops provide host continuity for AM fungi, an essential feature in a successful agroecosystem. The lack of a host for long periods of bare fallow (more than 12 months) may lead to decreased propagules and yield, a condition known as “long fallow disorder” (Thompson, 1987). To ensure AM fungal diversity and viable infectivity, it is vital to use strongly mycorrhizal host plants as cover crops; the lack thereof may have a more detrimental effect on AM fungal populations than tillage or P fertilization (Gavito and Miller, 1998a). However, exceptions have been found in studies where non-mycorrhizal cover crops did not have a detrimental or lasting effect on soil fungal infectivity levels (Ocampo and Hayman, 1981; Powell, 1982; Sorensen et al., 2005), or increased yield, but these results are not yet understood (Ryan and Graham, 2002).

Leguminous cover crops can increase available N in the soil by N-fixation via its symbiosis with *Rhizobium* bacteria in root nodules (Touchton et al. 1984; Smith et al.,

1987) which can reduce the need for application of organic fertilizers. AM fungi have been found to enhance nodulation and N-fixation by legumes (Ganry et al., 1985; Arias et al., 1991; Ibibijen et al., 1996; Vázquez et al., 2002). Most legumes are highly dependent on AM fungi (Owusu-Bennoah and Mosse, 1979; Plenchette et al., 1983; Thompson, 1987; Schweiger et al., 1995; Kahiluoto et al., 2001) due to the high P requirements for adequate nodulation and N-fixation (Crush, 1974; Barea and Azcón-Aguilar, 1983). Therefore, the legume–rhizobium symbiosis is strongly influenced by AM fungi. Furthermore, some studies suggest that legume nodules contain AM fungal communities quite distinct from those found in the roots of legumes (Scheublin et al., 2004). Some studies report that AM fungi increase host plant N uptake in both legumes and non-legumes (Gaur and Adholeya, 2002). Nitrogen may also be transferred from legumes to non-legumes through the mycelial network (He et al., 2003), thus having further impacts in organic systems where polycropping is employed.

1.4 Control of pests

AM fungal suppression of crop pests. AM fungi alter root exudates and thereby reduce infection by root rot pathogens, nematodes, and may physically occupy roots and reduce potential colonization sites for other root endophytes (Graham 2001). AM fungi may confer increased plant resistance to soil pathogens (particularly fungal) (Newsham et al., 1995; Lingua et al., 2002; Pozo et al., 2002) and to foliar-feeding insects (Gange and West, 1994). Pathogen infection reduces AM fungal colonization (Torres-Barragán et al., 1996; Dar et al., 1997; Karagiannidis et al., 2002), and vice versa. Pathogen reduction occurs due to several mechanisms, including competition for colonization space (Sylvia and Chellemi, 2001), changes in root exudates, improved nutrition status of the host, limited soil nutrients, especially P, changes in rhizosphere

microbial communities, changes in leaf defense chemicals, or changes in host root architecture (Azcón-Aguilar and Barea, 1996). Furthermore, AM colonization has been found to induce the host plant to respond faster to infection by pathogenic fungi (Whipps, 2004). However, the degree to which AM fungi assist pest suppression depends on many other factors such as soil nutrient status, species of AM fungi, and host or pest specificity (Xu et al. 2012).

Effects of pesticides, solarization on AM fungi. Pesticides are generally detrimental to the AM fungal association, although the effects of individual chemicals on the fungi can be unpredictable, sometimes temporary, and even neutral or beneficial in some cases, based on their chemical composition and host specificity (Koide and Mosse, 2004). While most synthetic pesticides are prohibited in organic agriculture systems, “natural” pesticides and approved synthetic pesticides are allowed as per USDA standards. Copper-based fungicides may be used in organic production, but they can severely damage AM fungi even at levels below label recommendation (Sreenivasa and Bagyaraj, 1989). Gosling et al. (2006) reviewed the effects of different pesticides, and notes that many studies do not accurately simulate realistic application regimes, which may account for such varied results for their impacts. Solarization by covering the ground with clear plastic mulch is a common pest control strategy in organic farms. The plastic traps heat which kills soil pests with increased temperatures as opposed to chemical means. However, its efficacy may be variable, and more research is needed.

2. Transitioning Management Strategies: Conventional to Organic

Several studies have examined the differences in AM fungal responses between conventional and organic systems. An excellent review by Ryan and Tibbett (2008) describes the role and reaction of AM fungi in organic agroecosystems. It is important to

keep in mind that a comprehensive understanding of organic systems cannot come from studies that examine only one or two factors in the agroecosystem, or attempt to extrapolate conclusions based on a very narrow and artificial simulation of an organic farm. Organic farming is not a static or monolithic practice, but rather a set of practices and a philosophical framework that espouses management practices that maximize biological functioning and diversity in order to maintain the health of the broader ecosystem. An active soil microbial community in particular is vital to agroecosystem functioning (Lampkin, 1990) with AM fungi as a key component (Oehl et al., 2003; Harrier and Watson, 2004).

As previously discussed, AM fungi provide the most benefit to host plants when tillage is minimal, nutrients are slowly released from organic matter, and a strongly mycorrhizal host is consistently available. However, when nutrient applications are in excess of crop needs and build to high levels in the soil, and when tillage is a frequent occurrence, functioning of the plant-fungal symbiosis is diminished (Rasmann et al., 2009). Transitioning from a conventional or high-input system to some form of lower-input or organic system is of great interest and concern to most organic farmers and recently, more conventional farmers. A transition period of three years is required by the USDA in order for a farm to become certified organic. Due to so many factors in the agroecosystem changing at once and at different rates, it can be hard to discern the exact effects on AM fungi or yield in a factorial cause-effect experimental design, especially when attempting to predict the future conditions of the farm. Decreased yields due to nutrient limitation and pest incidence in the early stages of transition are a major concern for farmers, and are thus a barrier to transitioning to organic production (Tu et

al., 2006). For example, the incorporation of organic fertilizers to reduce soil-borne pests has been shown to be variable during the transition period (Baysal et al., 2008) but may also provide sustained soilborne disease suppression (Chellemi et al., 2012). It is therefore important to study this transition period in order to help determine what strategies should be employed and what effects can be expected. Rasmann et al. (2009) concluded that AM fungi are capable of persisting and recovering from detrimental management practices; the effect of tillage is only transitory, and that the creation of a P-bank in the soil is a prominent condition in transitional systems that can suppress AM fungi.

Very high levels of AM fungal colonization may not always be beneficial to crops in an organic system, as previously mentioned; Dann et al. (1996) found that yields may decrease due to the C drain by very high colonization. The level of AM fungal benefit or detriment to crops depends heavily on the type of management the farm has experienced historically. For example, long-term conventional, high input management reduces AM fungal diversity (Helgason et al., 1998; Daniell et al., 2001) and may favor inefficient species of AM fungi (Johnson et al., 1992; Johnson, 1993). Thus, soon after conversion to organic, the community of AM fungi may be reduced in diversity and shifted to species that are more tolerant of intensive farming practices, a condition that may persist even after several years (Scullion et al., 1998). Implementing farming practices to promote species diversity may be necessary to stabilize the AM fungal community (Johnson et al., 2010). Still, the early stages of recolonization of soils may be characterized by significant fungal species heterogeneity (Boerner et al., 1996) and low infectivity, until propagules of latent AM fungi reemerge as disturbance decreases.

While uncommon, some propagules can arrive from adjacent natural areas or unmanaged land; these propagules are transported at differing rates depending on vector type, such as wind, water erosion, animals, growing roots, or agricultural machinery (Warner et al., 1987).

Notably, organic systems often increase diversity of many organisms in the agroecosystem, and an increase in soil-borne biodiversity specifically is an important component of soil health, potentially enhancing plant productivity and ecosystem sustainability (Brussaard et al., 2007; van der Heijden et al., 2008). Furthermore, results from a 21-year study of agronomic and ecological performance of biodynamic, bioorganic, and conventional farming systems in Central Europe by Mäder et al. (2000) found that crop yields were 20% lower in the organic systems, but input of fertilizer and energy was reduced by 34 to 53% and pesticide input by 97%. The enhanced soil fertility and higher biodiversity found in organic plots may cause organic systems to be less dependent on external inputs.

3. Research Methods for Studying AM Fungi

There are many different methods to quantify and identify AM fungi and their propagules in field soil or host roots. Since their discovery in the mid-1800's, study of AM fungi has increased in-step with improving technologies to describe them. The utility of a method depends on what is intended to be measured or understood, as well as the method's inherent limitations. Classical, biochemical, and molecular tools have been developed to measure colonization levels, metabolic activity, exudates, mycelial structure, spore identification, rates and efficiency of nutrient uptake, and relative amounts of fungal biomass or genes present in soil or hosts. Due to their obligate symbiotic nature, however, studying AM fungi has been challenging since they cannot

be cultured axenically, thus further hindering the development of identification tools (Douds and Millner, 1999). Other main challenges to measuring AM fungal diversity include difficulty of identifying field collected spores, the detection of non-sporulating members of the community, and the complex genetic condition of AM fungi. In addition to the two primary methods used in this study, other ways to quantify AM fungi include: detection of levels of chitin, campesterol, 24-methylenecholesterol (Schmitz et al., 1991), ergosterol (Frey et al., 1994), phospholipid fatty acid profiles (Bossio et al., 1998; Buyer 2010), and yellow carotenoid pigments found in some mycorrhizal roots (Bothe et al., 1994). Other techniques such as quantification of extraradical hyphae, assays for functional activity, and identification using immunological methods are discussed in a good overview by Millner and Wright (2002).

Spore identification is a commonly employed method of studying AM fungal diversity. However, there is a considerable lack of relationship between the morphological diversity of spores used to delineate species, and their functional diversity in an agroecosystem (Douds and Millner, 1999). Fungi have historically been classified based on spore morphology, giving rise to a “morphological species concept,” but in the absence of reproductive structures, endophytic fungi are often described as “morphotype,” which is a “group of morphologically differentiated individuals of a species of unknown or of no taxonomic significance” (Hawksworth et al., 1995). There is substantial variation in functioning among morphotypes (Munkvold et al., 2004) and their functional role can be very plastic depending on their host and soil environment (Johnson et al., 1997). Spore counting and identification may not reflect the active symbiosis in the field (Clapp et al., 1995; Jansa et al., 2003), the true species diversity,

the relative abundance, which AM fungi are the source of extraradical hyphae, or the total contributed biomass by each species (Douds and Millner, 1999). However, these parameters are more feasibly and better estimated using molecular or immunological tools (Treseder and Allen, 2002; Redecker et al., 2003). AM fungal diversity may relate more to the sustainability of an agroecosystem rather than its functioning (Sieverding, 1990), or increased diversity may indeed provide benefits to crops due to a wider range of functions (Koide, 2000). Using trap culture methods can only identify and enumerate sporulating species in field soil, but again, does not give an idea of that species' relative abundance or functioning in the field due to different conditions in the pot culture which causes increases or decreases in sporulation, depending on a particular species' lifecycle (Brundrett et al., 1999). Furthermore, non-sporulating species of AM fungi are completely unaccounted for using the trap culture approach necessitating the use of molecular methods to identify and study the distribution of these fungi in the AM community (Rosendahl and Stukenbrock, 2004).

Similar limitations exist even in the best field-simulating mycorrhizal inoculum potential (MIP) assays, creating a "filter effect" on the indigenous AM fungal communities by favoring those aggressive enough to colonize and sporulate in a fast growing host under artificial conditions and short time span (Leal et al., 2009). The percent of colonized root in an MIP assay may not be representative of the colonization level on host crops in the field due to different conditions in the greenhouse or characteristics of the host plant, such as its level of dependence on AM fungi or its preference for particular fungal species. For this reason, assay host plants used are those that tend to quickly and indiscriminately form associations with AM fungi, such as

sudangrass *(Sorghum sudanense*) and maize (*Zea mays*). The level of magnification used to view roots and assess presence or absence of fungal structures can affect identification accuracy, although there are advantages and disadvantages to using dissecting and compound microscopes for different reasons. However, if approached with awareness of their limitations, spore-based community analysis and colonization levels in MIP assays are still useful because community diversity and structure of AM fungi are highly relevant to AM fungal susceptibility to changes in agricultural practices (Oehl et al., 2003), and since species can have strong and varied effects on host crops.

MIP studies are an effective method for evaluating the amount of viable fungal propagules and inoculum in the soil by growing a different host plant in samples of the experimental field soil and examining the amount of fungal colonization in harvested roots. Field soil can be extracted primarily from the rhizosphere, or bulk soil, depending on the research focus. Samples must be kept refrigerated in order to prevent decay of fungal propagule tissues, which could decrease their viability. Field soil may be diluted with sand in MIP assay containers in order to reduce soil nutrient status and increase plant reliance on AM fungi, thus promoting colonization of the assay host. However, in this study, the soil is used undiluted in order to obtain information about propagules as they exist in the field environment, without manipulation to encourage their activity. Similarly, while fertilizer is often used during part of the MIP growing period in order to supply the assay plant with nutrition until the symbiosis can be formed, it was not used in this study so that the fertilizer effects of the edaphic site conditions could be expressed, especially since the nutrient effects of these various OM treatments are among the main variables being studied.

By using a crop with fast-growing roots that become rapidly colonized, such as sudangrass (*Sorghum sudanense*) and maize (*Zea mays*), it is possible to assess the level of initial AM fungal infection that may occur on a mycotrophic host. Twenty-eight days is considered approximately the time required for AM fungi to establish primary infections and colonize a host before developing secondary infections (Moorman and Reeves, 1979), which may complicate evaluation of percent colonization on a particular host. In order to visualize fungal structures under a microscope, the host root needs to be cleared of tannins, cytoplasmic components, and other compounds that are dark pigmented. This is accomplished by boiling or autoclaving roots in 10% KOH liquid to clear the cytoplasm, and bleaching further if necessary, depending on the type of roots. Trypan blue is a common staining solution used to enhance visualization of AM fungi, although other root endophytes may take up the stain as well, causing increased difficulty in identifying structures in the field of view, and possible overestimation of AM fungal colonization.

Quantifying MIP can be accomplished by estimating either the percentage colonization by AM fungi or total mycorrhizal root length. In this study, the percent colonization was determined using the procedure developed by Newman (1966) now known as the line intersect method, wherein stained roots are spread out on a petri dish that is lined with a grid which serves to create a background to visualize “intersections” between lines and the root. At each intersection the AM fungal status of the root can be observed under a dissecting microscope. Percent of mycorrhizal root length can also be calculated if the length of root is based on the distance between the gridlines. The line-intersect method was tested for accuracy, and compared to several other techniques by

Giovannetti and Mosse (1980). They determined that increasing the number of observations from 100 to 200 decreased the standard error of measurements only from ± 4 to ± 3, which pertains to the main advantage of using a dissecting microscope; i.e., an estimation of colonization with minimal time and allowance for adjustment of the number of observations according to desired level of accuracy.

The precision of the line-intersect method is limited by the lower magnification compared to compound microscopes. AM fungal structures can be difficult to discern from other root endophytes, especially when the latter have also become stained, and if the observer has little experience identifying AM fungal morphological features. Also, even though precautions are taken when clearing and staining roots, fragile hyphae or root cortical tissue can become chemically over processed and damaged by excessive physical agitation at many points in the procedure. McGonigle et al. (1990) described a technique known as the magnified intersections method which examines intersections between the eyepiece crosshairs of a compound microscope and roots at a magnification of 200x. This method can increase the level of accuracy in identifying mycorrhizal structures such as arbuscules, but requires a considerable more sample preparation and observation time, and a marginal improvement in the assessment of the root system's colonization level (McGonigle et al., 1990).

Several molecular methods have emerged in recent decades, but are still nascent and limited in their efficacy due to the lack of a comprehensive understanding of the AM fungal genome and its complexity. A method that has been found to be particularly successful is the amplification of the nuclear DNA encoding for the small subunit rRNA and the attendant internal transcribed regions known to vary at the species level (Douds

and Millner, 1999). By creating primers that select for unique phylotypes, quantitative polymerase chain reaction (qPCR) can provide a more accurate assessment of the active AM fungal community, their spatial distribution, and individual quantification. However, it has been difficult to create primers that do not amplify other non-target microorganisms and have a high enough level of phylotype specificity (Simon et al., 1993). The challenge is accounting for the multinucleate nature of AM fungi, multiple copies of the targeted genes, and blurry lines among morphotypes and phylotypes which necessitates further research.

AM fungi genetics have proven difficult to study due to the inability to culture AM fungi axenically, multinucleate spores, interconnected and multinucleate "cells," an unclear mode of reproduction, and an undefined phylogenetic concept. Multiple genomes can co-exist within individual AM fungi (Kuhn et al., 2001; Rodriguez et al., 2001), and individual spores have been shown to hold a high number of nuclei of differing genomes (Raab et al., 2005). A vague definition of AM fungal "species" further complicates the considerably underestimated 200 described species (Schüßler, 2006), which is also due to the shortage of rigorous taxonomic studies of AM fungi. Attempting to compare AM fungi to determine diversity at the species level may only provide an evaluation of morphological diversity because "the life-history traits important to the functional symbiosis (e.g., amount and architecture of external hyphae; proportional fungal biomass as arbuscules versus vesicles, hyphae, auxillary cells, or spores; and absorptive and transport capacity of hyphae) are not linked to any apparent character traits used to distinguish species" (Morton and Bentivenga, 1994).

Classical methods of measuring AM fungal diversity and community structure include visual identification of spores or intraradical structures, but these methods are limited due to morphological similarities at the genus and species level, and due to some AM fungi not staining within roots (Morton and Redecker, 2001). To avoid the strong selection biases of using trap cultures to identify and describe AM fungal species diversity based on spore morphological characteristics, PCR-based molecular methods have been increasingly used to characterize AM fungi and other soil microbial communities in recent decades (Anderson and Cairney, 2004). Molecular analysis of spore DNA has been widely performed, but the same sporulation bias arises as when using greenhouse trap cultures (Oehl et al., 2005): the proportion of infrequently sporulating species or dormant species is increased, and the relative proportion of species within the community may not be representative of those which are actively colonizing the roots of hosts in the field (Oehl et al., 2010). The current symbionts of the crop are not necessarily represented in the spore population, which is an accumulation of the site's sporulation history (Hijri, 2006). This is one reason why performing molecular analysis on crop roots may be more accurate. However, root colonizing fungal structures and spores are only part of the AM fungal community; the majority of the fungal biomass exists in the mycelial network in the soil (Johnson et al., 2010), representing a large portion of the host-fungus interaction by playing the important role of acquiring mineral nutrients for the plant host. By extracting DNA from whole soil (including spores, roots, and extraradical hyphae), a more extensive and inclusive assessment of AM fungi in an agroecosystem can be obtained (Johnson et al., 2010). Biochemical analysis of fatty acid composition has also been employed to characterize

soil fungal communities, but these assessments often yield general fungal biomass estimations with only a low level of taxonomic specificity when they rely on the quantification of sterols or chitin, neither of which are exclusive to AM fungi (Alkan et al., 2004). However, signature fatty acids exist that are AM fungal specific, and have been used as an effective tool to determine biomass of multiple AM fungal morphotypes (Rasmann et al., 2009). Therefore, unique PCR primers may be used to detect individual species with greater certainty.

Quantitative real-time PCR (qPCR)-based methods may provide rapid, accurate, and sensitive quantification of AM fungi. This method amplifies and simultaneously quantifies a specific targeted DNA sequence by employing sequence-specific oligonucleotide probes that are labeled with a fluorescent reporter (flourophore) that hybridizes with its DNA target. Detection occurs by measuring amounts of fluorescence to provide a relative amount of DNA copies present (Heid et al., 1996). By comparing a measured DNA sample to a linear regression of a standard curve, the amount of DNA target in a sample can be calculated, thus producing a comparison between different experimental treatments. This process overcomes the limitations that typify the endpoint detection methods used in conventional PCR (Alkan et al., 2004), specifically by providing increased sensitivity and ability to easily quantify target DNA concentration.

Molecular methods offer a distinct advantage due to the unique ability to identify AM fungi based not only on spore DNA, but any type of fungal cell or propagule present such as hyphae or structures attached to the host root (Clapp et al., 1995; Read, 2000). Additionally, since AM fungal species can be identified by their DNA, this circumvents the problem of the inability to culture AM fungi axenically. However, DNA-based qPCR

analyses cannot enumerate propagules or determine total biomass due to previously described issues specific to AM fungi, but do provide more detailed information on the AM fungal species present in a given soil sample by allowing relative comparisons between samples. qPCR profiling is not a sufficient method of gaining understanding of the physiology and function of the AM fungus detected because linking genomic diversity with functional diversity in an agroecosystem remains a challenge (Torsvik and Øvreås, 2002; Kirk et al., 2004; Anderson and Cairney, 2004). However, if significant community structure differences are found, qPCR can identify AM fungi that may be good targets for functional studies. Nevertheless, phylotype profiling is presently one of the more powerful tools to study the population genetics, evolution, diversity, and community composition of AM fungi with a high degree of specificity and accuracy. It is most useful when targeting specific phylotypes, due to the ability to design specific primers and probes, and when highly accurate quantification is desired. The use of molecular identification methods in field settings has produced new insights into AM fungal ecology (Opik et al., 2006).

To select for a particular fungal species, a primer must be designed for a unique sequence within its genome. Many conventional PCR primers have been described based on highly conserved regions of nuclear and mitochondrial ribosomal DNA (rDNA) (Simon et al., 1993; Abbas et al., 1996; van Tuinen et al., 1998; Lanfranco et al., 1999; Redecker, 2000; Millner et al., 2001; Schussler et al. 2001) to amplify two variable non-coding regions: the internal transcribed spacers (ITS) and the intergenic spacers (IGS) such as the 18S and 28S genes. The nuclear ribosomal genes are currently the only available molecular markers for identification of AM fungi. These genes comprise highly

conserved as well as variable regions, therefore are useful for a wide range of purposes, including designing group-specific primers (Redecker, 2000). The 18S gene is frequently used in AM fungal community studies because it can reliably discriminate different groups of AM fungi with a relatively short sequence.

Despite the immense utility and precision of qPCR, there are several limitations to this method that are related to the genetic nature of AM fungi. The variable regions of the genes most useful for species identification also show some variation within the organism (Jansa et al., 2002). Specifically, unlike most other fungi, the AM fungal rDNA sequence is variable, even within individual spores (Sanders et al., 1995; LloydMacGilp et al., 1996; Lanfranco et al., 1999). These variable rDNA sequences are also not always identical within a morphotype (Redecker et al., 2003), which makes it impossible to assign a single sequence to a given fungal isolate (Hijri et al., 2006). The identification of closely related morphotypes and the differentiation of isolates within a morphotype are also compromised. Given that there are often 1,000-5,000 nuclei in the same spore (Becard and Pfeffer, 1993), and there are about 75 copies of ribosomal genes for each nucleus (Hosny et al., 1999), a considerable amount of sequence variability exists. However, the ITS regions have been determined to have more variation than the more conserved regions of genes. Jansa et al. (2002b) found that ITS sequences within a single spore isolate of *Glomus intraradices* were as different as sequences from other spores. It has been proposed (Kuhn et al., 2001; Hijri and Sanders, 2005) that this genetic heterogeneity is linked to polygenetic structure of the glomeromycotan genome, although this proposal is disputed (Pawlowska and Taylor, 2004). The identity of unique glomalean markers is difficult to establish due to the

numerous microorganisms that live on AM fungal spores, and it has been difficult to acquire alternative sequence markers to rDNA (Redecker, 2002).

Since there is no simple correlation between sequence identity and morphotype identity, it has been necessary to group taxa based on shared ecological or physiological characteristics (Redecker, 2003). Due to the problematic nature of defining AM fungal species by molecular methods, similar sequence groups, not species, are identified in molecular studies of AM fungal communities (Redecker, 2003), and are known as “phylotypes.”

Agricultural soils are often dominated by *Glomus* species (Daniell et al., 2001; Jansa et al., 2003; Oehl et al., 2003; Troeh and Loynachan, 2003; Sjoberg et al., 2004). This dominance may be due to the extremely low diversity of hosts in monoculture cropping systems (An et al., 1993; Burrows and Pfleger, 2002; Oehl et al., 2003), or due to the strong selection pressure imposed by agricultural practices. These practices tend to select for a predominance of species that colonize roots quickly (Oehl et al., 2004) and those that are able to tolerate repeated disruption of the hyphal network, periodic absence of a host, and the application of fertilizers and fungicides (Gosling et al., 2006). *Glomus mosseae* and *G. intraradices* have been classified as generalists (Oehl et al., 2003) due to their abundance across disturbed and more mature ecosystems (Sykorova et al., 2007a), and being widespread geographically across a range of habitats (Opik et al., 2006). Monoculture may also select for AM fungal species that provide limited benefits to the host plant (Johnson, 1993), and sporulate rapidly (Oehl et al., 2003). While AM fungi have traditionally been considered to be non-host specific (Klironomos, 2003), the host plant may play an active role in selecting which AM fungal species

colonize its roots (Sanders and Fitter, 1992; Helgason et al., 2002; Davison et al., 2011), and this concept will be evaluated further in this study.

In an agroecosystem, the activity and diversity of AM fungi have vital functions relating to crop nutrition, soil water retention, soil aggregation and stabilization, and stress tolerance (Graham and Miller, 2005, Rosendahl, 2008, Gianinazzi, 2010). In order to fully understand how AM fungi are affected by agricultural practices, it is important to examine AM fungal diversity and community structure in addition to abundance measurements such as colonization level, infection potential, or number of spores. Due to varying physiological characteristics, different species of AM fungi respond in diverse ways to changes in the environment. For example, soil temperature and moisture changes may occur due to the amount of organic amendment, causing some AM fungi to sporulate more frequently than others (Nemec, 1987), although imposed treatments have been found to have a more significant effect in controlling microbial biomass and community structure than temperature and moisture (Buyer et al., 2010). Such physical and chemical properties of soil markedly change when land use transitions are imposed on a system when transitioning from conventional to organic agriculture, altering not only the activity of AM fungi, but also the number and relative abundance of species. AM fungal community structure has been known to vary between different soil ecosystems and plant communities across a region (Aldrich-Wolfe, 2007; Galvan et al., 2009; Oehl et al., 2010). However, geographically separated study sites can obfuscate the cause of these community differences due to the dominating effects of soil type and soil disturbance on AM fungal communities (Galvan et al., 2009). While many studies have compared AM fungal community variation under

different cropping systems or land use gradients (Galvan et al., 2009; Lumini et al., 2010; Santos-Gonzalez et al., 2011), few studies have examined the relationship between agricultural practices and soil properties, and linked them to AM fungal diversity and community structure (Hijri et al., 2006; Verbruggen et al., 2010; Johnson et al., 2010; Wu et al., 2011).

Effects of different land management practices on soil fungal diversity and community composition are studied far less frequently than soil bacterial communities (Anderson and Cairney, 2004). Only a few studies have examined the degree to which AM fungal species richness and composition differ between organic and conventional farming systems (Oehl et al. 2003, 2004; Hijri et al. 2006). Specific environmental conditions have been found to have developed differing associated AM fungal communities (Dumbrell et al., 2010; Koenig et al., 2010; Johnson et al., 2010; Dumbrell et al., 2011). Diversity of AM fungal communities has been shown to be substantially lower in conventional agroecosystems when compared to organic systems (Helgason et al., 1998; Daniell et al., 2001) and natural ecosystems (Menendez et al., 2001), although significant overlap can occur (Galvan et al., 2009). Communities of AM fungi in organic agroecosystems have been found to be more similar to those of natural grasslands than to conventional agroecosystems, and less uniform (Verbruggen et al., 2010). When farming systems transition to a different management strategy, the time since transition, the duration of organic management, and the conditions of the previous history of conventional management can impact AM fungal communities differently (Rasmann et al., 2009). Verbruggen et al. (2010) found that AM fungal richness increased significantly with the time since conversion to organic management. They

found that the duration of organic management explained 15-50% of the variance in richness.

Changes in AM fungal diversity and community structure are of notable importance for study due to the interactive effects with plant health, yield, and soil quality. In one study (Muthukumar and Udaiyan, 2002), growth and yield responses of cowpea were affected by the responses of AM fungal populations to organic amendments. Specifically, changes in relative abundance of AM fungi within the fungal community may have augmented host crop growth and yield found with organic amendments. In contrast, long-term conventional, high input management tends to favor inefficient species of AM fungi (Johnson et al., 1992; Johnson, 1993), thus facilitating a more parasitic association (Ryan and Graham, 2002). Since AM fungal morphotypes affect crops in differing ways, oftentimes depending on the conditions induced by management strategy, it is important to investigate how relative abundance changes for commonly found AM fungal species.

CHAPTER 2
EVALUATION OF MYCORRHIZAL INOCULUM POTENTIAL OF COVER CROPS AND TOMATO AS AFFECTED BY SOIL ORGANIC AMENDMENTS

Background

Measuring the percent of root colonized by AM fungi is one of the most common methods for assessing the mycorrhizal symbiosis under contrasting conditions for many reasons. Although this method does not reveal information about the species diversity of AM fungi in the soil, their spatial variation, or their functioning, it provides an effective, inexpensive, and accurate description of the degree to which AM fungi are interacting with host plants. While technologies for studying the mycorrhizal symbiosis have greatly improved over the past 40 years, measuring percent colonization is the primary parameter for assessment of AM fungal activity under field conditions because it is significantly affected by many soil and environmental factors. Soil nutrient levels (Smith and Read, 1997; Kelly et al., 2005), amount of tillage (Galvez et al., 2001), pathogen infection (Karagiannidis et al., 2002), and plant host presence or absence (Ryan and Graham, 2002) are primary factors affecting root colonization.

In agricultural systems that are transitioning from high input conventional to organic or low-input management, increases in colonization have been documented in several studies (Douds et al., 1993; Ryan et al., 2000; Bending et al., 2004), in one case up to 60% (Mader et al., 2000). For example, Werner et al. (1990) found increased colonization in strawberry crops after two years that transitioned from conventional to biodynamic management. Likewise, Limonard and Ruissen (1989) measured a three- to fourfold increase in root colonization of wheat in a field that had been rotated with potato during the transition from conventional to low-input management. The increased root colonization during and after this transition is generally thought to be related to reduced

applications of fertilizers and lower available soil P status that increases crop dependency on the AM fungi for nutrient uptake (Ryan, 1999; Mäder et al., 2000; Ryan et al., 2000).

However, higher colonization does not always occur in organic management because these systems often employ more frequent tillage, or higher rates of soil inorganic and organic P (Ryan and Tibbett, 2008). Phosphorus is the nutrient most often found to affect AM fungal colonization (Bending et al., 2004). High soil P may exist in organic farms due to previous conventional practices of over fertilizing with soluble P which leads to a residual storage or nutrient "bank" of P in the soil, or due to large amounts of soil amendments containing organic P.

Although it is the most commonly used measure of AM fungal activity, root colonization may not necessarily be the best predicator of the physiological status of AM fungi, the effectiveness of the symbiosis, or the host plant growth response (McGonigle, 1988; Jakobsen et al., 2001; Lekberg and Koide, 2005a). Other parameters, such as the quantity and distribution of extraradical mycelium, are possibly more important characteristics to measure the nutrient acquisition capacity of AM fungi (Abbott et al., 1992; Miller and McGonigle, 1992).

For various reasons, it may not be feasible or ideal to measure AM fungal colonization of host roots from the field, or the research may be focused more so on fungal infectivity in relation to soil conditions rather than presence of colonization in crop roots. In these cases, measuring the mycorrhizal inoculum potential (MIP) of soil from the root zone (i.e. rhizosphere soil) can provide a different measure of the AM fungal population activity. This assessment may be broader due to not only indicating which

AM fungal species that are capable of actively colonizing crop roots, but also the propagules that exist as hyphal or root fragments, or as spores. The MIP assay measures the ability of the propagules present in the field soil to form primary infections on host crop roots. This is useful when considering how prevailing soil colonization potential affects future symbioses and success of crops in upcoming rotations or plantings. Also, active symbioses may be highly variable and unpredictable during times of agricultural transition, causing certain AM fungal species and the total available AM propagule population to not emerge as root colonists until the system stabilizes under new management practices. Soils from organic and low-input systems have been found to have much higher MIP when compared to conventional systems (Mader et al., 2000; Menendez et al., 2001; Oehl et al., 2003). The inclusion of green manures and cover crop rotations has also shown to increase MIP as well as the growth and yield of subsequent crops (Dodd and Jeffries, 1986; Galvez et al., 1995; Boswell et al., 1998). However, under conditions of very high P inputs, including from organic sources, a state of plant sufficiency can cause decreased colonization and spore incidence (Rasmann et al., 2009). Thus, the effect of organic management on AM fungi varies depending on crop nutrient needs and fertilizer application rates.

The objective of this study was to use the MIP assay to assess AM fungal activity in the rhizosphere at different rates of soil amendments, types of amendment (urban plant debris and poultry broiler litter), and two host crop types (cover crop or tomato) compared to conventional soil management. The control treatment was synthetic fertilizer to simulate conventional management. I hypothesize that organic amendments will either increase or have no effect on MIP compared with conventional mineral

fertilizer management. Crop type is predicted to have an effect depending on plant nutritional status in response to treatments. Soil treatments are expected to affect MIP through changes in soil moisture and increased organic N and P.

Methods

Field Experiment Site and Design

A field microplot experiment was established in 2006 at the USDA, ARS, USHRL Picos Road Farm in Fort. Pierce, FL. Ninety microplots were constructed from 31 x 31cm ceramic flues 0.9m in length that are open at both ends and buried in the ground with a 5cm lip protruding to minimize contamination of adjacent plots by wind driven rain. In 2006, plots were filled with a Riviera fine sand (loamy, siliceous, active, hyperthermic Arenic Glossaqualfs), fumigated with methyl bromide:chloropicrin (67:33), and then inoculated with the following pests: *Fusarium oxysporum* f. sp. lycopersici race 3, root-knot nematodes (*Meloidogyne incognita*), and purple nutsedge (*Cyperus rotundus*). Two tomato *(Solanum lycopersicum*) cultivars (cv Tiny Tom or Tigris) were cultivated under conventional conditions for two years. Osmocote was used as the conventional fertilizer. Baseline soil conditions were obtained in February 2008 from samples taken from 10 plots (plots number 3, 25, 26, 41, 43, 45, 47, 58, 72, 78). Eight soil cores of 144.4cm^3 each were taken from each plot and mixed thoroughly in an 18.9L bucket. The sample analyzed was from the combined sample, the analysis of which is presented in Table 2-1.

In April of 2008, experimental treatments to begin the 3-year transitional period were established in the microplots using a 3 x 3 x 2 factorial design consisting of 3 rates of urban plant debris (0, 30, and 60 g Kg^{-1} soil (wt:wt)), 3 rates of broiler litter (0, 10, and 20 g Kg^{-1} soil (wt:wt)), and two planting regimes: tomato and cover crop (Fig. 2-1).

Amendment rates for each treatment are listed in Table 2-2. These 9 treatments were randomly assigned to the plots and replicated 5 times per crop type (Fig. 2-2). Broiler litter (BL) was obtained from broiler houses in the Live Oak, FL region, and consisted of a mixture of pine shavings and chicken manure that was dry stock, aged between 6 and 12 weeks in static row piles. Urban plant debris (UPD) was tub ground and partially composted. Corresponding fertility rates are presented in Table 2-3. Instead of methyl bromide fumigation to eliminate pests, plots were solarized using clear plastic film.

To simulate conventional agriculture conditions, osmocote (10-2-12), was applied at a rate equivalent to 224-19-139 kg/ha of N-P-K (Treatment 1, Table 2-2). Organic amendments (Treatments 2 to 9, Table 2-2) were applied on April 22-25, 2008. Tomatoes (cv Florida 91) were planted in half of the plots, and cover crops in the other half. Cover crops consisted of Sunn hemp (*Crotolaria juncea*) and Japanese millet (*Echinochloa crusgallii*) and were planted alternately after each harvest. All plants and their root systems were removed during the incorporation of amendments, and the roots were reincorporated after amendment materials had been thoroughly mixed into the microplot soil to a depth similar to that of a plow. In July 2010, after two years of transitional period cropping, tomato (cv Florida 91) was planted in all 90 microplots for the final year of the experiment. Table 2-4 contains the chronology of amendment applications, planting, and other treatments executed on the microplots.

Soil Sampling

Sampling took place in 2010, the fourth year of the microplot experiment, in three months: February, April, and October. Soil samples were collected for MIP assay on February 20-21 and April 28 during one cropping cycle when tomato and millet hosts were planted, and October 21 during another cropping cycle when only tomato was

planted (Table 2-4). Four cores to a depth of about 20 cm were extracted from random locations within each microplot. Soil cores were combined by plot and refrigerated in plastic bags before later use for MIP assays. Later, all 5 samples from each treatment-crop combination in October were combined due to inadequate total volume of each plot sample to fill MIP assay containers.

Cores were also taken separately by researchers at the USDA research center and analyzed for three soil parameters. Nitrogen (N) was measured using the Kjeldahl method to obtain Total Kjeldahl Nitrogen (TKN); Phosphorus (P) was measured using the Mehlich III method; and moisture content was measured gravimetrically.

Infection Potential Study

The MIP assays were conducted in a temperature controlled greenhouse (diurnal temperatures ranged from 30-35ºC in July/August 2010 in the first period of assays, and 23-31ºC in the last assay in October/November 2010) at the University of Florida in Gainesville, FL. Soil samples were sieved to homogenize the soil and to remove root tubers of purple nutsedge (*Cyperus rotundus*), and the soil was divided into subsamples to fill three 150mL containers. However, since there was not enough soil from the October sampling date to fill three containers from each plot, soil from plots of the same treatment and crop type were bulked and homogenized. For example, all soil samples from the 5 plots that had Treatment 1 and tomato were combined. Although in October, all plots contained tomato plants, soil samples were still kept separate according to the type of crop that the plot had contained previously, thus allowing testing for crop effects on soil that had transitioned from previously having a cover crop. This soil was then used to fill five containers and labeled according to treatment number and crop type. For all three sampling date sets, three maize (*Zea mays*) seeds were planted in each

container, and thinned to one plant after seedling emergence (Fig. 2-3). Since the maize seeds were treated with a fungicide, they were washed several times with soap and water to remove the fungicide residue. Plants were not fertilized, but watered as necessary, and harvested after 28 days. Aboveground plant growth was cut and removed, and soil was gently washed from the roots. A small amount of fine roots were cut from the midsection of each plant's root system and placed into a nylon screen-lined tissue cassette.

Clearing and Staining Roots

Roots were cleared and stained for observation of AM fungal colonization according to the protocol described by Brundrett et al. (1996). Cassettes were immersed in 10% (w/v) KOH solution and autoclaved for 10 minutes. After rinsing with tap water, cassettes were briefly immersed and stirred in diluted Clorox bleach (5% NaClO) for less than one minute to remove any remaining pigment. After repeated rinsing with water, cassettes were immersed in 2% HCl solution for approximately 10 minutes, then drained and covered completely in trypan blue 0.05% (w/v) staining solution (1:2:2 lactic acid - glycerol - water) for approximately 24 hours. Staining solution was poured off, and roots were stored in water and refrigerated until prepared for observation.

Determination of Percent Colonized Root

AM fungal colonization was quantified by the grid-line intersect method (Giovannetti and Mosse, 1980). Cleared and stained maize roots were spread onto a Petri dish lined with grid paper. The presence or absence of AM fungal structures (arbuscules, vesicles, and coarse hyphae) was observed at 100 root intersections with the gridlines using a dissecting microscope (Wild Heerbrugg M5-51522, Switzerland) at

a magnification of 40 x. The percent of root length colonized by AM fungi was calculated as the total number of intersections of infected roots divided by the total of 100 intersections. (Fig. 2-4, 2-5, and 2-6)

Statistical Analysis

Using the statistical software R (R Development Core team, Murray Hill, New Jersey, United States, version 2.15.0), a 3-way analysis of variance (ANOVA) test was performed on February and April datasets to assess differences between treatments, fertilizer type, crop type, and between the three sampling dates. The relationship between percent colonization and each of the three measured soil parameters (percent moisture, N, and P) was plotted and evaluated visually for correlation.

Results

MIP Assays

Colonization of maize roots in the MIP assay varied greatly by crop type, within each treatment, and across sampling dates. Overall, colonization levels were substantial despite very high levels of nutrients (especially P) applied in the organic fertilizers, and high levels of disturbance. At all three sampling times, the conventional plots (0UPD/0BL) had slightly lower levels of colonization, although not statistically significant. Soil planted with tomato had slightly higher levels of colonization in the MIP assay than cover crop soil in 5 of the 9 treatments in February (Fig. 2-7) and October (Fig. 2-9), and 8 of the 9 treatments in April (Fig. 2-8). No significant treatment, crop, or amendment level effects were found in February (P = 0.9524) or April (P= 0.4275).

Colonization levels in the MIP assay were clearly affected by the time the assay was conducted (Fig. 2-10). The levels across all treatments were substantially lower in

April than in October and February, although colonization levels varied more among plots in October than in February and April.

To more closely examine the effect of amendment type and rate of application of amendment type, data was separated and analyzed according to amendment type (BL or UPD), rate of application (0, 10, or 20 g Kg^{-1} soil for BL; 0, 30, or 60 g Kg^{-1} soil for UPD), and crop (Fig. 2-11, 2-12, and 2-13). No significant effects were found.

Soil Nutrient Parameters

Soil parameter data for each plot within the nine treatments are presented in Appendix A. There were no evident trends in the relationship between AM fungal colonization and soil N, P, or moisture levels. There was a high level of variation in AM fungal colonization, which did not correlate significantly with any of the soil parameters. However, the overall data showed slight changes in soil parameters by month that were similar to those evident in the colonization vs. treatment data, i.e., levels of soil N, P, and moisture generally decreased in April and rebounded in October.

Discussion

Overall levels of colonization were quite high despite very high levels of nutrients (especially P) applied in the organic fertilizers. This may be due to the soil conditions established with the soil amendments that are beneficial to AM fungi, such as improved soil moisture and soil structure, and possibly due to P and N being less bioavailable in their organic form, reducing levels of these nutrients in the plant and soil environment. It is difficult to ascertain if there were any effects of any remaining fungicide residue on the maize seeds on mycorrhizal colonization levels; if a suppressive effect was present, it was likely minimal.

The decrease from February to April is likely due to new and recent amendment material incorporation into the plots right before soil sampling took place, causing very recent disturbance to the hyphal network, and possibly a dilution effect of propagules in the soil by the amendment. These plots can be considered highly disturbed due to the removal and replanting of host crops, and incorporation of organic matter multiple times per year. It is again surprising that percent colonization levels in the MIP assay were so high despite repeated disturbance to the mycelial network. This lack of effect of disturbance has been attributed to the resilience and tolerance of the predominant AM fungi in this type of agroecosystem (Rasmann et al., 2009). Also, the six weeks without a host could explain the increased variability in MIP for the October sampling time (Fig. 2-10). Not only were the AM fungal communities adjusting due to the conversion from conventional to organic management in 2008, but were also responding to and recovering from various disturbance, fertilization, and pest stressor events. These factors may impose differing selective pressures on the relative amounts of propagules in the soil, and AM fungi may respond in different ways depending on their biological differences (e.g. some sporulate more frequently in times of environmental stress).

A major change in the microplots occurred between April and October, and is also an important component of this study: tomato plants were planted in all microplots, which introduced a different host to the AM fungi in the half of the plots which had previously had a cover crop. If there is a host specificity effect on AM fungal communities and propagules, this change in hosts could be expected to affect AMF populations. The rebounding levels of colonization in October may have been related to the change in host, implying that the AM fungal communities in the cover cropped soil

benefited from the change to tomato. Alternatively, there may have been a host-mediated change in those AM fungal communities, in which the tomato plant allocated more C to the fungus, thereby increasing the presence of AM fungal propagules, or had an increased need for AM fungal assistance in nutrient uptake compared to millet, thus also causing it to allocate more C and foster the symbiosis. Tomato plants may have also had an increased need for assistance in N acquisition since millet was growing in plots that had previously received elevated N levels in the soil due to having an N-fixing legume in recent cropping cycles, thus high levels of N may have persisted in the microplots and were available to the millet. However, the rebound in colonization levels in October may have simply been caused by a readjustment to normal levels after enough time had passed since the disturbance event (incorporation of amendments) in April. It is interesting to note that colonization levels are very high in October despite the many applications of supplemental organic fertilizer from July to October (Table 2-4), which may have been expected to contribute to lower colonization levels due to plant sufficiency.

Soil from tomato plots yielded slightly higher average levels of colonization in a vast majority of the treatments when compared to soil from cover cropped plots. It is possible that this was due to a decreased need for N in the cover-cropped soil, and thus a decreased C allocation to AM fungi. However, if spores were the primary component of the total AMF propagules in the soil, and if there was a unique effect of cover crops on AMF sporulation, they may have played a bigger role in colonizing greenhouse maize plants, which would in turn affect inoculum potential in ways that were not measured in this study.

Table 2-1. Soil nutrient status in the microplots in February 2008, before soil organic amendments were applied.

N (kg/ha)	P (kg/ha)	K (kg/ha)	C:N ratio	Organic Matter (%)	Bulk density[a] (g/cm^3)	pH	Cation Exchange Capacity
543.75	139.2	134.85	32	1.2	1.45	6.3	3.5

[a]Bulk density calculated from samples taken 1/10/2008 from 10 plots: 3, 10, 20, 31, 44, 54, 65, 70, 77, and 87.

Figure 2-1. Rows of microplots at the USDA research station in Fort Pierce, FL, in October 2010, at which point all plots are planted with tomato. Photo courtesy of Megan Smith.

Table 2-2. Nutrient status of broiler litter and urban plant debris of an aggregated sample from 10 plots (plots number 3, 25, 26, 41, 43, 45, 47, 58, 72, 78) in 2010. All units are kg/ha. Absent data fields were either due to data being unavailable or not applicable.

	Broiler Litter (10 g Kg^{-1} soil rate)	Broiler Litter (20 g Kg^{-1} soil rate)	Urban Plant Debris (30 g Kg^{-1} soil rate)	Urban Plant Debris (60 g Kg^{-1} soil rate)
Total Solids	11192	22384	-	-
Total Ash	483	967	-	-
Total N	680	1360	1.76	3.51
Ammonia N	159	319	-	-
Est. Avail N	646	1292	-	-
Total P	301	602	0.21	0.41
Total K	653	1306	11.27	22.53

Table 2-3. Amount of each fertilizer amendment type in each treatment, in kg/ha.

Treatment number	Urban Plant Debris (UPD)content (g Kg^{-1} soil)	Broiler Litter (BL) content (g Kg^{-1} soil)	Treatment identification
1 -control	0	0	0UPD/0BL
2	0	10	0UPD/10BL
3	0	20	0UPD/20BL
4	30	0	30UPD/0BL
5	30	10	30UPD/10BL
6	30	20	30UPD/20BL
7	60	0	60UPD/0BL
8	60	10	60UPD/10BL
9	60	20	60UPD/20BL

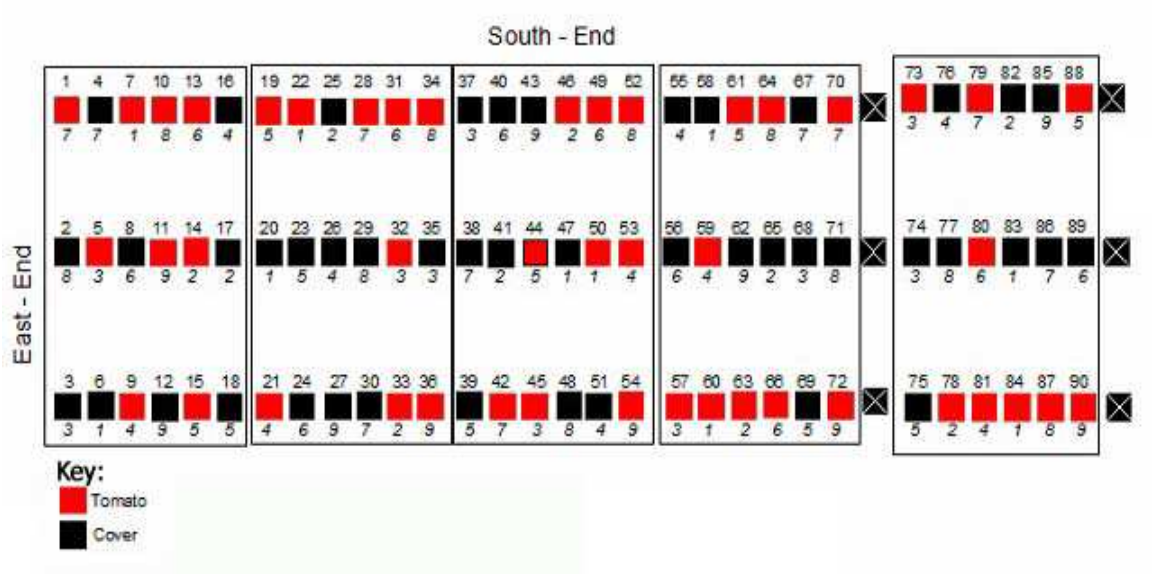


Figure 2-2. Schematic of microplots and experimental design. Numbers above microplot squares are plot number; those below the squares are treatment number (see Table 2-2 for treatment identification).

Table 2-4. Chronology of selected activities and treatments applied to the microplots in 2010. Tomatoes were replanted due to losses from bacterial wilt.

Date	Activity
January 11	Planted tomatoes or millet
February 2	Sampling for DNA analysis
February 20-21	Soil sampling for MIP assay. Plots included tomato or millet.
April 20-23	Sampling for DNA analysis
April 28-30	Amended plots with fertilizers
April 28	Soil sampling for MIP assay. Plots contained no crop plants.
May 3	Solarization with plastic sheets
July 12	Planted Tomato 'FL-91' in all plots
July 16	Due to plant stress, fertilized w/ 150 mL 20-10-20 per plot for conventional (treatment 1) and organic treatments received fish emulsion and seaweed kelp at 11.23g (ea.) per liter per plot
July 20	Applied supplemental fertilizers (as above)
July 22	Due to worm damage, sprayed plants with Copper, Bt, and Entrust.
July 30	Sprayed plants with Copper, Bt, and Entrust.
August 6	Sprayed plants with Copper, Bt, and Entrust.
August 9	Applied supplemental fertilizers (as above)
August 18	Sprayed plants with Copper, Bt, and Entrust.
August 19	Applied supplemental fertilizers (as above)
August 23	Replanted plots #72, 84, 88, & 4
August 26	Sprayed plants with Copper, Bt, and Entrust. Applied supplemental fertilizers (as above)
September 1	Replanted plots #4, 35, 42, 44, 61, 79, 88
September 7	Sprayed plants with Copper, Bt, and Entrust.
September 8	Applied supplemental fertilizers (as above)
September 9	Replanted plots #4, 21, 28, 34, 36, 42, 61, 87, and 90
September 15	Sprayed plants with Copper, Bt, and Entrust. Applied supplemental fertilizers (as above)
September 17	Replanted plots #28, 34, 36, 44, 53, 59, 83, 84, 87, 88, and 90
September 22	Sprayed plants with Copper, Bt, and Entrust.
September 23	Replanted plots #4, 21, and 42
September 30	Replanted plots #28, 34, 44, 61, 79, 81 and 87
October 1	Sprayed plants with Copper, Bt, and Entrust. Applied supplemental fertilizers (as above)
October 8	Sprayed plants with Copper, Bt, and Entrust. Applied supplemental fertilizers (as above)
October 12-21	Sampling for DNA analysis
October 21	Soil sampling for MIP assay. All microplots contained tomato.

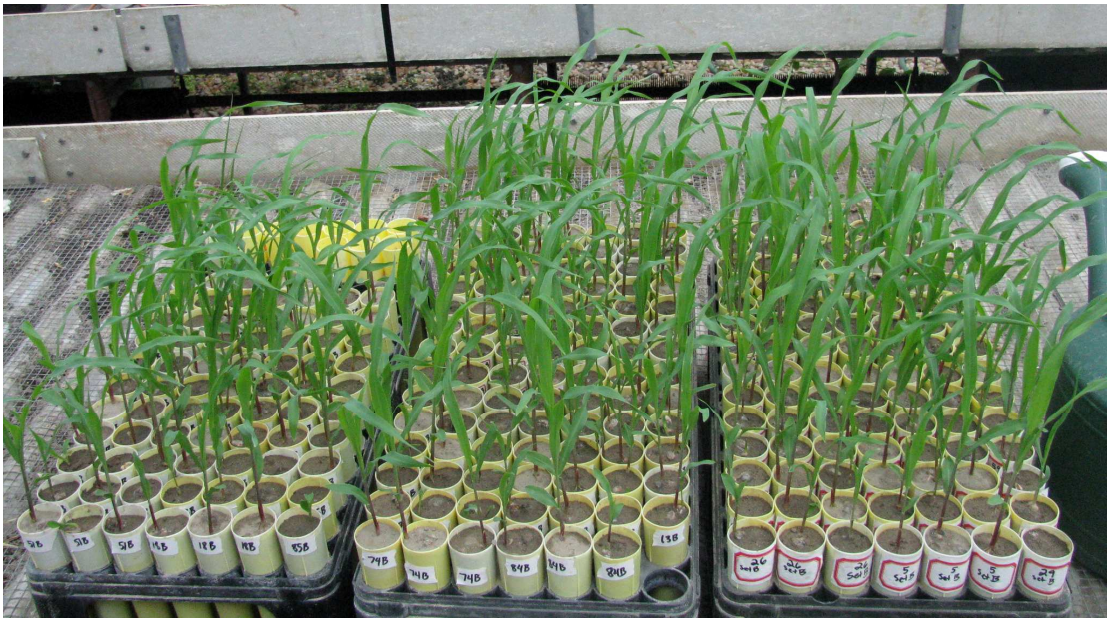


Figure 2-3. MIP assay in June 2010 with soil samples from February 2010, and maize plants. Photo courtesy of Megan Smith.

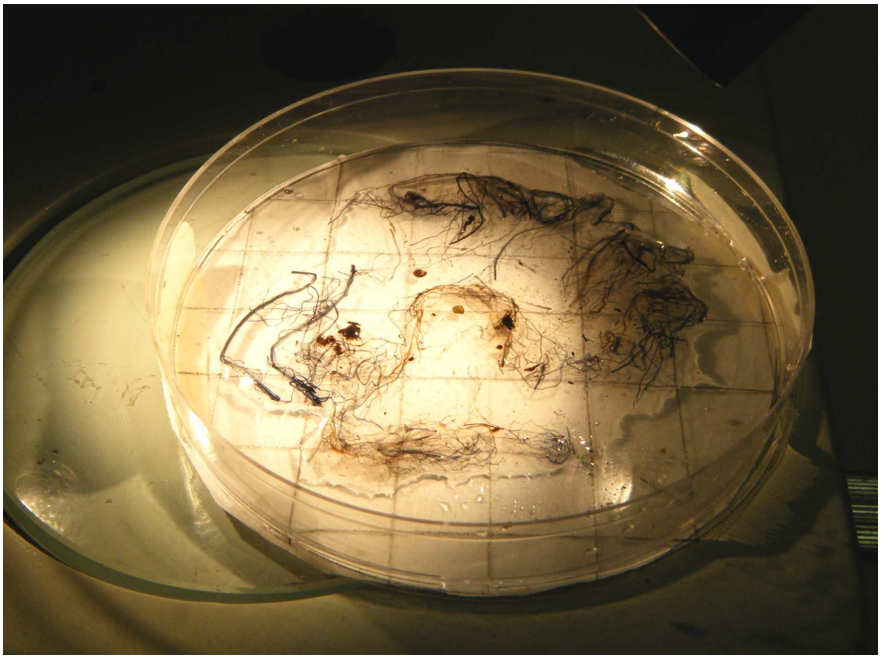


Figure 2-4. Fine maize roots spread out in a Petri dish backed with grid paper. Points at which roots intersected with lines were examined for presence of AM fungal structures. Photo courtesy of Megan Smith.

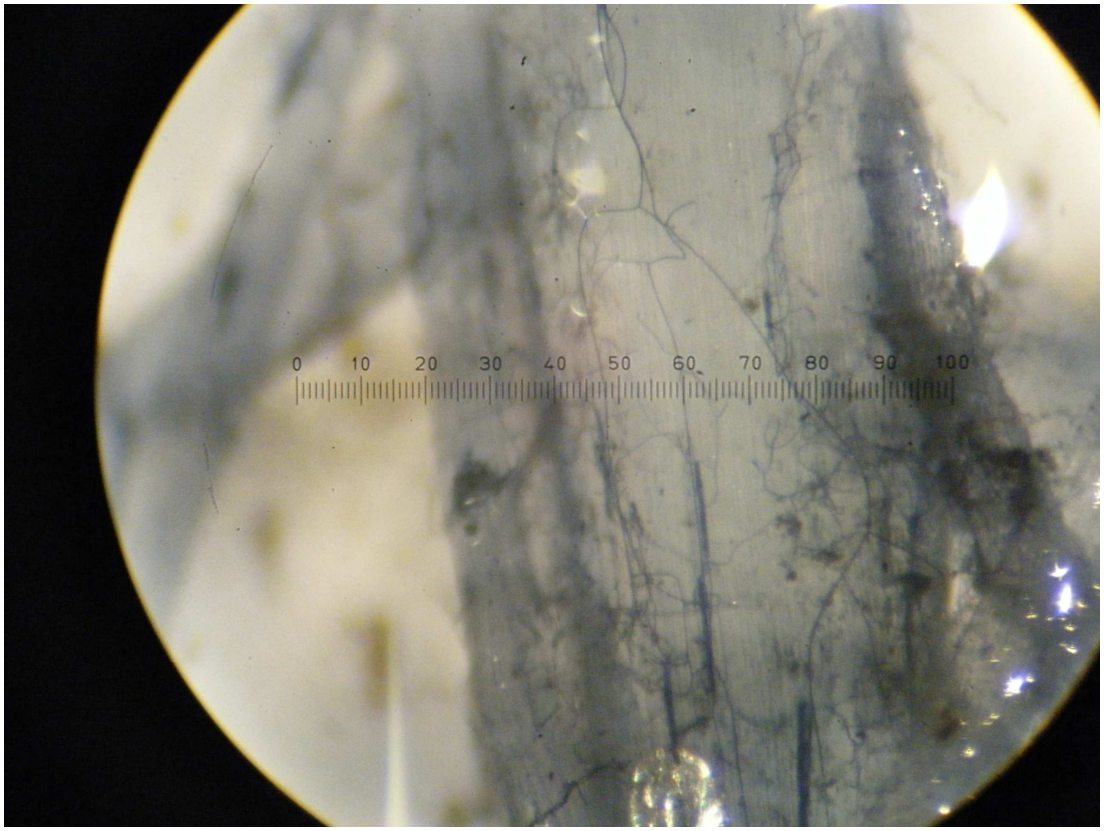


Figure 2-5. A view through the dissecting microscope, showing maize root with mycorrhizal colonization, stained with trypan blue. Approximate magnification is 40x. Photo courtesy of Megan Smith.

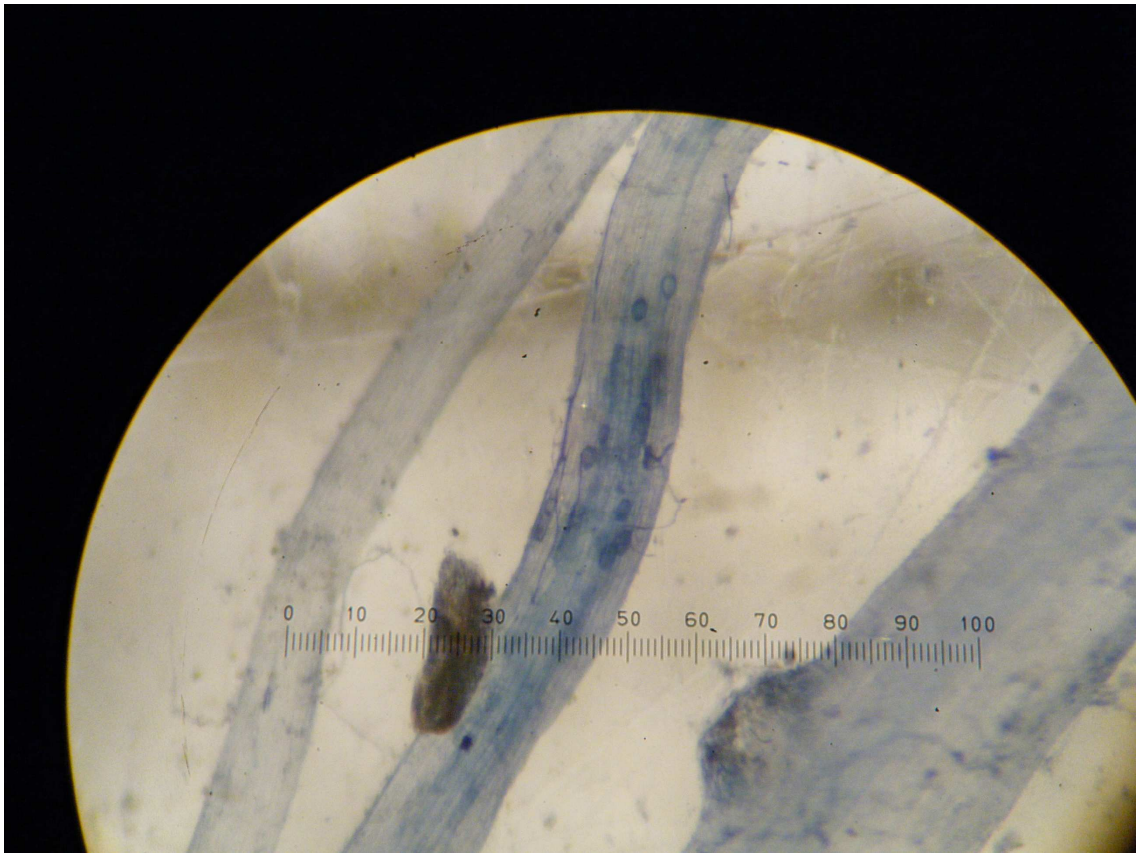


Figure 2-6. Maize roots colonized with AM fungal hyphae and vesicles. Photo courtesy of Megan Smith.

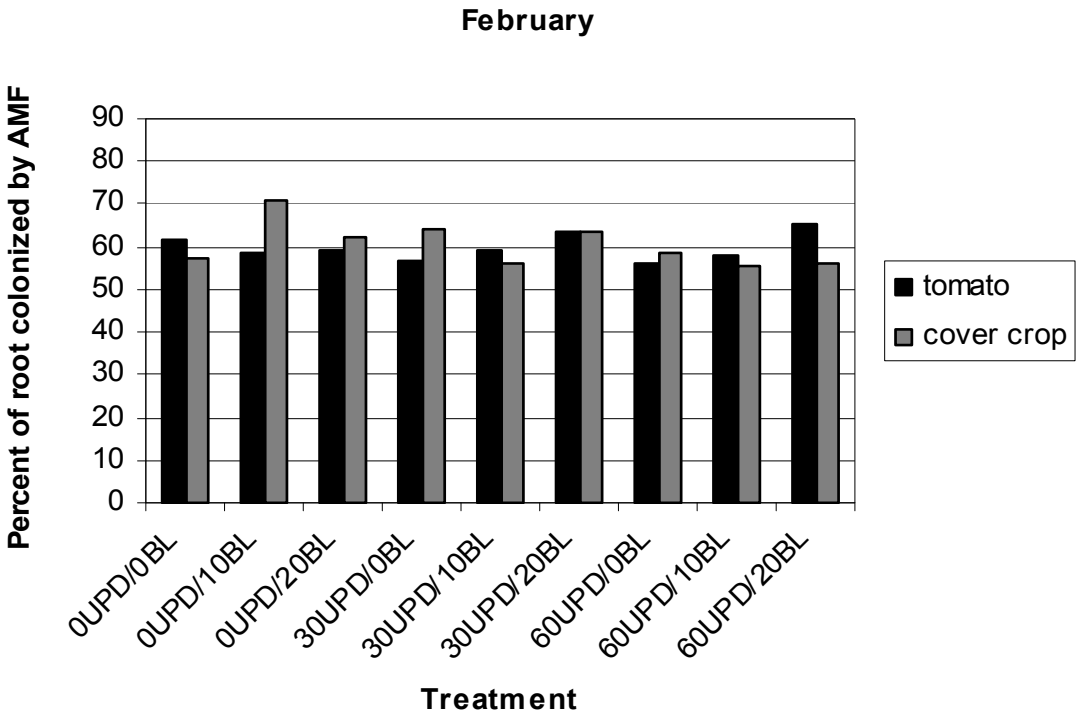


Figure 2-7. Average percent colonization of arbuscular-mycorrhizal fungi (AMF) in a mycorrhizal inoculum potential assay of soil sampled in February 2010 from plots amended with different rates of urban plant debris and poultry broiler litter compared to conventional fertilizer management (control) and planted with two host crop types, cover crop (millet) or tomato. Means were obtained from 5 replicates. See Table 2-2 for treatment identification.

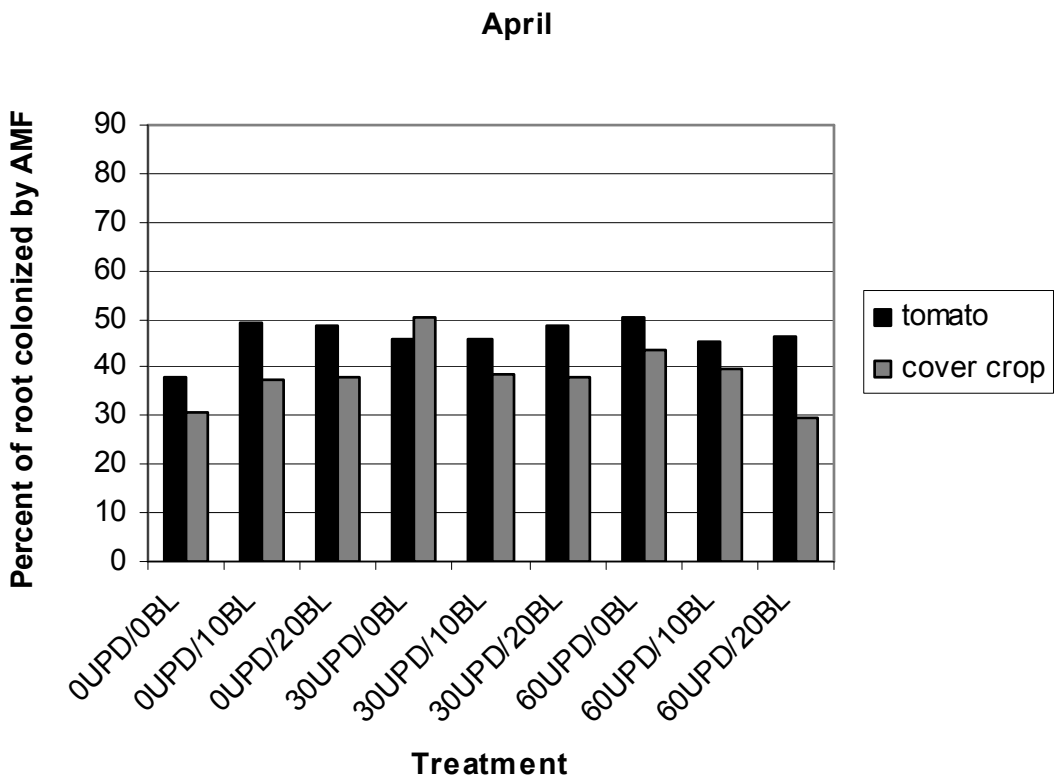


Figure 2-8. Average percent colonization of arbuscular-mycorrhizal fungi (AMF) in a mycorrhizal inoculum potential assay of soil sampled in April 2010 from plots amended with different rates of urban plant debris and poultry broiler litter compared to conventional fertilizer management (control) and planted with two host crop types, cover crop (millet) or tomato. Means were obtained from 5 replicates. See Table 2-2 for treatment identification.

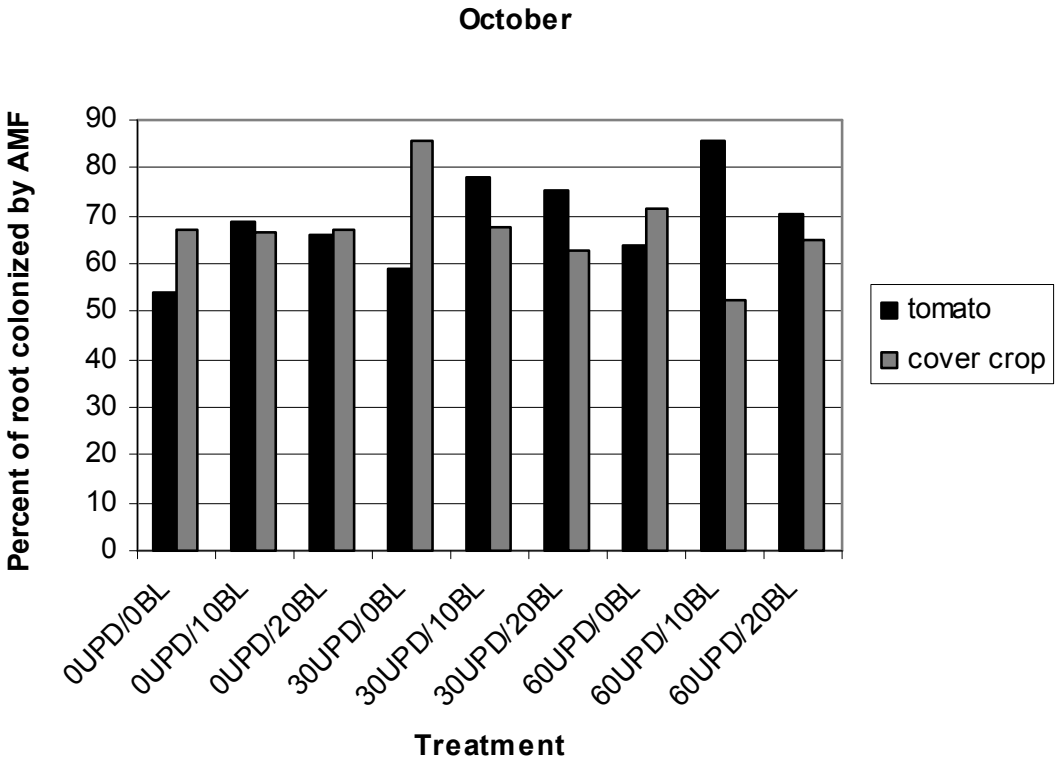


Figure 2-9. Percent colonization of arbuscular-mycorrhizal fungi (AMF) in a mycorrhizal inoculum potential assay of soil sampled in October 2010 from plots amended with different rates of urban plant debris and poultry broiler litter compared to conventional fertilizer management (control) and planted with tomato. Treatments labeled as cover crop were plots previously planted with millet or sunn hemp before the tomato crop cycle began. Percent colonization numbers from the October samples are from the results from one MIP assay per treatment. See Table 2-2 for treatment identification.

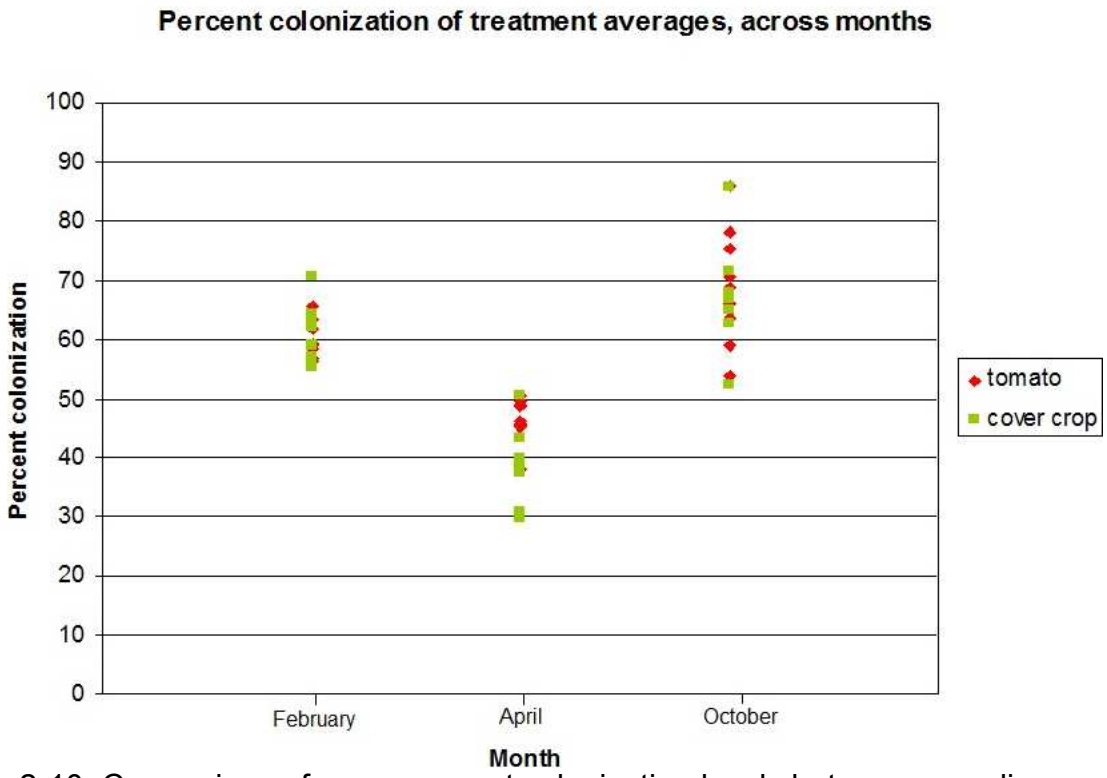


Figure 2-10. Comparison of mean percent colonization levels between sampling months, separated by crop types in each month. Means are shown for each treatment, for a total of 18 data points per month.

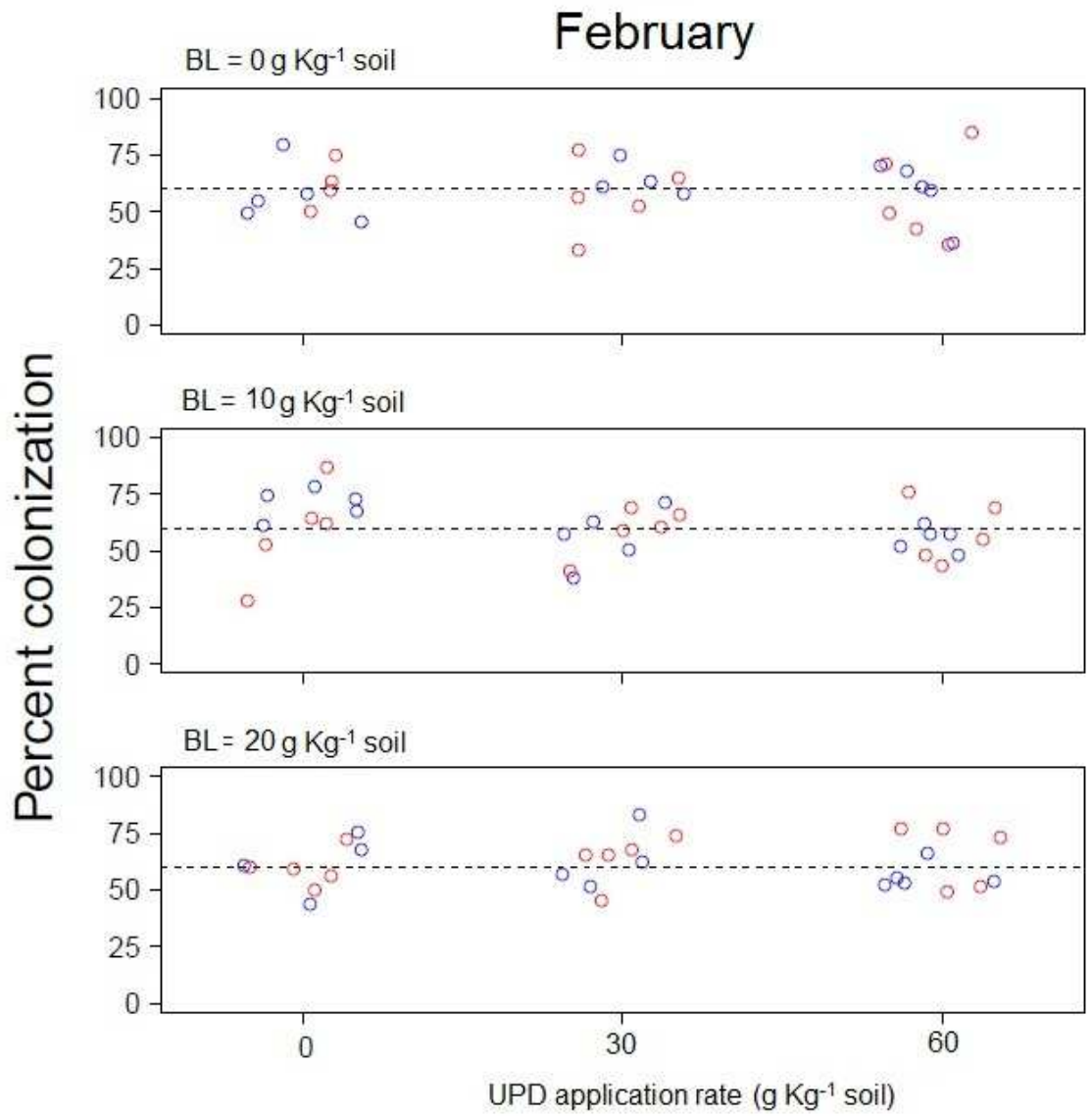


Figure 2-11. Percent colonization for each microplot in February, separated by application rate of BL and UPD. Red circles represent tomato cropped soil, and blue circles represent cover cropped soil. The dotted line is the mean predicted by the statistical model.

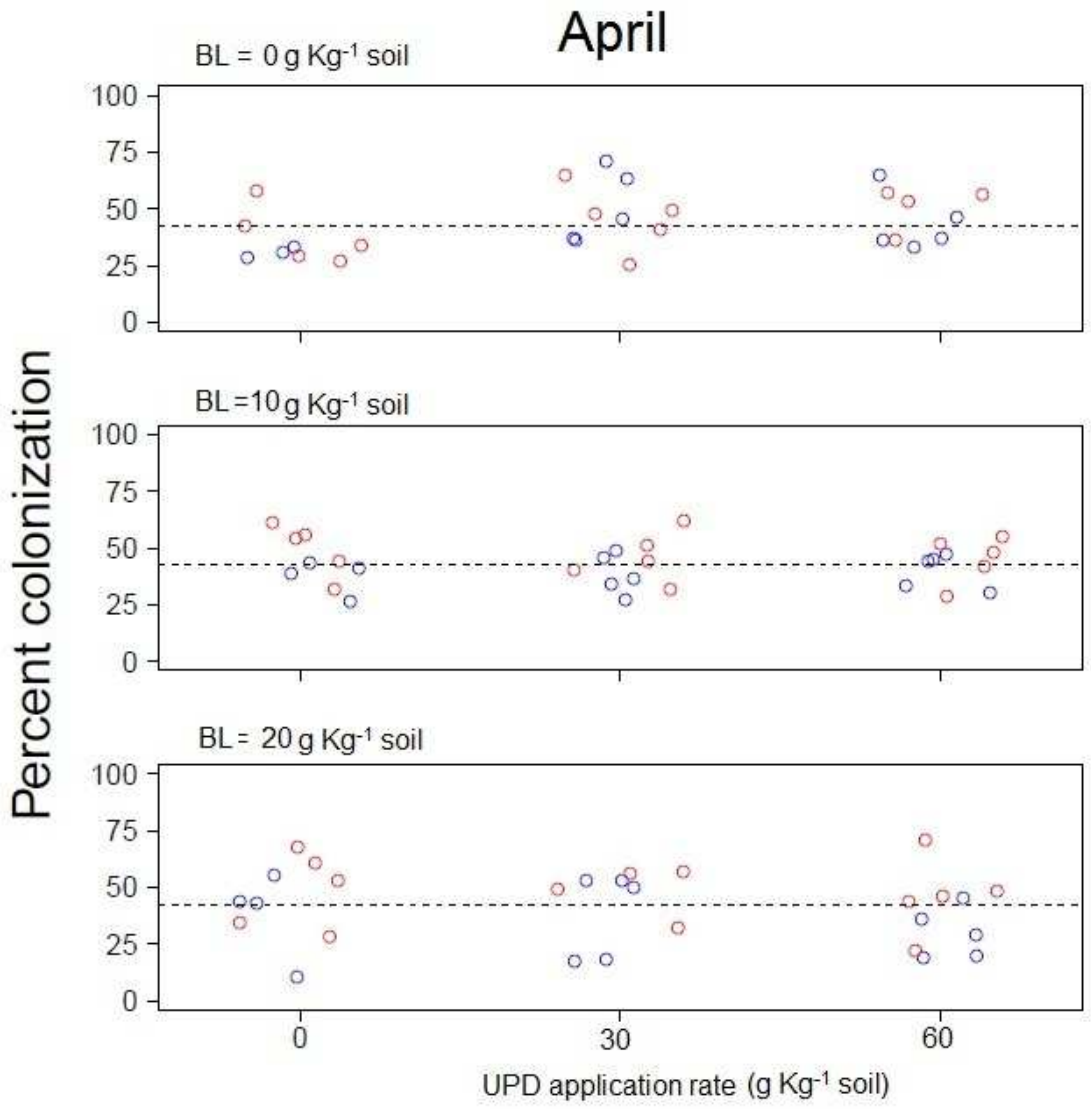


Figure 2-12. Percent colonization for each microplot in April, separated by application rate of BL and UPD. Red circles represent tomato cropped soil, and blue circles represent cover cropped soil. The dotted line is the mean predicted by the statistical model.

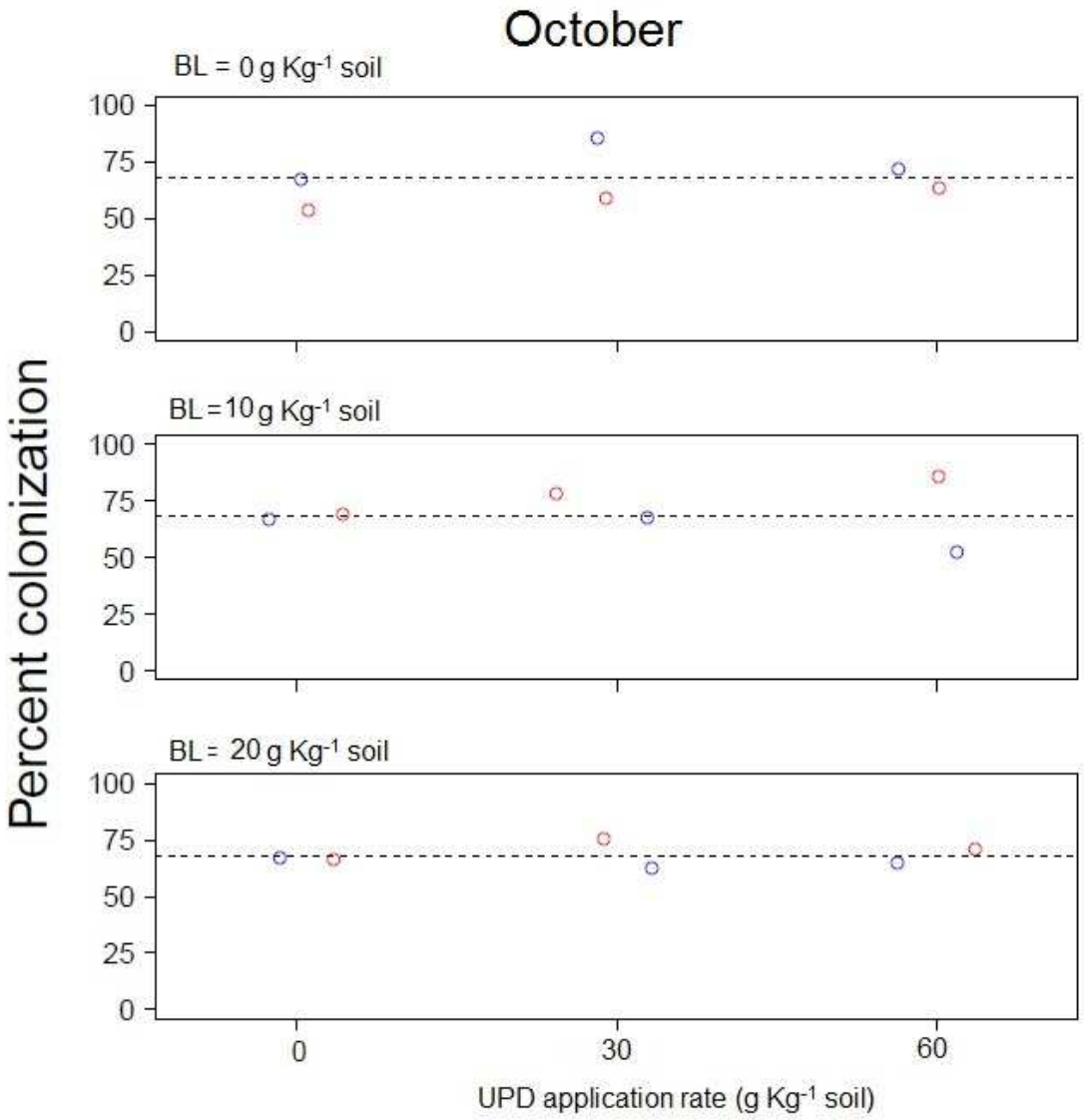


Figure 2-13. Average percent colonization for each treatment-crop combination in October, separated by application rate of BL and UPD. Red circles represent tomato cropped soil, and blue circles represent cover cropped soil. The dotted line is the mean predicted by the statistical model.

CHAPTER 3
EVALUATION OF THE ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITY COMPOSITION USING SELECTED GLOMEROMYCOTIN DNA SEQUENCES

Background

Since AM fungal morphotypes affect crops in differing ways, oftentimes depending on the conditions induced by cultural management practices, it is important to investigate how relative abundance changes for prevalent AM fungal species. Due to varying physiological characteristics, different species of AM fungi respond in diverse ways to changes in the environment. Quantitative real-time PCR (qPCR)-based methods can provide rapid, accurate, and sensitive enumeration of fungal propagules. With this method, the amount of DNA target in a sample can be calculated, thus producing a comparison between different experimental treatments. This process eliminates the limitations that typify the endpoint detection methods used in conventional PCR (Alkan et al., 2004) by providing increased specificity and sensitivity to quantify the target DNA. qPCR offers a distinct advantage due to the unique ability to identify specific AM fungi based on any type of fungal propagule present such as hyphae or structures attached to the host root (Clapp et al., 1995; Read, 2000).

This study aims to assess how the relative abundance of three AMF phylotypes are affected by different sources and rates of organic amendment and host crop, during the transition period from conventional to organic management. qPCR is a highly effective method for accurate quantification of morphotypes for which specific primers are available. Thus, an evaluation of changes in AM fungal community structure in these experimental microplots was based on a selection of three phylotypes for AM fungi detected in a tomato agroecosystem study conducted in a nearby area in the Florida Everglades Watershed (FEW) (E. J. Johnson, unpublished data). This study

seeks to understand the ways organic amendments and host crop affect the relative responses of the selected AM fungal phylotypes under the experimental conditions of the transition from conventional to organic management. The 90 microplots contain either tomato plants or a cover crop, receiving nine treatments: either synthetic fertilizer (simulating conventional management as a control) or one of eight combinations of organic amendments comprised of 3 rates of urban plant debris (0, 30, and 60 g Kg^{-1} soil) and 3 rates of broiler litter (0, 10, and 20 g Kg^{-1} soil). The microplots had been managed conventionally using synthetic fertilizer and tomato monoculture for two years, then began a transitional period during which the nine treatments were applied for two years prior to the three sampling months in this study: February, April, and October 2010. The hypothesis is that organic amendments will affect the relative incidence and proportion of the three phylotypes depending on host species, rate of organic amendment, and time since transition. The expectation is that host specificity will have the most prominent role in determining the AMF phylotype response.

Methods

Sampling and DNA Extraction

Soil samples were collected in February, April, and October 2010 at the USDA Picos Road Farm in Fort Pierce, FL. Two cores per microplot were taken using soil augers, yielding a homogenized sample of 144.4 cm^3 per plot. Samples were sieved to count nutsedge tubers, but they were not removed. DNA was extracted from 1g of soil per plot using a soil DNA extraction kit (UltraClean™ Soil DNA Isolation Kit, Mo Bio Laboratories, Solana Beach, CA, USA) following the manufacturer's protocol, and stored at -20 ºC until analyzed.

Quantitative Real Time PCR

Due to the presence of inhibitory compounds in soil, an inhibition test of the PCR reaction was performed on select DNA samples at different dilutions to determine the threshold for the dilution necessary to eliminate the effect of inhibitors on the qPCR reaction. To test for inhibition, samples were diluted to 10^{-1} and 10^{-2} and spiked with either 1µL water or 10^{-5} copies/µL of the plasmid DNA standard. Amplification plots were examined to compare the expected and observed quantifications in order to determine the minimum dilution necessary to eliminate PCR inhibition. A 1:10 dilution of each DNA sample was used for qPCR quantification of AM fungal phylotypes.

qPCR reactions were conducted using Taqman Universal PCR Master Mix (Applied Biosystems) following the manufacturer's protocol. Reactions were run with 1µL of sample DNA (diluted 1:10) in a final reaction volume of 20 µL, which included 7.8 µL Ultrapure water, 10 µL 2x Taqman® Master Mix, 0.4 µL each of forward primer, reverse primer, and probe. Three technical replicate samples were made per microplot, and each plate included non-template controls containing water to check for contamination in the reaction components, and a series of plasmid standards. Each sample was tested with each primer/probe set (Table 3-1). Plates were sealed with optical film, centrifuged, and run in an Applied Biosystems™ ABI PRISM® 7000 Sequence Detection System (Perkin-Elmer Biosystems, Norwalk, CT, USA) according to the following program: 2 minutes at 50ºC and a 10 minute hot start *Taq* activation step at 95 ºC followed by 50 cycles of 95ºC for 15 seconds and 60ºC for 1 minute.

Primers were designed for three AMF phylotypes (Johnson 2011, unpublished data) that were expected to be present in this type of agroecosystem and region based on previous sequencing of an AM fungal community in the Florida Everglades

Watershed (Rasmann et al., 2009; Johnson et al., 2010). The 18S gene from the large ribosomal subunit was used due to the robust reference AM fungal sequence library for this gene, thus increasing the likelihood that the targeted AM fungal sequences occurred in the study site.

The standard curve was based on plasmids of the PCR product cloned into pDRIVE vector (Qiagen® PCR cloning Kit) and transformed into *E. coli* XL1Blue strain and extracted with the Qiagen Miniprep Kit. Plasmid DNA was quantified with a nanodrop 1000 and the ng/µL was used to convert to copies/µL. Plasmid was then diluted to 1,000,000 copies/µL. 10-fold serial dilutions were used to construct the standard curve down to 10 copies/µL.

Data were collected and organized in the ABI 7000 SDS software (version 1.1). The standard curve plot was assessed for R^2 value and slope, and the threshold was manually adjusted to indicate significant fluorescence signals that rose above background based on negative controls and the standard curve. The output amplification plot of the experimental samples was examined for consistency of amplification curves, and outlier wells and non-specific or partial amplifications were deleted. Threshold cycle (Ct) was converted to copies of 18S gene per reaction based on the standard curve by the ABS software. Since the
original 50µL DNA sample (one per microplot) was extracted from 1g of soil, and 1 µL of a 1:10 dilution of the original sample was used for a reaction, the analyzed sample was 1/10 of 1/50 of the total sample (1/500 of the total sample). Therefore assuming an even distribution of DNA in the sample, the measured value was multiplied by 500 to calculate the number of 18S copies in 1 g of soil sample. The log was taken of these

numbers, and averaged across triplicate wells. Means were then averaged within treatments and crop type for each sampling date.

Statistical Analysis

Using the statistical software R: A Language and Environment for Statistical Computing (version 2.15.0, GNU General Public License), a Generalized Linear Mixed Model (GLMM) test was performed to assess differences between treatments, fertilizer type, between crop type, and between the three sampling dates. The model transformed the data to binomial data and assessed for incidence (presence or absence) of each of the three phylotypes.

Results

The measurement of number of copies of the mycorrhizal 18S gene per g of soil varied greatly among crop and treatment, and did not present many discernable trends. Due to the generally low incidence and lack of obvious trends, interpretation of the quantitative PCR data was determined to be less productive than interpreting the data in the context of incidence. Also, the interaction with crop prevents pooling of the data to observe soil amendment effects on phylotypes. Adding zeros to quantitative data can mask any significance because of the inherent violation of normality, especially when the cause for the zeros is not certain. For general analysis, the variability is too high in number of gene copies per g of soil to be able to easily analyze or graph it in a meaningful way.

There was no significant treatment effect or amendment type effect found on the incidence of the three phylotypes, but there appeared to be a cropping effect. Therefore, crop effect was evaluated for the presence or absence of each phylotype, over all months.

The incidence of each phylotype by crop and by month are presented in Figures 3-1 and 3-2. The extremely low incidence of Phylotype G precluded meaningful interpretation for its relationship with treatments (either crop or soil amendment), although when it was detected, the relative counts were high compared to phylotypes A and B. No trend for Phylotype B across soil treatments was apparent, but a possible temporal effect was observed. In February and April but not October, average incidence of Phylotype B is slightly greater in the tomato cropped soil than in the cover cropped soil across the soil amendment treatments, but is not statistically significant (P=0.1209). Phylotype A was more prevalent in the cover cropped soil than in the tomato cropped soil across all the soil amendment treatments. This trend was detected for the February and April crop cycles, but not for October. There was a significant effect (P = 0.0004) of tomato on Phylotype A; incidence was lower in tomato than cover crop.

Discussion

It appears that there is a difference in the response of Phylotypes A and B to tomato and cover cropped soil. Phylotype A was more prevalent under the cover cropped soil, while Phylotype B was favored by tomato cropping. This trend is more apparent in February and April samples, when each of the two crops were growing in their respective microplots. In October, after all plots transitioned to tomato cropping, the host effect was less apparent. Tomato cropping appeared to mediate a shift to Phylotype B compared to February and April, resulting in detection of both Phylotypes A and B in eight of the nine treatments compared with only three of the cover crop treatments in February (treatments 2, 7, and 8), and in April (treatments 1, 2, and 8). If this trend continues, it could be expected that with tomato cropping, Phylotype B will

become dominant in the AM fungal community due to the selection for this phylotype by tomato.

The incidence of Phylotype G was too sporadic to draw conclusions about its relationship with soil amendment treatments or with crop type. However, when it was detected, the number of gene copies per g of soil was relatively high compared to Phylotypes A or B. This is an interesting finding because it suggests a potentially different lifestyle or growth pattern of Phylotype G. That is, low frequency of detection yet high levels of gene copies may be due to a highly variable distribution of this fungus in rhizosphere soil, such as the clustering of external hyphae and spores. Alternatively, it may suggest that Phylotype G has fewer propagules, but when present in the rhizosphere aggressively colonizes tomato roots, potentially outcompeting other root endophytes.

Overall, there was substantial variation in detection of the three phylotypes, which raises an issue about the interpretation of non-detections. The lack of phylotype detection does not necessarily mean that the target was not present in the sample, i.e., these non-detections are not “true zeros.” Many factors may have caused such variation in detection, including sample dilution of the DNA, and the presence of DNA-inhibiting compounds despite dilution of the samples. Variable detection of target DNA in these samples also raises the issue of minimum qPCR detection limits. The theoretical detection limit in qPCR is one DNA template per PCR reaction. When accounting for dilution and sampling of 1 µL per reaction, the limit is 1000 copies of 18S rDNA genes per g of soil. The detection limit is likely about 10,000 copies per g of soil because qPCR is unreliable for quantification below 10 copies for sampling and other reasons.

Also, soil sampling techniques may disproportionately select for AM fungi with relatively high amounts of vegetative mycelium (Smit et al., 1999).

The fact that the original DNA extraction was performed on a small soil sample size (1g of field soil derived from 2 soil cores), and size of the microplots raises the issue of spatial heterogeneity of AMF communities. A phylotype may not be detected due to patchy root colonization that was not adequately assessed with the soil core taken, or the small sample size of 1g of soil may not be representative of the whole microplot. While replication of treatments in the experimental design somewhat addresses the spatial heterogeneity in the plots, it may be necessary to increase the number of soil cores per plot.

The PCR amplifications showed a considerable number of abnormally shaped curves that did not match standard or positive samples, but showed increases in fluorescence above the non-template control. This may have been caused by interaction between primers, interaction between primer-probe, or nonspecific primer-probe activity leading to a partial amplification. Thus, these values were not included in the calculation of averages within treatments and crops, and statistical analysis of the data.

Based on current taxonomy of AM fungi, it is essentially not possible to determine to what extent AM fungal species or morphotypes actually correspond with the phylotype groups chosen for study. Johnson et al. (2010) found that the sequences comprising Phylotypes A and B were the most difficult to group of all phylotypes detected in their agro-ecosystem in a nearby field site in the Florida Everglades

Watershed. However, in a similar experiment, Rasmann et al. (2009) detected them under differing treatments, so there is likely a biological difference.

All nine treatments had high levels of nutrient input which suppresses AM fungal activity (Chapter 2; Rasmann et al., 2009), although these levels are consistent with many existing commercial organic farms (Chellemi, personal correspondence). Since all plots were set up in the same soil type and were treated with high levels of nutrients and soil disturbances, there is evidence to suggest the effect of host plant on AM fungal community structure is stronger than the soil treatment effects. Apparently a host-mediated shift in dominant phylotypes occurred since tomato and millet crops were frequently associated with different phylotypes. Similar variation between legumes and non-legumes in AMF communities reported by Scheublin et al. (2004) is consistent with differences in response to the two functionally distinct host crops in this study.

The phylotype responses for October may provide further support that the AM fungal community was shifting along with host since tomato had been recently planted in the plots where millet or sunn hemp had previously been grown. Other cropping events in the plots between April and October may have also contributed to the shift in addition to host effects. The plots were solarized in May and were without host plants for approximately 6 weeks. The tomato crop in the microplots experienced stress in the form of worm damage and bacterial wilt, necessitating spraying with organic pesticides (Copper, Bt, and Entrust). Due to losses due to disease and pests, the plots were replanted and amended multiple times with supplemental organic fertilizer (fish emulsion and seaweed kelp). These cropping events could have affected total propagule levels or selected for phylotypes that better resist the various host stresses

and management activities. For example, if tomato roots changed their allocation of C to the fungi under these conditions, they could have selected for more aggressive, parasitic or resilient phylotypes (Rasmann et al. 2009). An additional stressor to the plants was the purple nutsedge growing in high amounts in all plots, which may have increased root competition for resources. This may have in turn indirectly influenced the AM fungal community by affecting resource availability and allocation between the plant and fungi, and potentially selecting for more resilient phylotypes.

Since the plots had only been transitioned into organic management relatively recently, the AM fungal community may still be in the early stages of transition as well. Significant fungal species heterogeneity has been found in soils in the early stages of recolonization (Boerner et al., 1996), and a high degree of variation in community composition has been found in agro-ecosystems that are undergoing major land management changes (E. J. Johnson, unpublished data). It is important to note the degree of spatial and temporal variability of AM fungal communities in transitioning agroecosystems may vary with season, host species, and even between parts of a root system (Scheublin et al., 2004). AM fungal communities from different agroecosystems have been found to vary in species composition and seasonal and successional sporulation dynamics (Oehl et al., 2009); the successional dynamics are especially important to evaluate in the context of transitioning organic farms.

Further experiments examining AMF community composition and structure changes could determine whether this apparent shift due to host crop continues. Because in the present study only selected phylotypes were followed, they may not give a complete picture of the AMF community shift as a whole. If primers for more

phylotype groups were employed, it is likely that more variation among members of the AM fungal population could be detected in response to the transition to organic tomato cropping. Studying phylotype groups with a wider diversity of lifestyle traits could yield a more comprehensive understanding of the major drivers, i.e. host species and land management practices, for the shift in AM fungal communities.

Table 3-1. Primer and probe names, sequences, and the phylogenetic-based group to which they correspond.

Phylotype	Corresponding morphotype	Type of Reagent	Reagent name	Sequence
		Forward primer	EJ062 A F1	GGGTCAGTAGATTGGTCGTGCCA
	Closely related to	Reverse primer	EJ063 A R1	AAAGTAACAGTCCAGTCTCCCCGCA
A	*G. intraradices* [a]	Probe	EJ064 A P1	TCCTCCTTCCTGATGAACCGTAATGCCA
		Forward primer	EJ065 B F1	GGGGTTAGTAGGTTGGTCATGCCTCT
	G. intraradices	Reverse primer	EJ066 B R1	CAAAGTAACAGTCCAAATTCCCCGCA
B	*G. fasiculatum*	Probe	EJ067 B P1	CTCACTGATTCCTCCTTCCTTATGAACCGT
		Forward primer	EJ068 G F1	TTCGGTCATGCCGTTGGTATGC
		Reverse primer	EJ069 G R1	ACAGTCCTAATTCCCCGTAACACCA
G	*G. mosseae* [b]	Probe	EJ070 G P1	CTCACCTTCTAAAGAACCGTAATGCCA

[a] The sequence databases cannot be linked to a described morphotypes for phylogenetic group A

[b] Primers for group G may also detect closely related species such as *G. geosporum*, *G. coronatum*, and *G. fragilistratum*.

Table 3-2. Summary of GLMM test on separate phylotypes.

	Crop	Estimate of model parameter	Standard Error	p-value
Phylotype A	Cover crop	-0.3154064	0.2182364	[a]
	Tomato	-0.9877666	0.2733304	0.0004
Phylotype B	Cover crop	-1.7061608	0.2870076	[a]
	Tomato	0.4845959	0.3114097	0.1209
Phylotype G	Cover crop	-2.6390573	0.3463179	[a]
	Tomato	-0.6190392	0.5737406	0.2816

Crop type over all months had a significant effect on the incidence of all three phylotypes. A was more prevalent in tomato (p =.0004), B and G were more prevalent in the cover crop (p = 0.0000).

[a] Since p-values correspond to the test of whether each parameter is significantly different from zero, p-values for cover crop are not reported. The tomato parameter is the effect of tomato crop relative to the incidence of the cover crop; it reflects the amount by which mean incidence in tomato crop is different from that in the cover crop, on the logit scale.

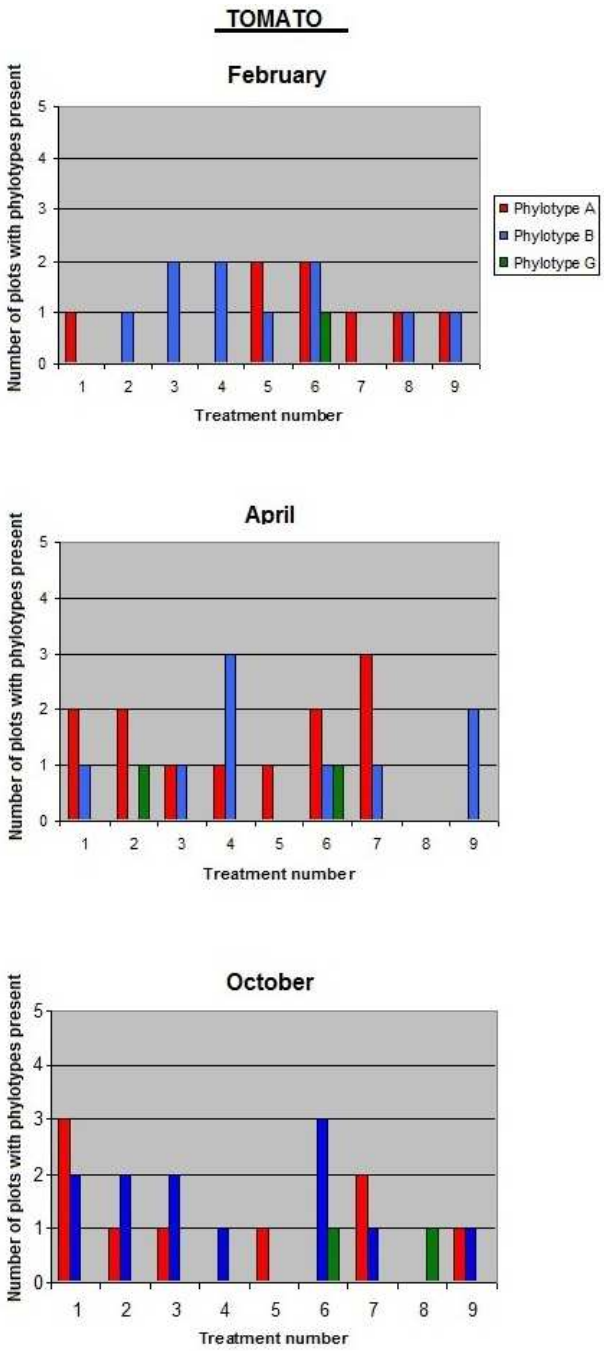


Figure 3-1. Incidence of phylotypes A, B, and G by month in tomato crop. Due to there being 5 replications of each treatment and crop combination in the experimental design, phylotype detection could have occurred potentially up to 5 times, as presented in the y-axis. Treatment codes are as follows: 1 – control, 2 - 0UPD/10BL, 3 - 0UPD/20BL, 4 – 30UPD/0BL, 5 – 30UPD/10BL, 6 – 30UPD/20BL, 7 – 60UPD/0BL, 8 – 60UPD/10BL, 9 – 60UPD/20BL. UPD = urban plant debris (0, 30, and 60 g Kg^{-1} soil) and BL = broiler litter (0, 30, and 60 g Kg^{-1} soil).

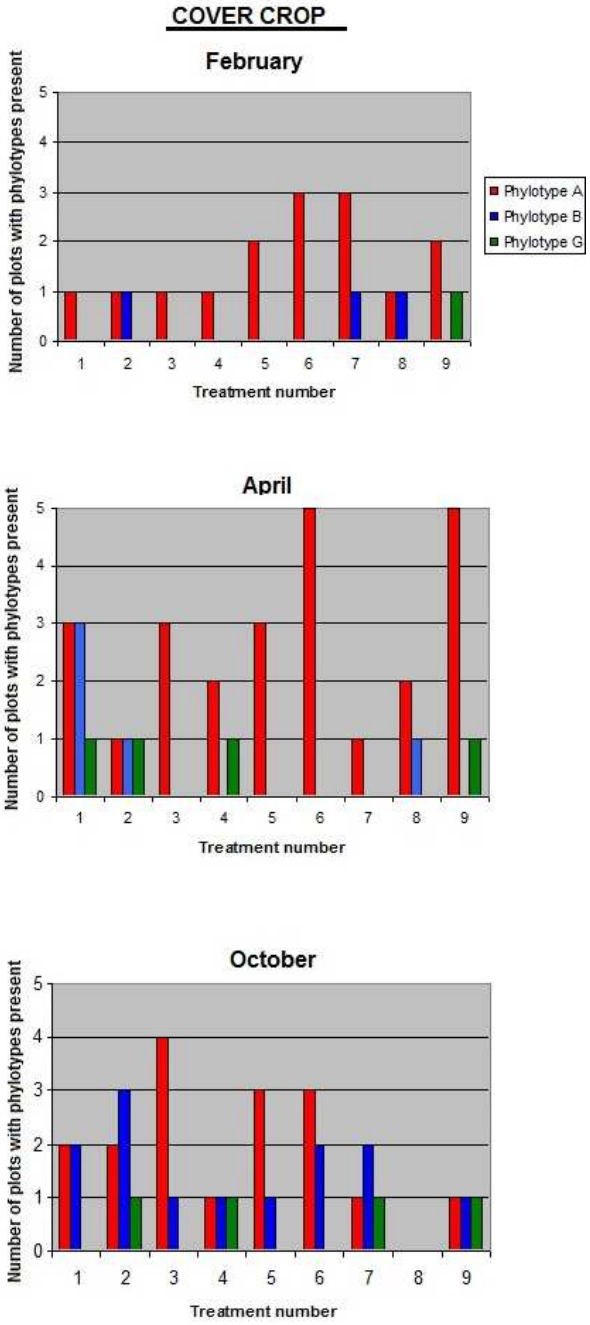


Figure 3-2. Incidence of phylotypes A, B, and G by month in cover crop. Due to there being 5 replications of each treatment and crop combination in the experimental design, phylotype detection could have occurred potentially up to 5 times, as presented in the y-axis. Treatment codes are as follows: 1 – control, 2 - 0UPD/10BL, 3 - 0UPD/20BL, 4 – 30UPD/0BL, 5 – 30UPD/10BL, 6 – 30UPD/20BL, 7 – 60UPD/0BL, 8 – 60UPD/10BL, 9 – 60UPD/20BL. UPD = urban plant debris (0, 30, and 60 g Kg^{-1} soil) and BL = broiler litter (0, 30, and 60 g Kg^{-1} soil).

CHAPTER 4
CONCLUSIONS

The experimental microplots at the USDA Picos Road farm represented a range of treatments and conditions that are generally understood to strongly affect AM fungal presence and functioning. The array of variables present had the potential to both benefit and harm AM fungal communities, and were designed to simulate realistic agroecosystem practices in the region. Treatments were implemented across a single soil taxonomic unit, effectively minimizing the substantial influence of soil type on AM fungal communities.

Despite very high levels of P and N input, the AM fungal communities appeared to be quite resilient; inoculum potential colonization levels were consistently high across all treatments, in contrast to studies with similar high levels of nutrients and disturbance (Rasmann et al., 2009). AM fungal communities also showed resilience in the apparent rebound effect in frequency of overall phylotype incidence between April and October, where levels increased across all treatments to a level equal to or higher than that which was measured in February. Microplots were highly disturbed by frequent incorporation of organic amendments into the soil, and by the seasonal removal and planting of host plants. Soil mixing and incorporation of organic amendments mimic tillage and its damaging effects on the mycelial network. However, the suppressive effects of this disturbance and high nutrient levels may have been mitigated by the benefits provided by the amendments, such as improved soil moisture retention and structure, which are known to be conditions that promote AM fungal activity.

A major factor that likely affected colonization levels over time was the possible dilution effect of the amendments, when samples were taken from the microplots shortly

after in April. This idea is supported by the finding that N and P levels changed over time in the same way that percent colonization changed. Also, high nutrient levels may have affected rates of AM fungal sporulation, or there may have been a temporary increase in soil nutrient availability. Nutrients in organic matter are mainly in a form that is inaccessible to the plant until metabolized by soil microbes, releasing a more labile form into the soil matrix or delivering it directly to the plant, facilitating its uptake. High levels of labile nutrients in the form of synthetic fertilizers have frequently been found to suppress AM fungal activity (Elmholt and Kjøller, 1989; Sivapalan et al., 1993; Fliessbach and Mäder, 2000; Shannon et al., 2002), but high levels of organic fertilizers seem to have variable impacts. This may be due to the slow availability of nutrients as released by microbes over time under different conditions, thus affecting their availability, uptake, and sufficiency in the host plant. Due to the plant's dependency on AM fungi to obtain and deliver these unavailable nutrients, the plant may be benefiting from supporting a high level of colonization on its roots. Organic sources of fertilizer have been shown to stimulate AM fungi (Harinikumar and Bagyaraj, 1989; Ryan et al., 1994; Baby and Manibhushanrao, 1996; Dann et al., 1996; Douds et al., 1997; Kabir et al., 1998; Miller and Jackson, 1998; Joner, 2000; Alloush and Clark, 2001), although not in all cases.

The effects of rotating tomato with a leguminous cover crop on AM fungal communities are difficult to discern on such a short time scale. Only three months had passed since planting tomato in plots that formerly had millet in this study. Using a consistent legume cover crop (as opposed to alternating legume and non-legume: sunn hemp and millet), may also produce clearer changes in the AM fungal communities.

Since average colonization levels were highest in October, it is possible that replacing the host crop with tomato had a beneficial effect on AM fungal populations. Alternatively, the cause of this increase may have been due to the effects of a leguminous plant on soil nutrient status, which in turn benefited the AM fungal population with a new and different host. Due to the complex nature of the relationship between host crop, AM fungi, and nutrient sources and supply, it is likely that many of their effects on each other are indirect and may not be immediately observed. In a similar future study, measurements of colonization and AMF community structure should be taken at larger intervals of time past the point of transition from conventional to organic management to better illustrate changes that occur immediately after transition, and long after. The same should be considered for duration of time after cover cropping ends and organic mono-cropping begins. Also, data should be collected pre-transition, mid-transition, and post-transition.

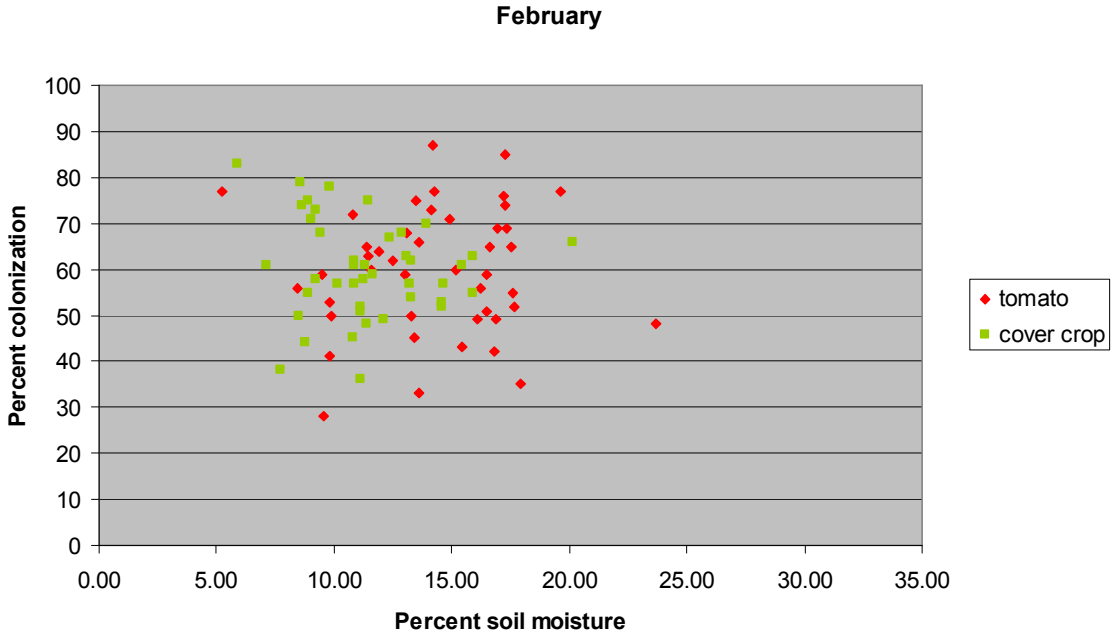
Considering the amount of samples and technical replicates required to assess all nine treatments and other variables in this study, a wider scope is unfortunately not feasible within the bounds of the current project. Similarly, while measuring the relative abundance of three indicative phylotypes of AMF can be illustrative of changes within the AMF community, this is a very narrow and reductionist view of the AMF community as a whole. Some agroecosystems have been found to have 26 species present (Ellis et al., 1992), which implies that observing changes in only three of them may not provide the most comprehensive understanding of the ways in which the AMF community is changing.

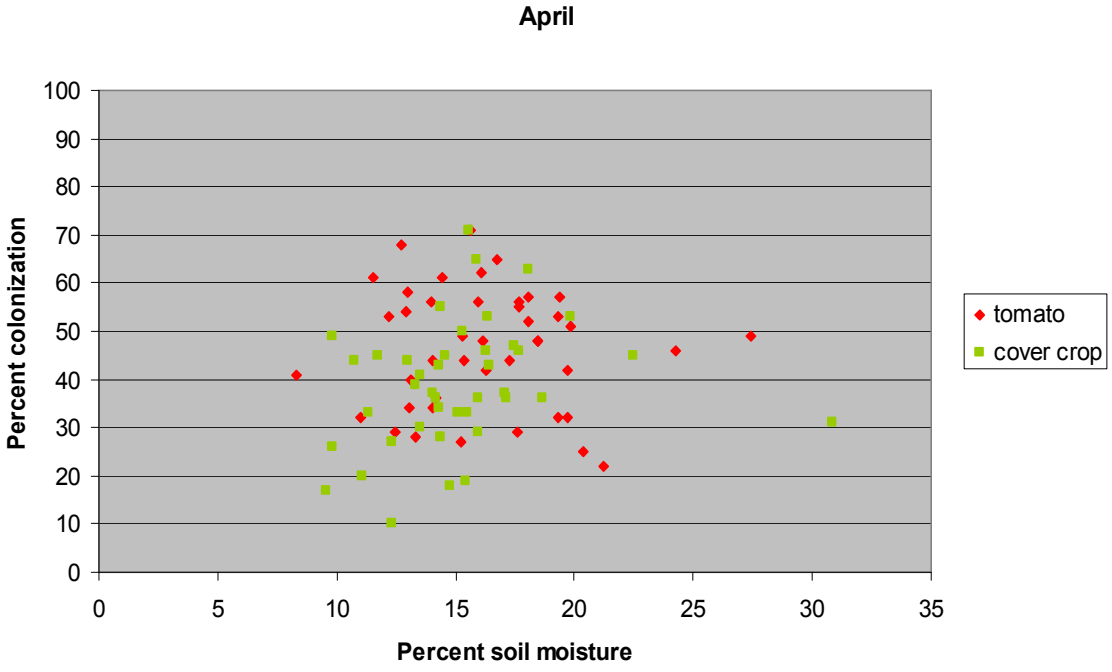
The results of this study suggest some degree of host-mediated community shift in the dominant AM fungal phylotypes; however, due to limitations in qPCR and AM fungal genetic understanding, five binomial measurements of phylotype incidence are not enough to perform a robust statistical analysis or make strong conclusions about their response to treatments. Additionally, while examining changes in prevalent or dominant phylotypes is useful, their functioning may not represent the AM fungal community as a whole, especially when other phylotypes may likely react differently from those selected for assessment (i.e. differing rates of colonization, frequency of sporulation, resilience to stressors, etc) (E. J. Johnson, unpublished data).

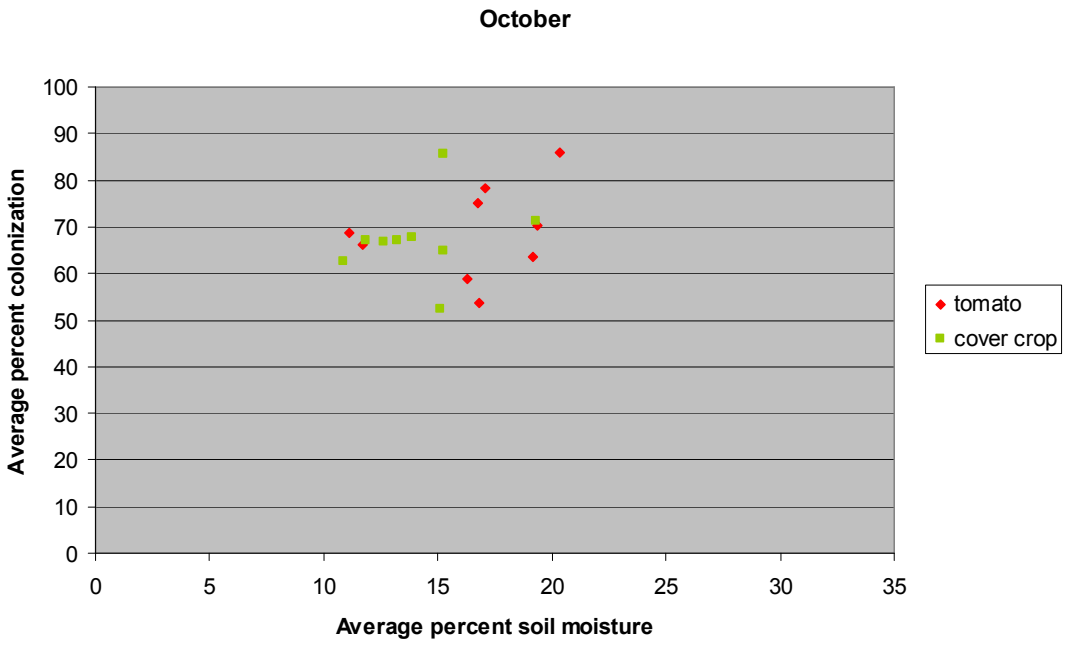
It would be interesting and useful to measure the effects of these fluctuating AM fungal communities on crop yield, as it has been proposed (Ryan and Graham, 2002) to determine whether high colonization levels can be detrimental to the plant due to a shift in the association toward parasitism when the AMF becomes a carbon sink, diverting it from fruit production (i.e. yield) or from its total biomass. Unfortunately, yields of tomato were not measured in this study of the soil microbial ecology, but were observed to be substantially low and damaged apparently due to persistent herbivory and pathogen distress. An ideal study would also include measurements of the holistic functioning of many aspects of the agroecosystem, including AMF diversity and community structure, bacterial and fungal pathogens, soil nutrient retention, and crop yield, all of which are elements of high concern to farmers.

APPENDIX
MEASURES OF SOIL PARAMETERS VS. COLONIZATION

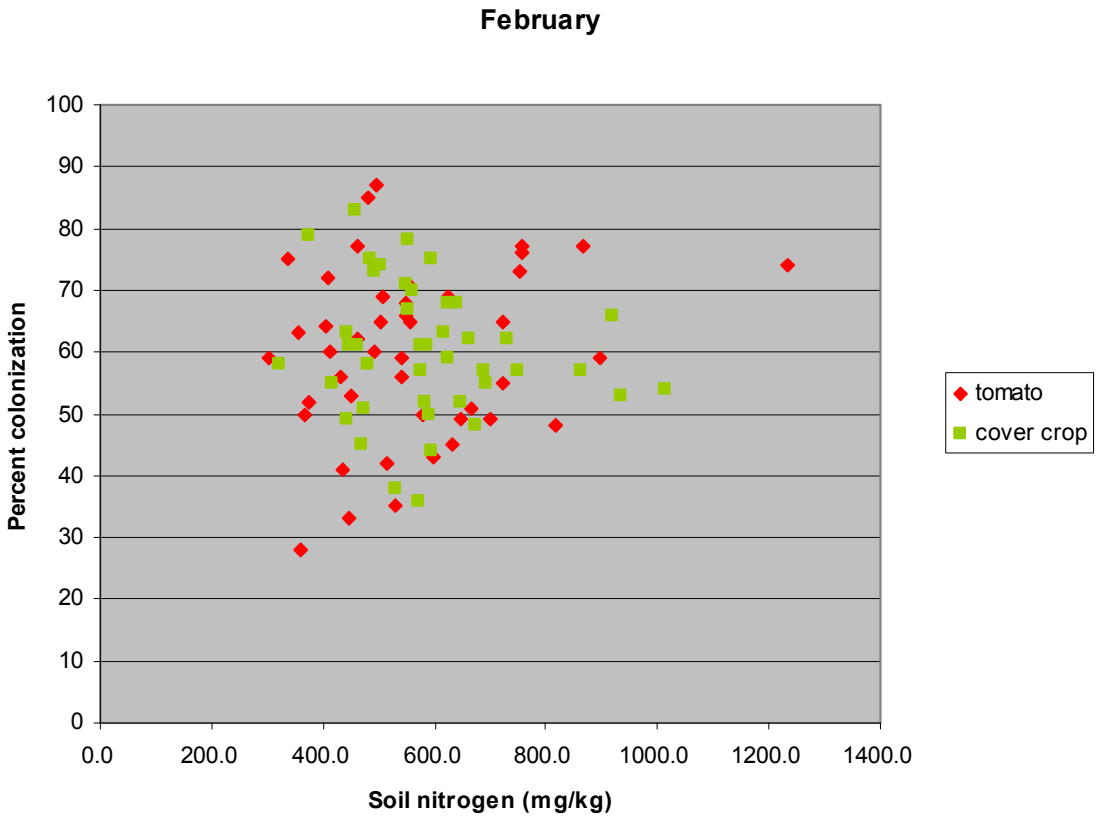
Soil Moisture

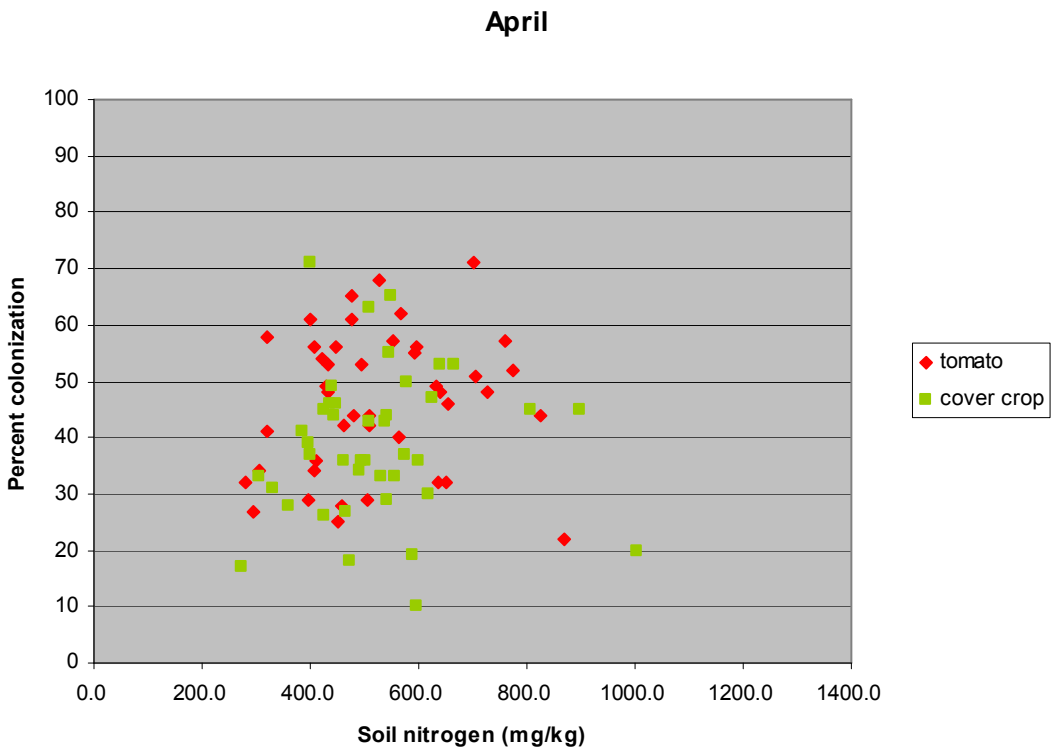


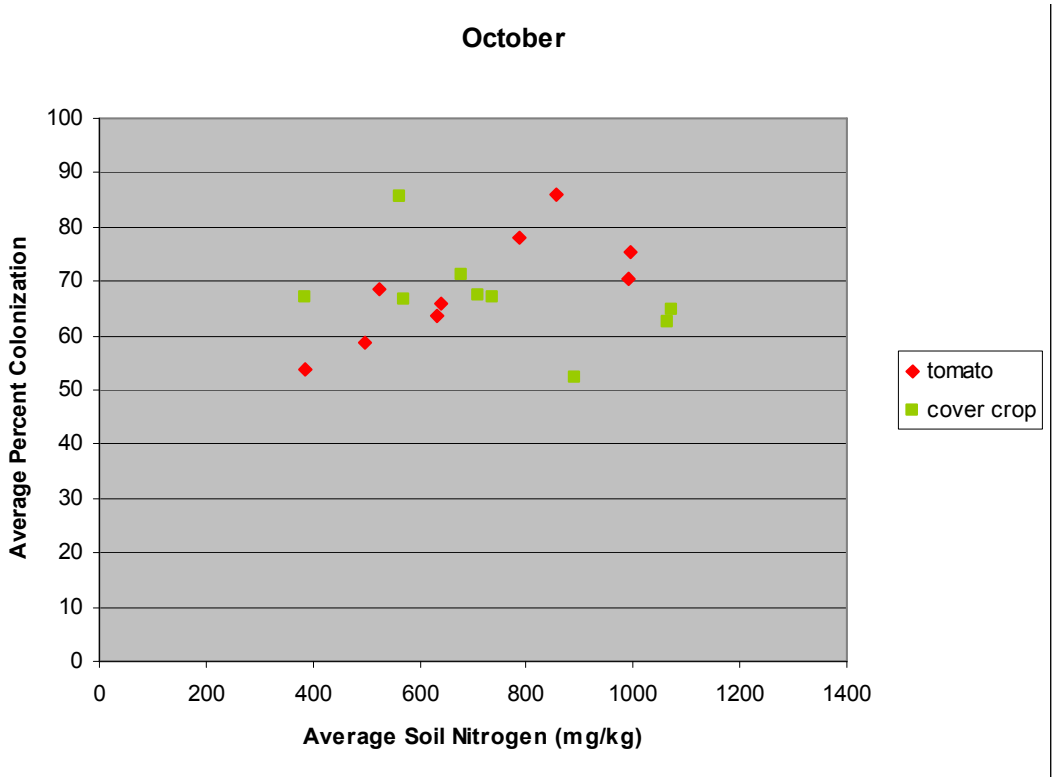




Soil Nitrogen

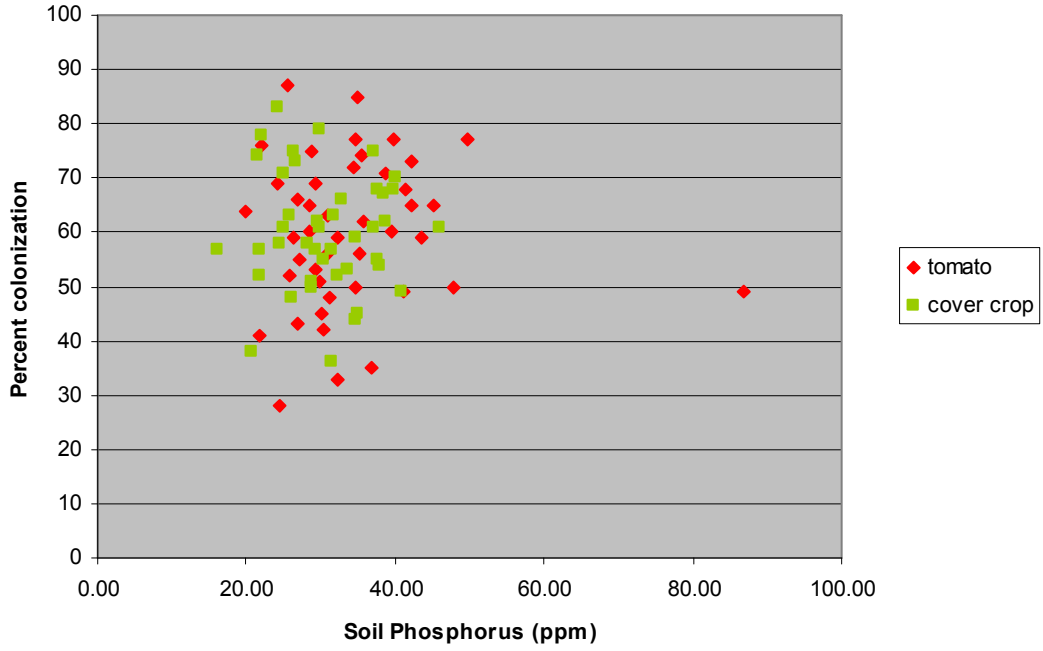



April
Percent colonization
100
90
80
70
60
50
40
30
20
10
0
0.0
200.0
400.0
600.0
800.0
1000.0
1200.0
1400.0
Soil nitrogen (mg/kg)
tomato
cover crop

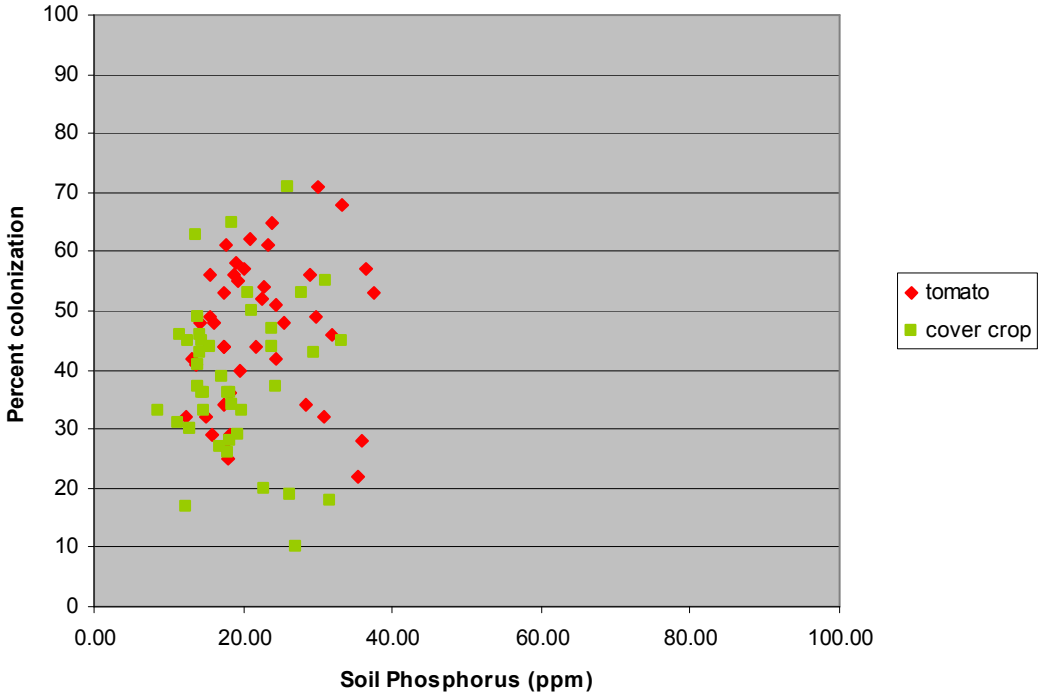

October
Average Percent Colonization
100
90
80
70
60
50
40
30
20
10
0
0
200
400
600
800
1000
1200
1400
Average Soil Nitrogen (mg/kg)
tomato
cover crop

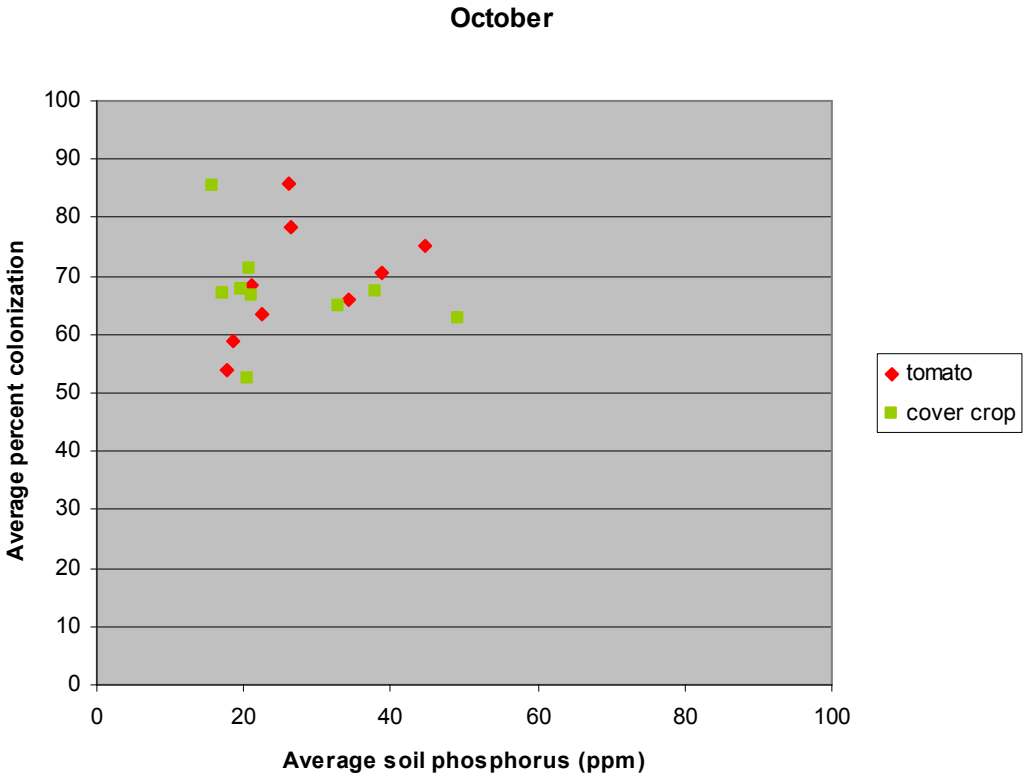
Soil Phosphorus

February



April




October
Average percent colonization
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
Average soil phosphorus (ppm)
tomato
cover crop

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BIOGRAPHICAL SKETCH

Megan Smith received her Master of Science in soil and water science from the University of Florida, with a concentration in tropical conservation and development. She received her Bachelor of Science in environmental science with a minor in biology from the University of Michigan. Her passion for conservation issues is applied in her studies of sustainable agriculture, food systems, resource management, and tropical ecology. She enjoys hiking, reading, dance, and performance art.