IMPACT OF WINTER COVER CROPS AND TILLAGE ON SOIL MICROBIAL POPULATIONS AND MYCORRHIZAL COLONIZATION IN DRYLAND COTTON

A Thesis

by

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ABSTRACT

Cotton producers are interested in adopting conservation tillage for potential soil health benefits such as increasing soil microbial biomass. However, little is known about the resulting impacts of cover crops and tillage practices on soil microorganisms, especially plant beneficial microbes such as AMF abundance and interactions with cotton plants under dryland conditions. The objectives of this study were to evaluate soil microbial biomass and soil parameters in different tillage systems and cover crop rotations with dryland cotton, and to evaluate root colonization and AMF diversity in these treatments. A multi-year field study was conducted with a randomized-complete block design, with four replicates. Treatments included conventional tillage, no-till, and no-till with a variety of different cover crops. Prior to planting in year 2, soil samples (0-7.5 cm depth) were collected and characterized for microbial biomass using phospholipid fatty acid analysis (PLFA). Mycorrhizal colonization of cotton roots was determined at multiple time points during the growing season. Individual root fragments were isolated from cotton roots, DNA extracted and used to identify mycorrhizal community structure in cotton roots by ribosomal RNA gene sequencing. The PLFA results showed little difference in microbial biomass levels between conventional tillage and no-till samples Inclusion of a cover crop increased microbial biomass by up to 2-fold. In August, the use of cover crops increased percentage of mycorrhizal colonization of cotton, as higher root colonization was observed in hairy vetch, Austrian winter field

pea and crimson clover. Root colonization was lowest in conventionally tilled plots. In October, the differences in root-colonization among the treatments had largely disappeared. Principal coordinate analyses of relative abundance of AMF species (operational taxonomic units) indicated that different cover crop treatments influenced AMF community structure in cotton roots. In August, AMF species colonizing cotton roots were similar in most treatments. The AMF community structure appeared distinct between conventional tillage and cover crop treatments by October. The results indicated that cover crops demonstrated positive legacy effects by increasing soil microbial biomass and AMF colonization of cotton roots, especially at the initial growth stages of cotton. These impacts could translate to higher drought tolerance and productivity under dryland conditions.

DEDICATION

To my sister Halime Özal, my father Ahmet Zekeriye Özal, my mother Mümtaze Özal. I can always rely on their love, support, and encouragement to follow my dreams.

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Contributors

This work was supervised by a thesis committee Professor Terry J. Gentry, Assistant Professor Anil Somenahally, Associate Professor Paul Delaune of the Department of Soil and Crop Science and Associate Professor Raghupathy Karthikeyan of the Department of Biological and Agriculture Engineering.

Professor Gentry and Assistant Professor Somenahally helped significantly with the research, preparation, writing, and reviewing the manuscripts, which led to the contents of this thesis. Associate Professor Delaune and Karthikeyan reviewed the manuscripts, which led to the contents of this thesis.

The data analyzed for Chapter II was provided by Dr. Jeff A. Brady.

All other work conducted for the thesis was completed by the student independently in the joint laboratory of Professor Gentry.

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NOMENCLATURE

AMF	Arbuscular Mycorrhizal Fungi
AM	Arbuscular Mycorrhizae
VAM	Vesicular Arbuscular Mycorrhizae
P	Phosphorus
N_2	Nitrogen
FAO	Food and Agricultural Organization
T	Tillage
CT	Conventional Tillage
NT	No Tillage
MT	Mulch tillage
C	Carbon
TX	Texas
RCBD	Randomize Complete Block Design
NRCS	Natural Resources Conservation Services
mm	Millimeter
°C	Centigrade
Ib	Pound
ha	Hectare
L	Liter
PLFA	Phospholipid Fatty Acid

DNA	Deoxyribonucleic Acid
KOH	Potassium Hydroxide
HCl	Hydrochloric Acid
rRNA	Ribosomal Ribonucleic Acid
PCR	Polymerase Chain Reaction
OTU	Operational Taxonomic Unit
NMDS	Nonmetric Multidimensional Scaling
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis

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1. INTRODUCTION AND LITERATURE REVIEW

1.1 Arbuscular Mycorrhizal Fungi Symbiosis

One of the main components of the soil microbiota in most agro-ecosystems is the arbuscular mycorrhizal fungi (AMF). These obligate mutualistic symbionts colonize the roots of the vast majority (around 80%) of plant families (Smith, et al., 1997). AMF is one of the most abundant organisms in the rhizosphere and more than 200,000 species of host plants can be colonized by AMF (Schüßler and Walker, 2010).

Arbuscules (Figure 1) are the intercellular tree-like branching structures that AMF form within root cortical cells of the majority. In some cases, the arbuscule structures appear as coils of hyphae (Figure 2). Arbuscules are considered to be essential locations for the exchange of carbon and mineral nutrients between AMF and plants, particularly the phosphorus (P) from the fungus. Some of the fungi also form large oil-filled vesicles in the intercellular spaces (Figure 3). The fungi produce hyphae that explore intercellularly within the plant and also the extraradical mycelium, that grows into soil and can extend several centimeters from the root surface.

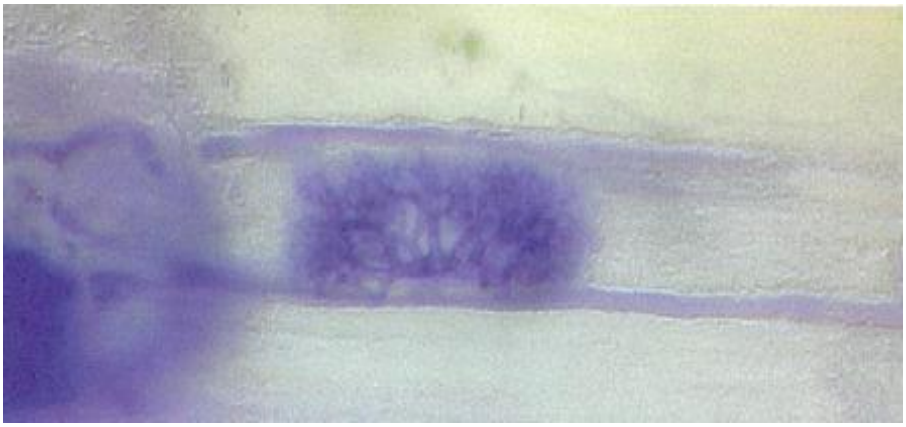

Figure 1: Typical Arbuscule Structure of AMF in Root Cell After Staining (400X)*.

Figure 2: Typical Hyphae Structure of AMF in Root Cell After Staining (200X)*.

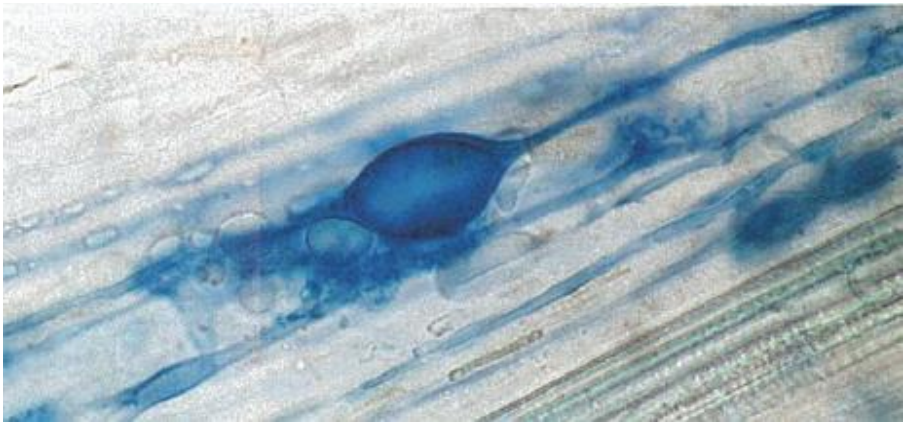

Figure 3: Typical Vesicle Structure of AMF in Root Cell After Staining (200X)*.

*Figures adapted from (Goss, et al., 2017)

1.2 The Influence of AMF on Plants

The main benefit of the mycorrhizal symbiosis for plants is believed to be facilitated nutrient uptake, particularly with respect to immobile nutrients like phosphorus (P) (George, et al., 1995, Miller and Jastrow, 1992). AMF can considerably increase P acquisition by the plants, both from inorganic sources (Cardoso and Kuyper, 2006) and from organic P (Jayachandran, et al., 1992). Hawkins, et al. (2000) showed that mycorrhizal symbiosis could improve the fitness of their host plant under low soil fertility. AMF can also enhance resistance to root pathogens (Borowicz, 2001) or abiotic stresses such as drought (Augé, 2001, Augé, 2004, Miransari, 2010, Ruiz-Lozano, 2003), metal toxicity (Clark, et al., 1999, Meharg and Cairney, 1999), salinity (Evelin, et al., 2009, Miransari, 2010, Porcel, et al., 2012), heat (Compant, et al., 2010), and cold (Charest, et al., 1993).

1.3 The Influence of AMF on Soil Chemical and Physical Properties

AMF may also play a role in the formation of stable soil aggregates, building up a macro porous structure of soil that allows penetration of water and air and prevents erosion (Miller and Jastrow, 1992).

Some studies related the occurrence of specific AMF species to soil physical and chemical characteristics such as soil texture, organic matter content, and nutrient contents; in particular to the availability of phosphorus. Mycorrhizae and fungal hyphae play a significant role as binding agents between aggregates (Tisdall, 1994). Glomalin produced by AMF can promote soil aggregate stability, especially at the micro aggregate level (Rillig, 2004). Oehl, et al. (2010) found that land use intensity and soil type

strongly affected AMF community composition as well as the presence and prevalence of many AMF. Regardless of soil types, grassland generally has more AMF species and genera than arable soils. It seems likely that the inputs of nutrients either organic or inorganic is compatible with sustained and abundant AMF population if other elements of cropping systems – such as the use of no-till, reduced tillage, and diversified cover-cropping sequence (Galvez, et al., 2001) – are also supportive.

1.4 Cotton

Archeological evidence shows that humans have used cotton for more than 4000 yr. The history of cotton cultivation is at least 3000 yr old. There are four domesticated species of cotton. *Gossypium arboreum* L. and *G. herbaceum* L., both diploids, are native to the Old World (India and drier area of Africa and Asia). *Gossypium hirsutum* L. and *G. barbadense* L., both allotetraploids, evolved in the New World (drier areas of Middle America, northern South America, the West Indies, north Africa and Southern Asia). *Gossypium hirsutum,* known most widely as Upland cotton, contributes about 95% of the current world production of 118 million bales of fiber weighing about 225kg/bale (Fang and Percy, 2015).

Cotton (*Gossypium hirsutum* L.) remains an important product in the agricultural economy of the United States. Its scope and economic impact extend well beyond the approximately 19,000 farmers that plant between 4 and 6 million hectares of cotton each year in 17 states across the southern United States (Mauney, 2015).

1.5 The Influence of Land Practices on Cotton Production

Since cotton produces low amounts of residue, crop rotation with green manure cover crops is essential to produce residue and its management with tillage is often required to maintain adequate surface coverage. Naudin, et al. (2010) compared cotton treatments with tillage (T), no tillage (NT), and no tillage with mulch (NTM) in a 2-year rotation in multiple farmer fields in North Cameroon and found that cotton yields were 12 % lower for tillage (T) and 24 % lower for no-till (NT) than for no tillage with mulch (NTM). Similarly, Nyakatawa, et al. (2000) compared cotton yield with tillage, cropping system and N source from 1996 to 1998 in northern Alabama and found that cotton lint yield under NT was 24% and 18% greater than that under conventional tillage (CT) and mulch-till (MT) treatments, respectively. Also, both no-till and a winter rye (*Secale cereal* L.) cover crop improved seedling emergence in 2 years and lint yield in one year of a 2 year study (Nyakatawa, et al., 2000). Also, between 1983 and 1986, cotton yields under conservation tillage were always higher than conventional tillage in Texas (Harman, et al., 1989). Keeling, et al. (1989) reported that conservation tillage cotton systems enhanced net returns compared to conventional tillage at two different locations in the Texas Southern High Plains.

1.6 Crop Rotation Effects on AMF

The importance of crop rotation is increased under reduced or no-till systems where the crop residues accumulate on the soil surface and are an essential element of conservation agriculture systems (FAO, 2015). To improve the potential contribution of AMF in the agricultural systems, the design of crop rotations must also take into account

the impact of the plant host in maintaining AMF colonization. Black and Tinker (1979) report that the amount of mycorrhizal spores decreased after leaving the land fallow in 2 years. Another study notes that fallow treatment and growing a non-mycorrhizal host diminished the mycorrhizal propagules by 40% and 13%, respectively (Harinikumar and Bagyaraj, 1988); similarly, extending length of the fallow period decreased AM efficiency by 33 % in a pot experiment (Kabir, et al., 1999).

1.7 Cover Crops

Cover crops are "herbaceous crops grown to create a favorable soil micro-climate, decrease evaporation, protect soil from erosion and also to produce biomass that can be used as forage and to improve the soil"(Bayer and Waters-Bayer, 1998). Cover crops are also known as living mulches and green manures. Cover crops have been shown to provide many environmental and agronomic services within agro-systems. These include reduced soil erosion, increased biological diversity as well as nutrient cycling and nitrogen (N_2) fixation, increased soil organic matter content, improved weed control, and increased crop yield (Higo, et al., 2010, Lal, 2004, Locke and Bryson, 1997, Mallory, et al., 1998, Oka, et al., 2010, Parkin, et al., 2006, Sainju and Singh, 1997, Varco, et al., 1999)

1.8 The Influence of Cover Crops on AMF Abundance and Diversity

Cover crops should be considered as a key element for the management of AMF colonization and diversity, as long as they are mycotrophic, which are in association with a mycorrhiza. On the other hand, some cover crops are considered non-mycotrophic, such as canola and oilseed rape. Cover crops with mixed species or

rotations, including legumes, cereals, and other groups of cover crops, can increase the diversity as well as number of individual AMF (Lehman, et al., 2012). García-González, et al. (2016) found the effect of winter cover crops (barley and vetch) on AMF parameters such as length of hyphae, enzymatic activity etc. They found that hyphae length of AMF increased to 80% with barley used as a cover crop compared with the fallow. Säle, et al. (2015) reported that AMF spore density and species richness increased in the topsoil under reduced tillage compared to the plowed plots by 55% and 20%, respectively. Similarly, Oehl, et al. (2003) found that increased land use intensity such as crop rotation and mono-cropping was correlated with a 40% decrease in AMF species richness.

The integration of cover crops, such as wheat, rapeseed, or crimson clover, in crop rotation systems reduces seasonal fallow and thus provides many benefits to subsequent cash crop and soil fertility (Clark, 2008). Furthermore, Wang, et al. (2007) claim that cover crops increase soil microbiological biomass through the decomposition of organic carbon (C).

Also, in a four-year study published by Isobe, et al. (2014), the number of AMF spores in winter crop wheat, an AMF host plant, was approximately twice as high as rapeseed, an AMF non-host plant, in 2008, 2010, and 2011. After rapeseed cultivation, the AMF colonization rate in soybean roots was detected at 2.9% to 12.7% in each of 4 years. The AMF colonization rate was significantly higher in wheat cultivation, was detected at 6.7% to 16.3% in each of 4 years. Galvez, et al. (1995) reported, that, hairy vetch as an overwintering cover crop, increased the number of *Glomus* spp. spores and

the overall AMF colonization potential of the spring soil samples. In a study by White and Weil (2010), a rye cover crop increased AMF colonization of subsequent corn as a cash crop in three of six site-years; and a wheat cover crop increased AMF inoculum within 2 years in a study by Boswell, et al. (1998).

1.9 The Influence of Land Practices on AMF Diversity and Colonization

AMF abundance or diversity is important for soil biodiversity, soil fertility, and functioning of terrestrial ecosystems (Douds, et al., 1995). About 240 AMF species have been identified so far (Oehl, et al., 2011). Most of these species belong to the genera of *Glomus, Acaulospora, Scutellospora, and Gigaspora*.

Farming practices such as soil tillage and fertilization have effects on AMF diversity and abundance (Jansa, et al., 2003, Kabir, 2005, Oehl, et al., 2010, Oehl, et al., 2003). AMF abundance has been noted to increase under low-input systems (Mäder, et al., 2000, Njeru, et al., 2015). Some studies revealed that community structure and diversity of AMF in soils differ between tilled and reduced or no-till soils (Jansa, et al., 2002, Köhl, et al., 2014, Maurer, et al., 2014, Wetzel, et al., 2014, Yang, et al., 2012). Brito, et al. (2012) reported that conventional tillage decreased AMF diversity by 40 % compared to no-till. Another study showed that under a no tillage wheat-oat-wheat rotation, the number of AMF spores was higher than under conventional tillage, ranging from 158 to 641 spores per 100 cm^3 (Castillo, et al., 2006). *Glomus* spp. are believed to survive perturbations and hence they prevail in highly disturbed agricultural systems (Dodd, et al., 2000) and become more abundant under conditions of environmental stress such as tillage disturbance (Jansa, et al., 2003). Öpik, et al. (2006) reported that *Glomus*

fasciculatum was present in all soil types. Therefore, this genus can be considered a 'generalist'. In contrast, other AMF species such as *Glomus badium* and *Glomus microcarpum* were present in grassland, with *Glomus caledonium* in arable lands. Those species can be characterized as 'specialists' as they are only present in grassland or specific soil types. At the population level, one of the studies on *Glomus intraradices* did not find any significant tillage treatment effects on its diversity (Koch, et al., 2004), suggesting that this species may be relatively more tolerant to disturbance than other AMF.

1.10 The Influence of Plants on AMF Diversity and Colonization

Even though many AMF species are thought to be generalists, which are able to thrive in a wide variety of environmental conditions, there is evidence that plant species composition influences the structure of AM fungal communities (Harley and Smith, 1983). AMF populations are greatest in ecosystems with higher plant diversity such as temperate grasslands and tropical rainforests where they have many potential host plants to colonize (Smith and Read, 2010). There is evidence to show both under experimental conditions (Van der Heijden, et al., 1998) and natural conditions (Moora, et al., 2004) that higher AMF diversity can induce a range of growth responses in plants. Hart and Reader (2002) proved that colonization by diverse families of AMF results in substantially different benefits to the host plants. As such, Gigasporaceae may be more 'mutualistic' since they provide the most nutritive benefits for their hosts due to heavy investment into primarily absorptive hyphae. Further, such differences in mycelium sizes may tell that cost of colonization can be higher than the others. That is, AMF with large

mycelia may be better at nutrient uptake but may pose a larger carbon sink than AMF with small mycelia.

1.11 The Influence of AMF on Cotton

Cotton (*Gossypium hirsutum* L.) is a mycotrophic plant in which growth and nutrient uptake is usually increased by mycorrhizal colonization (Pugh, et al., 1981, Torrisi, et al., 1999). Several studies have shown that the association of root systems of crop plants, including cotton, with AMF can increase the ability of plants to absorb water and nutrients and can result in increased biomass production and yield (Afek, et al., 1991, Linderman, 1992, Price, et al., 1989, Rich and Bird, 1974, Smith and Roncadori, 1986). As the root system of cotton has a low density per unit soil volume, producing an extensive mycelial network system outside of the root by AM can be beneficial for cotton (McMichael, 1990). Reduced AM colonization in cotton has been linked to reduced nutrient uptake such as, P and Zn and growth disorder of cotton (*Gossypium hirsutum* L.) where early growth in the crop is stunted, crop maturity delayed and yield decreased (Nehl, et al., 1996). However, the lack of mycorrhizal development was not due to a lack of AM fungi in the soil, suggesting that edaphic factors can have substantial impacts on mycorrhizal development in cotton (Nehl, et al., 1996, Torrisi, et al., 1999). Therefore, cotton may be more dependent on mycorrhizal associations for exploring soil for water and nutrients.

Introduction of mycorrhizal crops during the winter season can be an effective management strategy to increase and sustain AMF diversity in soils and provide sufficient inoculum for subsequent crops. In the Texas Rolling Plains, wheat and rye, are

the most popular cool season cover crop species because of their high persistence, tolerance to grazing, and biomass (Keeling, et al., 1996). These cover crops have been successfully implemented as rotation with dryland cotton production. It has been noted that mixture of two or more cover crops is often more effective for soil health than planting a single species (Zarea, et al., 2009). Kabir and Koide (2002) reported that the combination of cover crops (rye and oats) was significantly better than single species of cover crops for increasing mycorrhizal colonization. Evidence also exists for many crops, including cotton, to show that cover crop rotations can increase yields (Isobe, et al., 2014, Oka, et al., 2010). Hence, rotations of mixed cover crops are anticipated to enhance AMF diversity and enhance root colonization of the following main crop through legacy effects. However, there is no data on AMF diversity and abundance of mixed cover crops and their legacy effects on cotton rotation in dryland Texas cropping systems.

1.12 Study Hypothesis and Objectives

We hypothesize that cool season mixed cover crop rotations will produce larger benefits on microbial biomass and AMF diversity and colonization of cotton roots than compared to single species of cover crop rotation. Cover crop treatments will include wheat (*Triticum aestivum* L.), Austrian winter field pea (*Pisum sativum* L.), crimson clover (*Trifolium incarnatum* L.), hairy vetch (*Vicia villoa Roth*), and mixed cover crops in rotation with cotton. We also hypothesize that legume cover crops of Austrian winter field pea and hairy vetch will produce larger benefits than other individual cover crops due to their higher biomass and lower C/N ratio.

These hypotheses were tested on field study by investigating the diversity of AMF communities in cotton roots of an established five-year winter cover crop cotton rotational system and no till treatments. Following objectives were established to test the hypotheses.

Objective 1. To evaluate soil microbial biomass and soil parameters in different tillage systems and cover crop rotations with semi-arid cotton

Objective 2. To evaluate root colonization and AMF diversity in cotton roots in different tillage systems and cover crop rotations.

2. MATERIALS AND METHODS

2.1 Research Location

The field experiment was conducted in 2016 and evaluated in one growing season of multiple years at the Texas A&M AgriLife Chillicothe Research Station (34.25° N, 99.51° W, 447 m above sea level) near Chillicothe, TX (Figure 4). Dryland cover crop treatments with no-till plots were established in Fall 2011. Soils at the site are Grandfield Series fine sandy loam. The initial range of Soil Organic Carbon (SOC) and pH for each treatment's plot in 2015 were between 3990 to 5102 mg/kg and 6.31 to 6.67, respectively. There were 4 demonstration areas each taking 8 rows of cotton that are 15 m x 12 m. There were 4 rows and 6 m. between each treatment, (Table 1).

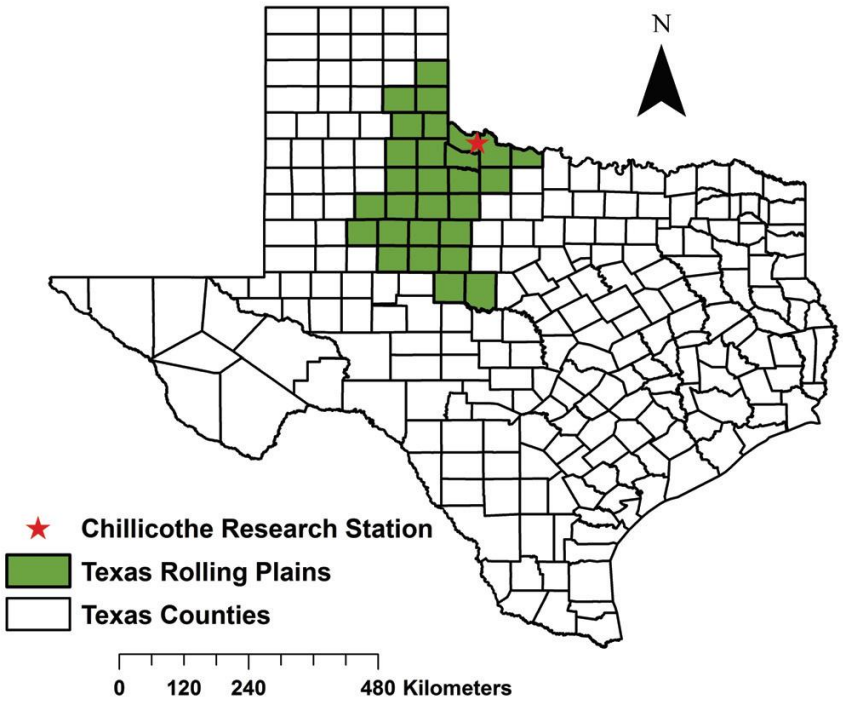

Figure 4: Project Location Within The Texas Rolling Plains.

Table 1: Field Demonstration for the Cropping System at Texas A&M AgriLife Chillicothe Research Station, Chillicothe, TX, USA.

	8 rows	4 rows	8 rows	4 rows	8 rows	4 rows	8 rows
12.2 m	CT		CC		MC		HV
6.1 m							
12.2 m	CC		AP		NO-TILL		WHEAT
6.1 m							
12.2 m	NO-TILL		HV		AP		CC
6.1 m							
12.2 m	HV		CT		WHEAT		CT
6.1 m							
12.2 m	WHEAT		MC		HV		AP
6.1 m							
12.2 m	AP		WHEAT		CC		MC
6.1 m							
12.2 m	MC		NO-TILL		CT		NO-TILL

2.2 Experimental Design

The study was arranged as Randomize complete black design RCBD with 4 replicate plots per treatment. The entire testing site did not have any irrigation and relied solely on rainfed precipitation under semi-arid environmental conditions. Average rainfall and temperature are shown Table 2 for the region.

Table 2: Monthly Rainfall, High and Low Temperature for Chillicothe in 2016.

Months	High Temperature °C	Low Temperature °C	Rainfall mm
January	12.1	-2.4	30
February	14.4	-0.3	36
March	19.2	4.3	56
April	24.3	9	57
May	28.9	15.3	85
June	33.1	20.1	108
July	35.9	22.3	53
August	35.7	21.8	62
September	30.9	16.9	80
October	25.3	10.6	71
November	18.3	4.1	42
December	12.4	-1.7	31

The Dryland system consisted of seven treatments:

- Conventional tillage with no cover crop (CT)
- No-till with no cover crop (NO-TILL)
- No-till & Austrian Winter field pea (AP)
- No-till & Wheat (WHEAT)
- No-till & Hairy vetch (HV)
- No-till & Crimson clover (CC)
- No-till & Mixed cover crops (MIXED)

The mixed cover crop (cereal rye 6.7kg/ha, hard red winter wheat 10.1kg/ha, austrian winter field pea 13.4kg/ha, and hairy vetch 3.4kg/ha) consisted of a seed mix as recommended by NRCS & Haney Soil Health Tool. Each cover crop (MBS seed

Denton-TX) was planted in the fall following cotton harvest. Cover crop treatments applied for planting and biomass production are shown Table 3 for the region. In the late spring, cotton (NG1511- Americot cotton seed company / TX) was planted after termination of the cover crops by chemical burn on June 10, 2016 and harvested on October 22, 2016. There was no fertilizer application. Herbicide applications during the treatment are shown Table 4.

Table 3: Planting Densities C:N Ratio, and Biomass Production for Each Cover Crop Treatment, with Statistical Significant Denoted by Different Letters ($p<0.05$).

Cover Crops Treatments	Densities kg/ha	C:N Ratio	Biomass Production kg/ha
Crimson Clover	22.4	18.4	1776^{c}
Hairy Vetch	22.4	14.6	2908^{abc}
Austrian W. Field Pea	39.2	13.2	3856^{ab}
Wheat	33.6	37.6	1947^{bc}
Mixed	33.6	22.0	4033^{a}

Table 4: Herbicides Application for Each Cover Crop Treatment.

Herbicides	Applied Amount	Date
Glyphosate	2.34 l/ha	25 April 2016
2,4-D	1.17 l/ha	5 May 2016
Glyphosate	2.34 l/ha	6 June 2016

2.3 Soil and Cotton Root Sampling

Soil samples (0-10cm) were collected using soil cores (2.5 cm in diameter) from randomly selected locations in each of the four plots per replicate sampled in May of 2016. Soil samples analyzed for pH (1:2 soil: deionized water; (Schofield and Taylor, 1955)), soil organic carbon (OC) according to (McGeehan and Naylor, 1988). Samples

were run for phospholipid fatty acid (PLFA), which analyses for total bacterial biomass, total fungal biomass, and mycorrhizal fungi biomass. Soil collected was transported in the cooler to the lab, and then transferred in a zip lock freezer bag. They were frozen and shipped immediately to WARD Laboratories, Inc. (Kearney, NE) for PLFA analysis.

To measure AMF colonization, cotton root samples (3 replicates) were collected from randomly selected second or seventh rows in each plot, both in August and October 2016. Cotton roots were excavated with a shovel and roots separated from the stem. Roots were gently washed with tap water three times to remove soil particles and divided in two subsamples, one was frozen at -80 °C for subsequent DNA extraction and the other was transported on ice to the lab for determining AMF colonization.

2.4 Fungal / Bacterial Biomass

Total fungal biomass, total mycorrhizal fungal biomass and total bacterial biomass were determined using phospholipid fatty acid (PLFA) analysis. PLFA were analyzed according to the methods of White and Zelles (White, et al., 1979, Zelles, 1997). PLFA standard markers are shown in Table 5 for each microbial group. The abundance of individual PLFAs was expressed as ng PLFA g^{-1} dry soil.

Table 5: PLFA Standard Markers For Each Microorganism.

Target Microorganism	Marker	References
Total Bacteria	15:0, i15:0, a15:0, i16:0 16:0ω9, i17:0, a17:0 cy17:0, 18:1ω7, cy19:0	(Frostegård and Bååth, 1996) (Zelles, 1997, Zelles, 1999)
Total Fungi	18:2ω6c & 16:1ω5	(Frostegård and Bååth, 1996, Petersen and Klug, 1994)
Total Mycorrhizal Fungi	16:1ω5	(Balser, et al., 2005, Spring, et al., 2000)

2.5 Detection of Mycorrhizal Colonization

Cotton roots were collected to measure mycorrhizal colonization by trypan blue staining (Phillips and Hayman, 1970). Roots were separated from plants and treated in 10 % KOH for 45 min at 65 C°. The KOH treatment removes the host cytoplasm and then nuclei. Then the roots were washed three times with distilled water and placed in 0.7N HCl for 45 min, at 65 C°. The HCl treatment decolorizes root pigments. After the HCl was poured off, roots were washed three times with distilled water and roots were stained for 20 min at 65 C° with 0.05 % trypan blue. As a last step, roots were treated with lactic acid for 10 min. Then 10 root segments were cut into 0.8 to 1 cm lengths, aligned within a 24 x 50 mm area on a microscope slides, then covered with the same size cover slip and viewed under a stereomicroscope at 4X (objective lens) x 10X (ocular-eyepiece) total magnification. The proportion of the length of each root segment, which contained vesicles, arbuscules, or hyphae, was used to assess colonization levels. Data was expressed as percentages of root length infected.

2.6 DNA Extraction from Roots, PCR and Sequencing

DNA was extracted from cotton root fragments using the Qiagen DNAeasy Plant Minikit (Qiagen Inc.) following the manufacturer's instructions. The extracted DNA was quantified using a NanoDrop ND-1000 (Thermo Scientific, USA) and kept frozen at -20 C° until used. The PCRs were performed in 50µl reaction mixtures at the Texas A&M AgriLife Research and Extension Center at Stephenville, TX. PCR thermocycling protocol was 95 degrees for 3 minutes, then 35 cycles of 95 degrees for 10 seconds, 55 degrees for 30 seconds, 72 degrees for 30 seconds. The total PCR reaction volume was

15 µl, and it contained 7.5 µl of 2X Kapa HiFi PCR master mix, forward and reverse primers each at 250 nM concentrations. DNA was normalized to 10 ng/µl before PCR thermocycling protocol. Primers used to amplify an 18S rRNA gene fragment for arbuscular mycorrhizal fungi are listed in Table 6.

Table 6: PCR Primers for Amplification of Arbuscular Mycorrhizal Fungi.

Primer	Sequence	Target	Reference
AMV4.5NF	5'AAGCTCGTAGTTGAATTTCG 3'	arbuscular mycorrhizal fungi	Sato et al. 2005
AMDGR	5'CCCAACTATCCCTATTAATCAT3'		

Paired-end sequence data was generated on an Illumina MiSeq instrument using v3 600 cycle kits (Illumina, San Diego, CA) as described in the Illumina 16S Metagenomic Sequencing Library Preparation protocol, except that dual 6 bp instead of 8 bp index sequences were attached to each amplicon during indexing PCR.

The raw sequencing reads were processed with a combination of QIIME (Caporaso, et al., 2010) and USEARCH (Edgar, 2010) software packages, as well as custom python scripts. Individual AMF sequences were compared to the Silva database (Quast, et al., 2013) using UCLUST (Edgar, 2010) in order to pick referenced-based Operational Taxonomic Units (OTUs) at 97% similarity, and to provide taxonomic assignments for each sequence read. The sequencing dataset was rarified to an equal sequence count for each sample by randomly subsampling sequences without replacement to provide even measures of microbial alpha- and beta-diversity and for statistical analyses.

2.7 Statistical Analysis

The mycorrhizal colonization data and soil organic C data obtained were analyzed for normality using JMP® Pro v.12.2 (SAS Institute, 2015). Significant differences were detected by using Fisher's least-significant-difference (LSD) test at a *P* value of <0.05 after a one-way analysis of variance (ANOVA).

Unweighted UniFrac distance metrics were used in the calculation of diversity measures (Lozupone and Knight, 2005). In order to determine if microbial community composition was significantly different between samples, PERMANOVA was conducted on OTU abundance data. The same matrix was used to perform one-way analysis of similarity (ANOSIM) to double check the result of PERMANOVA. Both analyses use the same interpretations with different calculations. AMF community structure was compared by estimating the bray-curtis distances and then visualized by Non-metric Multidimensional Scaling analysis (NMDS) or – PCoA – Principal coordinates analysis using PAST 3.X (University of Oslo 2016). Since some of the samples did not amplify with the AMF primer, missing values occurred and are replaced by their column average (Mean value imputation).

3. RESULTS AND DISCUSSION

3.1 Soil Chemical Properties

Soil organic carbon (SOC) levels observed in surface soil samples are presented in Figure 5. SOC levels were significantly higher in wheat treatment (4227 mg/kg) than the crimson clover treatment (3152 mg/kg). There were no significant differences in other treatments compared to wheat and clover treatments (Figure 5). Cover crops biomass production ranged from 1176 kg/ha to 4033 kg/ha (Table 3). Mixed cover crop biomass was significantly higher than both wheat and crimson clover, while Austrian winter field pea was significantly higher than only crimson clover treatments ($p<0.05$). Although both winter cover crop and crimson clover cover crop biomass were lower than the rest of cover crops, SOC between wheat and crimson clover was significantly different, due to C:N ratio. Lu, et al. (2000) reported that legume cover crops also decompose more rapidly due to the low C:N ratio. In general, organic residue decomposition rate is considered negatively related to C:N ratios (Melillo, et al., 1989, Melillo, et al., 1982). Since crimson clover belongs to the legume family, C/N ratio was 18.4, while wheat cover crop's C:N ratio was 37.6 as expected (Table 3). We found significant difference only between wheat and crimson clover. If it is assumed that these results occur annually for five years of cotton/cover crops cropping systems, then this possible reason for the difference may be due to a difference in crop residue quality, or possibly due to difference in C:N ratio. Also, the amount of SOC changed little from year to year during the study. With more time, larger differences in SOC may be observed under different cover crop treatments.

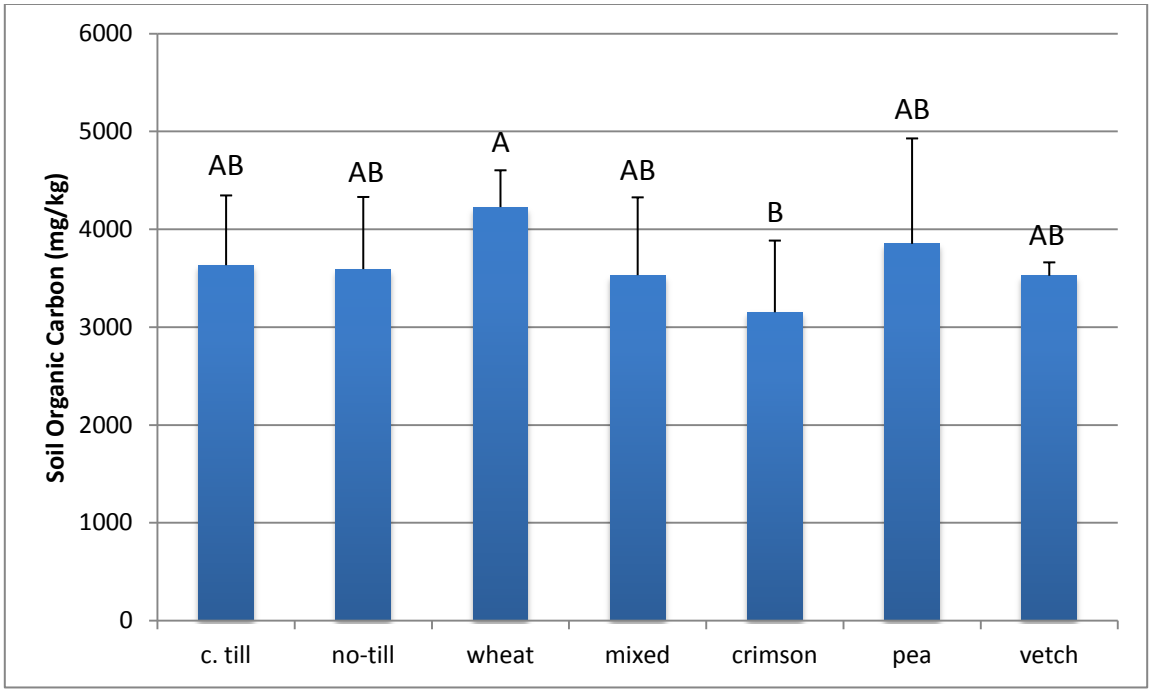

Figure 5: Soil Organic Carbon as Affected By Tillage and Cover Crop Treatments, with Statistical Significant Denoted by Different Letters ($p<0.05$).

3.2 Microbial Biomass Estimates Based on Phospholipid Fatty Acid (PLFA) Analysis

Phospholipid Fatty Acid analysis (reported in ng/g soil PLFA) was used to characterize the total bacterial biomass, total fungal biomass, and mycorrhizal fungal biomass. Figure 6 shows total bacterial biomass as affected by tillage and cover crop treatments. Total bacterial biomass was significantly higher in hairy vetch and Austrian pea treatments than in conventional tillage and no-till treatments ($P<0.05$). However, crimson clover, mixed species, and wheat were not statistically different than conventional tillage and no till treatments. The highest total bacterial biomass among cover crops was noticed in hairy vetch with 738ng/g soil of PLFA markers detected,

while the lowest total bacterial biomass of 436ng/g soil was detected in crimson clover plots.

Total biomass of cover crops seems to be the major factor driving bacterial biomass, as positive correlation was observed between the two variables. It is generally expected that higher plant residue can support higher decomposer communities including bacterial biomass. The most significant differences were observed between treatments with cover crops and those without cover crops, no till and conventional tillage (Figure 6). The total bacterial biomass results are in agreement with Reddy, et al. (2003) who examined the effect of rye and crimson clover residues on weeds, soil microbial populations, and soybean yield in conventional tillage and no-tillage systems. Their result showed that total fungal and bacterial populations were higher in soil with crimson clover (5.41 $\log_{10}$ CFU/g, 8.34 $\log_{10}$ CFU/g), followed by rye (5.25 $\log_{10}$ CFU/g, 8.32 $\log_{10}$ CFU/g), and no cover crop (5.12 $\log_{10}$ CFU/g, 7.97 $\log_{10}$ CFU/g). Similarly, Balota, et al. (2003) found that averaging all crop rotations in the surface soils, (0-5 cm), there was 100% increase in microbial biomass for no-till over conventional tillage.

The general increase of microbial biomass under no-till over conventional tillage could be based on several factors including higher moisture content and greater soil aggregation. Lower disturbance as a result of no-tillage can conserve SOC and hyphal networks, and support the microbial community structure compared to conventional tillage. Moreover, no-tillage and cover crop rotations favors formation and stabilization

of macro aggregates to improve and protect habitat for microbiota (Balota, et al., 2003, Powlson and Jenkinson, 1981, Sørensen, et al., 1996).

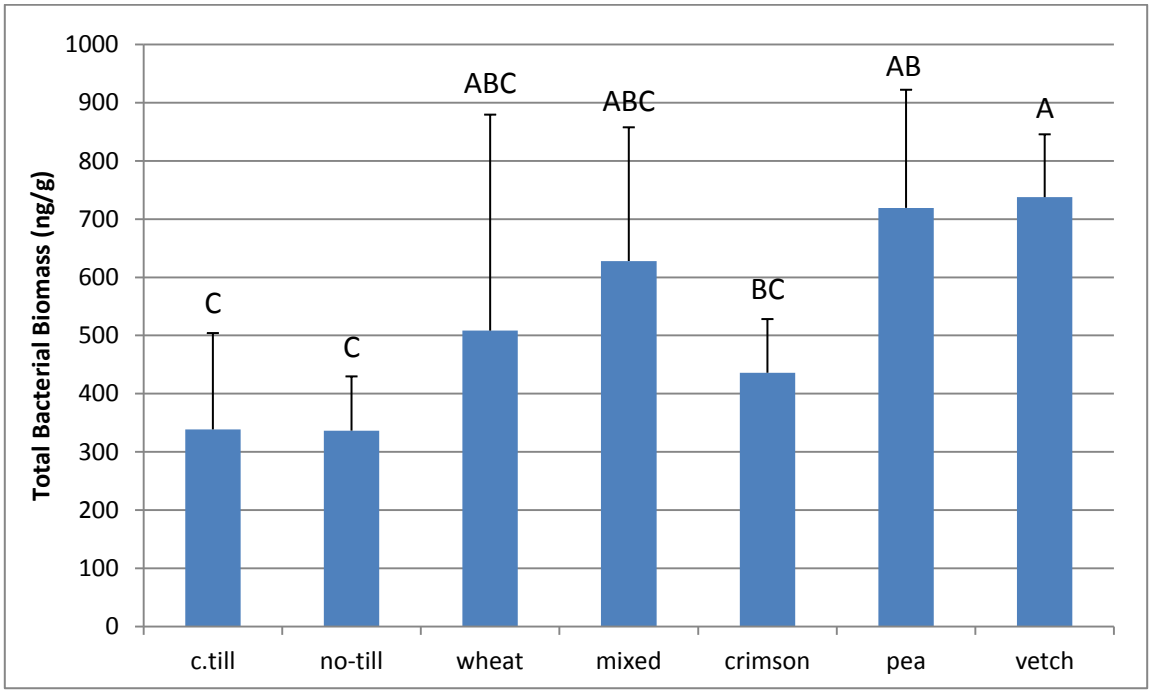

Figure 6: Total Soil Bacterial Biomass as Affected by Tillage and Cover Crop Treatments, with Statistical Significant Denoted by Different Letters ($p<0.05$).

In this study, no statistical differences were observed for total fungal biomass as affected by tillage, no-till, and cover crop treatments (Figure 7). Plots under Austrian pea rotation had highest fungal biomass at 159 ng/g soils, while the plots with crimson clover rotation had the lowest total fungal biomass at 83.7 ng/g soil. Results of our study also indicated that total fungal biomass did not significantly increase in no-till compared to conventional systems although the cover crop treatments trended higher for fungal biomass than in the conventional till and no-till treatments without cover crops (Figure

7). Although, it has been confirmed by many studies that fungal populations increase when soil is less disturbed by tillage (Govaerts, et al. (2008) and Helgason, et al. (2009), although many of those studies were conducted on longer-term tillage plots than this study sites. No-tillage systems and cover crop treatments may take longer timeframe (probably more than 5 years) to significantly alter fungal populations.

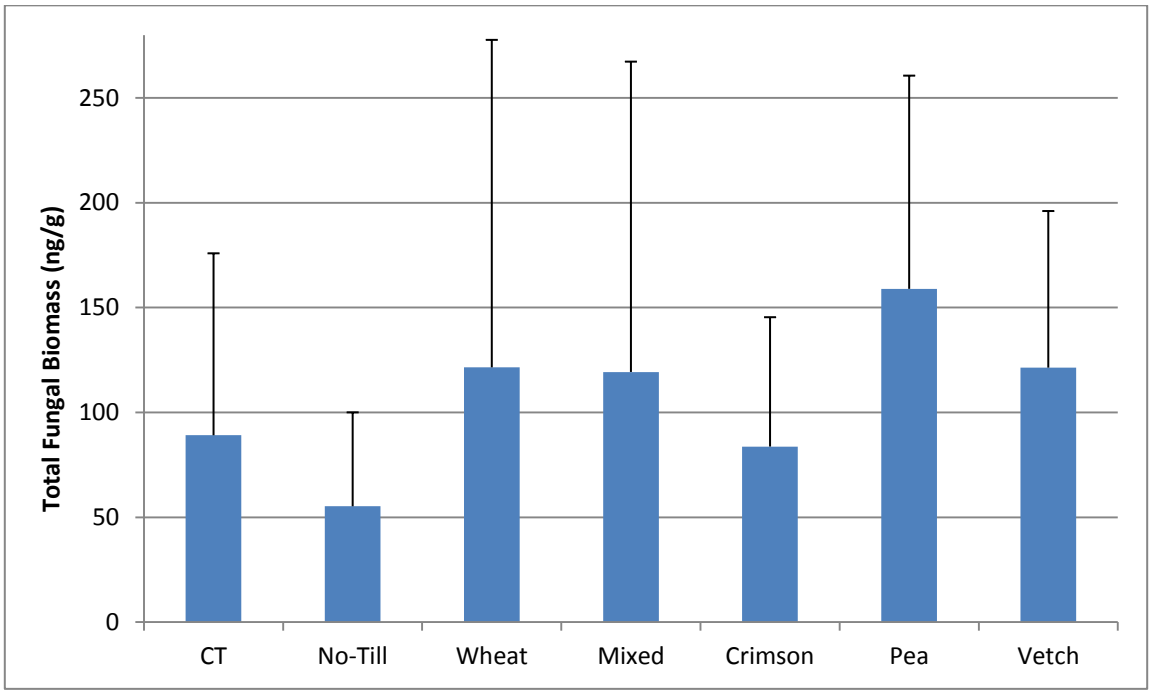

Figure 7: Total Soil Fungal Biomass as Affected by Tillage and Cover Crop Treatments.

Similar to total fungal biomass, there was no statistical difference for total mycorrhizal fungi biomass among the treatments, although Austrian pea, mixed cover crops, wheat, and hairy vetch plots trended higher than conventional tillage and no-till with no cover crop (Figure 8). Helgason, et al. (2010) reported that in the 5-10 cm

depths, biomass of AMF decreased by 25% in no-till compared to conventional tillage. However, Tiemann, et al. (2015) studied the impact of crop rotational diversity on microbial communities in an agro-ecosystem and found almost double AMF abundance in the corn monoculture compared to rotations, such as Soy-Wheat-Corn-1 cover crop, Soy-Wheat-Corn-2 cover crops, and Soy-Corn rotations. Buyer, et al. (2010) studied soil microbial community structure in tomato cropping systems with 9 different treatments: bare soil, black polyethylene mulch, white polyethylene mulch, rye cover crop, rye roots only, rye shoots only, vetch cover crops, vetch roots only, and vetch shoots only and three different sampling time: July, August, and September. The results of the study showed that rye and vetch cover crops increased the absolute amount of AM in bulk soils, and AMF levels in the tomato rhizosphere were highest under the cover crop treatments, while lowest under no cover crop treatments. However, our results showed that AMF biomass did not significantly increase under cover crop treatments. Probably this was due to shorter duration of these rotations, which suggest that under semiarid cotton systems, the cover crops and tillage systems may take longer time frame to produce noticeable differences in mycorrhizal fungi biomass.

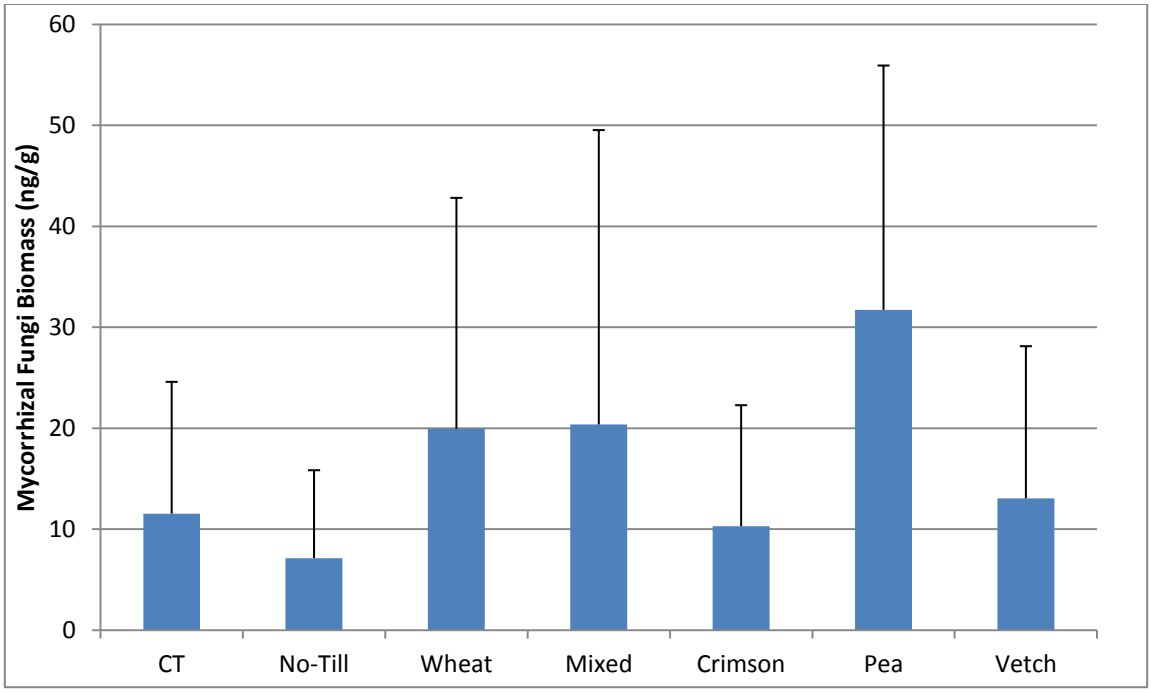

Figure 8: Total Mycorrhizal Fungi Biomass as Affected by Tillage and Cover Crop Treatments.

3.3 AM Fungal Colonization

At mid-season, mycorrhizal colonization in no-till treatments was as follows: crimson clover (98%), hairy vetch (95%), and Austrian pea (97%). These colonization rates were significantly higher than both conventional tillage (65%) and no-till (75%) treatments. The root colonization rate in wheat (88%) and mixed species (85%) were significantly higher than conventional tillage (Figure 9). The highest mycorrhizal colonization among no-till with cover crops was noted in crimson clover (98%), while no-till with mixed cover crops produced the lowest mycorrhizal colonization (85%). According to Evans and Miller (1990), Kabir, et al. (1998), and McGonigle and Miller (2000) plowing and other disturbances of the soil can decrease the extent of AMF

mycelial networks. Our result showed that AM colonization under several cover crops treatments was significantly higher than conventional tillage and no-till treatments. This is in agreement with Kabir and Koide (2002) who conducted an experiment on the influence of cover crops (oats, rye, and combination of oats and rye) on AMF colonization of sweet corn. AMF colonization under combination of rye and oats (60%), only rye (50%), and only oats (48%) were significantly higher than fallow (15%) treatments.

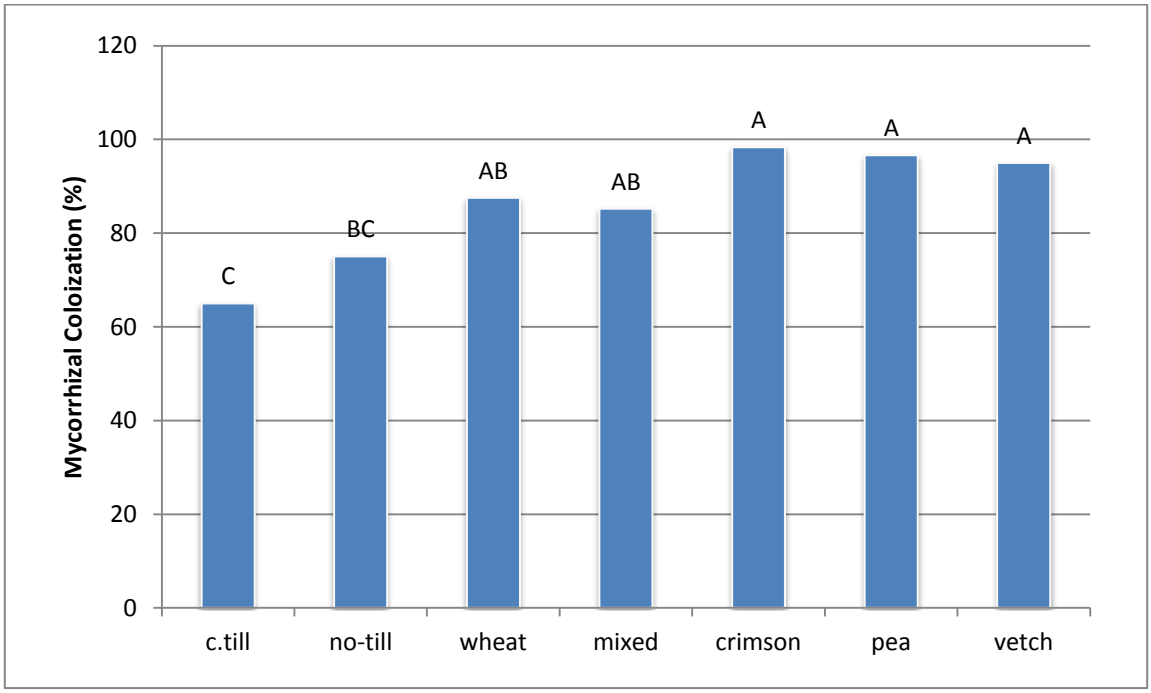

Figure 9: Percentage of Mycorrhizal Colonization of Cotton at Mid-Season (August) as Affected by Tillage and Cover Crop Treatments, with Statistical Significant Denoted by Different Letters ($p<0.05$).

However, statistically significant differences between treatments disappeared by the end of the season (October; Figure 10). This was mostly as result of higher mycorrhizal colonization in conventional tillage and no-till at 82% and 80%, respectively. While the root colonization decreased in the no till with cover crop treatment to 80%, 80%, 93%, 84%, and 89% in crimson clover, hairy vetch, Austrian pea, wheat, and mixed species, respectively. In a study at a similar semi-arid location on the Southern High Plains of Texas, mycorrhizal colonization of cotton was detected at the higher rate in early season, at around 50% and then declined to around 15% at the end of the growing season (Zak, et al., 1998).

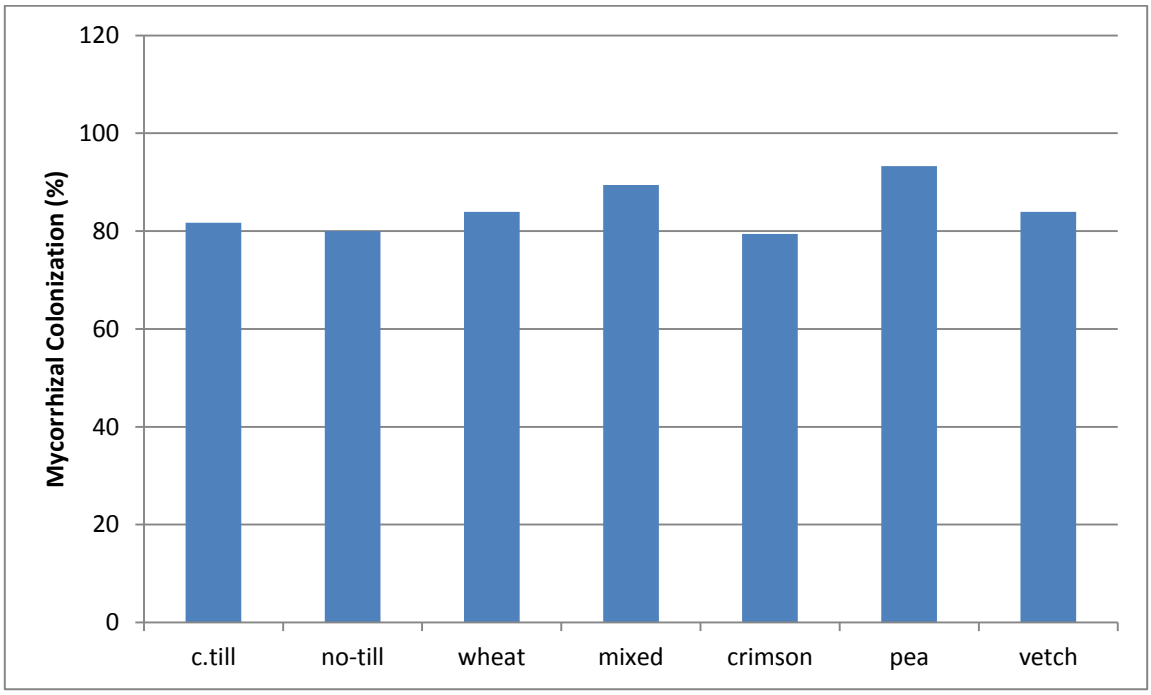

Figure 10: Percentage of Colonization of Cotton at the End of the Season (October) as Affected by Tillage and Cover Crop Treatments.

Similarly in our study, AMF colonization of cotton roots was higher during the early part of the growing season, which suggest that nutrient acquisition and drought tolerance through AMF associations may be critical during initial stages of cotton-plant development. As winter cover crops colonize mycorrhizae (Boswell, et al., 1998, Cade-Menun, et al., 1991), the existence of cover crop roots will enable persistence of AMF hyphal networks and spores, which may probably support higher colonization of developing cotton seedlings. Despite the significant effects of cover crops on mycorrhizal colonization at mid-season, the effect was diminished by the end of the season (October). These results are similar to other studies, which observed high colonization during the peak growing season and low colonization during the late season in comparable habitats (DeMars and Boerner, 1995, Escudero and Mendoza, 2005, Kabir, et al., 1997, Mandyam and Jumpponen, 2008, Mullen and Schmidt, 1993, Reinhardt and Miller, 1990).

Tillage disturbances have been noted to negatively affect AMF abundance, and the persistence of their active propagules such as spores, hyphae and colonized roots. Roldan, et al. (2007) noted the highest levels of mycorrhizal propagules in maize and bean crop soil under no-till compare to tillage. Soil tillage has been noted to extensively damage hyphal networks, and detrimental to AMF hyphae, especially when the soil is tilled before planting of cover crops in the fall (Kabir, 2005). Our result at the mid season under no-till with no cover crop trended higher than conventional tillage based on the AMF colonization in cotton roots, but this trend disappeared by the end of the growing season.

3.4 AMF Community Characterization in Cotton Roots

3.4.1. 18S rRNA Sequence Analysis

The AMF communities in the cotton roots were evaluated by obtaining 1180 partial 18S rRNA gene sequences for each root sample DNA. The number of AMF OTUs ranged between 98 and 175 in the treatments, while the highest number and the lowest numbers were observed in the hairy vetch cover crop treatment and conventional tillage treatment in August, respectively (Table 7). In October, the number of AMF OTUs ranged between 96 and 205 in the treatments, while the highest number and the lowest numbers were observed in the Austrian pea cover crop treatment and no tillage treatment, respectively (Table 8). There were no noticeable differences among the treatments based upon the number of OTUs, Chao1 richness estimates, or Shannon or Simpson's diversity indices both in August and October. The cover crops trended higher compared to conventional tillage and no-till treatments for most measurements based on the number of OTUs, Chao1 richness, or Shannon or Simpson's diversity indices.

Table 7: Diversity and Richness Estimates for AMF Communities in Cotton Roots under Different Cover Crop Treatments in August. Total number of Sequences Per Sample Is 1180.

Treatments	Number of OTUs*	Chao1 Richness	Shannon	Simpson (1/D)
Conventional Tillage	98 (∓28.2)	191 (∓38.9)	3.11 (∓0.89)	0.71 (∓0.13)
No-Tillage	133 (∓17.4)	272 (∓45.1)	3.92 (∓0.4)	0.80 (∓0.07)
Wheat	126 (∓64.8)	266 (∓141.3)	3.39 (∓1.57)	0.68 (∓0.25)
Mixed	130 (∓28.4)	306 (∓34)	3.55 (∓0.68)	0.73 (∓0.01)
Crimson	111 (∓18.6)	245 (∓47.7)	3.33 (∓0.14)	0.72 (∓0.02)
Pea	166 (∓39.7)	398 (∓127.8)	4.40 (∓0.45)	0.86 (∓0.02)
Vetch	175 (∓61.5)	469 (∓173.5)	4.26 (∓0.99)	0.81 (∓0.1)

Table 8: Diversity and Richness Estimates for AMF Communities in Cotton Roots under Different Cover Crop Treatments in October. Total number of Sequences Per Sample Is 1180.

Treatments	Number of OTUs*	Chao1 Richness	Shannon	Simpson (1/D)
Conventional Tillage	122 (∓4.1)	235 (∓8.1)	3.76 (∓0.06)	0.78 (∓0.05)
No-Tillage	96 (∓35.5)	191 (∓62.3)	3.10 (∓0.88)	0.74 (∓0.10)
Wheat	153 (∓31.3)	348 (∓115.8)	4.02 (∓0.99)	0.78 (∓0.16)
Mixed	110 (∓54.2)	243 (∓144.4)	3.56 (∓0.97)	0.77 (∓0.1)
Crimson	151 (∓10.1)	404 (∓36)	4.19 (∓0.45)	0.84 (∓0.07)
Pea	205 (∓2.3)	565 (∓20.7)	5.14 (∓0.06)	0.92 (∓0.00)
Vetch	140 (∓40.5)	363 (∓107.9)	3.88 (∓0.60)	0.80 (∓0.04)

* Obtained from rarefied OTU table.

Based on ANOSIM results, we did not find statistically significant differences in AMF community composition (R= 0.15, p = 0.11) in cotton roots of target species both at mid-season and at end of growing season (Tables 9 & 10). Similarly, PERMANOVA results showed (Table 11-12) no significant effect at mid-season (August) and the end of the growing season (October) (PERMANOVA p=0.586 and p=0.077 respectively).

Table 9: Result of ANOSIM Pairwise Comparison of AMF Associated with Different Tillage and Cover Crop Treatments, at the Mid-Season (August). P<0.05

	CT	No-till	Wheat	Mixed	Crimson	Pea	Vetch
CT	-						
No-till	0.500	-					
Wheat	0.101	0.101	-				
Mixed	0.097	0.606	0.302	-			
Crimson	0.104	0.097	0.394	0.097	-		
Pea	0.101	0.204	0.202	0.204	0.100	-	
Vetch	0.103	0.306	0.305	0.497	0.499	0.294	-

Table 10: Result of ANOSIM Pairwise Comparison of AMF Associated With Different Tillage and Cover Crop Treatments, at the End of the Season (October). P<0.05

	CT	No-till	Wheat	Mixed	Crimson	Pea	Vetch
CT	-						
No-till	0.696	-					
Wheat	1	1	-				
Mixed	0.90	0.607	0.902	-			
Crimson	0.80	0.804	1	1	-		
Pea	0.19	0.693	1	0.600	0.701	-	
Vetch	0.097	0.097	0.194	0.799	0.298	0.102	-

Table 11: Result of PERMANOVA Pairwise Comparison of AMF Associated With Different Tillage and Cover Crop Treatments, at Mid-Season (August).

	CT	No-till	Wheat	Mixed	Crimson	Pea	Vetch
CT	-						
No-till	0.798	-					
Wheat	1	1	-				
Mixed	0.603	0.7	0.7	-			
Crimson	0.599	0.698	0.703	0.806	-		
Pea	0.496	1	0.798	0.396	0.2	-	
Vetch	0.104	0.199	0.205	0.297	0.104	0.098	-

Table 12: Result of PERMANOVA Pairwise Comparison of AMF Associated With Different Tillage and Cover Crop Treatments, at the End of Season (October).

	CT	No-till	Wheat	Mixed	Crimson	Pea	Vetch
CT	-						
No-till	0.8	-					
Wheat	0.098	0.101	-				
Mixed	0.096	0.496	0.590	-			
Crimson	0.098	0.106	0.195	0.102	-		
Pea	0.337	0.409	0.603	0.896	0.293	-	
Vetch	0.339	0.496	0.605	0.706	0.297	1	-

Nonmetric multidimensional scaling (NMDS) plot was created using the Bray-Curtis distance matrices for relative abundances of AMF OTUs in the cotton roots under different tillage and cover crops treatments at mid-season (Figure 11). AMF community composition in cotton roots under conventional tillage displayed slight dissimilarity compared to other treatments. Also, among the cover crop treatments, only hairy vetch AMF community was slightly dissimilar.

Towards the end of the cotton-growing season (October), AMF community in cotton roots under cover crops treatments were distinct from those under conventional tillage and no-till with no cover crop (Figure 12). Communities were also somewhat distinct between the conventional tillage and no-till treatments.

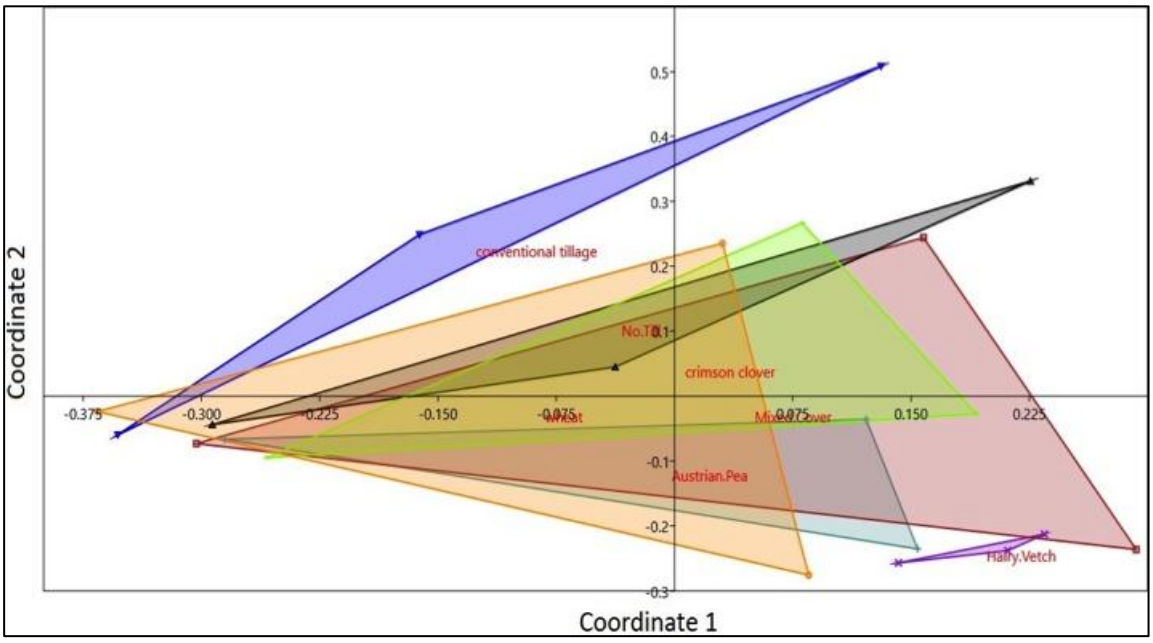

Figure 11: Principal Coordinate Analysis (PCoA) of Mycorrhizal Species Colonizing Cotton Roots, Under Different Tillage and Cover Crop Treatments, at Mid-Season (August).

In addition, AMF species in cotton roots among cover crops showed dissimilarities at the end of the season, but not to the extent observed between the cover crops and no cover crop treatments. Different level of AMF diversity were reported under various agricultural management practices, where tillage (Jansa, et al., 2002) decreased diversity, while conservation management (Oehl, et al., 2004, van der Gast, et al., 2011), or cover crop systems (Oehl, et al., 2003) increased diversity. Lehman, et al. (2012) suggested that multiple cover crops could build affirmative environment for AMF inoculum in soils compare to individual cover crops, and winter cover crops in rotation would provide diverse, year-round hosts to continue or enhance AMF community composition. Previous studies also suggested that fallow rotations without cover crops

declined AMF diversity in both soil and roots (Hijri, et al., 2006, Oehl, et al., 2003, Sasvári, et al., 2011).

Our results also suggested that the effects of cover crop treatments on mycorrhizal community were more evident at the end of season (October) than in the growing season. It has been noted that AMF may exhibit distinct seasonal patterns (Pringle and Bever, 2002) and that the abundance of some AMF taxa in plants may change seasonally (Mathimaran, et al., 2007, Merryweather and Fitter, 1998, Turrini, et al., 2016). From this perspective our results support that introduction of winter cover crops in rotation with dryland cotton can chance AMF community composition and support the hypothesis that no-tillage and crop diversification and maintaining continuous cover may be beneficial for increasing AMF abundance in semi-arid cotton.

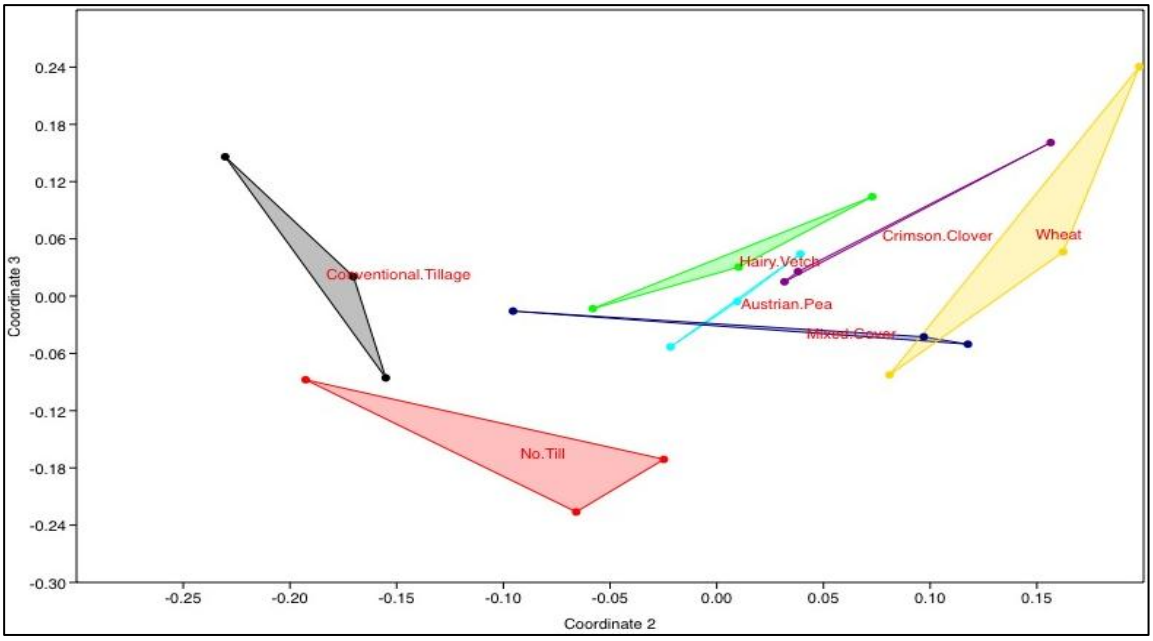

Figure 12: Principal Coordinate Analysis (PCoA) of Mycorrhizal Species Colonizing Cotton Roots, Under Different Tillage and Cover Crop Treatments, at the End of the Season (October).

3.4.2 Identifying Groups of AMF Communities

Principal Component Analysis (PCA) was performed on OTU abundance data for revealing AMF species associations with treatments and time of sampling. AMF communities in cotton roots varied by treatments similar to that seen in the PCoA results. First principal component (PC1), accounted for 61% of the variability and PC2, accounted for 25.4% of the variability in the mid-season (August) samples (Figure 13). At the end of the season (October), PC1 explained 62.3% of the variability, and PC2 explained 19.6% of the variability (Figure14). Bi-plot vectors within the scatter plots represent AMF OTUs, plotted in accordance to their loading values, which in turn represents their relative contribution to overall variability of the community between the

treatments (commonly used to identify major drivers of variability). Major vectors were labeled with respective OTU id to identify the AMF species. Three OTUs that had loading values for PC 1 or 2 in excess of 0.67, 0.74, and 0.92 were strongly correlated to *Glomus intraradices, Glomus* sp. G27, and *Glomus clarum,* respectively, based on BLAST search. *Glomus* sp. G27 was significantly higher under hairy vetch treatments, while *Glomus intraradices* was significantly higher under no-till in the mid-season (Figure 15A-B). However, *Glomus clarum* was not significantly different among the treatments (Figure 15-C). Percentage of *Glomus clarum* sequence ranged from 6% to 34% in the treatments, while the highest percentage and the lowest percentage were observed in the wheat treatment and vetch treatment, respectively. In October samples, AMF communities were similar in cotton roots among all cover crop treatments, but were different from no-till and conventional tillage treatments (Figure 14). Results showed that *Glomus sp. G27* under wheat and crimson clover treatments were significantly higher than mixed, no-till and conventional tillage treatments. Also, *Glomus sp. G27* in hairy vetch treatments were significantly higher than both conventional tillage and no-till treatments (Figure 16-A). *Glomus intraradices* and *Glomus clarum* were significantly higher under conventional treatments and no-till treatments, respectively (Figure 16-B, C).

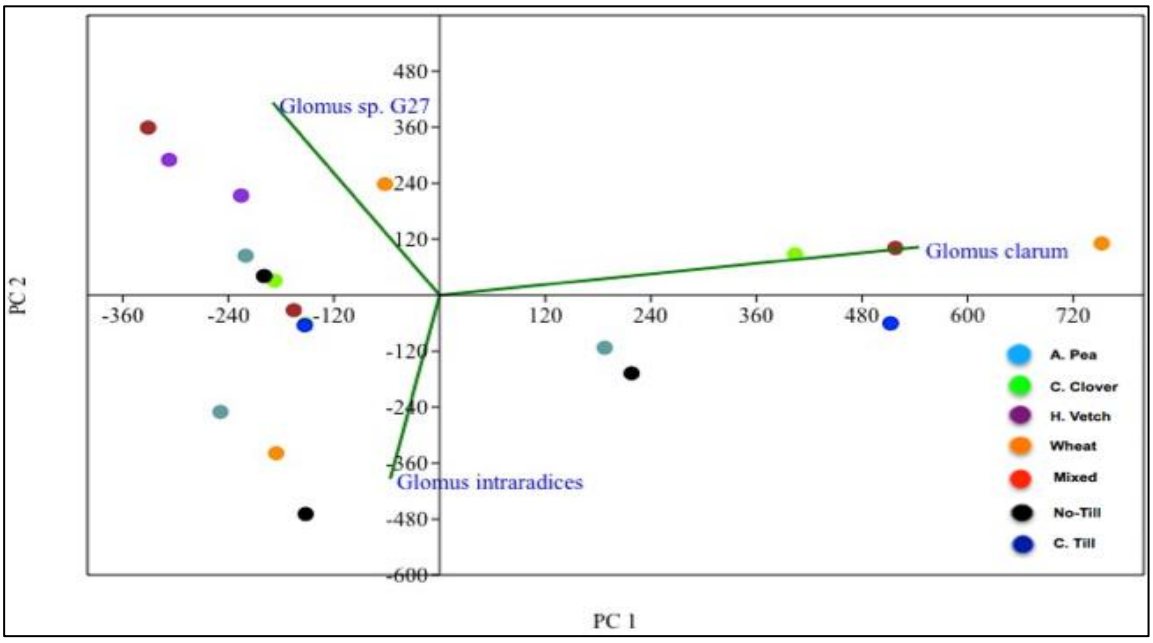

Figure 13: Principal Component Analysis (PCA) on Mycorrhizal Species Colonizing Cotton Roots, Under Different Tillage and Cover Crop Treatments, at Mid-Season (August).

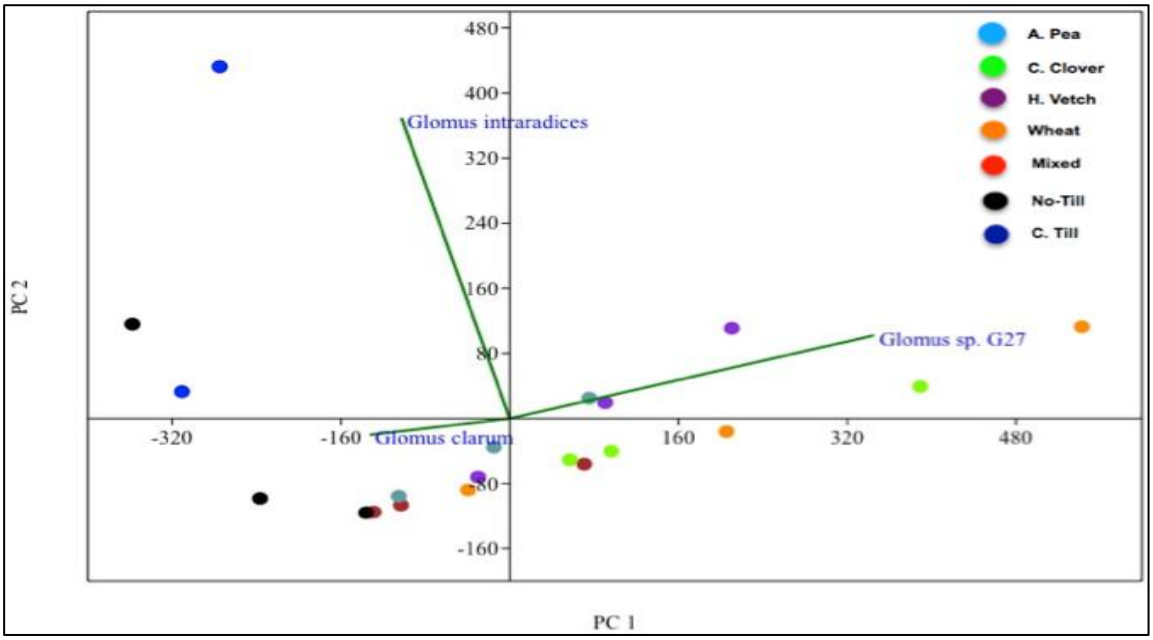

Figure 14: Principal Component Analysis (PCA) on Mycorrhizal Species Colonizing Cotton Roots, Under Different Tillage and Cover Crop Treatments, at the End of the Season (October).

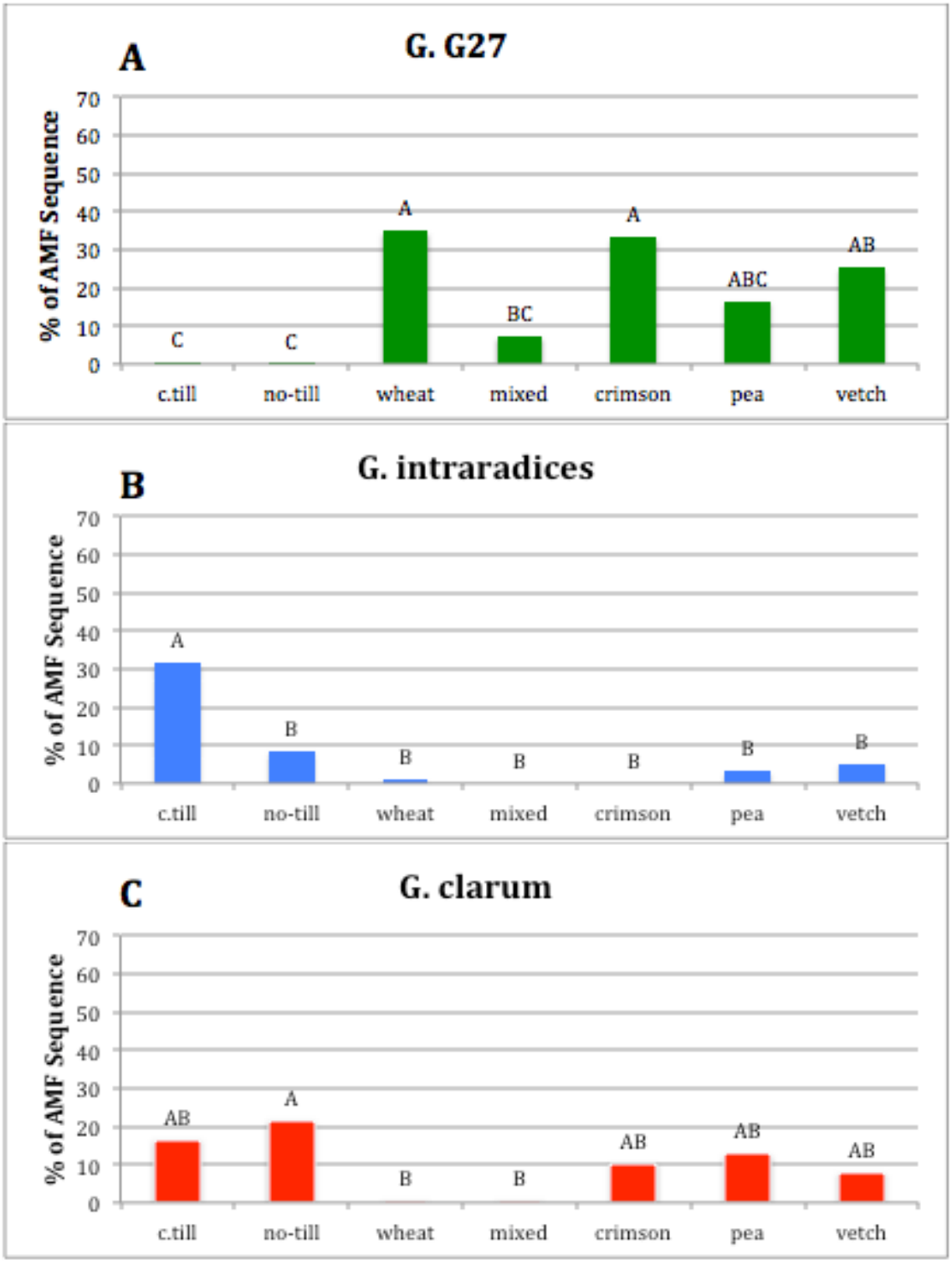

Figure 15: Percentage of AMF Sequences as Affected at Mid-Season (August) by Tillage and Cover Crop Treatments, with Statistical Significant Denoted by Different Letters ($p<0.05$).

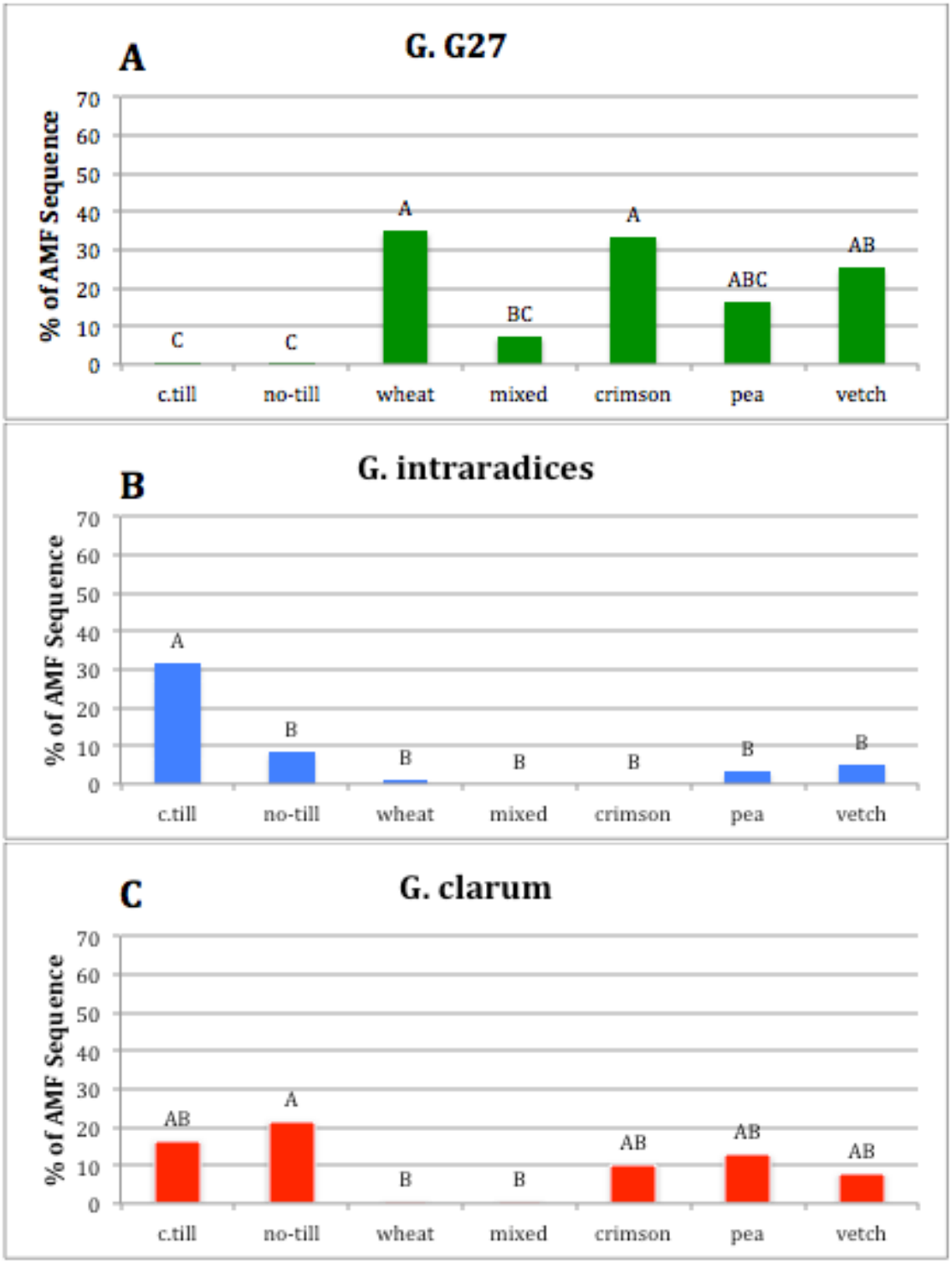

Figure 16: Percentage of AMF Sequences as Affected at the End of the Season (October) by Tillage and Cover Crop Treatments, with Statistical Significant Denoted by Different Letters ($p<0.05$).

At both sampling times, *Glomus sp. G27* was higher in hairy vetch than in both conventional tillage and no-till. As a leguminous AMF host crop, hairy vetch may have been more supportive to AMF during the winter. Although, Njeru, et al. (2015) reported that hairy vetch did not increase AMF species richness and diversity in organic tomato farming, while it consistently enhanced spore abundance. Higo, et al. (2011) reported that the difference in crop types (wheat vs. red clover), and season (winter vs. spring wheat), affected AMF community composition in an andosol. Furthermore, Higo, et al. (2013) showed that the shifts in AMF community composition were observed due to winter cover crop management.

Seasonal variability of AMF communities has been observed in soil (Dumbrell, et al., 2011) and plant roots (Bever, et al., 2001, Daniell, et al., 2001, Husband, et al., 2002, Liu, et al., 2008, Öpik, et al., 2003). Some species of AMF may be more sensitive to seasonal changes than others according to many reports (Dumbrell, et al., 2011, Helgason, et al., 1999, Mathimaran, et al., 2007, Sommerfeld, et al., 2013, Turrini, et al., 2016). However, some studies reported no such variability and showed that similar AMF community colonized plants roots of soybean at all time points of growth stages (Higo, et al., 2014, Higo, et al., 2015, Rosendahl and Stukenbrock, 2004, Santos-González, et al., 2007). Results of this study showed that the taxon composition of AMF community in cotton roots was relatively stable during the growing season.

Highly infective AMF species, such as many *Glomus* spp., become more abundant under conditions of environmental stress (Oehl, et al., 2004) and tillage

disturbance (Jansa, et al., 2003). However, some studies found no significant effect on *Glomus* spp. genetic diversity under tillage treatments (Koch, et al. (2004). Since *Glomus intraradices* produces more external structures early stages of inoculation and has a fast colonization rate (Hart and Reader, 2005), we expected this species to dominate under tillage treatments.

Data from this study support our second hypothesis, that cover crop rotations will increase AMF root colonization and change AMF community composition in cotton. Results revealed significantly different abundance of AMF compositions among the sampling periods of the year (Figures 9, 10). We detected *Glomus* sp. G27, *Glomus intraradices*, and *Glomus clarum* species both mid-season (August) and end of the growing season (October). In another study of andosolic soils in Japan to determine whether there is an impact of cover crops on AMF community composition in subsequent soybean (Higo, et al., 2014). They did not find a significant change in the patterns of the AMF community composition as a whole. However, Higo, et al. (2011) reported that the difference in crop type (wheat and red clover) and sowing date (winter and spring wheat) affected AMF community composition in its roots. In addition, Dumbrell, et al. (2011), Higo, et al. (2013) confirmed that the change of compositions of AMF communities were observed based on winter cover crop management.

Taxonomic association of AMF community was evaluated by comparing proportional class distribution of the AMF sequence types in cotton roots (Figure 17). Vetch cover crop treatments markedly enhanced the abundance of *Glomeromycetes* (almost 3-fold) and reduced *Ambiguous* (approximately 6-fold), while other cover crop

treatments barely decreased with time. Similarly, conventional tillage treatments remarkably increased the abundance of *Glomeromycetes* (around 2-fold), and reduced *Ambiguous* (around 4-fold) from August to October. Conversely, crimson cover crop treatment markedly reduced the amount of *Glomeromycetes* 3-fold and increased around 2-fold, while the amount of *Incertae sedis*, which is called unknown or undefined, increased 8-fold during the growing time. For no-till treatment, the amount of *Incertae sedis* increased 6-fold, but *Ambiguous* decreased around 2-fold from August to October; while *Glomeromycetes* did not change.

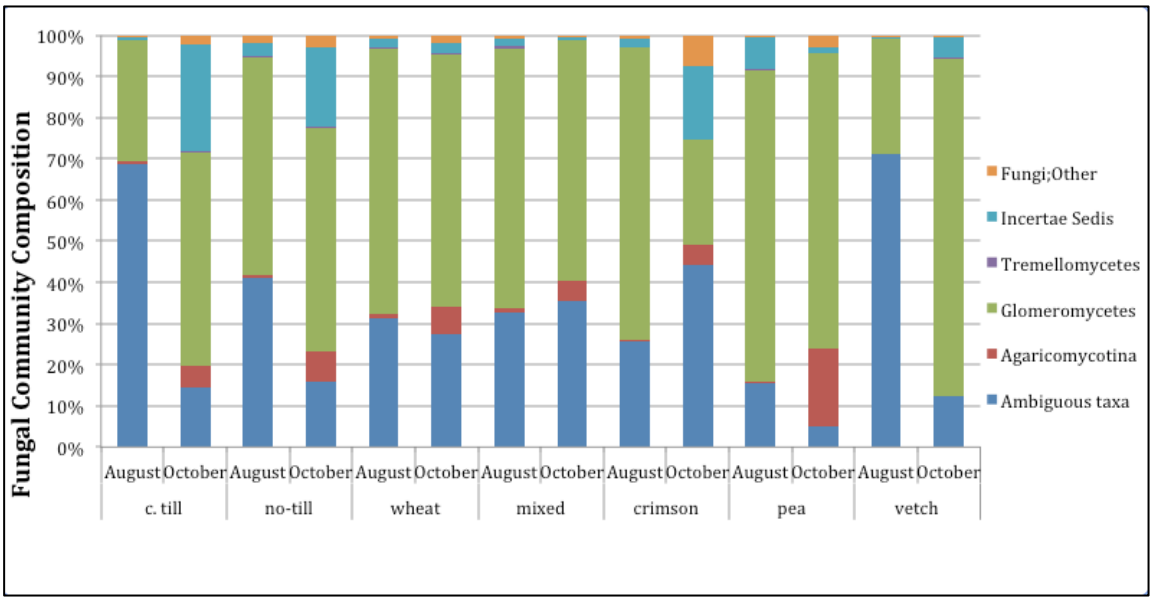

Figure 17: Proportional Class Distribution of the AMF Sequences Types in Cotton Roots at the Mid-Season (August) and at the End of the Season (October).

Overall, *Glomeromycetes* dominated all cover crop treatments except in vetch treatment in August. It is not surprising that most of the AMF class detected belonged to

Glomeromycetes given this is the most prevalent class in agricultural soils among AMF species described (Daniell, et al., 2001, Jansa, et al., 2003).

We studied influence of winter cover crops on soil microbial population and mycorrhizal colonization of semi-arid cotton in Texas High Plain region. Cover crops significantly increased bacterial biomass compared to conventional tillage and no-till treatments. We did not find any significant difference in fungal biomass and AMF biomass among the treatments, but they trended higher under cover crop treatments than conventional tillage and no-till treatments.

Mycorrhizal colonization of cotton was significantly higher at mid-season (August), but differences disappeared at the end of the growing season (October). Conversely, impacts of treatments on mycorrhizal community composition were more apparent at the end of the growing season (October) than in middle season (August).

4. CONCLUSIONS

The aim of this study was to investigate the impacts of tillage practices and use of cover crops on soil microbial populations and mycorrhizal colonization in a dryland cotton system. Of the factors analyzed, cover crop treatments had a positive impact on total bacterial biomass, while tillage systems proved to have no significant impact. Even though the data did not show significant effects on fungal biomass and mycorrhizal fungal biomass, values for both parameters trended higher under cover crop treatments except crimson clover compared to conventional or no-till with no cover crop. Our results suggest that Austrian winter field pea and hairy vetch are the most effective cover crops for positively influencing microbial biomass and mycorrhizal root colonization under the systems of dryland cotton.

The second aim of this study was to investigate the effect of tillage systems and cover crop treatments on the enhancement of AMF diversity in cotton. AMF root colonization was significantly higher among the treatments early in the growing season, but by the end of the growing season there were no significant differences between the treatments. However, AMF community composition between the treatments was similar during early season, but was dissimilar at the end of the growing season. The result does not conclusively identify the most effective cover crops treatments for enhancing AMF diversity and colonization in dryland cotton. Although, some cover crops may be more beneficial for increasing root colonization at early stages of cotton and establishment.

The results of this study, while not conclusive, are promising and elucidate the most effective treatment factor, specifically cover crop, on mycorrhizal community colonization within a rotational agricultural system. Furthermore, cover crops contributed to increased AMF population and enrichment of soil microbial biomass. These benefits of cover crops may translate higher drought tolerance, establishment and nutrient acquisitions. However, it is not clear from our findings whether 5 years cotton/cover crop cropping systems is long enough to significantly increase mycorrhizal community colonization. Therefore, further research needs to be carried out in order to validate the complex interactions between cotton and AMF interactions in response to land management practices of cover crop rotations and tillage systems.

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