COVER CROP IMPACTS ON SOIL MICROBIAL POPULATIONS AND

NITROGEN CYCLING OF DRYLAND AGRICULTURAL PRODUCTION IN

THE TEXAS ROLLING PLAINS

A Thesis

by

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ABSTRACT

The Rolling Plains of Texas are characteristic of a low input, high risk environment with semi-arid conditions and sporadic, high intensity storms followed by periods of long drought. Agricultural practices that increase input costs are not readily adopted in the region; however, better management practices might be needed to tolerate global climate change. Cover crops in no-till agriculture have been used to increase soil health under environments with low precipitation and serve as an alternative to fallow management. The first study took place in a dryland cotton system with evaluated treatments that include 1) conventional tillage (CT); 2) no-till (NT); and no-till with the following cover crops 3) wheat; 4) Austrian winter pea (AP); 5) crimson clover; 6) hairy vetch (HV); and 7) mixed species (MC). It was predicted that cover crops would improve soil health by increasing carbon mineralization (CMIN) and increasing soil carbon (C) and nitrogen (N) pools. Soil organic carbon (SOC) and total N (TN) were highest in AP and MC treatments. The water extraction technique, which measures the smaller, labile subset of the nutrient pool, proved to be a more sensitive measure of soil substrate. Total NO_3^-, water-extractable organic nitrogen (WEON), water-extractable organic carbon (WEOC), and CMIN levels were significantly higher in no-till treatments with cover crops (AP or HV) when compared to no-till without cover crops. The total phospholipid fatty acid (PLFA) biomass analysis shared this same trend, but there were no significant treatment differences. Ammonia-oxidizing bacteria, responsible for nitrification, was highest in AP, and was highly correlated to total NO_3^-. The mixed-species cover crop improved soil health more than CT and NT, but this study concluded that the single-species legume crop, AP, had the highest overall soil health improving benefits. Both AP and MC could be used as an alternative to fallow management.

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My second study took the alternatives to fallow a step further and tested a dryland continuous winter wheat with fallow management with two different cover crop management strategies. One subset of mix-species cover crops treatments were chemically terminated and left in the field to serve as ground cover and decomposable organic matter. Those treatments included in the mix-species treatments were divided in two more categories depending on seeding rates, which were 16.8 kg/ha (Low Mix) and 22.4 kg/ha (High Mix). The Low Mix and High Mix were further divided by termination timing, which were early maturity (55-70 days) and late maturity (75-90 days). A separate subset of treatments, cowpea, mung bean, and guar, were harvested to serve as a double crop for additional production income. The final treatments were a wheat-fallow and a wheat-canola (no cover crop) management as a control. Different offseason cover crop treatments were analyzed for CMIN, PLFA biomass abundance, water-extractable organic nutrients, inorganic N rates, and soil water storage to determine if the cover crop would be viable as a harvested double crop. Harvested guar reported the highest WEON, NO_3^- and inorganic N values in the soil compared to the wheat-fallow management, while having statistically the same amount of stored water at the time of winter wheat being planting. The CMIN and WEOC did not statistically increase or decrease for any treatment when compared to fallow, which implies that CMIN and WEOC were not negatively impacted from changing management practices to fallow alternatives. Throughout all the experiments implemented, CT and NT never once indicated a significant difference between them at any date or depth, so the addition of cover crops to no-till cotton systems could potentially enhance no-till in regard to soil function. This research suggests that double crops could be a competitive strategy in the Southern Great Plains dryland wheat system.

DEDICATION

This thesis is dedicated to Chris Hulsey, Kim Hux, Jerry Frank Jr. Hux., Matthew Attalai, Lyndsey Ivy, Roy H. Williams, & Buddha.

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NOMENCLATURE

amoA	Ammonium Monooxygenase Gene
ANOVA	Analysis of Variance
AOA	Ammonia-Oxidizing Archaea
AOB	Ammonia-Oxidizing Bacteria
AP	Austrian Winter Field Pea
ATP	Adenosine Triphosphate
$BaCl_2$	Barium Chloride
$BaCO_3$	Barium Carbonate
$^{\circ}C$	Centigrade
C	Carbon
CC	Crimson Clover
CEC	Cation Exchange Capacity
CT	Conventional Till
C_t	Cycle Threshold
cm	Centimeter
CMIN	Carbon Mineralization
C_2H_2	Acetylene
$cmol_c$	Centimole
CO	Colorado
CO_2	Carbon Dioxide
CO_2-C	Carbon Dioxide Carbon
DAP	Days After Planting
DCM	Dichloromethane
e.g.	"for example"
et al.	"and others"

ft	Feet
g	Gram
Gram$^-$	Gram-negative Bacteria
Gram$^+$	Gram-positive Bacteria
kg	Kilogram
K	Potassium
KCl	Potassium Chloride
KS	Kansas
ha	Hectare
HCl	Hydrochloric Acid
HV	Hairy Vetch
IAC	Internal Amplification Control
L	Liter
LSD	Least Significant Differences
M	Mass conversion from cmol$_c$ to g C
M	Molar
MC	Mixed Species Cover
MCL	Maximum Containment Level
MeOH	Methanol
min	Minute
mm	Millimeter
mL	Milliliter
µg	Microgram
µL	Microliter
MT	Montana
N	Normality of Acid
N	Nitrogen

N_2	Dinitrogen or nitrogen gas
NaOH	Sodium Hydroxide
NE	Nebraska
ND	North Dakota
NO_2^-	Nitrite
NO_3^-	Nitrate
NH_4^+	Ammonium
NT	No-Till
NTC	No Template Control
P	Phosphorus
PLEL	Phospholipid Ether Lipid
PLFA	Phospholipid Fatty Acid
pH	Hydrogen Ion Concentration
qPCR	Quantitative Polymerase Chain Reaction
rcf	Relative Centrifugal Field
rpm	Revolutions Per Minute
S	Soil Weight
s	Second
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SON	Soil Organic Nitrogen
TN	Total Nitrogen
TX	Texas
USA	United States of America
W	Winter Wheat
WEOC	Water Extractable-Organic Carbon
WEON	Water Extractable-Organic Nitrogen

WEN   Water Extractable Nitrogen

ω   Aliphatic End of a Molecule

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<center>INTRODUCTION AND LITERATURE REVIEW</center>

1.1 INTRODUCTION

1.1.1 Climatic Issues

Most arid areas of the world occur approximately along the subtropical belts, 30° latitude north and south of the equator, where dry winds are known to descend (Lydolph, 1985; Lane and Nichols, 1999). More than a third of the world's land surface is either arid, generally receiving less than 250 mm of annual precipitation, or semi-arid receiving between 250 mm or 500 mm of annual precipitation (Lane and Nichols, 1999). The Southern Great Plains are near this subtropical, latitudinal boundary, and are known for the semi-arid climate that is often stressed with extreme drought conditions, including: hot temperatures, low precipitation, and generally unfavorable growing conditions for agricultural production (Guerro, 2011). High levels of temporal and spatial climate variability with recurring periods of severe droughts have led to widespread crop failure with little residue cover (Fannin, 2012; Hansen et al, 2012). The limited precipitation that does occur usually comes in the form of intense and localized rainstorms in the late spring and summer. These intense precipitation events can cause losses of soil and nutrients in runoff (Blanco-Canqui et al., 2013). It has been difficult to achieve residue buildup and increased soil organic carbon in fields that have been converted to no-till due to that fact that crop residues rapidly degrade (Rasmussen et al., 1998; Novara et al., 2016).

1.1.2. Soil Water

Due to limited precipitation, a common practice with semi-arid production fields is to set them fallow, or unseeded during a growing season, in an attempt to store soil water. Producers in

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the semi-arid central Great Plains of the United States use a fallow period that last approximately 16 months, and is intended to store water for the subsequent cash crop (Blanco-Canqui et al., 2013). However, producers in the southern Great Plains use a shorter fallow period lasting between 3-6 months long. The primary reason for summer fallow is to stabilize crop production and reduce the chances of crop failure by forfeiting production in one season in anticipation that there will be at least partial compensation by increased crop production the next season (Nielson and Vigil, 2010). Nielson and Vigil (2010) analyzed the precipitation storage efficiency between conventional tillage and no-till during a 10 year wheat-fallow rotation with each fallow period lasting 14 months. The average precipitation storage efficiency for the entire fallow period with conventional tillage was 20% compared to no-till with 35% (Nielson and Vigil, 2010).

The weeds that occupy the fallow bare soil are often managed with tillage. Also, the low biomass return of cotton, high tillage rates, and fallow periods have been shown to decrease soil organic matter (SOM) and aggregate stability, leading to soil degradation and erodibility (Peterson et al., 1998; Acosta-Martinez et al., 2004; Blanco-Canqui et al., 2013). However, maximizing soil water storage during the growing season and fallow periods potentially increases the chances of crop survival between the erratic precipitation events. Many of the high intensity precipitation events in the Southern Great Plains exceed the soil's water infiltration capacity (Blanco-Canqui et al., 2013). Using no-till management and growing cover crops as a replacement to fallow management could improve soil properties by increasing water infiltration, increasing water storage, soil organic carbon (SOC) sequestration, soil erosion protection, and agricultural sustainability and productivity in semi-arid regions.

1.1.3. Cover Crops

An alternative to fallow management is the use of cover crops, which are grown for a specific duration in the offseason and then chemically or mechanically terminated. The terminated cover crop is then left in the field to decompose over time, and release nutrients for the cash crop. Adding cover crops to no-till management may provide an affordable on-site management option to shield the soil from evaporative losses due to high temperatures associated with direct sunlight and wind, and at the other extreme, erosion losses with heavy rainfall (Wright et al., 2007). Cover crops can improve soil structure, SOC, water infiltration, water retention, and root penetration while enhancing soil microbial communities and nutrient cycling; however, these benefits are heavily influenced by soil moisture (Wright et al., 2007; Clark et al., 2009; Blanco-Canqui et al., 2013). Cover crops can also reduce soil crusting, soil erosion, runoff, and nutrient leaching (Wright et al., 2007).

The first step in increasing water storage is water capture, which is limited by the water infiltration rate (Blanco-Canqui et al., 2013). No-till management with cover crops increasing the water infiltration rates of the soil can be a solution to efficiently capturing and storing more precipitation from the sporadic and unreliable storms that are common in the Southern Great Plains (Blanco-Canqui et al., 2013). These semi-arid regions are characteristically viewed as low input, high risk environments, meaning that practices having increased input costs, such as cover crops, must be carefully monitored and evaluated in order to expect widespread adoption. The adoption of conservation tillage, and particularly no-till was not widely adopted in the southern Great Plains, which was less than 5% of cultivated land area in northwest TX and southwest KS, as compared to central and northern Great Plains, which was greater than 20% of cultivated land in northeast CO, southwest NE, northeast MT, and northwest ND (Hansen et al, 2012). In

general, farmers in the Southern Great Plains have not readily adopted soil health promoting

practices or water quality improvement strategies due to major concerns that cover crops could

potentially reduce soil water and thereby affect the yield of subsequent cash crops (Unger and

Vigil, 1998; Unger et al., 2006; Adhikari et al., 2017). For example, Baughman et al. (2007)

showed that cotton yields were reduced in the Southern Great Plains when a cover crop was used

in combination with no-till cotton. However, interest in cover crops has been increasing, as

evidence has begun to show improved soil health under drought and heat stress. For example,

DeLaune et al. (2012) found no significant impact of a wheat cover crop on subsequent cotton

fiber yield in both dryland and irrigated systems. Sij et al. (2004) found no significant difference

in cotton lint yields as a result of rye cover crops over a three-year period. Keeling et al. (1996)

determined agricultural production in the Southern Great Plains could obtain satisfactory ground

cover if the proper species is sown and that fall rainfall is adequate for germination and plant

survival. They concluded that wheat and rye were the most dependable species, and that several

legume crops failed due to low moisture characteristic of the Southern Great Plains. Other

grower concerns leading to hesitation to adopt strategies that solely rely on no-till and use of

cover crops include concerns that soils are often susceptible to compaction due to lack of

disturbance, livestock grazing, and field equipment traffic (Hansen et al., 2012). Moreover,

because semi-arid regions are typically chronically short of water for stable dryland crop

production, there may be significant costs associated with cover crop water use and reductions in

subsequent cash crop yields that will make successful implementation of cover crops difficult to

achieve (Nielsen, 2016).

Cover crop systems often lead to an increase in SOC over time due to the increased C

input from cover crop biomass. This increase in SOC may add resilient properties to soil and

provide a buffer against compaction (Blanco-Canqui et al., 2010). Integrating cover crops with existing cropping systems has the potential to enhance ecosystem services such as soil nutrient and water cycling, improving resource (soil, water, and air) quality, and increasing the range of markets that producers can potentially reach (Blanco-Canqui et al., 2015). Grasses have been used as cover crops because they are tolerant over a wide range of environmental conditions, and can reduce nitrate leaching due to their ability to rapidly establish root systems (Meisinger et al., 1991). Legumes as cover crops are important because of their ability to increase soil N and supply it to the next crop (Meisinger et al., 1991). However, not every cover crop will provide adequate biomass or N contributions to justify the seed and planting cost. For example, attempts to employ several legume species have failed due to inadequate moisture in the Southern Great Plains (Keeling et al., 1996; Dozier et al., 2008).

Additionally, studies have focused on the potential advantages and disadvantages of using cover crop mixtures. Legume cover crops tend to decompose more rapidly than non-legume cover crops, which can reduce legume cover crop residue effectiveness at protecting the soil surface and moderating soil temperatures compared with grass cover crops (Blanco-Canqui et al., 2013). Cellulose-rich plants or plant parts degrade far more rapidly than if they were mature grasses with a higher lignin content. Hence, leafy portions of the shoot system degrade far more rapidly than the supportive stems (Edwards and Burney, 2005). Mixtures of two or more cover crops that combine the benefits of grasses and legumes are often more effective than planting a single species. A mixture of cover crop species can increase SOC more than a single-species treatment due to a greater biomass production above and below the soil (Faé et al., 2009; Blanco-Canqui et al., 2015). The grass component scavenges residual N effectively, while the legume adds biologically fixed N that is more readily available to the cash crop (Meisinger et al.,

1991; Clark, 2008). Disadvantages of cover crop mixtures may include higher seed cost, too much residue, more complicated management, and a difficulty to seed (Clark, 2008). There are virtually no published data on the effectiveness of mixed cover crops for semi-arid Texas cropping systems. Thus, there is a demand for a comprehensive evaluation and demonstration of the impact of conservation cropping systems. However, understanding what to measure in order to appraise benefits remains unclear.

1.1.4. Potential for Using Soil Biological Properties as Indicators of Health under Different Cropping Systems

The major challenge within sustainable soil management is to conserve ecosystem service delivery while optimizing agricultural yields. Soil testing services at government-sponsored and private laboratories have historically focused on total SOM, chemical soil indicators of inorganic N, phosphorus (P), potassium (K), soil pH, and various other macro- and micronutrients to assess nutrient availability to crops. These are important indicators, but inorganic nutrient availability alone does not offer a complete assessment of soil fertility or of soil biological influences on important soil properties and processes that affect crop yield and environmental quality (Franzluebbers, 2016). This has led to the development of commercially viable soil health testing focused on biological properties as an essential step for improving the sustainability of no-till production systems (Kibblewhite et al., 2008). Biological indicators of soil quality for the function of sustaining plant growth might include parameters such as microbial biomass and/or respiration, mycorrhizal associations, nematode communities, enzymes, or fatty-acid profiles (Karlen et al., 1997).

Soil health is presented as an integrative property that reflects the capacity of soil to respond to agricultural intervention, so that it continues to support both the agricultural

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production and the provision of other ecosystem services. One parameter currently used to

analyze soil health is determination of microbial biomass and community structure through

phospholipid fatty acids (PLFA) analysis. This is a culture-independent approach analyzing the

PLFA composition of the soil microbial community, since different subsets of microorganisms

have unique PLFA membrane patterns (Tunlid and White, 1992; Frostegård et al., 1993; Kujur

and Patel, 2014). The unique PLFA biomarkers can be monitored for shifts in the PLFA

signature, and then recorded as microbial biomass changes resulting from different management

practices (Feng et al, 2003).

Another approach is measuring the burst of microbial respiration following the rewetting

of air-dried soil, or the "flush of CO_2 on rewetting" (Franzleubebers et al. 2000), also known as

carbon mineralization (CMIN). The latter parameter has been widely accepted as a metric of soil

health and soil quality; however, there is not a widely accepted standardized protocol to obtain

results (Wade et al., 2018). Franzluebbers (2016) proposed that soil testing could be elevated to a

more complete evaluation of soil fertility and health with the adoption of a test for biological

activity by using the flush of CO_2 during 1 to 3 day following rewetting of dried soil and

incubation period to measure CMIN. The flush of CO_2 is related to soil microbial biomass

carbon and has repeatedly been shown to be strongly related to net N mineralization during

standard aerobic incubations. The rapid increase in soil water potential associated with the

rewetting of a dry soil causes microbes to experience osmotic shock (Fierer and Schimel, 2003).

Microbial cells either lyse completely or adjust to the water potential shock by releasing

intracellular osmoregulatory solutes (Halverson et al., 2000). The compounds released into the

soil environment are taken up by surviving microbes and mineralized, producing a respiration

pulse (Fierer and Schimel, 2002). The CO_2 burst that is measured corresponds to nutrient

availability for the cash crop that will follow the cover crop (Haney et al., 2012). Incubation-based methods have traditionally been lengthy for a routine test, such as 14-210 day incubation experiment to measure CMIN (Keeney and Nelson, 1982). Franzluebbers (1999) found that the C mineralized in 3 days from soils dried and ground for laboratory analysis that were rewetted was strongly correlated with 24-day C mineralization from undisturbed soil.

The CMIN rates have be used to provide an estimate of soil N mineralization potential (Luxhøi et al., 2005). The CMIN rates have also been shown to be correlated to N mineralization; however, the experiments suggest that differences in gross N immobilization and mineralization rates between the soils were more related to the respiration rate and adenosine triphosphate (ATP) content than to the C/N ratio (Bengtson et al., 2003). This means that respiration alone is not an indicator of N immobilization and does not accurately predict net N mineralization/immobilization. High soil microbial activity does not always lead to high N mineralization due to the fact that microbes can decrease plant-available nitrogen through immobilization; however, determining the C:N ratio from a smaller and more active pool of C and N to soil microbial activity could increase the accuracy of predicting new mineralization/immobilization (Wade et al., 2012). The smaller fractions of water-extractable organic carbon (WEOC) and water-extractable organic nitrogen (WEON) are likely to be a more sensitive measurement than the larger SOC and total nitrogen (TN) values, and therefore can be a better measurement of the impact of management inputs (Haney et al., 2012).

Thus, one of the objectives of my study was to determine the impact of no-till cover crop treatments on soil microbial biomass (PLFA) and activity (measured by CMIN) and the influence of these microbes on soil health (measured as nitrogen mineralization). This study

explored the link between the release of CO_2 following rewetting of dried soil and the WEOC:WEON labile nutrient ratio. The C:N ratio calculated from the labile nutrient fraction may become an additional tool in conjunction with the flush of CO_2 to better predict plant available N and possible N immobilization (Wade et al., 2012). If research data supported the hypothesis that no-till cover crop usage would enhance microbial activity leading to improved nitrogen mineralization, one outcome of the research would be to use these findings to promote wider adoption of soil health promoting practices within semi-arid environments.

CHAPTER II

WINTER COVER CROP IMPACT ON SOIL MICROBIAL POPULATIONS AND NITROGEN CYCLING IN DRYLAND COTTON PRODUCTION IN THE SEMI-ARID SOUTHERN GREAT PLAINS OF TEXAS

2.1. INTRODUCTION

2.1.1. Cotton Production Systems

Today, roughly 2.2 million hectares of Texas land has been developed for cotton (*Gossypium hirsutum)* cultivation, which is significant because it makes up about 50% of the overall cotton acreage in the United States (USDA, 2018a). For decades, the Southern Great Plains, including southern Kansas and Colorado, eastern New Mexico, the panhandle of Oklahoma, and northern Texas (Figure 2.1), has seen cotton monoculture development due to low grain prices, increased cost of irrigation, favorable U.S. government farm programs of 1985 and 1990, and historically a lack of insect pests (Allen, 2008). However, recent changes in agricultural policy have raised concerns about the profitability and financial viability of current cropping practices (Allen, 2008). The mechanical disturbance of soil, commonly referred to as conventional tillage, serves multiple functions, such as preparing the seedbed, killing encroaching weeds, incorporating nutrients or amendments, reducing soil compaction, managing crop residues for disease control, providing a proper environment for seed germination, encouraging root growth for crop production, and simply following the traditions of previous generations (Gebhardt et al., 1985). Tillage systems have changed as new technologies have become available and as the price of fuel has risen relative to the price of agricultural chemicals (Epplin et al., 1982). The development of cost-effective herbicides and advanced equipment has

influenced the overall adoption of no-till practices by a growing number of producers (Hansen et al, 2012). Strict no-till systems rely on herbicides instead of tillage for weed control, and nearly all soil disturbing operations (other than mechanical planting) are avoided (Lyon et al., 2004; Hansen et al., 2012). No-till practices used over a long term have been shown to significantly increase soil carbon (C), nitrogen (N), and microbial biomass compared to conventional-till practices (Feng et al., 2003; Mathew et al., 2012).

2.1.2. Impact of Tillage Practices and Cover Crops on Populations of Ammonia-Oxidizing Soil Microorganisms and Predictions on How This Influences Nitrogen Cycles in Semi-arid Regions

Ecological and commercial concerns have generated an increased demand to understand the fate of N in agricultural systems due to it being a limiting factor in crop production, and it is often applied as a soil amendment to increase crop yield. Substantial amounts of N are lost from the soil system through crop removal, accounting for a majority of the N loss from the soil system. Soil erosion and runoff can occur as a potential source of N loss. Best management practices can minimize N loss and can potentially increase N with effective crop rotation strategies.

Soil microbes also play a strong role in the N cycle. Saprophytic microbes decompose organic matter, converting organic N into inorganic forms, ammonium (NH_4^+) and nitrate (NO_3^-), which are plant available (Lamb et al., 2014). The positive charge from NH_4^+ makes it physically attracted (or held) by negatively charged soil and organic matter. The NH_4^+ that is not taken up by plants may be converted through nitrification to NO_3^-. This aerobic biological process occurs in warm, moist soils. The negative charge from NO_3^- makes it less susceptible to binding to soil particles. Water soluble NO_3^- moves easily in the soil profile, usually downward

below the root zone. This generally happens after an excessive water event occurs, such as extended rainfall or a generous irrigation application (Lamb et al., 2014).

A second focus of my research is to examine how cover crops influence ammonia-oxidizing soil microbial populations as a possible source for the NO_3^- contamination in the groundwater in these semi-arid areas of Texas. My interest in this topic stems from the involvement of my committee member, Dr. DeLaune in association with the Texas AgriLife Research station in a project entitled *Groundwater Nitrogen Source Identification and Remediation in the Texas High Plains and Rolling Plains.* The project seeks to identify the source of NO_3^- present in the area groundwater and evaluate and demonstrate strategies and practices for reducing these levels ("Groundwater Nitrogen Source Identification and Remediation" 2017). According to a Texas groundwater study by Hudak (2000), more than 50% of the observations in north-central and west-central Texas counties showed levels of NO_3^- in the groundwater that exceed the maximum containment level (MCL) of 44 mg/l. The highest NO_3^- concentrations were found in the Seymour Aquifer, which supplies water to the Texas AgriLife Research and Extension Center at Vernon, and had a median nitrate concentration value of 59.9 mg/l. Farmers in the Rolling Plains region may not be accounting for the increased levels of NO_3^- in the Seymour Aquifer. Irrigating with high-NO_3^- groundwater and not accounting for NO_3^- in the irrigation water while deciding fertilizer application rates can lead to significant source water contamination (Chaudhuri et al., 2010).

Microbial ammonia oxidation is the first and rate-limiting step in nitrification. It has long been believed that microbial ammonium oxidation was performed solely by chemolithoautotrophic ammonia-oxidizing bacteria (AOB) that possess the ammonia

monooxygenase (*amoA*) gene, the key enzyme of nitrification (Kowalchuk and Stephen, 2001). However, more recently chemolithoautotrophic ammonia-oxidizing archaea (AOA) have been identified as other microorganisms responsible for the nitrification of NH_4^+. In fact, AOA are so widespread that they numerically dominate AOB in ocean waters and many natural soil ecosystems (Karner et al., 2001; Francis et al., 2005; Schleper et al., 2005). As of yet, there are no other known microbial metabolic pathways that oxidize NH_4^+ to NO_3^-. Nitrification by AOA and AOB are important to consider when managing N cycles because they produce NO_3^-, which can be lost through leaching, although the exact contribution of each to the nitrification process remains unclear.

There is evidence that suggests AOA may be more important than AOB in many soils (Nicol et al., 2008; Tourna et al., 2008); however, studies indicate that this might not be true for agricultural soils. According to Jia and Conrad (2009), AOA gene copies were more numerous than AOB gene copies in both surface and deep soil layers of agricultural soils when NH_4^+ fertilizer was added, but they concluded AOB had a larger role in ammonia oxidation activity due to DNA stable-isotope probing showing that NH_4^+ fertilization only stimulated CO_2 assimilation by nitrifying bacteria but not archaea. It was also shown in that same study that AOA gene copy number increased when nitrification activity was inhibited by acetylene (C_2H_2) showing that ammonia oxidation alone did not support the growth of archaeal populations (Jai and Conrad, 2009). Concentrations of organic substrates, which might be an alternative carbon and energy source for archaea, are much higher in soils than aquatic environments (Jai and Conrad, 2009). Recent studies demonstrated that plant-induced organic substrates stimulated the increase of AOA gene transcripts, while fertilizer amendment with either NH_4^+ or NO_3^- had no effect on the change in AOA gene transcripts in rhizosphere and bulk soils (Chen et al., 2008).

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Population numbers of AOA remain relatively stable from surface to depth across tillage and no-till treatments, but AOB populations increase in tilled soil (Catão et al. 2016). The tillage disruption releases C and N substrates from disrupted soil aggregates. Studies have reported AOA populations are predominant in low-nutrient environments, so they should populate deeper in the soil profile where nutrients become scarce at lower depth (Nicol et al., 2008; Catão et al. 2016; Mushinski et al., 2017). Ouyang et al. (2016) showed that AOB were more responsive to NH_4^+ than AOA in agricultural soils. The abundance of AOA was always greater than AOB but was unaffected by N treatments. In contrast, AOB abundance and community structure were changed significantly by NH_4^+ fertilizers (Ouyang et al., 2016). This indicates that AOB populations could be higher than AOA populations when nutrients become available (Wessén et al 2010; Banning et al., 2015). This study examines how tillage practices and types of cover crops impact soil health parameters, the nitrifying microbial populations of AOB and AOA in a semi-arid climate, and how the N cycle is influenced by these agricultural inputs.

2.1.3. Hypotheses

- (H1) Cover crops with legumes will increase inorganic N in the soil, and show higher densities of nutrients in the bulk soil.

- (H2) NH_4^+ will increase following the cover crop termination and decrease thereafter while NO_3^- will increase inversely due to ammonia oxidation, or nitrification.

- (H3) No-till practices and cover crops will improve soil quality as measured by increased CMIN.

- (H4) The added benefits of both grasses and legumes in the mix species blend will improve soil health more than the other treatments.

- (H5) PLFA biomass from the soil microbial community can be used as a proxy to help distinguish which management practices can promote the highest PLFA biomarkers for bacteria, fungi, or rhizobia.

- (H6) AOA populations would dominate the ammonia-oxidizing community as proxied by *amoA* gene copy abundance.

- (H7) AOA abundance would not be negatively affected by soil depth while AOB abundance would be reduced at lower depth.

2.2. MATERIALS & METHODS

2.2.1. Experimental Design

The evaluation of various cover crop options within a continuous cotton cropping system occurred at the Texas A&M AgriLife Chillicothe Research Station near Chillicothe, TX. The test site is on a Grandfield soil series with a soil type that is described as a fine sandy loam. This location has been conducting a multi-year continuous cotton study testing no-till and conventional tillage as well as various cover crop treatments with no-till practices. Soil samples were collected during the 5th year of cover crop establishment. The cover crops in this study were planted on November 22, 2016 and later terminated on April 20, 2017. The cotton cash crop was planted on May 30, 2017 and was not harvested due to total crop failure. The fallow period for this study was approximately 6 months.

A randomized complete block design with four replicates was used with rainfed cotton in semi-arid environmental conditions. Evaluated treatments included: 1) conventional tillage (CT); 2) no-till (NT); and no-till with the following cover crops 3) winter wheat (W); 4) Austrian winter field pea (AP); 5) crimson clover (CC); 6) hairy vetch (HV); and 7) mixed species cover (MC; Table 2.1 & Table 2.2). All treatments were managed with an initial shred stalker to remove any cotton stalks left after harvest, and cotton debris was scattered in in the plot. The cool-season cover crops were planted with a no-till drill with 25 cm spacing.

The conventional tillage received the initial stalk shredding to disperse any cotton debris, then was followed by tillage with a 4-row offset disc implement to a depth of approximately 10-15 cm (4-6 in). The tillage occurred two different times during the winter. Before planting cotton, the conventional plot was reshaped with a bedder. After the cotton was planted, a field cultivator with 41 cm sweeps was used for cultivation between cotton rows.

All of the treatments received an herbicide application to terminate cover crops or weeds. Glyphosate was applied at 2.3 L/ha and dicamba was applied at 0.6 L/ha. No fertilizer or irrigation was applied to any treatment.

2.2.2. Soil Sampling

Soil sampling times were planned around the herbicide termination of cover crops and cotton planting. The first soil sampling date was April 20, 2017 (0 weeks after the herbicide termination), then May 9, 2017 (3 weeks after the herbicide termination), and May 30, 2017 (6 weeks after the herbicide termination and before cotton planting).

Soil samples (~400 g) were collected with handheld 2.54-cm diameter soil core sampler tools at two depths (0-10 cm and 10-20 cm) from three replicates of all seven treatments. Soil was homogenized by hand and collected in paper bags suitable for oven drying. A subsample (20 g) of each soil sample was immediately stored in separate 50 mL centrifuge tubes and stored in a -80º C freezer for later DNA analysis. A second subsample (20 g) from the topsoil (0-10 cm) only was taken for the PLFA analysis. The PLFA subsamples were shipped immediately to Ward Laboratories, Inc. (Kearney, Nebraska, USA) for PLFA analysis. The remaining soil for each treatment sample was placed in an oven at 60º C for 3 days to reduce water content and subsequently halt microbial activity; the dried soil was stored at room temperature until it was processed for further analysis.

2.2.3. Soil Physiochemical Analysis

After drying soil at 60 ºC for 3 days, soil was passed through a 2-mm sieve to remove large organic debris. An aliquot of oven-dried, sieved soil (25 g) from each treatment sample was used to determine SOC and TN concentration using an Elementar Vario Max elemental analyzer (Elementar, Langenselbold, Germany) by combustion elemental analysis (Mushinski et al., 2017).

Soil inorganic-N was extracted from a 2 g aliquot of oven-dried soil with 20 mL of 1M potassium chloride (KCl). The soil + KCl solution was shaken for 1 hour at 160 oscillations per minute and then filtered with Whatman No. 42 filter paper into 20 mL plastic scintillation vials (Keeney and Nelson, 1982). The filtrate was analyzed using a Skalar SANS^{++} segmented flow analyzer (Skalar, Breda, The Netherlands) for NH_4^+ and total nitrite + nitrate ($NO_2^- + NO_3^-$) concentrations (Keeney and Nelson, 1982; Dorich and Nelson, 1983).

2.2.4. CO_2 Flush Experiment

Another subset of oven-dried soil samples that were passed through the 4.75 mm sieve was then used to determine soil microbial respiration as an indicator of soil health (Franzluebbers, 2016). First, 100 g soil from each sample was weighed and placed into a 100 mL volume-delimited glass Wheaton bottle. Porosity was derived from weight and volume, and then deionized water added to achieve 50% water-filled pore space. This was determined from the pore space percentage equation: [100 – (bulk density/particle density x 100)] and then subsequently divided in half to achieve 50% water-filled pore space. Upon wetting, the volume-delimited glass Wheaton bottles containing 100 g of soil at 50% water filled pore-space was placed into a 1-L glass jar along with a screw cap vial containing 10.00 mL of 1 M NaOH to trap CO_2 and a vial of 10.00 mL of deionized water to maintain humidity. Jars were sealed and incubated for 3 days at 25 ºC (Franzluebbers, 2016).

At the end of the incubation, the vial of NaOH was removed and sealed until titration. In sequence, each vial of NaOH was opened and the following was added to the solution: sufficient 1.5 M $BaCl_2$ solution (3.5 mL) to precipitate bicarbonate as $BaCO_3$, 2 drops of phenolphthalein color indicator, and a small magnetic stir bar. The vial was placed on a magnetic stir plate and 0.5 M HCl was slowly added to solution until the pink color of the phenolphthalein disappeared. A screw cap vial of 1 M NaOH incubated without soil was used as a blank (Franzluebbers, 2016). The quantity of CO_2 evolved from a sample was calculated using the following formula:

CO_2-C (mg kg^{-1} soil) = (mL $_{[blank]}$ – mL $_{[sample]}$) x N x M/S

Where N = normality of acid (mol L $^{-1}$; e.g., 1), M = mass conversion from cmol$_c$ to g C (6000), and S = soil weight (g; e.g., 100 g)

The protocol in Franzluebbers (2016) called for 1 M NaOH to be used as the alkali trap. This was diluted to 0.5 M NaOH in order to increase the sensitivity of the procedure since preliminary analysis had indicated low levels of CO_2 emission from the soils in this study. Finally, the actual method for water addition to achieve 50% water-filled pore space was not described by Franzluebbers (2016). Wade et al. (2018) tested adding the 50% water-filled pore space from the top, from the bottom, and through capillary action from the bottom. Their results suggest that rewetting from above will optimize the sensitivity of the measurement for 24-hour incubation. The difference between adding water from the top or from saturating the bottom at 50% water-filled pore space is likely due to differences in water flow. For instance, wetting from above would fill all pores, followed by the draining of water from the macropores over a short time interval, whereas wetting from below is primarily driven by capillary action, which would result in slower and more unequal distribution of moisture toward the top of the soil column (McCoy et al., 1994). Given potential issues with either approach, the water that was added to the soil in this study was applied in three layers: bottom, middle, and top.

2.2.5. Water-Extractable Organic Carbon and Water-Extractable Organic Nitrogen

Water-extractable organic C (WEOC) and water-extractable organic N (WEON) was determined from 4 g of oven-dried soil with 40 mL of deionized water and shaking for 10 minutes on a mechanical shaker at 160 oscillations per minute. Samples were then centrifuged for 5 minutes at 2095 rcf (3500 rpm), filtered through Whatman 2 V paper (Haney et al., 2012), and analyzed for WEOC and WEON using Elementar TOC Select (Langenselbold, Germany).

2.2.6. Quantitative Polymerase Chain Reaction - Copy Number of *amoA* Genes

DNA was extracted using a Qiagen PowerSoil DNA extraction kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. DNA extracts were checked for quality and concentration following the NanoDrop protocol listed in Mushinski et al. (2017). Numbers of ammonia-oxidizing bacteria (AOB) were determined using quantitative polymerase chain reaction (qPCR) analysis targeting the *amoA* gene with primers amoA-1F (5' - GGG GTT TCT ACT GGT GGT -3'; Rotthauwe et al., 1997) and amoA-2R (5' - CCC CTC KGS AAA GCC TTC TTC -3'; Rotthauwe et al., 1997). Numbers of ammonia-oxidizing archaea (AOA) were determined using qPCR analysis targeting the *amoA* gene with primers Arch amoA-1F (5' -STA ATG GTC TGG CTT AGA CG-3'; Francis et al., 2005) and Arch amoA-2R (5' - GCG GCC ATC CAT CTG TAT GT -3'; Francis et al., 2005). The primers were selected because they have been extensively used for the study of soil ammonia oxidizers, and there is a well-established body of literature using these primers that target the *amoA* gene (Taylor et al., 2013; Banning et al., 2015; Mushinski et al., 2017). For bacterial and archaeal *amoA*, the qPCR was run with the following conditions: 95° C for 5 min; 94° C for 45 s, 56° C for 45 s, and 72° C for 1.5 min (30 cycles; Mushinski et al., 2017). The 25 µL reaction mixture contained 13 µL SYBR$^{®}$ Select Master Mix (Thermo Fisher Scientific, Waltham, MA), 2.5 µL of each primer (concentration 10 mM), 5 µL DNA template, and 2 µL molecular grade water. Each analysis run included a set of standards, negative controls, and replicated samples (n = 3 replicates) on a 96-well plate. Standard curves were developed from synthetic oligonucleotides (Integrated DNA Technologies, Inc., Coralville, IA) representing AOB (*Nitrosomonas europaea*; GenBank #L08050.1) and AOA (*Nitrosopumilus maritimus* isolate SF_AOA_A07; GenBank #HM345608.1). Standards were made from 10-fold dilutions of the fragment of the gene of interest. All qPCR assays were

performed using an Eppendorf Mastercycler® ep realplex thermal cycler (Eppendorf, Hamburg, Germany). Results were then be reported as *amoA* gene copies g^{-1} dry-weight soil (Mushinski et al., 2017). In order to increase the sensitivity of the assay, an internal amplification control (IAC) was used to check for PCR inhibitors (Shanks et al., 2014). Inhibition was determined when the mean IAC threshold cycle (C_t) value (qPCR cycle at which the IAC amplification curve crosses a threshold value) for a spiked sample was three or more C_t greater than the mean C_t value for spiked no template control (Hartman et al., 2005). If inhibition occurred, the soil DNA extraction was diluted (1:10), and the diluted sample was quantified again using qPCR until inhibition did not occur.

2.2.7. Phospholipid Fatty Acid Analysis

PLFA analysis was conducted by Ward Labs, Inc. according to Hamel et al., (2006) with slight modifications. Total soil lipids were extracted in test tubes by shaking 2 g (dry weight equivalent) of frozen soil in 9.5 mL dichloromethane (DCM): methanol (MeOH): citrate buffer (1:2:0.8 v/v) for 1 hour. Then 2.5 mL of DCM and 10 mL of a saturated KCl solution were added to each tube and shaken for 5 min. Tubes were then centrifuged at 1,008 rcf (3,000 rpm) for 10 min. The organic fraction was pipetted into clean vials. Lipid-class separation was conducted in silica gel columns and the vials washed twice with a small amount of DCM using a pipette. The neutral, glyco- and phospholipids fractions were eluted by sequential leaching with approximately 2 mL of DCM, 2 mL of acetone and 2 mL of methanol, respectively. The neutral and glycolipid fraction was discarded and the phospholipids fractions were collected in separate 4 mL vials. These fractions were dried under a flow of N$_2$ at 37 ± 1C in a fume hood. The dried fractions were dissolved in a few mL of MeOH for PLFA and stored at -20 °C. Samples were

analyzed using an Agilent 7890A GC with a 7693 autosampler and a flame ionization detector. The abundance of individual PLFAs was expressed as µg PLFA g^{-1} dry soil (Hamel et al., 2006).

Selected terminal-branched saturated PLFAs (i15:0, a15:0, i16:0, a16:0, i17:0, and a17:0) were used as markers for Gram-positive (Gram$^+$) bacteria (Federle, 1986; Zelles, 1997). Selected monounsaturated and cyclopropyl-saturated PLFAs 16:1ω5, 16:1ω9, 17:1ω9, cy17:0, 18:1ω11, and cy19:0 was used to represent Gram-negative (Gram$^-$) bacteria and the PLFA 14:0, 15:0, and 17:0 for unspecific bacteria (Federle, 1986; Frostegård et al., 1993; Zelles, 1997). The polyenoic, unsaturated PLFA 18:2ω6c was used as an indicator of fungal biomass (Federle, 1986; Frostegård and Bååth, 1996; Huang et al., 2011). The PLFA 16:1ω11 or 20:0 was used to represent arbuscular mycorrhizal fungi (Olsson et al., 1999; Huang et al., 2011). The biomarkers for PLFA 20:3 at 6 and 20:4 at 6 was used as an indicator for protozoa biomass (Cavigelli et al., 1995). The rhizobia PLFA biomarkers contained 16:0, 17:0, 18:0 and 19cycloω9C fatty acids (Jarvis and Tighe, 1994). Total bacteria was calculated as sum of Gram$^+$, Gram$^-$, and unspecific bacteria. The total PLFA biomass was calculated as the sum of all the extracted PLFAs, and reported as total ng PLFA biomass g^{-1}. Individual total ng PLFA biomass g^{-1} from each treatment was used to report which cover crop can support the highest total PLFA biomass.

2.2.8. Cover Crop Herbage Characterization

Cover crop herbage samples were randomly collected from the treatment plots before the cover crop termination. Two 1-m^2 quadrats of cover crop herbage were then clipped at the 5 cm height from each of no-till with cover crop treatments, weighed, and dried at 65°C for dry matter determination. Total C and N content was determined using combustion analysis using an Elementar Vario Max elemental analyzer (Elementar, Langenselbold, Germany).

2.2.9. Statistical Analysis

Treatment differences were evaluated using analysis of variance (ANOVA), followed by the Fisher's least significant differences (LSD) test, and linear regression analysis. Unless otherwise noted, only significant ($p < 0.05$) interactions are discussed. Analyses were conducted with the use of JMP® Pro 13.2.1 (SAS Institute; Cary, NC).

Main effects were cover crop treatments, date, and depth with randomized replicates. To meet the assumptions of normality and homogeneity of variance, *amoA* copy numbers were $\log_{10}$ transformed. Relationships among selected variables were examined by pairwise correlation analysis (Haney et al., 2012; Taylor et al., 2013). Also, taxonomic domain (archaea or bacteria) was an effect that was also tested in the ammonia-oxidation population analysis.

2.3. RESULTS

2.3.1. Cover Crop Herbage Mass and Characterization

Cover crop biomass from the no-till treatments were highest in HV, AP, and MC plots. The biomass from CC (342 kg ha-1) was significantly lower than AP (3,148 kg ha-1), HV (2,950 kg ha-1), and MC (2,491 kg ha-1; Figure 2.2). The biomass from CC was numerically lower than W (1,766 kg ha-1), but it was not statistically different (Figure 2.2).

2.3.2. Soil Chemical Properties: Carbon

2.3.2.1. Combustion Analysis C

A three-way ANOVA did not detect main effect interactions for SOC, so all the replicates from each date and depth were combined (n = 18 replicates) to highlight the significant treatment effects that occurred (Table 2.3). The SOC values for AP (4,619 mg SOC kg^{-1} soil) were significantly higher than NT (3,529 mg SOC kg^{-1} soil) and CT (3,441 mg SOC kg^{-1} soil) by

24% and 26% respectively. The MC (4,263 mg SOC kg^{-1} soil) treatments indicated 19% higher

SOC values compared to CT (Figure 2.3). The remaining cover crop treatments were not

significantly different from NT or CT.

2.3.2.2. Water-Extractable Organic C

A three-way ANOVA did not detect main effect interactions for WEOC, so all replicates from

each sampling date and depth were combined for analysis (n = 18 replicates; Table 2.3). The

cover crop treatments indicated that WEOC values from AP (108 mg WEOC kg^{-1} soil) were

significantly higher than NT (90 mg WEOC kg^{-1} soil) , CC (87 mg WEOC kg^{-1} soil), and CT (85

mg WEOC kg^{-1} soil) by 16%, 19%, and 21% respectively. The WEOC values for W (104 mg

WEOC kg^{-1} soil) and MC (103 mg WEOC kg^{-1} soil) were roughly 17% higher than CC and CT,

but not different from NT. (Figure 2.4). The HV and CC treatments were not significantly

different from NT and CT.

A two-way interaction between treatment and depth occurred (Table 2.3). When grouped

by depth (n = 9 replicates), significant differences were observed in the upper 0-10 cm of soil.

The WEOC values for AP (130 mg WEOC kg^{-1} soil) was significantly higher than NT (99 mg

WEOC kg^{-1} soil), CC (91 mg WEOC kg^{-1} soil), and CT (84 mg WEOC kg^{-1} soil) by 23%, 30%,

and 35% respectively. Also, the WEOC values for W (114 mg WEOC kg^{-1} soil) were

significantly higher than CT by 25% (Figure 2.5A). In the lower 10-20 cm of soil, MC and W

trended the highest but the differences were not statistically significant (Figure 2.5B).

2.3.2.3. CO$_2$ Flush: 3-day Incubation

A three-way ANOVA did not detect main effect interactions for CMIN, but all main effects for

the CMIN values were highly significant ($p < 0.0001$; Table 2.3). All replicates from each

sampling date and depth were combined for analysis (n = 18 replicates) to observe the cover crop treatment effect. The CMIN values from AP (63 mg CMIN kg^{-1} soil) were higher than NT (37 mg CMIN kg^{-1} soil) and CT (33 mg CMIN kg^{-1} soil) by 41% and 48%, respectively. The CMIN values from HV (55 mg CMIN kg^{-1} soil) were 31% greater than NT and 40% greater than CT. MC (46 mg CMIN kg^{-1} soil) indicated 28% higher CMIN compared to CT, but was not statistically different from NT. The remaining cover crop treatments, W and CC, were not statistically different from NT or CT (Figure 2.6).

Also, the CMIN values from the cover crop treatments indicated two-way interactions with both date and depth (Table 2.3). CMIN was analyzed by separate depths by combining cover crop treatments and dates only (n = 9 replicates). In the upper 0-10 cm of soil, AP (82 mg CMIN kg^{-1} soil) indicated significantly higher CMIN values compared to NT (44 mg CMIN kg^{-1} soil) and CT (37 mg CMIN kg^{-1} soil) by 46% and 55% respectively. HV (68 mg CMIN kg^{-1} soil) indicated significantly higher CMIN values compared to NT and CT by 34% and 45% respectively. The remaining cover crop treatments (W, MC, and CC) in the upper 0-10 cm soil range were not significantly different than NT and CT (Figure 2.7A). In the lower 10-20 cm of soil, AP (44 mg CMIN kg^{-1} soil) and HV (42 mg CMIN kg^{-1} soil) were significantly higher than all other treatments and were roughly 30% higher than both CT (29 mg CMIN kg^{-1} soil) and NT (30 mg CMIN kg^{-1} soil; Figure 2.7B).

2.3.3. Soil Chemical Properties: Nitrogen

2.3.3.1. Combustion Analysis: Total N

A three-way ANOVA did not detect main effect interactions for TN, so all replicates from each sampling date and depth were combined for analysis (n = 18 replicates; Table 2.3).

The cover crop treatments indicated that TN values for AP (630 mg TN kg^{-1} soil) and MC (600 mg TN kg^{-1} soil) were both significantly higher than NT (448 mg TN kg^{-1} soil) and CT (451 mg TN kg^{-1} soil) by approximately 25% (Figure 2.8). The remaining cover crop treatments (HV, W, and CC) were not significantly different from NT and CT.

2.3.3.2. KCl Extraction: Total NO$_3^-$

A three-way ANOVA did not detect main effect interactions for NO$_3^-$, so all replicates from each sampling date and depth were combined for analysis (n = 18 replicates; Table 2.3). The cover crop treatments indicated that NO$_3^-$ levels were highest in the AP and HV treatments. The NO$_3^-$ values for AP (9.4 mg NO$_3^-$ kg^{-1} soil) were 36% higher than NT (6.3 mg NO$_3^-$ kg^{-1} soil) and CT (6.4 mg NO$_3^-$ kg^{-1} soil). The NO$_3^-$ values for HV (8.6 mg NO$_3^-$ kg^{-1} soil) were 26% higher than NT and CT (Figure 2.9). The remaining cover crop treatments (MC, W and CC) were not statistically different from NT or CT.

There was nearly a two-way interaction between the date and treatment effect at the $p < 0.05$ statistical threshold (Table 2.3). Significant differences were observed when setting the threshold to $p < 0.10$. After week 3, the NO$_3^-$ values for AP and HV continued to increase into week 6 while the other cover crop treatments began to decrease. At week 6, the NO$_3^-$ values for AP and HV were statistically higher than NT, W and CT ($p < 0.1$; Figure 2.10).

2.3.3.3. KCl Extraction: Total NH$_4^+$

A three-way ANOVA did not detect main effect interactions for NH$_4^+$. Total inorganic N is the sum of NH$_4^+$ and NO$_3^-$, and a majority of that total is comprised of NH$_4^+$ in this system. There were no treatment effects when observing NH$_4^+$ values (Table 2.3). There was a date effect with

the collective NH_4^+ values from all replicates during week 3 (n = 42 replicates) were statistically higher than week 6. Week 0 had the lowest collective NH_4^+ values (Figure 2.11)

2.3.3.5. Water-Extractable Organic N

A three-way ANOVA did not detect main effect interactions for WEON, so all replicates from each sampling date were combined for analysis (n = 18 replicates; Table 2.3). The cover crop treatments indicated that WEON values for AP (27.8 mg WEON kg^{-1} soil) were significantly higher than NT (24.3 mg WEON kg^{-1} soil), CC (24.4 mg WEON kg^{-1} soil) and CT (22.9 mg WEON kg^{-1} soil) by 13%, 14%, and 21%. The WEON values from the HV (26.1 mg WEON kg^{-1} soil) treatment indicated that it was 14% higher compared to CT (Figure 2.12)

A two-way interaction between treatment and depth occurred. When separating the replicates by depth (n = 9 replicates), significant differences can be observed in the upper 0-10 cm of soil. The WEON values for AP (32.2 mg WEON kg^{-1} soil) were significantly higher than W, CC, and NT (25.7 mg WEON kg^{-1} soil) by 25%, and CT (23.2 mg WEON kg^{-1} soil) by 39% (Figure 2.13A). In the lower 10-20 cm of soil, all treatments indicated roughly the same amount of WEON values, and none were statistically different (Figure 2.13B).

2.3.4. Microbial Biomass Estimates Based on Phospholipid Fatty Acid Analysis

The PLFA biomass values were analyzed for two-way ANOVA for treatment and date effects (Table 2.4). No treatment effect or interaction occurred. There was a date effect so all PLFA biomass values from each treatment were combined (n = 21 replicates) and separated by sampling date (Figure 2.14). Significant differences within treatments for any specific PLFA parameter were not observed, but all dates were combined (n = 9 replicates) in order to observe trends from the treatments. The mean values for PLFA biomass trended highest in the AP

treatment for total PLFA, bacteria PLFA, fungi PLFA, rhizobia PLFA, and protozoa PLFA, but

the differences were not significant (Table 2.5). The average total biomass PLFA for all

replicates was highest at week 0, decreased by 35% by week 3 (not significantly different) and

significantly decreased by 58% by week 6 (Figure 2.14A). The average bacteria biomass PLFA

for all replicates during week 0 had significantly decreased 38% by week 3. Then, the average

bacteria biomass PLFA for all replicates during week 3 significantly decreased 58% by week 6

(Figure 2.14B). The average fungi biomass PLFA for all replicates during week 0 had

significantly decreased 48% by week 3. Then, the average fungi biomass PLFA for all replicates

during week 3 significantly decreased 74% by week 6 (Figure 2.14C). The average rhizobia

biomass PLFA for all replicates during week 0 had decreased 47% by week 3, but it was not

significant. However, the average total rhizobia PLFA for all replicates during week 0

significantly decreased 92% by week 6 (Figure 2.14D).

2.3.5. Microbial Ammonia-Oxidizing Population Analysis

A three-way ANOVA did not detect main effect interactions for AOB, so all the

replicates from each date and depth were combined (n = 18 replicates) to highlight the significant

treatment effect that occurred (Table 2.6). The AOB *amoA* gene copy values for AP (5.8 *amoA*

$\log_{10}$ copies g^{-1} dry soil) and HV (5.7 *amoA* $\log_{10}$ copies g^{-1} dry soil) were significantly higher

than MC (5.4 *amoA* $\log_{10}$ copies g^{-1} dry soil) and CC (5.4 *amoA* $\log_{10}$ copies g^{-1} dry soil) by

106%, CT (5.3 *amoA* $\log_{10}$ copies g^{-1} dry soil) by 150%, and W and NT (5.2 *amoA* $\log_{10}$ copies

g^{-1} dry soil) by 230% (Figure 2.15). A depth effect occurred, so all treatment replicates and dates

were combined (n = 63 replicates) in order to observe these differences (Table 2.6). When

analyzing the total *amoA* gene copy abundance for AOB, there was a significant 62% decrease

from the 0-10 cm to 10-20 cm of soil (Figure 2.16A).

All samples were tested for AOA *amoA* gene copy abundance, but there were not any statistical differences among the main effects (date, depth, or treatment) or their interactions (Table 2.6). In contrast to AOB, AOA numbers did not differ between 0-10 and 10-20 cm soil depths (Figure 2.16B).

The AOA *amoA* gene copy numbers appeared to trend higher than AOB *amoA* gene copy numbers across all treatments, depths, and dates. All samples were tested for a taxonomic (Archaea or Bacteria) effect using the *amoA* gene abundance as a proxy to determine if AOA statistically dominated over AOB. Individual cover crop treatments were separated and tested for date, depth, and taxonomic effect. The NT treatment indicated a taxonomic effect, with AOA *amoA* gene copy numbers being statistically higher than AOB amoA gene copy numbers (p = 0.0312). No other treatment showed a significant difference (data not shown).

A three-way ANOVA did not detect main effect interactions for the ratio of AOA:AOB, but there was a depth effect. All treatments and dates were combined (n = 63 replicates) to observe a significant increase in the lower 10-20 cm depth compared to the upper 0-10 cm depth (Figure 2.17; Table 2.6). There was also a depth and date interaction so the AOA:AOB ratio was separated by date and depth (n = 21 replicates). At week 0, the AOA:AOB ratio was approximately 15 in the 10-20 cm of soil compared to 1 in the 0-10 cm of soil, and was statistically different. During week 3, the AOA:AOB ratio was approximately 6 in the 10-20 cm of soil compared to 4 in the 0-10 cm of soil and was statistically different. The AOA:AOB ratio during week 6, was approximately 5 for both depths (Figure 2.18).

2.3.6. Correlation Analysis

The SOC and TN were highly related ($r^2 = 0.64$; p <0.001) as were WEOC and WEON ($r^2 = 0.43$; $p < 0.001$; Figure 2.19A&B; Table 2.7). The C and N values from the combustion analysis and water extraction technique were below the mineralization/immobilization ratio threshold of 20, with and average C:N ratio of 7.27 and 3.75 for combustion analysis and water extraction, respectively. The SOC, TN, WEON values were significantly correlated to CMIN (Figure 2.20A&C; Table 2.7). The total inorganic N values, total NH_4^+, and total NO_3^- from the KCl extractions were not significantly correlated to the CMIN (Figure 2.20B; Table 2.7). The AOB *amoA* gene copy abundance was strongly correlated to SOC, WEOC, WEON, NO_3^-, and CMIN when all dates and depths were combined (Table 2.7). In contrast, the AOA *amoA* gene copy abundance was not significantly correlated to any of the soil chemical parameters tested. Total PLFA, bacteria PLFA, and fungi PLFA were all highly correlated to SOC, WEOC, and CMIN, but not significantly correlated to TN, WEON, or any inorganic N value (Table 2.8). Rhizobia PLFA was not significantly correlated to any soil chemical parameter tested. Protozoa PLFA was highly correlated to soil chemical parameters SOC, WEOC, CMIN, inorganic N, NO_3^-, and NH_4^+ (Table 2.8). Protozoa PLFA was also highly correlated to microbial parameters such as the PLFA biomarkers for bacteria, fungi, and rhizobia (Table 2.8).

2.4. DISCUSSION

Total N was highest in AP and MC treatments. Total WEON was highest in AP and MC treatments when analyzing both depths combined. When separating the treatments by depth, then AP and HV had the highest WEON values in the upper 0-10 cm of soil. The total NO_3^- levels were also highest in AP and HV treatments. A common trend in all these soil chemical parameters tested is the high performance of the AP cover crop treatment, which partially

supports the first hypothesis, which stated that cover crops with legumes will increase inorganic

N in the soil, and show higher densities of nutrients in the bulk soil. This is true for legumes that

performed well, such as AP and HV, but not CC. This was consistent with Doane et al., (2009)

from semi-arid production in California, which reported the use of a legume cover crop was

especially favorable to management of N fertility in a reduced tillage system. Blackshaw et al.,

(2010) from the semi-arid Canadian prairies also reported legumes, including AP, can be

successfully established as an offseason ground cover. Also, Ebelhar et al. (1984) from a no-till

corn production in Kentucky reported that HV produced more dry matter with higher N

percentage which resulted in higher N concentration in corn plants and substantially more

inorganic N in the soil than with other legumes tested. Furthermore, certain legume cover crops,

including HV, can provide a substantial portion of the N to a no-till corn system, thereby

decreasing the amount of N fertilizer needed (Ebelhar et al., 1984). This study tested CC, but the

stands that were established in the semi-arid environment were not good enough to produce

adequate biomass, and this was probably due to late planting dates.

The N input from the legume cover crop treatments would be readily available for the

cotton because NO_3^-, a water-soluble anion that does not bind to the negatively charged sites on

soil colloids and is very mobile in water, can be readily absorbed by the plant and do not need to

undergo any further conversion, as is the case with urea, before plant uptake (Follett, 1995).

During week 6, which is when the cotton was planted, the AP and HV ranked the highest of the

treatments in regard to NO_3^- input. Something else to consider is that NH_4^+ is a cation, and can

be sorbed to the cation exchange capacity (CEC) and incorporated into clay and other complexes

within the soil (Follett, 1995). The NH_4^+ may accumulate in the soil, when nitrification is limited

or completely stopped (Mengel et al., 2001). This can happen due to certain environmental

conditions such as low soil pH conditions that can substantially suppress nitrification, lack of oxygen from waterlogged soils, lack of organic matter to serve as a source of carbon for bacteria, or even dry soils that halt microbial activity (Mengel et al., 2001). This study was able to observe the second hypothesis, which states that NH_4^+ will increase during the cover crop termination and decrease thereafter while NO_3^- increases inversely due to ammonia oxidation. This process was indicated with the rise in NH_4^+ after week 0 to week 3, followed by a decline in NH_4^+ from week 3 to week 6. The decline of NH_4^+ was most likely caused by microbial oxidation, which converts NH_4^+ to NO_3^-, also known as nitrification.

The AP and MC cover crops were the only treatment to indicate greater SOC inputs compared to the fallow treatments, NT and CT. When analyzing the smaller, labile subset of the C pool using WEOC as a proxy, then AP, MC and W indicated greater WEOC compared to CT, and these differences took place mainly in the upper 0-10 cm of soil. The metabolically active component of soil can be measured in its simplest form as emission of CO_2, or CMIN, which corresponds to nutrient availability, moisture, and temperature, and can be quickly quantified (Haney et al., 2008; Wade et al., 2018). When analyzing the CMIN, the AP and HV treatments were statistically higher than the fallow treatments, and this was significant at both depths. This indicates that AP and HV had the highest soil biological activity measured as CMIN, which is a key indicator of soil health (Franzluebbers, 2016). Ghimire et al., (2017) from a semi-arid research site in New Mexico reported that CMIN rates were highest in AP when compared to a fallow control. This supports the third hypothesis, which stated that no-till practices and cover crops will improve soil quality by increasing CMIN. A general decline in CMIN occurred as the weeks progressed, most likely suppressing microbial growth due to the lack of degradable carbon in the semi-arid Southern Great Plains.

The next thing to consider is which N measurement (TN, inorganic N, or WEON) is most significantly correlated to CMIN. The TN, which represents a larger nutrient pool, was significantly correlated to CMIN. When analyzing inorganic N, the plant-available subset of the N pool, the values were not significantly correlated to CMIN. However, the WEON values, which are the essential substrates for microorganisms, were significantly correlated to CMIN. The WEON compounds include easily degradable organic compounds such as amino sugars, proteins, and nucleic acids which would also be detected as CMIN (Marschner and Kalbitz, 2003). This supports the WEON and CMIN correlation because the utilization of organic compounds by soil microorganisms is quantified by the disappearance of dissolved organic matter or by the evolution of CO_2 (Marschner and Kalbitz, 2003).

It is well known that the plant family Fabaceae, commonly referred to as legumes, peas, or beans, foster a beneficial symbiosis through root nodulation and the subsequent bacterial colonization of those nodules by *Rhizobium* spp., which is capable of biological N_2 fixation (Zahran, 1999; Hirsh et al., 2001). This is the technology of the legume cover crop, which naturally promotes soil nutrients and microbial activity. Although CC is a legume, it did not show the same CMIN levels as AP and HV. This could be explained by the low cover crop biomass of CC, which did not establish a prominent stand, and had the lowest biomass production. This indicates that CC may be beneficial in some situations, but not in the cotton cropping systems of the semi-arid Southern Great Plains. The AP and HV plants are known for their winter hardiness (Clark, 2008; Wiering et al., 2018), but they also appear to be tolerant of the characteristic drought and heat of Southern Great Plains by trending higher than the other cover crop treatments.

The W treatment had significantly higher WEOC and CMIN values in the upper 0-10 cm of soil compared to CT. Although all residue and root mass variables were not measured in this experiment, it is widely known that winter wheat can be a plentiful source of straw and stubble, and the fine root system of winter wheat can provide erosion control and improved topsoil tilth (Clark, 2008). The MC crop treatment had higher TN values compared to CT, and higher SOC, CMIN, WEOC, and WEON values compared to both fallow treatments, NT and CT. The single-species legume treatments of AP did increase soil nitrogen reserves more than the MC treatment, thereby increasing the microbial capacity of the soil. This partially supports the fourth hypothesis, which stated that the added benefits of both grasses and legumes in the mix species blend will improve soil health more than the other treatments, but it was the single-species AP that indicated the greatest overall soil health improvements. However, the MC treatment still offers a valid alternative to fallow management.

Further soil chemical analysis showed that SOC and TN were highly related ($r^2 = 0.64$) as were WEOC and WEON ($r^2 = 0.43$). The combustion analysis for SOC and TN were both roughly 10-fold higher than the water extraction and KCl extraction values, as would be expected since WEOC, WEON, and KCl extractions are a subset of the much larger SOC and TN pools. This was similar to Haney et al., (2012), which stated that SOC and TN were highly related ($r^2 = 0.93$), as were WEOC and WEON ($r^2 = 0.84$). Since both substrate availability and SOC:TN have a strong influence on N mineralization rates, it has been shown that WEOC:WEON could be a better method for determining the state of potential N mineralization/immobilization as an alternative to SOC:TN (Booth et al., 2005; Haney et al., 2012). However, our data indicates that SOC:TN and WEOC:WEON were both significantly correlated ($r2 = 0.19$; $p < 0.0001$). This is contrast to Haney et al. (2012), which indicated that

SOC:TN and WEOC:WEON were poorly related and attributed that to the fact that SOC and TN were roughly 40 times larger than WEOC and WEON fractions. This might not be the same for soils from the semi-arid Southern Great Plains that have less than 1% SOC and TN. Haney et al. (2012) tested soils from across the United States with a wide variety of SOC, ranging from 3,630 to 41,310 mg SOC/kg soil. This study noted SOC values that ranged from 2,531 to 6,686 mg SOC/kg soil (Table 2.7). The C and N values from the combustion analysis and water extraction technique were below the mineralization/immobilization ratio threshold of 20, with and average C:N ratio of 7.27 and 3.75 for combustion analysis and water extraction, respectively. This indicates that a vast majority of the soil N would be plant available and not immobilized by soil microbial biomass, so the cotton being planted after these cover crop treatments will undoubtedly benefit from the plant-available N due to the combined surplus of NH_4^+ from low C:N ratios and the input of biologically fixed NH_3 from *Rhizobium* filled legume root nodules. The idea to use the soil C:N ratio to predict variations in N mineralization and immobilization rates among soils is well known, indicating that N can be immobilized if the soil C:N ratio is greater than 20 (Tate, 1995). Soils with a low C:N ratio will have a net N mineralization potential and a surplus of available NH_4^+, derived from deamination of organic carbon sources (Bengtsson et al., 2003).

Haney et al. (2012) suggests that water extractions are more sensitive than combustion analysis, and therefore can be a better measurement of the impact of management impacts. The KCl extraction analysis does provide NO_2^-/NO_3^- and NH_4^+ measurements (and combined together create a total inorganic N value), however KCl extractions can significantly underestimate nitrite (NO_2^-) concentrations (Stevens and Laughlin, 1995) because concentrated salt solutions release exchangeable acidity (Ponette et al., 1996), and can decompose NO_2^- (Nelson and Bremner, 1969). Due to the NO_2^- transformation from unbuffered KCl, more accurate

measurements can be found simply by extracting with deionized water (Haney et al., 2012; Homyak et al., 2015).

This study sought to use the PLFA biomass from the soil microbial community to distinguish which management practices can promote the highest PLFA biomarkers for bacteria, fungi, or rhizobia. (Feng et al, 2003). This would have supported the fifth hypothesis, but no treatment differences among PLFA biomarkers were observed. However, AP trended highest in total PLFA biomarkers, as well as bacteria, fungi, and rhizobia PLFA biomarkers, which is consistent with the high performance of AP in the other parameters mentioned. There was a date effect for all PLFA biomarker parameters tested, which indicated a general decline from week 0 to week 6. This could be due the microbial communities benefiting from the living cover crop and its active rhizosphere, which was indicated by the PLFA biomass values decreasing after the cover crop termination at week 0. Also, another contributing factor could be the semi-arid environment. Soil moisture could have been declining as the weeks progressed after the cover crop termination causing a decline in microbial activity.

Protozoa PLFA was highly correlated to soil chemical parameters SOC, WEOC, CMIN, inorganic N, NO_3^-, and NH_4^+. Protozoa PLFA was also highly correlated to microbial parameters such as the PLFA biomarkers for bacteria, fungi, rhizobia, but not AOA or AOB populations. The presence of protozoa has been shown to stimulate nitrogen fixation, CO_2 evolution, and nitrification (Rønn and Ekelund, 1994). Soil bacteria are kept at relatively lower levels when grazed by predacious protozoa and this prevents them from being limited by density-dependent factors, such as lack of nutrients, crowding, and/or extraction products (Fenchel, 1987). Grazing releases nutrients immobilized in inactive microbial biomass, and this enables the remaining

population to grow faster and maintain higher levels of activity (Fenchel, 1987). Protozoa PLFA were not correlated to ammonia-oxidizing microbial populations, which most likely could be limited by NH_4^+ being sorbed and fixed into the clay complexes within the soil (Follett, 1995), or the semi-arid conditions halting nitrification activity (Mengel et al., 2001). However, rhizobia, saprophytes, and mycorrhizae are stimulated by protozoan grazing, and could be providing those ecosystem services synonymous with soil health (Feng et al, 2003).

AOB populations were highly correlated with CMIN, as well as SOC, WEOC, WEON, and NO_3^- when both depths were combined. In contrast, AOA was not correlated to any soil chemical parameter tested. The results suggest that AOB have more copiotrophic characteristics than AOA (Catão et al. 2016). With the energy gained from ammonia-oxidation, AOB populations consume CO_2 and incorporate the C source into their microbial biomass (Kowalchuk & Stephen, 2001), which could help explain the strong correlation of AOB *amoA* gene copy abundance and CMIN. Due to the fact that AOA was not correlated to any inorganic N value, it might indicate that AOA populations have an oligotrophic lifestyle, which does not rely solely on ammonia-oxidation for an energy source, and other organic substrates might be an alternative C and energy source (Jia and Conrad, 2009; Hatzenpichler et al., 2012). The AOB populations are favored by higher concentrations of NH_4^+, while AOA population abundance is minimally affected (Jian and Conrad, 2009; Ouyang et al., 2017; Mushinski et al., 2017). This further supports that AOA are adapted to a wide range of conditions and may possess a more versatile metabolism than AOB (Leninger et al., 2006). It appears that AOA populations are independent of the soil chemical parameters tested in this study and generally trended higher than AOB populations. Wessén et al. (2010) showed that AOB can be more abundant in fertilized agricultural soils with high nutrient and substrate availability, but this was not seen in the semi-

arid Southern Great Plains. It is likely that our study lacked a certain threshold of NH_4^+ to observe AOB *amoA* gene population dominance over AOA. However, the NT treatment indicated that AOA *amoA* gene copy abundance had a statistical dominance over AOB *amoA* gene copy abundance. This was the only time in this study that the sixth hypothesis was supported, which stated that AOA populations would dominate the ammonia-oxidizing community as proxied by *amoA* gene copy abundance. Leininger et al. (2006) also studied agricultural soils and found *amoA* gene copies from archaea were more abundant than bacteria.

When analyzing the total *amoA* gene copy abundance for AOB, there was a significant 87% decrease from the 0-10 cm to 10-20 cm of soil. The lower total *amoA* gene copy abundance for AOA at the 0-10 cm of soil compared to the 10-20 cm of soil was not statistically different. This supports the seventh hypothesis for this study, which predicted AOA abundance would not be negatively affected by soil depth while AOB abundance would be reduced at lower depth. AOA are well adapted to conditions with low levels of available nutrients and oxygen (Jai and Conrad, 2009; Schleper and Nicol, 2010). This was also observed in the AOA:AOB ratio being higher in the lower 10-20 cm of soil compared to the upper 0-10 cm. Some AOA have been observed to oxidize NH_3 with a metabolic pathway that is different than AOB, and requires less oxygen to perform (Walker et al., 2010). Furthermore, AOA have cell volumes that are 10 to 100 times smaller than those of known AOB, which could allow for deeper colonization of the soil profile and more efficient utilization of smaller microsites within the soil (Hatzenpichler et al., 2012). Our study only analyzed the 0-10 and 10-20 cm of soil, but future research could analyze *amoA* gene copy abundance at even lower depths for AOA and AOB populations to investigate gene dominance at even lower depths. Archaeal *amoA* gene copy numbers being more abundant

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than bacterial *amoA* gene copy numbers across all soil depths and the AOA:AOB increasing with depth is consistent with Mushinski et al. (2017).

2.5. CONCLUSION

In this study, no-till practices with cover crops did in fact improve soil quality, indicated by increased plant available nutrients, microbial populations, and CMIN. The soil C and N nutrient pool was most improved by the AP treatment indicated by increased SOC and TN when compared to fallow treatments, NT and CT. The inorganic N values were most improved by the AP treatment indicated by NO_3^- levels rising until week 6 when the cotton was planted. The labile nutrient fraction that is readily available for soil microbes to utilize was most improved by AP indicated by increased WEOC and WEON values. Total PLFA biomass and the PLFA biomass for bacteria, fungi, and rhizobia trended highest in the AP treatment. The AP and HV treatments proved to be the most successful single-species legume cover crop treatment. The MC treatment was initially thought to combine the benefits of grasses and legumes, but the semi-arid Southern Great Plains did not select for that. The AP and HV treatments were also observed to promote the highest AOB, which likely increased net N mineralization. For all parameters tested, conventional till and no-till never once indicated a significant difference between them at any date or depth, so the addition of cover crops to no-till cotton systems could potentially enhance no-till in regard to soil function. A limitation of this study was that conventional till was not tested with cover crops to adequately compare all treatments, but conventional till without a cover crop acted as a control, especially since this is a common practice in the semi-arid Southern Great Plains. Ideally, the offseason ground cover provided by cover crops promotes greater erosion control and increased soil health. This can minimize the damaging effects of a

drought, which is characteristic of this region being studied. Future research into economic costs

might be beneficial to quantify how much financial investment it would take to adjust

management strategies to adopt cover crops, such as AP, however that is beyond the scope of

this research.

IMPACTS ON SOIL HEALTH RESULTING FROM USE OF DOUBLE CROPS OR COVER

CROPS INSTEAD OF FALLOW MANAGEMENT IN DRYLAND WINTER WHEAT

PRODUCTION IN THE SEMI-ARID TEXAS ROLLING PLAINS

3.1. INTRODUCTION

3.1.1. Semi-arid Winter Wheat Production

For decades, the predominant dryland cropping system in the Southern Great Plains and Rolling Plains of Texas (Figure 2.1) has been winter wheat–summer fallow management in a conventional tillage system (Peterson et al., 1998; Collins et al., 2012). However, this widely accepted practice has caused dramatic SOC losses at many Great Plains sites that utilize wheat-fallow systems with conventional tillage (Haas et al., 1957). Soil cultivation stimulates soil C loss because it accelerates oxidation of soil C by microbial activity, and tillage reduces aggregate size from mechanical disturbance and also exposes new aggregate surfaces to microbial attack, which stimulates further soil oxidation (Peterson et al., 1998). Despite the negative effects on SOC, the wheat-fallow agroecosystem has remained very popular to producers because it stabilizes short-term production, provides short-term sustainability, and farm program regulations have favored it (Peterson et al., 1998). The negative influence on soil health and environmental quality continues to build, even while economic advantages seem to be lessening due to low grain market prices and higher production costs (Peterson et al., 1998).

The Southern Great Plains are primarily made up of monoculture cropping systems with wheat and cotton accounting for more than million hectares. Depending on weather suitability, winter wheat offers flexibility for growth as a cover crop, forage crop, or cash crop for grain,

making it a popular choice to help growers minimize risk (Adhikari et al., 2017). Winter wheat can withstand extreme winter weather conditions, and it provides a good option to control wind erosion during early spring, when the Southern Great Plains region commonly experiences high wind speeds (Adhikari et al., 2017). Between 2016 and 2018, roughly 5 million acres of Texas was dedicated for winter wheat (*Triticum aestivum* L.) production which makes up about 15% of the overall wheat acreage in the United States (USDA, 2018b). The semi-arid Rolling Plains of west Texas is responsible for about 80% of wheat production in Texas (USDA, 2018b).

3.1.2. Cover Crop or Double Crop Alternatives

It is estimated that half of the wheat producers in the Southern Great Plains utilize a fallow period to store soil water for the subsequent wheat phase (Peterson et al., 1998; Blanco-Canqui et al., 2013). Cover crops can be used to replace the fallow period that leaves the soil bare, susceptible to weeds, and exposed to wind and erosion. Farmers will chemically or mechanically terminate the cover crop, and the residue left in the field to decompose over time, and release nutrients for the subsequent crop. The timing of termination can be important because it can significantly impact the persistence of the cover crop to remain on the soil surface as decomposing organic matter. Also, the timing of the termination becomes more critical as the probability of precipitation decreases (Unger et al., 1998).

Another alternative to fallow management is the use of a cover crop to be grown to maturity, then harvested, and sold as a double crop, where the aboveground biomass is harvested for profit rather than left on the soil as decomposing organic matter (Feyereisen et al., 2013). These double crops have historically been used as a pulse crop or catch crop after crop failure. Some wheat producers have reported the potential benefits from double crop systems. Begna et

al. (2017) reported that winter canola (*Brassica napus* L. *biennus*) has the potential to be a dual-purpose crop in the wheat fallow mono-cropping commonly found in the Southern Great Plains region. However, canola would not be a true double crop, but a rotational crop because winter wheat and winter canola have the same growing season. Nielson and Vigil (2018) identified that diversifying their wheat-fallow rotation by adding proso millet (M, *Panicum miliaceum*, L.) as an intensified rotation increased average wheat yields and yield stability. Blanco-Canqui et al. (2013) compared wheat-fallow and continuous wheat under no-till management with cover crops that included winter triticale (×*Triticosecale* Wittm.), winter lentil (*Lens culinaris* Medik.), spring lentil, spring pea (*Pisum sativum* L. ssp.), and spring triticale cover crops. Crops were either grown as cover, harvested for forage (annual forage crop), or harvested for grain and were all managed under no-till. After 5 years, results indicated that spring triticale and spring lentil increased soil aggregate size distribution, while spring lentil reduced soil's susceptibility to wind erosion. Cover crops also increased wet aggregate stability and reduced runoff loss of sediment, total P, and NO_3^- (Blanco-Canqui et al., 2013).

Crop sequences varying in the quantity and quality of residue production can result in changes in SOM and soil microbial biomass. Increasing cropping intensity (or wheat-forage legume rotations) in various wheat-fallow sequences resulted in greater SOM and soil microbial biomass after 30 years (Campbell et al., 1991). Continuous wheat and wheat-pea (*Pisum sativum L.)* sequences had greater SOM and soil microbial biomass than wheat-fallow after 58 years (Collins et al., 1992). These studies were valuable, but were conducted in northern latitudes where minimal decomposition of crop residue during the winter may have contributed to increased levels of SOM under intensive cropping (Franzluebbers et al., 1994). Only a few

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studies have been done comparing SOM and soil microbial biomass as an indicator for soil health in the southern United States (Franzluebbers et al., 1994).

Research is needed by producers in the region to make timely, well-informed decisions to help sustain farm families in the high risk, semi-arid environment. A comprehensive study detailing how double crops or cover crops affect soil health parameters would bring benefit to the producers in the region. Soil health is presented as an integrative property that reflects the capacity of soil to respond to agricultural intervention, so that it continues to support both the agricultural production and the provision of other ecosystem services.

Specific to my study, monoculture winter wheat with conventional tillage and a summer fallow period were tested against a winter wheat-summer cover crop rotation with no-till management. Different offseason cover crop treatments were analyzed for CMIN, PLFA biomass abundance, water-extractable organic nutrients, inorganic N rates, and soil water storage to determine if the cover crop would be viable as a harvested double. Virtually no published data on mixed cover crops exists for semi-arid agricultural production in Texas. My study focused on cover crop mixtures that combine the benefits of legumes and grasses, which can then be utilized to optimize water storage capacity and promote soil health for dryland farmers in the Southern Great Plains region (Faé et al., 2009; Blanco-Canqui et al., 2015).

3.1.3. Hypotheses

- (H1) Cover crops with legumes will increase inorganic N in the soil and show higher densities of nutrients in the soil.

- (H2) Wheat and cover crop management, will improve soil quality by increasing PLFA biomarkers and CMIN when compared to wheat-fallow management.

- (H3) Double crops can provide the same or better soil health benefits, measured by inorganic N rates or CMIN, as compared to cover crops.

3.2. MATERIALS & METHODS

3.2.1. Experimental Design

The evaluation of various cover crop options within a continuous wheat cropping system were conducted on a farm in Wilbarger County, Texas which is representative of the semi-arid Rolling Plains. Texas A&M AgriLife Research has worked closely with the Wichita County Crop Advisory Committee whose own members are progressive no-till farmers, and have been considered pioneers of no-till in the region. This study took place on private land that has been fully committed to no-till since 1999. This location began conducting a multi-year winter wheat rotation in the summer of 2016 by testing summer cover crop treatments and using no-till conditions. The first cover crops were planted on June 12, 2017, and different termination dates were tested. The winter wheat cash crop was planted November 9, 2017 and harvested May 30, 2018. The wheat seed is hard red winter wheat, and the variety is Bentley. A randomized complete block design with ten treatments and four replicates were used to analyze a rainfed, dryland wheat system under semi-arid environmental conditions. Continuous winter wheat with fallow management was tested against two different cover crop management strategies. One subset of mix-species cover crops treatments was chemically terminated and left in the field as decomposing organic matter. Those treatments included in the mix-species treatments were divided in two more categories depending on seeding rates, which were 16.8 kg/ha (Low Mix) and 22.4 kg/ha (High Mix). The Low Mix and High Mix were further divided by termination timing, which were early maturity (55-70 days) and late maturity (75-90 days) during the

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reproductive phase. Another subset of treatments were harvested instead of terminated to serve

as a double crop for additional production income. The final treatment was a wheat-fallow and a

wheat-canola (no cover crop) management as a control. These fallow treatments would last

approximately 6 months. Canola would be considered a rotational crop since it shares the same

growing season as winter wheat. The sampling time of 2017 for this experiment coincided with

the end of the canola phase.

The first treatment analyzed in this winter wheat system was a cool season crop rotation

with 1) winter wheat and fallow management and 2) winter wheat and cool-season canola as a

rotational crop with fallow in between rotations. Winter wheat was planted in fall and harvested

in spring and then canola planted the next fall and harvested in spring. A subset of warm-season

cover crops were grown to provide ground cover and later harvested as a double crop. These

treatments included: 3) wheat-mung bean; 4) wheat-cowpea; and 5) wheat-guar (Table 3.1).

Another subset of warm-season cover crops were grown as a mixture, and would act as ground

cover that would be later terminated before the winter wheat season. These treatments would

become decomposing organic matter during the winter wheat phase. These warm-season mixture

varieties that have differing seeding rates which were 16.8 kg/ha (low mix) and 22.4 kg/ha (high

mix). Each seeding rate also had differing termination dates which were 55-75 days after

planting (DAP; early), and 75-90 DAP (late). The treatments (before and after the reproductive

stage) were 6) Low Mix Early; 7) Low Mix Late; 8) High Mix Early; and 9) High Mix Late

(Table 3.2; Table 3.3). Another treatment included 10) broadleaf mix, which was terminated 75-

90 DAP with a 22.4 kg/ha seeding rate (Table 3.4). All cover crop treatments were planted with a

no-till drill with 19 cm spacing. The final treatment was the wheat-fallow control. Double crops

were harvested with a small plot combine. Cover crop termination were done with a CO_2 backpack sprayer with using a four-nozzle boom.

3.2.2. Soil Sampling

One year after the cover crop trial began in the summer of 2016, soil was sampled at the end of the second year of summer cover crops in November 9, 2017, which was after the late termination of the cover crop and just before the planting of the cash crop, winter wheat. Soil samples (~400 g) were collected with soil cores from two depths (0-10 cm and 10-20 cm) from three replicates of all ten treatments. Soil was collected in paper bags suitable for oven drying. A subsample (20 g) from the topsoil (0-10 cm) was collected, promptly frozen, and shipped immediately to Ward Laboratories, Inc. (Kearney, NE, USA) for PLFA analysis. The remaining soil for each treatment sample was placed in an oven at 60° C for 3 days to reduce water content and subsequently halt microbial activity, and the dried soil was stored at room temperature until the soil health analysis.

3.2.3. Soil Physiochemical Analysis

Soil inorganic-N was extracted from a 2 g aliquot of oven-dried, sieved soil with 20 mL of 1M potassium chloride (KCl). The soil + KCl solution was shaken for 1 hour at 160 oscillations per minute and then filtered with Whatman No. 42 filter paper into 20 mL plastic scintillation vials (Keeney and Nelson, 1982). The filtrate was analyzed using a Skalar SANS^{++} segmented flow analyzer (Skalar, Breda, The Netherlands) for ammonium (NH_4^+) and total nitrite + nitrate ($NO_2^- + NO_3^-$) concentrations (Keeney and Nelson, 1982; Dorich and Nelson, 1983).

3.2.4. CO$_2$ Flush Experiment

Oven dried soil samples that were passed through the 4.75 mm sieve was then used to determine soil microbial respiration as an indicator for soil health. First, 100 g soil were weighed and placed into 100 mL volume-delimited glass Wheaton bottles. Porosity was determined from weight and volume, and then deionized water was added to achieve 50% water-filled pore space. This was determined from the pore space percentage equation: [100 – (bulk density/particle density x 100)] and then subsequently divided in half to achieve 50% water-filled pore space. Upon wetting, the volume-delimited glass Wheaton bottles containing 100 g of soil at 50% water filled pore-space was placed into 1-L canning jar along with a screw cap vial containing 10.00 mL of 1 M NaOH to trap CO$_2$ and a vial of 10.00 mL of deionized water to maintain humidity. Canning jars were sealed and incubated for 3 days at 25° C (Franzluebbers 2016).

At the end of the incubation, the screw-cap vial of NaOH was removed and sealed until titration can begin. In sequence, each vial of NaOH was opened and the following was added to solution: sufficient 1.5 M BaCl$_2$ solution (3.5 mL) to precipitate bicarbonate as BaCO$_3$, 2 drops of phenolphthalein color indicator, and a small magnet stir bar. The vial was placed on a magnetic stir plate and 0.5 M HCl was slowly added to solution until the pink color of the phenolphthalein disappears. A screw cap vial of 1 M NaOH incubated without soil was used as a blank (Franzluebbers 2016). Quantity of CO$_2$ evolved from a sample was calculated by the following formula:

$$\text{CO}_2\text{-C (mg kg}^{-1}\text{ soil)} = (\text{mL }_{[\text{blank}]} - \text{mL }_{[\text{sample}]}) \times N \times M/S$$

Where N = normality of acid (mol L^{-1}; e.g., 1), M = mass conversion from cmol$_c$ to g C (6000), and S = soil weight (g; e.g., 100 g)

3.2.5. Water-Extractable Organic Carbon and Water-Extractable Organic Nitrogen

Water-extractable organic C (WEOC) and water-extractable organic N (WEON) was determined from 4 g of oven-dried soil with 40 mL of deionized water and shaking for 10 minutes on a mechanical shaker. Samples were then centrifuged for 5 minutes at 2095 rcf (3500 rpm), filtered through Whatman 2 V paper (Haney et al., 2012), and analyzed for WEOC and WEON using Elementar TOC Select (Langenselbold, Germany).

3.2.6. Soil Moisture

A neutron moisture meter (Model 503DR, CPN International Inc, Martinez, CA, Serial No. H350607921) was used to measure soil water storage (Hanson, 2009). Aluminum access tubes, about 5-cm diameter and 180 cm long were placed by the plant row in each plot to a depth of 150 cm. The installation was done using a Giddings hydraulic coring machine. Soil water stored in the profile was measured once every two weeks at 20 cm depth increments from 0 to 140 cm.

3.2.7. Phospholipid Fatty Acid Analysis

PLFA analysis was analyzed according to Hamel et al., (2006) with slight modifications. Total soil lipids were extracted in test tubes by shaking 2 g (dry weight equivalent) of frozen soil in 9.5 mL dichloromethane (DCM): methanol (MeOH): citrate buffer (1:2:0.8 v/v) for 1 hour. Then 2.5 mL of DCM and 10 mL of a saturated KCl solution were added to each tube and shaken for 5 min. Tubes were then centrifuged at 1,008 rcf (3,000 rpm) for 10 min. The organic fraction was pipetted into clean vials. Lipid-class separation was conducted in silica gel columns and the vials washed twice with a small amount of DCM using a pipette. The neutral, glyco- and phospholipids fractions were eluted by sequential leaching with approximately 2 mL of DCM, 2

mL of acetone and 2 mL of methanol, respectively. The neutral and glycolipid fraction was discarded and the phospholipids fractions were collected in separate 4 mL vials. These fractions were dried under a flow of N_2 at $37 \pm 1C$ in the fume hood. The dried fractions were dissolved in a few mL of MeOH for PLFA and stored at $-20 \, ^{\circ}C$. Samples were analyzed using an Agilent 7890A GC with a 7693 autosampler and a flame ionization detector. The abundance of individual PLFAs was expressed as μg PLFA g^{-1} dry soil (Hamel et al., 2006).

Selected terminal-branched saturated PLFAs (i15:0, a15:0, i16:0, a16:0, i17:0, and a17:0) was used as marker for Gram-positive ($Gram^+$) bacteria (Federle, 1986; Zelles, 1997). Selected monounsaturated and cyclopropyl-saturated PLFAs 16:1ω5, 16:1ω9, 17:1ω9, cy17:0, 18:1ω11, and cy19:0 was used to represent Gram-negative ($Gram^-$) bacteria and the PLFA 14:0, 15:0, and 17:0 for unspecific bacteria (Federle, 1986; Frostegård et al., 1993; Zelles, 1997). The polyenoic, unsaturated PLFA 18:2ω6c was used as an indicator of fungal biomass (Federle, 1986; Frostegård and Bååth, 1996; Huang et al., 2011). The PLFA 16:1ω11 or 20:0 was used represent arbuscular mycorrhizal fungi (Olsson et al., 1999; Huang et al., 2011). The rhizobia PLFA biomarkers contained 16:0, 17:0, 18:0 and 19cycloω9C fatty acids (Jarvis and Tighe, 1994). Total bacteria was calculated as sum of $Gram^+$, $Gram^-$, and unspecific bacteria. The total PLFA biomass was calculated as the sum of all the extracted PLFAs, and reported as total ng PLFA biomass g^{-1}. Individual total ng PLFA biomass g^{-1} from each treatment was used to report which cover crop can support the highest total PLFA biomass.

3.2.8. Statistical Analysis

Treatment differences were evaluated using analysis of variance (ANOVA), followed by the Fisher's least significant differences (LSD) test, and linear regression analysis. Unless

otherwise noted, only significant ($p < 0.05$) interactions are discussed. Analyses were conducted with the use of JMP® Pro 13.2.1 (SAS Institute; Cary, NC). The main effects were cover crop treatments and depth with randomized replicates.

3.3. RESULTS

3.3.1. Cover Crop Herbage Mass and Characterization

The range of cover crop biomass was between 2,027kg ha^{-1} (Low Mix Late) and 2,755kg ha^{-1} (Low Mix Early), but there were no statistical differences among treatments (Figure 3.1).

3.3.2 Soil Chemical Properties: Carbon

3.3.2.1. Water-Extractable Organic C

A two-way ANOVA did not detect main effect interactions for WEOC, so all replicates from each depth (n = 8 replicates) were combined to observe treatment effect trends (Table 3.5). High Mix Early reported ~95 mg WEOC kg^{-1} soil, which was approximately 30 mg WEOC kg^{-1} soil more than wheat-fallow and wheat-canola, but the differences were not statistically significant (Table 3.6)

3.3.2.2. CO$_2$ Flush: 3-day Incubation

A two-way ANOVA did not detect main effect interactions for WEOC, so all replicates from each depth were combined (n = 8 replicates) to observe treatment effect trends. Harvested cowpea reported 62 mg CO$_2$-C kg^{-1} soil, which was approximately 27 mg CO$_2$-C kg^{-1} soil more than wheat-fallow and wheat-canola, but the differences were not statistically significant (Table 3.6)

There was a depth effect for CMIN, so all treatments were combined (n = 40 replicates) to highlight this observation (Table 3.5). The average CMIN for the 0-10 cm of soil was 71 mg CO_2-C kg^{-1} soil compared to 25 mg CO_2-C kg^{-1} soil in the 10-20 of soil, which is a significant 65% decrease.

3.3.3. Soil Chemical Properties: Nitrogen

3.3.3.1. Water-Extractable Organic N

A two-way ANOVA did not detect main effect interactions for WEON, but there was a treatment and depth effect. To observe the treatment effect, all replicates from each depth (n = 8 replicates) were combined to observe. Harvest guar plots had 26 mg WEON kg^{-1} soil, and was approximately 6 mg WEON kg^{-1} soil higher than legume mix, harvested mung bean, wheat-canola, and harvest cowpea, which was a 25% difference (Table 3.6).

There was a depth effect for WEON, so all treatments were combined (n = 40 replicates) to highlight this observation (Table 3.5). The average WEON for the 0-10 cm of soil was 24.4 mg WEON kg^{-1} soil compared to 20.7 mg WEON kg^{-1} soil in the 10-20 of soil, which was a significant 15% decrease.

3.3.3.2. KCl Extraction: NO_3^-

A two-way ANOVA did not detect main effect interactions for NO_3^-, but there was a treatment and depth effect. All replicates from each depth (n = 8 replicates) were combined to observe this treatment effect. Harvested guar reported 12.2 mg NO_3^- kg^{-1} soil, and was approximately 5 mg NO_3^- kg^{-1} soil higher than wheat-fallow and wheat-canola, which was a 42% difference (Table 3.6). Also, harvested guar and High Mix Late were about 6 mg NO_3^- kg^{-1} soil

higher than harvested cowpea, harvested mung bean, and legume mix, which was roughly a 50% difference.

There was a depth effect for NO_3^-, so all treatments were combined (n = 40 replicates) to highlight this observation (Table 3.5). The average NO_3^- for the 0-10 cm of soil was 9.9 mg NO_3^- kg^{-1} soil compared to 4.8 mg NO_3^- kg^{-1} soil in the 10-20 of soil, which is a significant 51% decrease.

3.3.3.3. KCl Extraction: $NH_4^\pm$

A two-way ANOVA did not detect main effect interactions for NH_4^+, so all replicates from each depth (n = 8 replicates) were combined to observe treatment effect trends (Table 3.5). Harvested cowpea reported 20.5 mg NH_4^+ kg^{-1} soil and the wheat-fallow treatment reported 11.4 mg NH_4^+ kg^{-1} soil, which was numerically the highest and lowest NH_4^+ value, respectively, but they were not statistically different (Table 3.6).

3.3.3.4. KCl Extraction: Total Inorganic N

A two-way ANOVA did not detect main effect interactions for total inorganic N, but there was a treatment and depth effect. To observe the treatment effect, all replicates from each depth (n = 8 replicates) were combined to observe the treatment effect. Harvested guar reported 31.6 mg inorganic N kg^{-1} soil, and was approximately 10 mg inorganic N kg^{-1} soil higher than High Mix Late, harvested mung bean, and wheat-canola, which was approximately a 65% difference (Table 3.6). Also, harvested guar, High Mix Late, and High Mix Early were 10 mg inorganic N kg^{-1} soil higher than wheat-fallow and legume mix, which was also an approximate 65% difference (Table 3.6).

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There was a depth effect for inorganic N, so all treatments were combined (n = 40 replicates) to highlight this observation (Table 3.5). The average inorganic N for the 0-10 cm of soil was 26.5 mg inorganic N kg^{-1} soil compared to 21.3 mg inorganic N kg^{-1} soil in the 10-20 of soil, which was a significant 19% decrease.

3.3.4. Soil Moisture

Stored soil water from 0-140 cm was recorded from August 2016 to December 2017 (Figure 3.2). All treatments followed the same trend, however significant differences among treatments occurred during two sampling dates.

On September 22, 2016, the range of stored soil water was between 222.3 mm H$_2$0 (High Mix Late) and 310 mm H$_2$O (wheat-fallow). Harvested cowpea, Low Mix Late, and High Mix Late were all statistically lower than both wheat-fallow and canola-fallow. Low Mix Early, harvested guar, and legume mix were not statistically different than wheat-fallow or canola/fallow (Figure 3.2).

On October 20, 2017, the range of stored soil water was between 256.2 mm H$_2$0 (harvested guar) and 333.6 mm H$_2$0 (wheat-fallow), and wheat-fallow was significantly greater than harvested cowpea, wheat-canola, Low Mix, Late, High Mix Late, and harvested guar. Also, Low Mix Early (315 mm H$_2$O) was significantly greater than High Mix Late, and harvested guar. High Mix Early, legume mix, harvested mung bean, and Low Mix Early were not statistically different from the wheat-fallow treatment (Figure 3.2).

The winter wheat cash crop was planted 20 days later on November 9, 2017, and there were no statistical differences among treatments regarding stored soil moisture at this date.

3.3.5. Microbial Biomass Estimates Based on Phospholipid Fatty Acid Analysis

In the upper 10 cm of soil, the range of total biomass PLFA was between 1,603 (wheat-canola) and 2,747 ng total biomass PLFA g^{-1} soil (High Mix Late), but there were no statistical differences among treatments (Table 3.7). In the upper 10 cm of soil, the range of total bacteria biomass PLFA was between 796 (wheat-canola) and 1,195 ng bacteria biomass PLFA g-1 soil (High Mix Late), but there were no statistical differences among treatments (Table 3.7). In the upper 10 cm of soil, the range of total fungi biomass PLFA was between 57.33 ng fungi biomass PLFA g-1 soil (harvested cowpea) and 220.69 ng fungi biomass PLFA g-1 soil (legume mix), but there were no statistical differences among treatments (Table 3.7). In the upper 10 cm of soil, the range of total rhizobia biomass PLFA was between 37 ng rhizobia biomass PLFA g^{-1} soil (wheat-canola) and 0 ng rhizobia biomass PLFA g^{-1} soil (harvested cowpea and High Mix Late), but there were no statistical differences among treatments (Table 3.7).

3.4. DISCUSSION

The cover crop treatments did have an impact on the soil C and N pools. The double crop, harvested guar, reported the numerically highest WEON values, and the highest NO_3^- and total inorganic N values when compared to wheat-fallow. CMIN and WEOC did not statistically increase or decrease for any treatment when compared to fallow, which implies that CMIN and WEOC were not negatively impacted from changing management practices to fallow alternatives. This partially supports the first hypothesis, which states that cover crop alternatives to fallow management will improve soil quality by increasing C and N values when compared to wheat-fallow management.

The PLFA analysis was not able to support the second hypothesis, which states that cover crop alternatives to fallow management will improve soil quality by increasing microbial populations as measured by PLFA biomarkers. This does however suggest that these cover crop alternatives have statistically similar PLFA biomass results compared to wheat-fallow and wheat-canola.

Utilizing cover crops instead of fallow management has unavoidable consequences regarding stored soil water that can positively, neutrally, or negatively affect the soil water supply (Unger and Vigil, 1998). A winter wheat-fallow system stores soil water by remaining dormant in the field during the summer months while the next season of winter wheat waits to use that stored water due to inactivity. Nielson and Vigil (2005) quantified the effect of varying legume termination dates on available soil water content at wheat planting following an 18 month fallow period. Different legume treatments in place of fallow in a winter wheat-fallow system were analyzed, and reported that wheat yields were significantly reduced by the use of legume cover crops compared to conventional fallow management, regardless of legume type. Nielson and Vigil (2005) concluded that the cost in water use by legumes and subsequent decrease in wheat yield may be too great to justify use legumes as fallow cover crops in wheat-fallow systems in semi-arid environments. This fallow period in this winter wheat study was only 6 months, but during a year-long soil moisture storage analysis at Lalk farm, the wheat-fallow treatment indicated significantly greater amounts of stored soil water in the 0-140 cm of soil than other treatments at two different dates. October 20, 2017 was one such date where the stored soil water from the wheat-fallow treatment was significantly greater than cover crop alternatives. However, the winter wheat cash crop was planted 20 days later on November 9, 2017, and there was no statistical difference among treatments regarding stored soil moisture at

this date. These cover crops were likely influencing soil water relationships by decreasing

evaporation due to the mulch formed, increasing infiltration of rainfall, using stored water by

transpiration, and/or changing the soil water use pattern of the primary cash crop (Smith et al.

1987). The use of cover crops not only increased nutrient availability, but their unique root

structures prepared the soil to capture water more efficiently during the sporadic precipitation

events of the Southern Great Plains, thereby increasing the water storage capacity of the soil

(Wright et al., 2007; Clark et al., 2009; Blanco-Canqui et al., 2013). This can allow farmers in

the semi-arid Southern Great Plains to know that although cover crops may use some stored soil

water during the fallow season, but they are also recharging the soil moisture, which was

indicated by the soil moisture data from the cover crop treatments being statistically similar to

wheat-fallow and wheat-canola at the time of planting the winter wheat cash crop. This soil

moisture data and the previous C and N values reported above supports our third hypothesis

which states that double crops can provide the same or better soil health benefits, measured by

inorganic N rates, CMIN, and stored soil moisture as compared to cover crops and/or fallow

management. One concern for these double crops is that plant biomass would be harvested and

removed from the field, which could suggest that soil C and N values would have decreased due

to the organic matter removal. However, harvest guar reported the highest N values and all the C

values were similar to the fallow treatments. The inorganic N reported from the harvest guar

treatments provides plant-available N to the cash crop, and the WEON provides soluble N for the

microbial communities that live in soil, which are known to provide improved ecosystem

services (Blanco-Canqui et al., 2015). From an initial analysis of the first year, intensified

cropping does not seem to have a negative impact on soil moisture, CMIN, or WEOC.

3.5. CONCLUSION

Different offseason cover crop treatments were analyzed for CMIN, PLFA biomass abundance, inorganic N, and stored soil moisture values to determine if terminated cover crops would be as viable as a harvested double crop. Harvested cowpea trended the highest CMIN and NH_4^+ values in the soil, but was not significant. Harvested guar reported the highest WEON, NO_3^- and inorganic N values in the soil. Wheat-fallow treatment indicated the lowest total inorganic N and NH_4^+ values. Total biomass PLFA was evenly distributed and did not indicate a single significant difference among treatments. There were no statistical differences among the cover crop biomass that was collected. This study offers alternatives to the wheat-fallow management system by exploring intensified cropping systems, but remains in the early stages of development. One year in to the study may not be enough time to report significant differences among treatments regarding stored soil moisture, CMIN, or WEOC in the soil. Investing and effectively using cover crops can boost soil nutrients and microbial activity while also increasing water infiltration capacity due to a more diverse soil structure, but only the N pool in the soil had been improved by double crop harvested guar when compared to wheat-fallow. This might indicate a promising alternative for producers to consider when exploring more markets to compete in by using harvested guar as a double crop. This improvement of soil N parameters did not come at a soil water storage cost that would negatively affect the wheat cash crop. The stubble and root structures remaining after above-ground biomass from harvested guar can ideally help capture an increased amount of water from the sporadic rain events of the Southern Great Plains, while simultaneously increasing important soil nutrients. This double crop alternative can hopefully help farmers combat drought conditions in the region, which most assuredly will save them money by avoiding total crop failure due to desiccation.

CHAPTER IV

CONCLUSION

The effects of no-till management with cover crops as an alternative to fallow on soil health were evaluated in the semi-arid Southern Great Plains. Soil quality was improved by increased plant available nutrients, microbial populations, and CMIN. In the cotton study, AP and HV proved to be the most successful single-species legume cover crop treatments after 5 years into the trial. The AP and MC cover crop treatment showed the greatest SOC input in the 0-20 cm of soil when compared to CT, but they were not significantly higher than NT. However, when analyzing WEOC, which is the smaller, labile subset of the C pool, then AP and MC indicated a significant increase of WEOC compared to the other treatments. AP, HV, and MC also had the highest cover crop biomass production. Perhaps more importantly is the greater NO_3^- values from AP and HV at week 6 when compared to NT and CT. Throughout all of the experiments implemented, CT and NT never once indicated a significant difference in any of the measured parameters between them at any date or depth, so the addition of cover crops to no-till cotton systems could potentially enhance no-till in regard to soil function. Additional research in cover crop composition and termination timing is essential to maximizing these benefits. One concern with cover crops in West Texas is that cover crops need to be terminated early (March or early April) in order to ensure that N is not immobilized and result in N deficiency for cash crop. This study terminated the cover crop in late April and soil samples were taken until the planting of the cotton crop in late May. The C and N values from the combustion analysis and water extraction technique were below the mineralization/immobilization ratio threshold of 20, with and average C:N ratio of 7.27 and 3.75 for combustion analysis and water extraction,

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respectively. This indicates that a vast majority of the soil N would be plant available and not immobilized by soil microbial biomass. The cotton being planted after these cover crop treatments will likely benefit from the plant-available N due to the combined surplus of NH_4^+ from low C:N ratios and the input of biologically fixed NH_3 from *Rhizobium* filled legume root nodules.

In the winter wheat study, different offseason cover crop treatments were analyzed for CMIN, PLFA biomass abundance, and inorganic N rates to determine if the cover crop would be viable as a harvested double crop or terminated. The double crop, harvested guar, indicated the numerically highest WEON values, and reported significantly greater NO_3^- and inorganic N values. Soil moisture used by harvested guar did appear to have a negative effect because the soil water recharge at the time of planting the winter wheat was similar to wheat-fallow treatment. This study had only taken place for one year before the first set of soil sampling. Ideally, this study would continue to be tested to determine when the treatments effects start making significant changes to soil chemical properties and soil water storage.

This research helps to provide producers in the semi-arid Southern Great Plains future options to manage against water scarcity in an unpredictable climate, which is the main reason farmers do not readily adopt no-till with cover crop management. While some cover crops do use some stored soil moisture they also enhance soil water recharge, promote nutrient cycling, and increase the soluble fraction of the soil nutrients, which makes the soil microbial community more resilient and more apt to provide beneficial ecosystem services. This increased soil moisture may be essential to sustaining yields and maintain healthy soil communities during periods of drought, but still needs to be further evaluated in dryland production systems.

Finally, this study helped producers in the semi-arid production region of the Southern Great Plains understand soil health management systems, but more research is necessary for successful adoption throughout the region. By eliminating soil moisture as the limiting factor when using cover crops, and educating producers about the added benefits from these selected cover crops, then it is possible to see an adoption trend of these soil health promoting practices. Further investigations into pest mitigation, termination timing, water usage, and nutrient management would help build upon our results. With greater understanding and improvements in these areas, adoption of alternatives to fallow treatment may increase in a region susceptible to drought conditions and vitally important to agricultural production.

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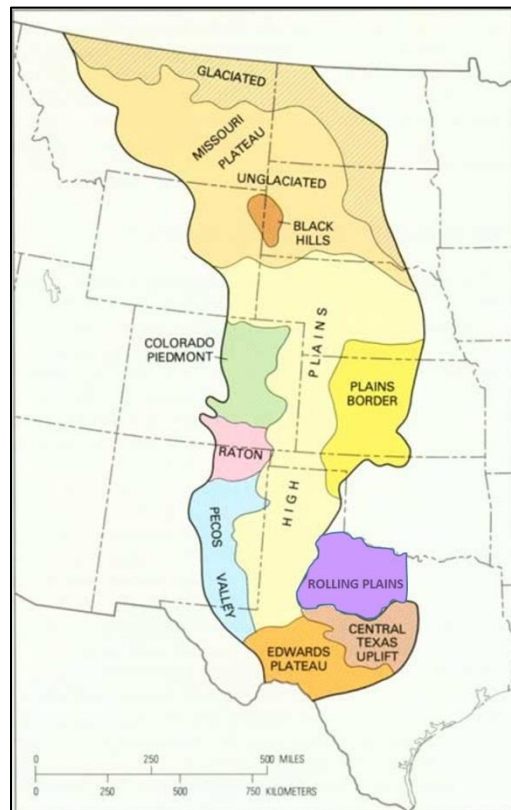
Figure 2. 1: The Great Plains region and its sections. Adapted from U.S. Geologic Survey Bulletin 1493 (Trimble, 1980)
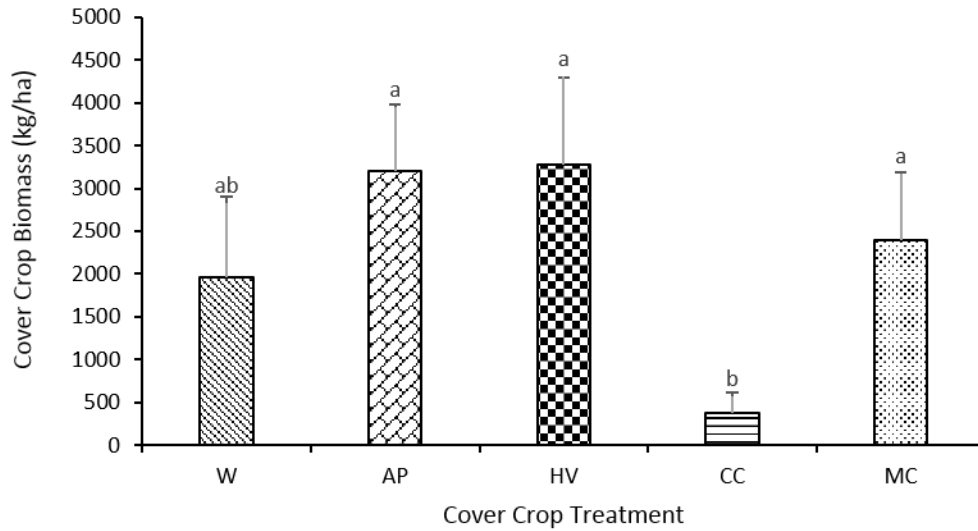
Figure 2. 2: Offseason cotton winter cover crop biomass from 2017 at Chillicothe Research Station as affected by treatments. Statistical significance denoted by different letters (p < 0.05). Error bars represent the standard deviation. W = winter wheat; AP = Austrian winter field pea; HV = hairy vetch; CC = crimson clover; MC = mixed species cover.
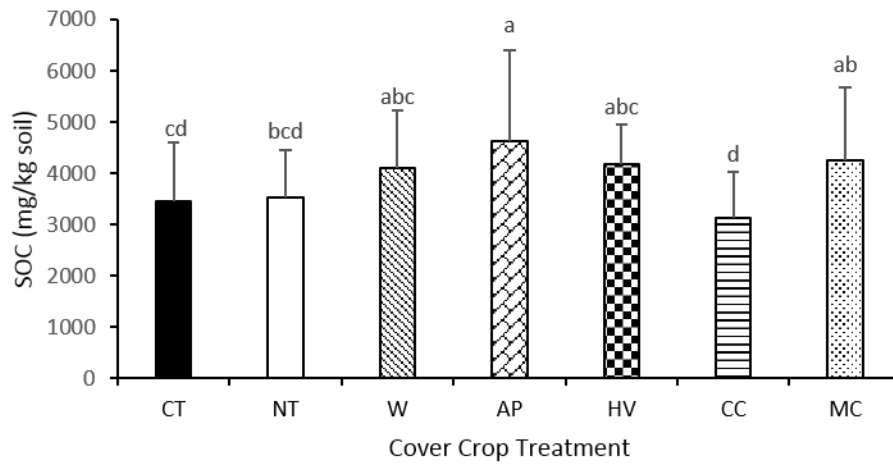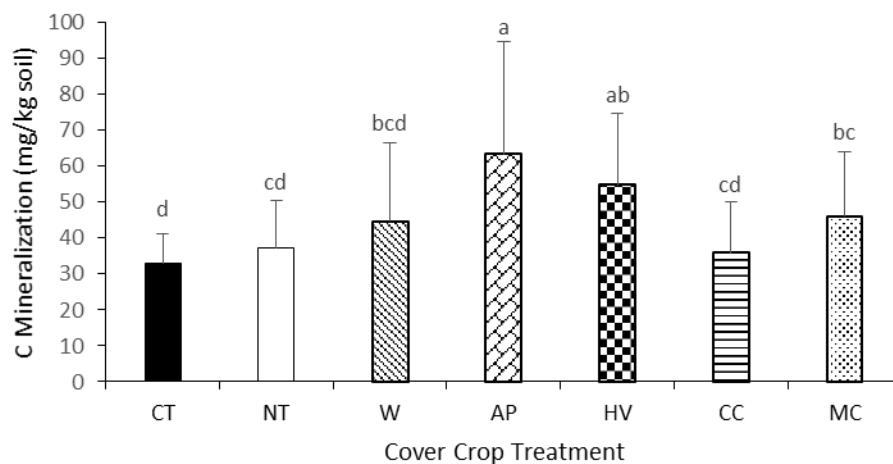
Figure 2. 3: SOC as affected by tillage and cover crop treatments from Chillicothe Research Station. Samples represent all dates and depths combined (n = 18). Statistical significance within treatment denoted by different letters (p < 0.05). Error bars represent the standard deviation. CT = conventional till; NT = no-till; W = winter wheat; AP = Austrian winter field pea; HV = hairy vetch; CC = crimson clover; MC = mixed species cover.
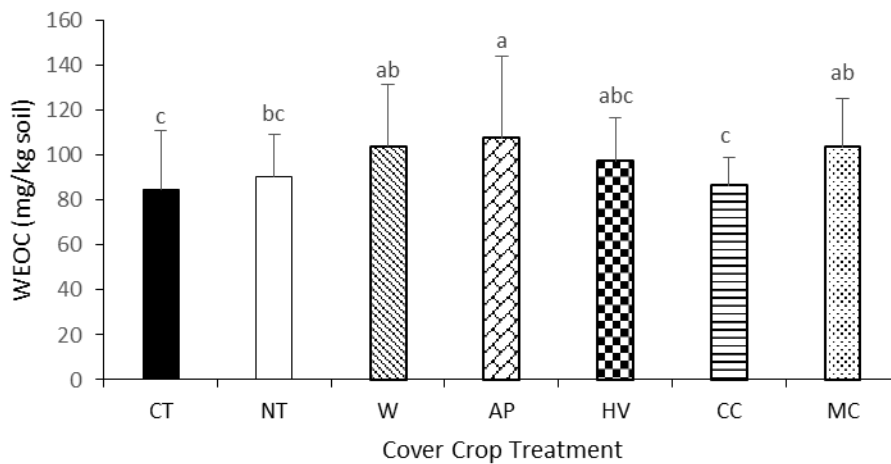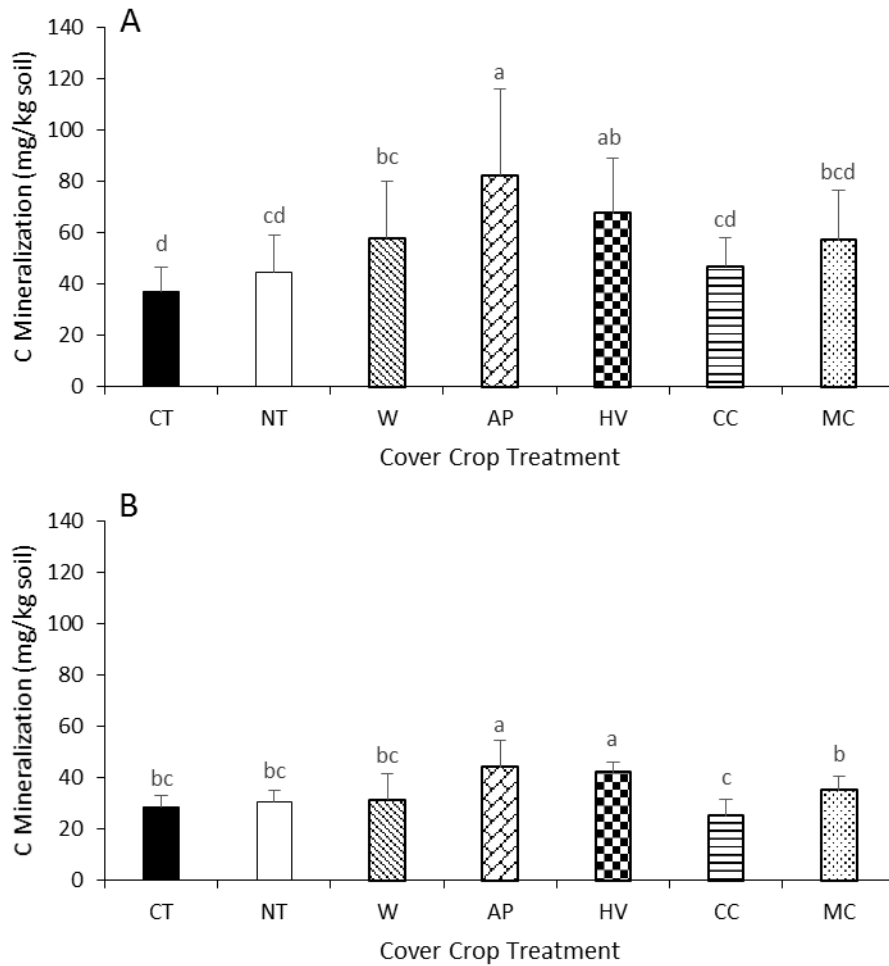
Figure 2. 4: Water extractable organic C as affected by tillage and cover crop treatments from Chillicothe Research Station. Samples represent all dates and depths combined (n = 18). Statistical significance within each treatment denoted by different letters (p < 0.05). Error bars represent the standard deviation. CT = conventional till; NT = no-till; W = winter wheat; AP = Austrian winter field pea; HV = hairy vetch; CC = crimson clover; MC = mixed species cover.
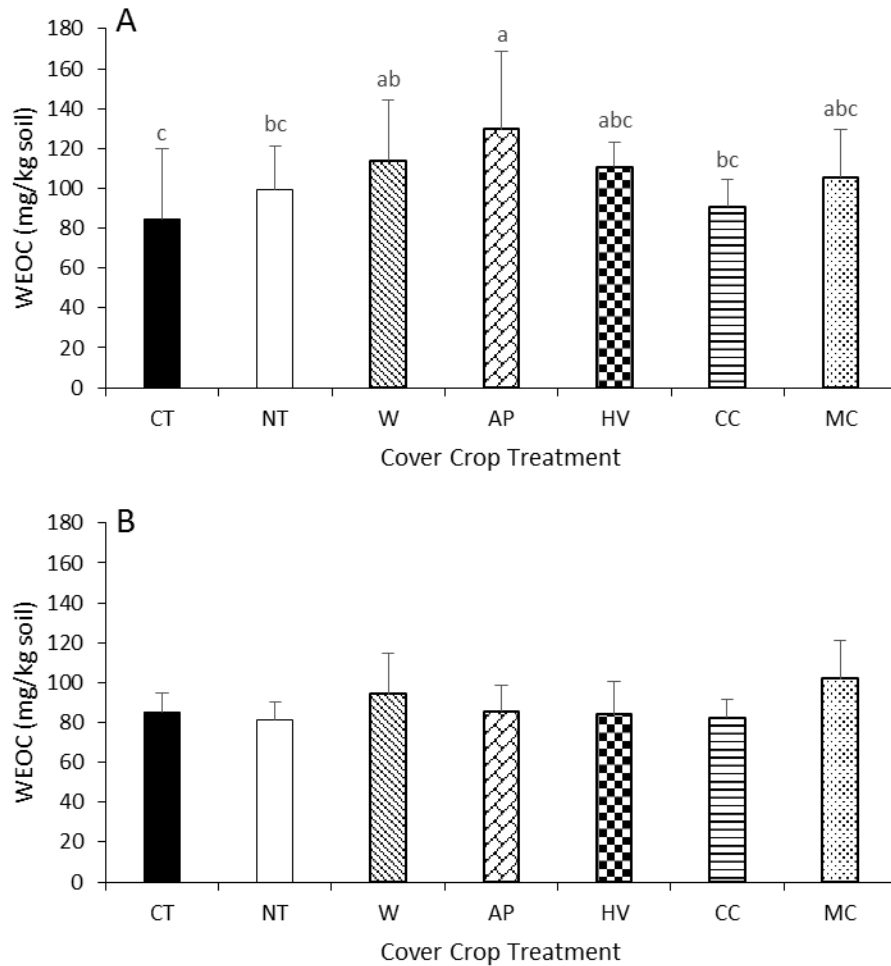
Figure 2. 5: Water-extractable organic carbon separated by depth as affected by tillage and cover crop treatment from Chillicothe Research Station. Samples represent all dates combined (n=9) and split by A) 0-10 cm depth; B) 10-20 cm depth. Statistical significance within each treatment denoted by different letters ($p < 0.05$). Error bars represent the standard deviation. CT = conventional till; NT = no-till; W = winter wheat; AP = Austrian winter field pea; HV = hairy vetch; CC = crimson clover; MC = mixed species cover.
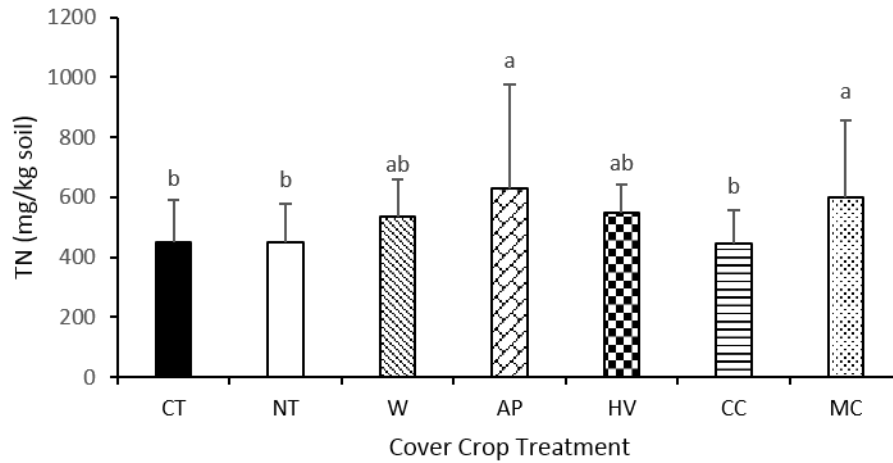
Figure 2. 6: CMIN as affected by tillage and cover crop treatments from Chillicothe Research Station. Samples represent all dates and depths combined (n = 18). Statistical significance within each treatment denoted by different letters (p < 0.05). Error bars represent the standard deviation. CT = conventional till; NT = no-till; W = winter wheat; AP = Austrian winter field pea; HV = hairy vetch; CC = crimson clover; MC = mixed species cover.
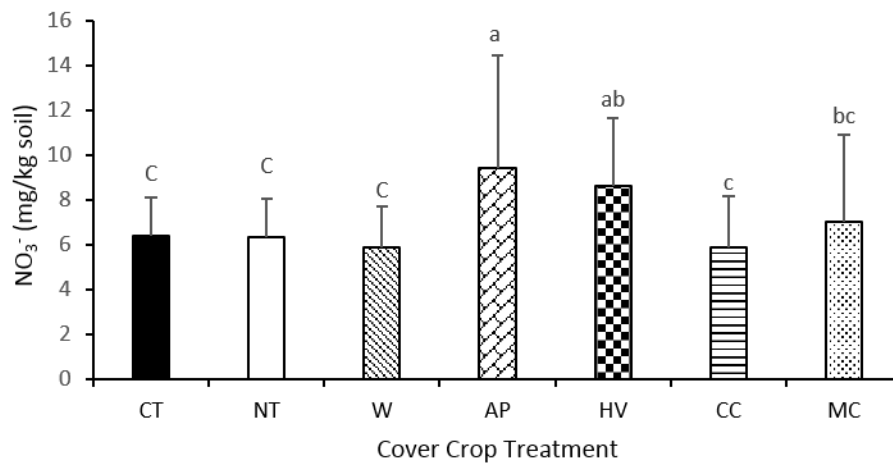
Figure 2. 7: CMIN as affected by tillage and cover crop treatment statistically analyzed over time and seperated by individual treatments from Chillicothe Research Station. Samples represent all dates combined (n=9) and split by A) 0-10 cm depth; B) 10-20 cm depth. Statistical significance within each treatment denoted by different letters (p < 0.05). Error bars represent the standard deviation. CT = conventional till; NT = no-till; W = winter wheat; AP = Austrian winter field pea; HV = hairy vetch; CC = crimson clover; MC = mixed species cover.
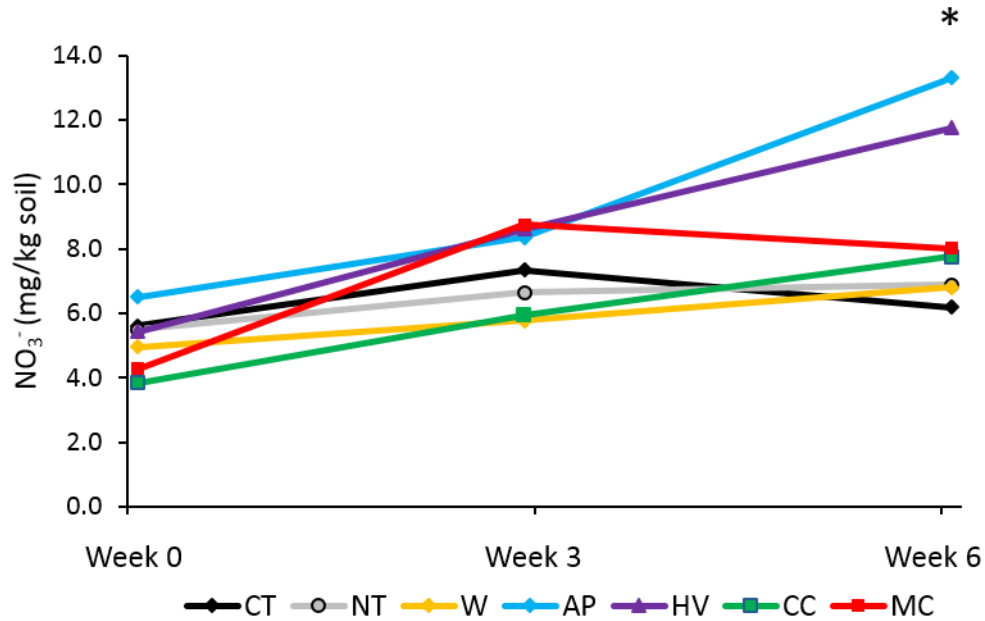
Figure 2. 8: Total N as affected by tillage and cover crop treatments from Chillicothe Research Station. Samples represent all dates and depths combined (n = 18). Statistical significance within each treatment denoted by different letters (p < 0.05). Error bars represent the standard deviation. CT = conventional till; NT = no-till; W = winter wheat; AP = Austrian winter field pea; HV = hairy vetch; CC = crimson clover; MC = mixed species cover.

Figure 2. 9: Total NO$_3^-$ as affected by tillage and cover crop treatment from Chillicothe Research Station. Samples represent all dates and depths combined (n = 18). Statistical significance within each treatment denoted by different letters (p < 0.05). Error bars represent the standard deviation. CT = conventional till; NT = no-till; W = winter wheat; AP = Austrian winter field pea; HV = hairy vetch; CC = crimson clover; MC = mixed species cover.
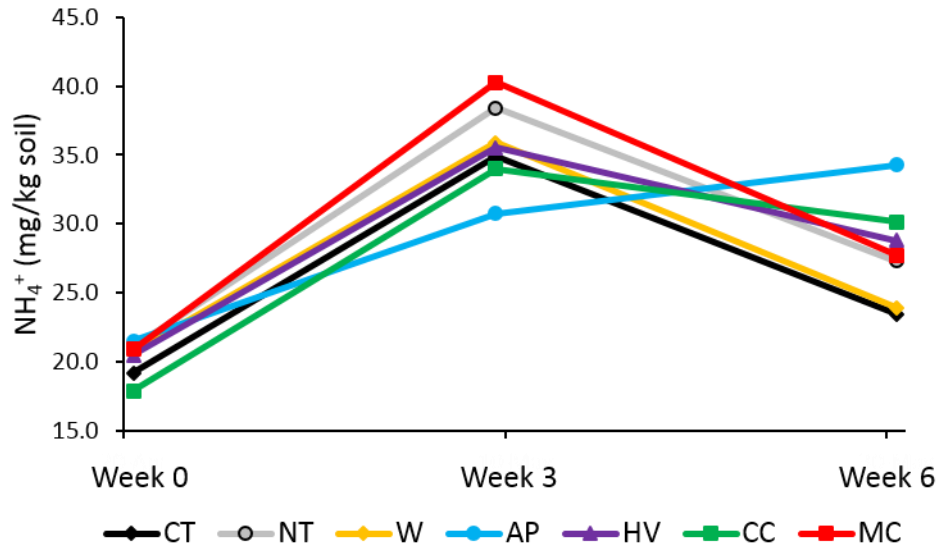
Figure 2. 10: Total NO₃⁻ as affected by tillage and cover crop treatments displayed across sampling time from Chillicothe Research Station. Samples represent both depths combined and separated by date (n = 14). (*) significance at $p < 0.10$. CT = conventional till; NT = no-till; W = winter wheat; AP = Austrian winter field pea; HV = hairy vetch; CC = crimson clover; MC = mixed species cover; Week 0 = April 20th, 2017; Week 3 = May 9th, 2017; Week 6 = May 30th, 2017.

Figure 2. 11: Total NH$_4^+$ as affected by tillage and cover crop treatments displayed across sampling time from Chillicothe Research Station. Samples represent both depths combined and separated by date (n = 14). CT = conventional till; NT = no-till; W = winter wheat; AP = Austrian winter field pea; HV = hairy vetch; CC = crimson clover; MC = mixed species cover; Week 0 = April 20th, 2017; Week 3 = May 9th, 2017; Week 6 = May 30th, 2017.
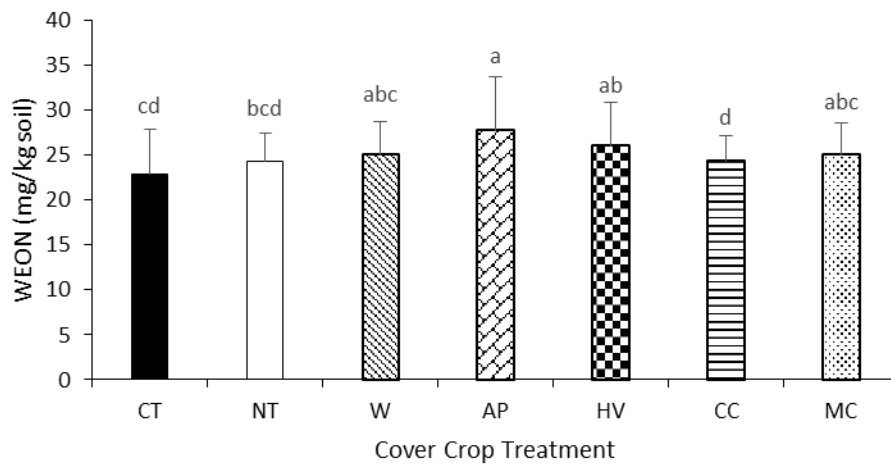
Figure 2. 12: Water-extractable organic N as affected by tillage and cover crop treatments from Chillicothe Research Station. Samples represent all dates and depths combined (n = 18). Statistical significance within each treatment denoted by different letters (p < 0.05). Error bars represent the standard deviation. CT = conventional till; NT = no-till; W = winter wheat; AP = Austrian winter field pea; HV = hairy vetch; CC = crimson clover; MC = mixed species cover.
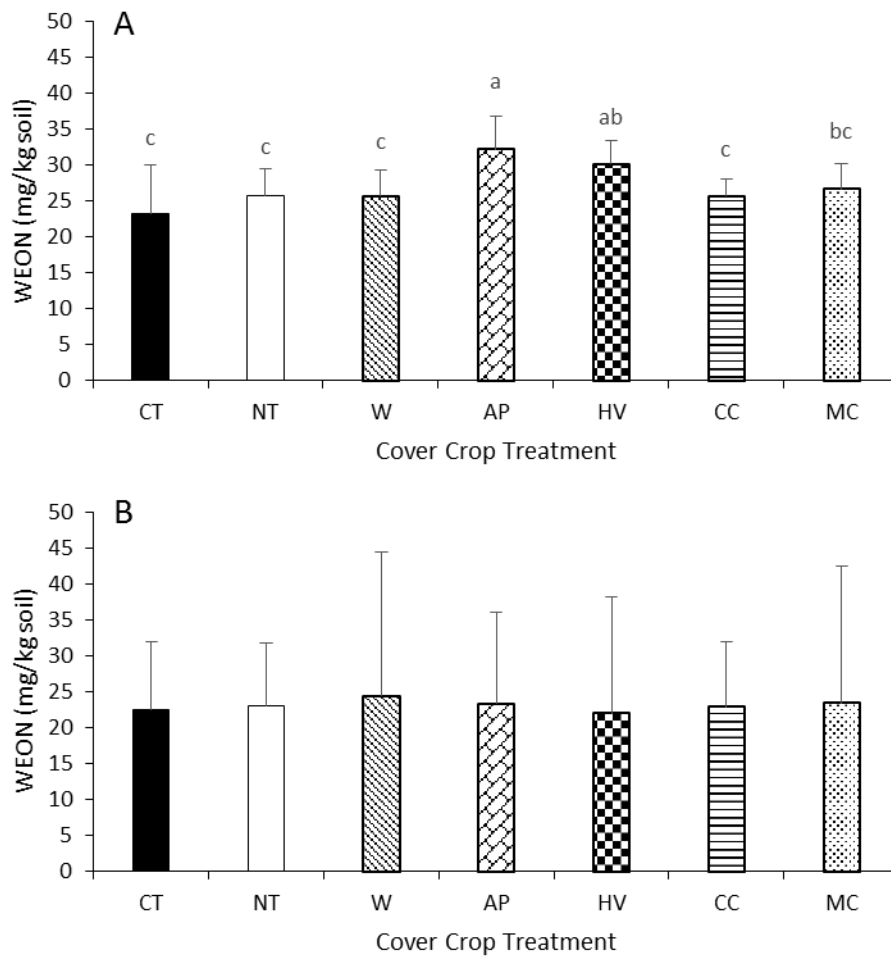
Figure 2. 13: Water-extractable organic nitrogen separated by depth as affected by tillage and cover crop treatment from Chillicothe Research Station. Samples represent all dates combined (n=9) and split by A) 0-10 cm depth; B) 10-20 cm depth. Statistical significance within each treatment denoted by different letters (p < 0.05). Error bars represent the standard deviation. CT = conventional till; NT = no-till; W = winter wheat; AP = Austrian winter field pea; HV = hairy vetch; CC = crimson clover; MC = mixed species cover.
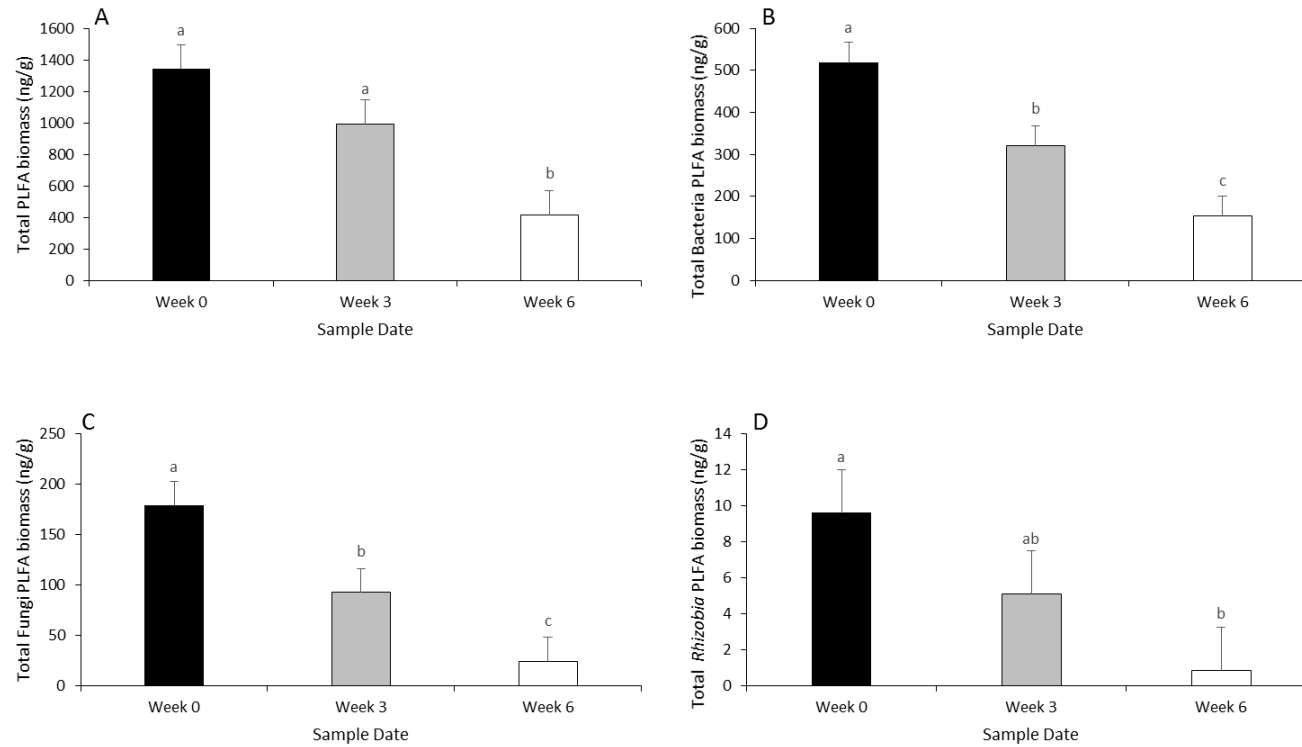
Figure 2. 14: PLFA biomass from 0-10 cm with treatments combined and separated by sampling date. Graphs represent A) total PLFA biomass, B) total bacteria PLFA biomass, C) total fungi PLFA biomass, and D) total rhizobia PLFA biomass. Statistical significance within each PLFA parameter denoted by different letters (p < 0.05). Error bars represent standard error. Week 0 = April 20th, 2017; Week 3 = May 9th, 2017; Week 6 = May 30th, 2017.
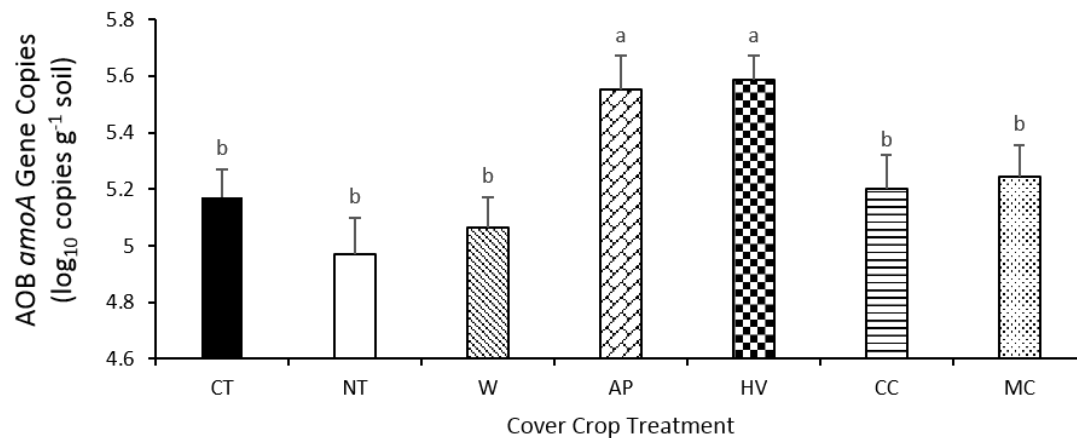
Figure 2. 15: Total Ammonia-Oxidizing Bacteria *amoA* gene copies as affected by tillage and cover crop treatment from Chillicothe Research Station. Samples represent all dates and depths combined (n=18). Statistical significance within each treatment denoted by different letters ($p < 0.05$). Error bars represent standard error. CT = conventional till; NT = no-till; W = winter wheat; AP = Austrian winter field pea; HV = hairy vetch; CC = crimson clover; MC = mixed species cover.
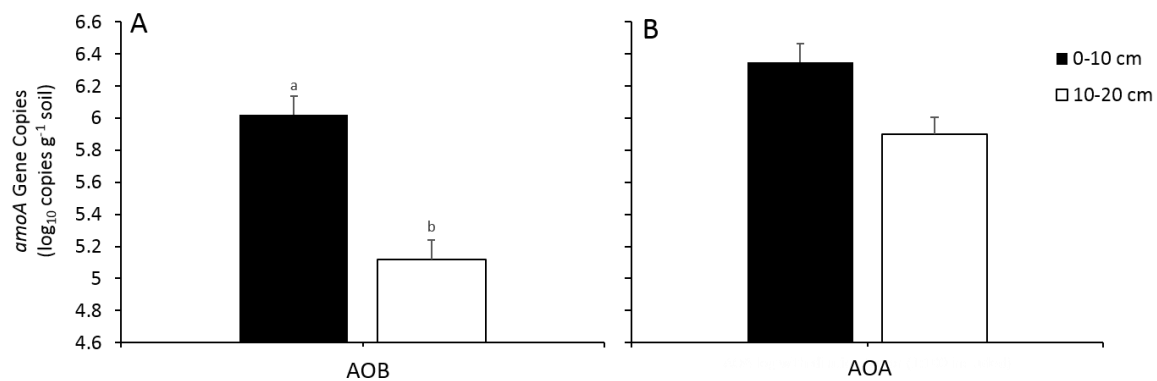
Figure 2. 16: Total *amoA* gene copies for AOB and AOA as affected by depth from Chillicothe Research Station. Samples represent all treatments and dates combined (n=63) from A) ammonia-oxidizing bacteria and B) ammonia-oxidizing archaea. Statistical significance within each treatment denoted by different letters (p < 0.05). Black bars represent 0-10 cm of soil; White bars represent 10-20 cm of soil; Error bars represent standard error.
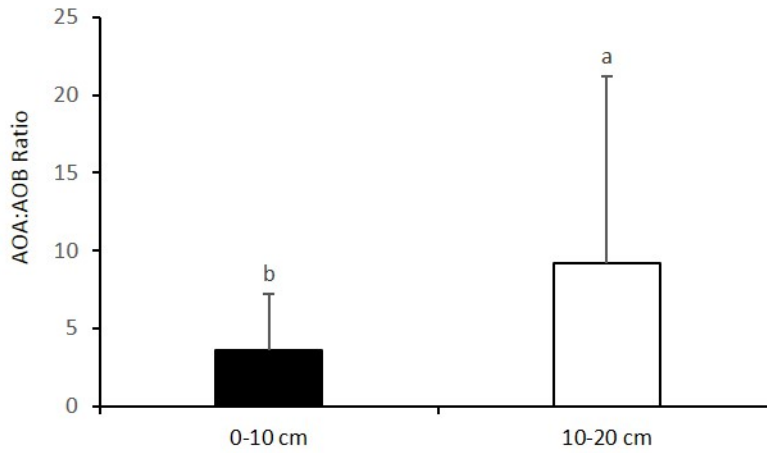
Figure 2. 17: The AOA:AOB ratio vs. depth. Samples represent all treatments and dates combined (n=63). Statistical significance within each treatment denoted by different letters (p < 0.05) and indicates a change in AOA population compared to AOB population. Black bars represent 0-10 cm of soil; White bars represent 10-20 cm of soil; Error bars represent standard error.
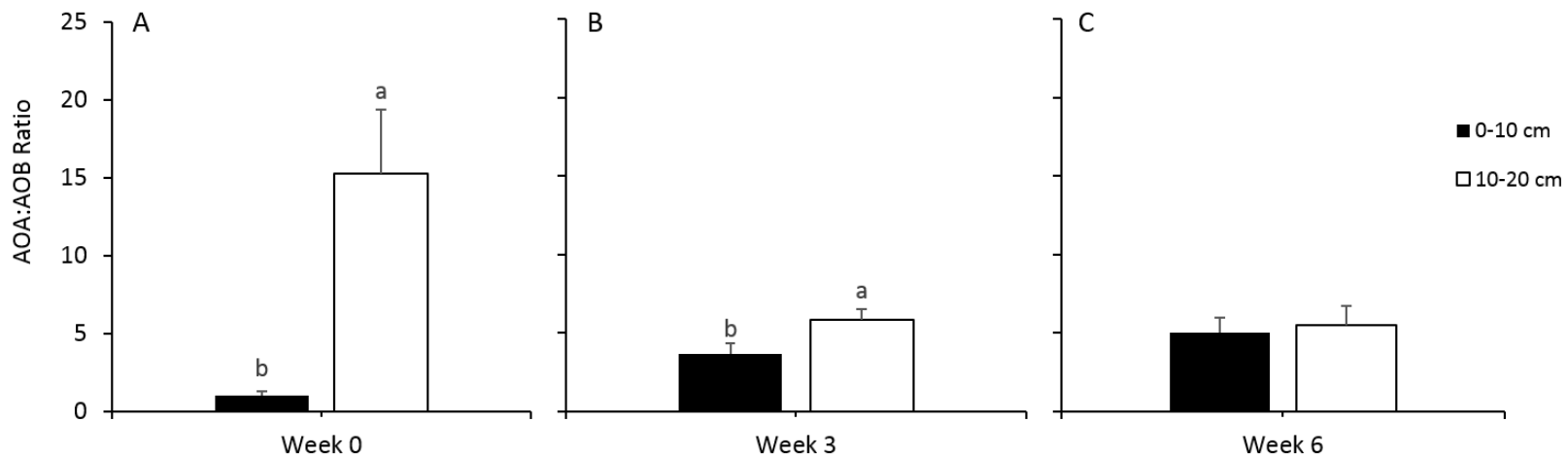
Figure 2. 18: The AOA:AOB ratio vs. date and depth. Samples represent all treatments separated by dates A) Week 0 = April 20th, 2017; B) Week 3 = May 9th, 2017; C) Week 6 = May 30th, 2017.and depth (n = 21). Black bars represent 0-10 cm of soil; White bars represent 10-20 cm of soil; Error bars represent standard error. Statistical significance within each treatment denoted by different letters (p < 0.05) and indicates a change in AOA population compared to AOB population.
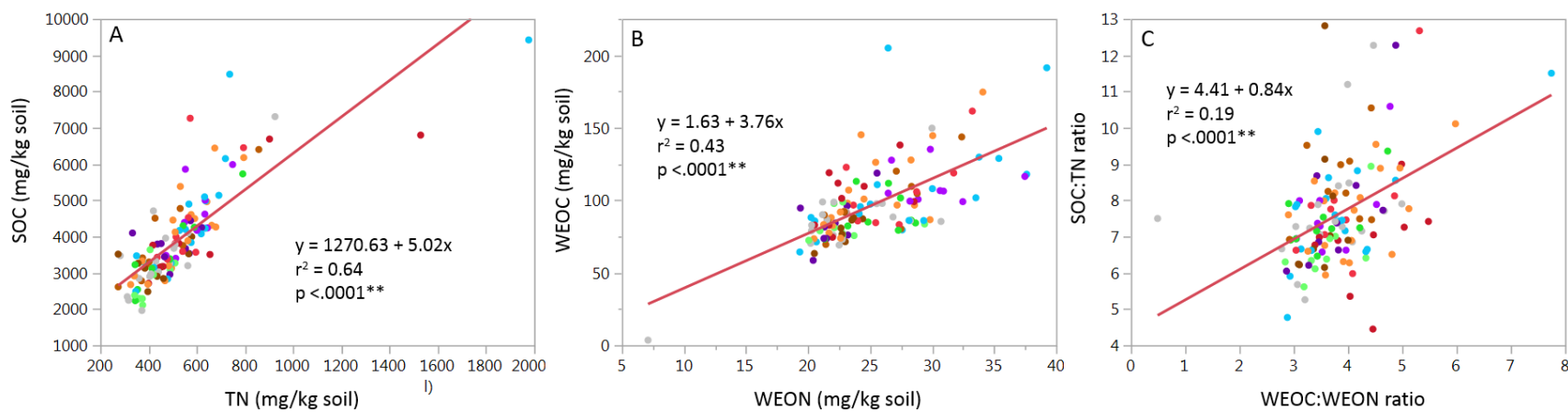
Figure 2. 19: Soil chemical C & N correlation analysis. A) SOC vs. TN, B) WEOC vs. WEON, and C) SOC:TN ratio vs. WEOC:WEON ratio. Statistical significance indicated by (*) denoting $p < 0.05$, (**) significance at $p < 0.01$

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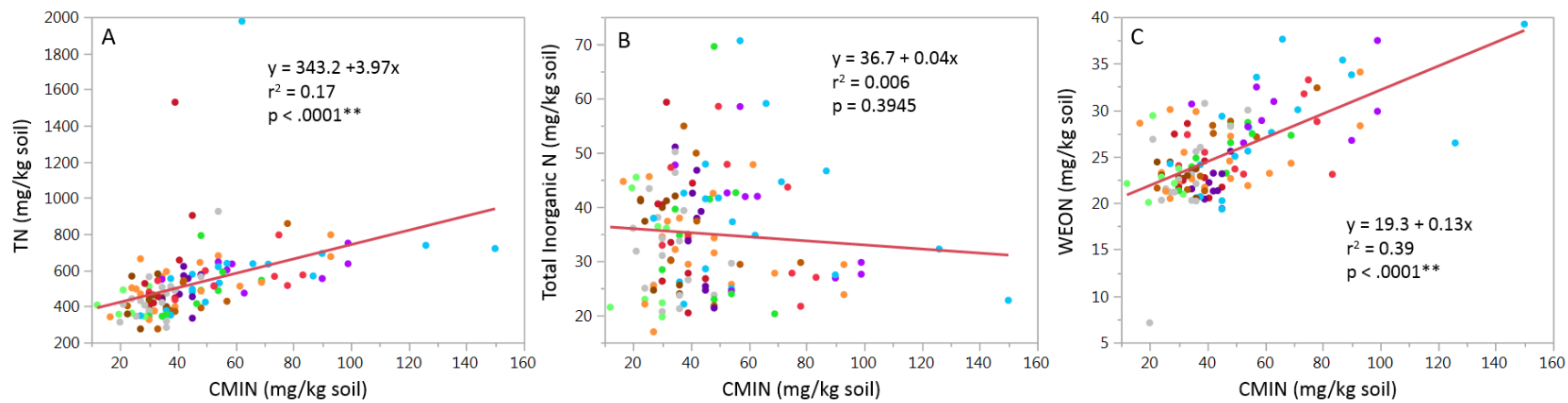
Figure 2. 20: CMIN and soil N correlation analysis. CMIN correlated with A) TN, B) Total Inorganic N, and C) WEON. Statistical significance indicated by (*) denoting $p < 0.05$, (**) significance at $p < 0.01$.
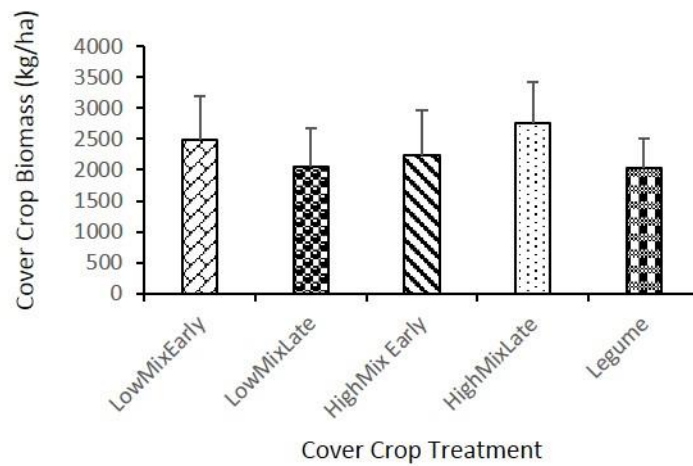
Figure 3. 1: Cover crop biomass as affected by treatments from 2017 at Lalk farm.
Statistical significant denoted by different letters (p < 0.05)
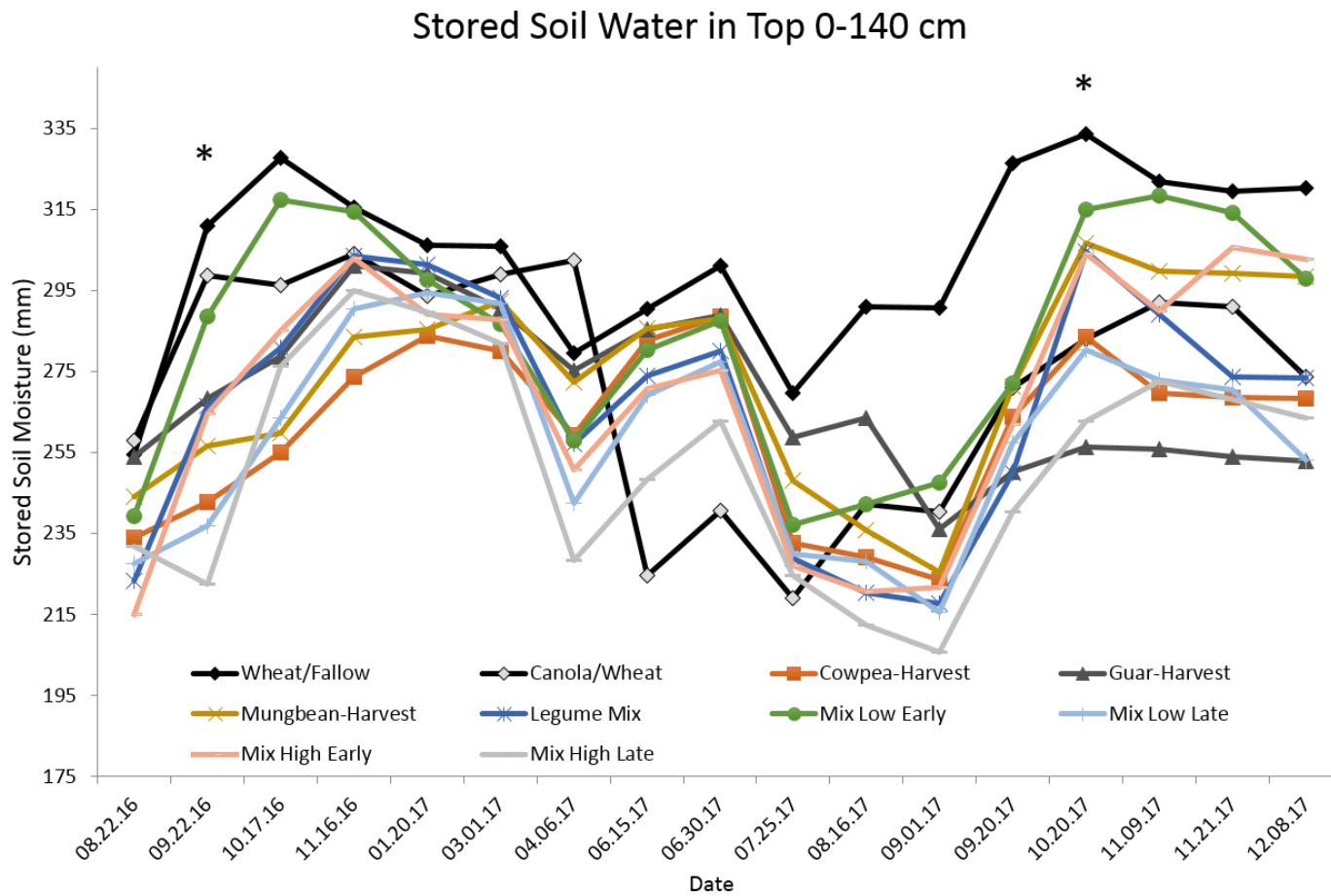
Figure 3. 2: Stored soil moisture (mm) in the upper 0-140 cm of soil at Lalk farm. (*) indicates significance at $p < 0.05$.

Table 2. 1: Seeding rate for offseason cotton winter cover crop treatments from Chillicothe Research Station

Cover crop treatment	Scientific name	Variety	Seeding rate (kg ha^{-1})
Hard red Winter Wheat	*Triticum aestivum*	Bentley	33.6
Austrian Pea	*Pisum sativum*	Austrian winter	39.2
Crimson Clover	*Trifolium incarnatum*	Alyce	22.4
Hairy Vetch	*Vicia villosa* ROTH	Hairy	22.4
Mixed Cover	see Table 2.2	-	33.6
No-Till	no crop	-	0
Conv. Till	no crop	-	0

Table 2. 2: Seeding rate for the offseason cotton winter mixed species cover crop from Chillicothe Research Station.

Mix Species Cover	Scientific name	Variety	Seeding rate (kg/ha)
Rye	*Secale cereale*	*Elbon*	13.4
Hard red Winter Wheat	*Triticum aestivum*	Bentley	10.1
Austrian pea	*Pisum sativum*	Austrian winter	6.7
Hairy vetch	*Vicia villosa*	Hairy	3.4
Total	-	-	33.6

Table 2. 3: Three-Way ANOVA p-values testing significance of soil nutrients measured from treatments, individual sampling dates, and different depths from Chillicothe Research Station.

Model Effects	SOC[†]	TN[†]	WEOC[‡]	WEON[‡]	CMIN[§]	NH$_4^+$	NO$_3^-$	Total Inorganic N[¶]
Date	<0.0001**	0.0004**	<0.0001**	0.0165*	<0.0001**	<0.0001**	<0.0001**	<0.0001**
Depth	0.0002**	0.5787	<0.0001**	<0.0001**	<0.0001**	0.1244	0.0159*	0.0331*
Treatment	0.0002**	0.0161*	0.0002**	0.0079**	<0.0001**	0.6395	<0.0001**	0.1402
Date*Treatment	0.7560	0.6131	0.3354	0.9128	0.0053**	0.2484	0.0531	0.0564
Depth*Treatment	0.9905	0.8294	0.0046**	0.0047**	0.0020**	0.9218	0.3165	0.8078
Depth*Date	0.1147	0.8826	0.0098**	0.1029	<0.0001**	0.5218	0.0787	0.6939
Treatment*Depth*Date	0.9990	0.5998	0.3368	0.9317	0.2568	0.9308	0.3449	0.9287

[†]Soil Organic Carbon (SOC) and Total Nitrogen (TN) obtained through combustion analysis

[‡]Water-Extractable Organic Carbon (WEOC) and Water-Extractable Organic Nitrogen (WEON)

[§]Carbon mineralization (CMIN)

[¶]Total Inorganic N obtained through KCl extractions and is the sum total of NH$_4^+$ and NO$_3^-$

(*) significance at p < 0.05; (**) significance at p < 0.01

Table 2. 4: Two-Way ANOVA p-values testing significance of main effects from PLFA measurements from treatments at 0-10 cm from Chillicothe Research Station.

Model Effects	Total PLFA[#] Biomass	Bacterial PLFA[#] Biomass	Fungal PLFA[#] Biomass	*Rhizobia* PLFA[#] Biomass	Protozoa PLFA[#] Biomass
Date	0.0005**	<0.0001**	0.0002**	0.0433*	<0.0001**
Treatment	0.1396	0.0568	0.2933	0.6162	0.6885
Date*Treatment	0.7304	0.8422	0.8950	0.9574	0.9053

[#]Phospholipid Fatty Acid

(*) significance at $p < 0.05$

(**) significance at $p < 0.01$

Table 2. 5: PLFA biomass from 0-10 cm at Chillicothe Research Station: Mean values from treatments. Statistical significance within each date denoted by different letters ($p < 0.05$).

Treatment	Total PLFA[#] biomass (ng/g)	Total bacteria PLFA[#] biomass (ng/g)	Total fungi PLFA[#] biomass (ng/g)	Total *Rhizobia* PLFA[#] biomass	Total Protozoa PLFA[#] biomass (ng/g)
CT	508	184	52	4	25
NT	921	305	87	4	47
W	763	292	78	3	51
AP	1,420	484	161	10	88
HV	1,145	460	129	9	74
CC	1,011	349	124	6	59
MC	650	243	58	1	29
p-value	0.2320	0.1612	0.4221	0.5891	0.6885

[#]Phospholipid Fatty Acid (PLFA)

CT = conventional till; NT = no-till; W = winter wheat; AP = Austrian winter field pea; HV = hairy vetch; CC = crimson clover; MC = mixed species cover

Table 2. 6: Three-Way ANOVA p-values testing significance of ammonia-oxidizing microbial gene abundance from treatments, individual sampling dates, and depth.

Model Effects	AOB[§§]	AOA[‡‡]	AOA:AOB Ratio
Date	0.4499	0.5180	0.1803
Depth	<0.0001**	0.2876	0.1389
Treatment	<0.0001**	0.5288	0.0003**
Date*Treatment	0.7227	0.5258	0.4888
Depth*Treatment	0.4109	0.6038	0.1718
Depth*Date	0.0614	0.0784	0.0004**
Treatment*Depth*Date	0.3703	0.3158	0.7207

[§§]Ammonia-Oxidizing Bacteria (AOB)

[‡‡] Ammonia-Oxidizing Archaea (AOA)

(*) significance at $p < 0.05$

(**) significance at $p < 0.01$

Table 2. 7: Total *amoA* gene abundance and soil chemical parameter correlation analysis from both depths combined at Chillicothe Research Station.

	SOC[†]	WEOC[‡]	CMIN[§]	TN[†]	WEON[‡]	Inorganic N[¶]	NH$_4^+$	NO$_3^-$	AOA[‡‡]	AOB[§§]	AOA:AOB
SOC[†]		<0.0001**	<0.0001**	<0.0001**	<0.0001**	0.4246	0.2138	0.4280	0.2488	0.0013**	0.8571
WEOC[‡]			<0.0001**	<0.0001**	<0.0001**	0.2086	0.1770	0.6918	0.9246	<0.0001**	0.2173
CMIN[§]				<0.0001**	<0.0001**	0.3946	0.1158	0.1300	0.3537	<0.0001**	0.0037**
TN[†]					<0.0001**	0.7919	0.4033	0.1544	0.6728	0.0545	0.5575
WEON[‡]						0.3047	0.9216	0.0019**	0.0964	<0.0001**	0.0177*
Inorganic N[¶]							<0.0001**	<0.0001**	0.6561	0.5016	0.1482
NH$_4^+$								<0.0001**	0.5038	0.9146	0.2512
NO$_3^-$									0.7058	0.0126*	0.1202
AOA										0.7813	0.7005
AOB											0.0017**
AOA:AOB											

[§§]Ammonia-Oxidizing Bacteria (AOB)

[‡‡]Ammonia-Oxidizing Archaea (AOA)

[†]Soil Organic Carbon (SOC) and Total Nitrogen (TN)

[‡]Water-Extractable Organic Carbon (WEOC) and Water-Extractable Organic Nitrogen (WEON)

[§]Carbon Mineralization

[¶]Total Inorganic N obtained through KCl extractions and is the sum total of NH$_4^+$ and NO$_3^-$

(*) significance at $p < 0.05$, (**) significance at $p < 0.01$

Table 2. 8: PLFA biomass and soil chemical parameter correlation analysis from 0-10 cm of soil at Chillicothe Research Station.

	Total PLFA[#]	Bacteria PLFA[#]	Fungi PLFA[#]	*Rhizobia* PLFA[#]	Protozoa PLFA[#]	SOC[†]	WEOC[‡]	CMIN[§]	TN[†]	WEON[‡]	Inorganic N[¶]	NH$_4^+$	NO$_3^-$
Total PLFA[#]		<0.0001**	<0.0001**	<0.0001**	<0.0001**	0.0042**	0.0009**	0.0012**	0.2419	0.4527	0.9158	0.8790	0.3904
Bacteria PLFA[#]			<0.0001**	<0.0001**	<0.0001**	0.0002**	<0.0001**	<0.0001**	0.0604	0.1621	0.3911	0.4960	0.3472
Fungi PLFA[#]				<0.0001**	<0.0001**	0.0005**	0.0002**	0.0024**	0.1563	0.4484	0.4440	0.6426	0.1878
Rhizobia PLFA[#]					<0.0001**	0.0674	0.0591	0.2556	0.5024	0.7722	0.7140	0.5216	0.4879
Protozoa PLFA[#]						<0.0001**	<0.0001**	<0.0001**	0.0014**	0.4641	0.0032**	0.0071**	0.0409*
SOC[†]							<0.0001**	<0.0001**	<0.0001**	<0.0001**	0.3048	0.3160	0.5917
WEOC[‡]								<0.0001**	<0.0001**	<0.0001**	0.1754	0.2171	0.3262
CMIN[§]									<0.0001**	<0.0001**	0.0684	0.0432*	0.8264
TN[†]										<0.0001**	0.9071	0.7079	0.4516
WEON[‡]											0.6975	0.6326	0.0028**
Inorganic N[¶]												<0.0001**	<0.0001**
NH$_4^+$													0.0118*
NO$_3^-$													

[#] Phospholipid Fatty Acid Analysis (PLFA)

[†] Soil Organic Carbon (SOC) and Total Nitrogen (TN)

[‡] Water-Extractable Organic Carbon (WEOC) and Water-Extractable Organic Nitrogen (WEON)

[§] Carbon Mineralization

[¶] Total Inorganic N obtained through KCl extractions and is the sum total of NH$_4^+$ and NO$_3^-$

(*) significance at $p < 0.05$, (**) significance at $p < 0.01$

Table 3. 1: Seeding rates for the offseason winter wheat summer cover crop treatments

Treatment	Scientific name	Variety	Seeding rate (kg/ha)	Termination time (DAP)
Low Mix Early	see Table 3.2	-	16.8	55-70
Low Mix Late	see Table 3.2	-	16.8	75-90
High Mix Early	see Table 3.3	-	22.4	55-70
High Mix Late	see Table 3.3	-	22.4	75-90
Broadleaf Mix	see Table 3.4	-	22.4	75-90
Canola	*Brassica napus*	Hyclass 225W	5.6	75-90
Guar	*Cyamopsis tetragonoloba*	Lewis	9.0	75-90
Mung bean	*Vigna radiata*	Berkens	22.4	75-90
Cowpea	*Vigna unguiculata*	Black eyed	28.0	75-90
Fallow	no crop	-	0	-

Table 3. 2: Seeding rates for the offseason winter wheat low mix species cover crop treatments

Low Mix Species	Scientific name	Variety	Seeding rate (kg/ha)
Cowpea	*Vigna unguiculata*	(Iron and Clay)	3.4
Mung bean	*Vigna radiata*	Berkens	3.4
Guar	*Cyamopsis tetragonoloba*	Lewis	2.2
Forage Sorghum	*Sorghum bicolor*	Hegari	1.1
Pearl Millet	*Pennisetum glaucum*	Hybrid pearl (Leafy 22)	2.2
Proso Millet	*Panicum miliaceum*	Dove proso	1.1
Foxtail Millet	*Setaria italica*	German Foxtail	1.1
Buckwheat	*Fagopyrum esculentum*	Mancan	1.1
Sunflower	*Helianthus annuus*	Peredovik type	1.1
Total	-	-	16.8

Table 3. 3: Seeding rates for the offseason winter wheat high mix species cover crop treatments

High Mix Species	Scientific name	Variety	Seeding rate (kg/ha)
Cowpea	*Vigna unguiculata*	Iron and Clay	3.36
Mung bean	*Vigna radiata*	Berkens	3.36
Guar	*Cyamopsis tetragonoloba*	Lewis	3.36
Forage Sorghum	*Sorghum bicolor*	Hegari	2.24
Pearl Millet	*Pennisetum glaucum*	Hybrid pearl (Leafy 22)	3.36
Proso Millet	*Panicum miliaceum*	Dove proso	2.24
Foxtail Millet	*Setaria italica*	German Foxtail	2.24
Buckwheat	*Fagopyrum esculentum*	Mancan	1.12
Sunflower	*Helianthus annuus*	Peredovik type	1.12
Total	-	-	22.4

Table 3. 4: Seeding rates for the offseason winter wheat broadleaf mix species cover crop treatment

Mix Species	Scientific name	Variety	Seeding rate (kg/ha)
Cowpea	*Vigna unguiculata*	Iron and Clay	11.2
Mung bean	*Vigna radiata*	Berkens	5
Guar	*Cyamopsis tetragonoloba*	Lewis	2.8
Buckwheat	*Fagopyrum esculentum*	Mancan	1.7
Sunflower	*Helianthus annuus*	Peredovik type	1.7
Total	-	-	22.4

Table 3. 5: Two-Way ANOVA p-values testing significance of carbon and nitrogen measured from treatments and different depths at Lalk farm

Model Effects	WEOC[‡]	WEON[‡]	CMIN[§]	NH$_4^+$	NO$_3^-$	Total Inorganic N[¶]
Treatment	0.3738	0.0460*	0.1787	0.1858	0.0165*	0.0389*
Depth	0.3160	0.0002*	<0.0001**	0.9616	<0.0001**	0.0105*
Treatment*Depth	0.9618	0.8165	0.6358	0.6827	0.4090	0.3830

[‡]Water-Extractable Organic Carbon (WEOC)

[‡]Water-Extractable Organic Nitrogen (WEON)

[§]Carbon Mineralization (CMIN)

[¶]Total Inorganic N obtained through KCl extractions and is the sum total of NH$_4^+$ and NO$_3^-$

(*) significance at $p < 0.05$

(**) significance at $p < 0.01$

Table 3. 6: Soil chemical measurements from Lalk wheat farm: Mean values from treatments. Sample represent both depths combined (n = 8) and separated by treatment. Statistical significance within each date and depth denoted by different letters (p < 0.05).

Treatment	WEOC[‡]	WEON[‡]	CMIN[§]	NH_4^+	NO_3^-	Total Inorganic N[¶]
Wheat/Fallow	67.0	22.2 abc	34.0	11.4	6.9 bc	18.2 c
Wheat/Canola	71.0	20.8 abc	38.1	14.1	6.7 bc	20.9 bc
Harvested Mung bean	73.1	21.2 bc	47.1	16.4	5.3 c	21.6 bc
Harvested Cowpea	67.3	19.6 c	61.7	20.5	5.3 c	25.9 abc
Harvested Guar	88.9	26.0 a	44.1	19.4	12.2 a	31.6 a
Low Mix Early	81.7	22.2 abc	43.0	16.7	6.9 bc	22.6 bc
Low Mix Late	67.6	22.4 abc	47.1	15.7	6.9 bc	23.6 abc
High Mix Early	94.5	25.0 ab	54.2	19.3	8.1 abc	27.4 ab
High Mix Late	75.8	25.1 ab	50.1	18.6	10.5 ab	29.1 ab
Legume Mix	72.2	21.4 bc	60.0	13.2	4.7 c	17.8 c
p-value	0.3738	0.0460*	0.1787	0.1858	0.0165*	0.0389*

[‡]Water-Extractable Organic Carbon (WEOC)

[‡]Water-Extractable Organic Nitrogen (WEON)

[§]Carbon Mineralization (CMIN)

[¶]Total Inorganic N obtained through KCl extractions and is the sum total of NH_4^+ and NO_3^-

Table 3. 7: PLFA biomass in the 0-10 cm of soil from Lalk Farm: Mean values from treatments. Statistical significance within each date denoted by different letters (p < 0.05).

Treatment	Total PLFA[#] biomass (ng/g)	Total bacteria PLFA[#] biomass (ng/g)	Total fungi PLFA[#] biomass (ng/g)	Total *Rhizobia* PLFA[#] biomass (ng/g)
Wheat/Fallow	1,835	915	151	7
Wheat/Canola	1,603	797	145	37
Harvested Mungbean	1,758	830	136	10
Harvested Cowpea	2,365	1,118	57	0
Harvested Guar	2,108	1,000	212	26
Low Mix Early	1,911	868	116	7
Low Mix Late	1,802	929	142	31
High Mix Early	2,211	1,112	164	0
High Mix Late	2,474	1,195	157	10
Legume Mix	2,226	1,107	221	31
p-value	0.9536	0.9254	0.9611	0.7384

[#]Phospholipid Fatty Acid (PLFA)

(*) significance at p < 0.05

(**) significance at p < 0.01