# COVER- AND DOUBLE-CROPPING IMPACTS ON SOIL HEALTH AND MOISTURE OF A DRYLAND WINTER WHEAT SYSTEM IN THE TEXAS ROLLING PLAINS

A Thesis

by

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# ABSTRACT

Constraints on food, land and water resources pose a need for conservation practices to maintain viable agricultural lands. Wheat - a staple cereal grain for humans and animals worldwide - has been heavily cultivated in the Great Plains of the United States well before the 1900s. Historically in this region, tillage and fallow periods were implemented as a means to conserve soil moisture during summer months. However, almost 100 years ago, these practices in conjunction with long periods of drought followed by large wind events resulted in the Dust Bowl of the 1930s and brought immense erosion and loss of topsoil to the southern Great Plains. Advances in conservation management to provide crop cover to soil surfaces and reduction in tillage have helped protect the soil in the Great Plains; however, adoption of these practices remains low in part due to concerns for soil moisture preservation.

Although the potential for no-tillage and cover crops to protect the soil, build organic matter, and improve soil health has been demonstrated across much of the USA, little research has focused on the application of these techniques to the Texas Rolling Plains region. The objective of this study was to evaluate cover and double cropping practices in a wheat monocropping system under no-till management and their effects on soil health and soil moisture.

A wheat-fallow control was compared to seven treatments with various cover crops in rotation including legumes and mixtures treated as cover crops or double crops. Soils were collected and analyzed for biological, chemical and physical properties including microbial biomass, mineralizable carbon, soil carbon and nitrogen fractions, soil aggregate distribution and water content. After three years of cover crops in rotation, there was generally greater soil microbial biomass and activity with treatments receiving cover. In addition, by implementing cover and double crops, soil moisture was not negatively impacted during the study period and those treatments had greater volumetric water content following precipitation events compared to the wheat-fallow control. Some parameters of soil health did not respond to the intensified wheat system; however, many soil characteristics (such as soil organic carbon) change on decadal time scales, and longer-term studies may be needed to more fully evaluate their potential responses. Results indicate cover and double crops can be a viable method to build soil health and sustainability without reducing soil moisture in wheat-monocropping systems in the Texas Rolling Plains.

# DEDICATION

To the stewards of land and of science.

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## Contributors

This work was supervised by a thesis committee consisting of Dr. Paul DeLaune (advisor) and Dr. Terry Gentry (co-advisor) of the Department of Soil and Crop Sciences and Dr. Thomas Boutton of the Department of Ecosystem Sciences and Management.

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# NOMENCLATURE

| | |
|---|---|
| NT | No-tillage; no-till |
| PET | Potential evapotranspiration |
| USDA | United States Department of Agriculture |
| NRCS | Natural Resources Conservation Service |
| CASH | Cornell Assessment of Soil Health |
| HTSH | Haney Test for Soil Health |
| SOM | Soil organic matter |
| CA | Conservation agriculture |
| C | Carbon |
| N | Nitrogen |
| OM | Organic matter |
| SOC | Soil organic carbon |
| CC | Cover crop |
| TRP | Texas Rolling Plains |
| DC | Double crop |
| FSA | Farm Service Agency |
| AMF | Arbuscular mycorrhizal fungi |
| TX | Texas |
| DAP | Days after planting |
| PLFA | Phospholipid fatty acid analysis |

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|---|---|
| qPCR | quantitative Polymerase Chain Reaction |
| WFPS | Water filled pore space |
| POxC | Permanganate oxidizable carbon |
| ANOVA | Analysis of variance |
| TC | Total carbon |
| POC | Particulate organic carbon |
| MBC | Microbial biomass carbon |
| MWDA | Mean weight diameter of aggregate |
| VWC | Volumetric water content |
| $CO_2$ | Carbon dioxide |
| $NH_4^+$ | Ammonium |
| $NO_3^-$ | Nitrate |
| mm | millimeter |
| kg | kilogram |
| ha | hectare |
| Pg | petagram |

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# 1. INTRODUCTION AND LITERATURE REVIEW

## 1.1. Introduction

Dating back to the beginnings of civilization, wheat (*Triticum aestivum*) has been a staple agricultural commodity worldwide, being the third most cultivated cereal grain and second most consumed (FAO, 2012), accounting for 20% of our consumed proteins (Tilman et al., 2011). Globally, wheat production spans six continents and between 2017 and 2018 was estimated to be 760.3 billion kilograms (FAO, 2019).

In the United States, six types of wheat are grown across 42 states (NAWG, 2019). In 2017, about 15.2 million hectares of wheat were harvested, of which winter wheat accounted for 10.2 million hectares and $5.5 billion in production (NASS, 2019). The Great Plains spans far north into Canadian provinces from Manitoba to Alberta down through Texas, where the majority of wheat production within the Great Plains of the United States includes Hard Red, Hard White and Soft Red Winter wheat classes (NAWG, 2019). Wheat is the second largest field crop in Texas in regards to acreage (NASS, 2019). Specifically, the state is the 5th highest in winter wheat production in the US (NASS, 2019), with an estimated 2.4 million hectares planted and with farmers harvesting around 1.3 million hectares for grain annually (Texas Wheat, 2019). Much of this harvested wheat as well as the remaining acreage is used over the winter for grazing in dual-purpose systems, making wheat an admirable crop for its utility as both forage and a grain.

Production of wheat in the Great Plains began as a continuous monocropping system as settlers of the area practiced this previously in eastern, more humid areas of the United States (Hansen et al., 2012). With drier conditions in the Great Plains, use of a fallow period, where the soil is devoid of crop production, for precipitation storage was adopted (Hansen et al., 2012) and this annual wheat-fallow system is still practiced in the region today. Fallow periods may vary across the Great Plains. For instance, in regions throughout such as the Texas Panhandle, western Kansas and eastern Colorado, producers typically grow wheat with another crop in rotation, resulting in two crops within three years and a fallow period of about 11 months between rotations. However, in the Texas Rolling Plains (TRP) towards the south, producers plant wheat in a continuous monocropping rotation with about three to five months of fallow between each wheat crop.

Prior to the Dust Bowl of the 1930s, Shaw (1911) noted that the primary goal of "dry farming" was to increase soil moisture, doing so through the use of deep plowing. Tillage continued to be encouraged in the southern Great Plains semi-arid region during this time (Stewart et al., 2010); however, the resulting absence of crop residues to protect the soil in conjunction with the Dust Bowl saw disastrous consequences of loss of soil and organic matter (Unger et al., 2010).

From here, rotation in cropping systems and a change in tillage practices, such as stubble-mulch tilling, were encouraged (Stewart et al., 2010). More recently, no-tillage (no-till, NT) practices began to take hold in the region (Stewart et al., 2010) yet most producers using NT are those also intensifying monocropping systems to include a rotation of crops; those continuing use of fallow periods have low NT adoption rates (Hansen et al., 2012). While in the U.S., practice of no-till farming increased between 1990 and 2008 from about 6% to 24% (Baumhardt et al., 2015), variations of adoption rates are noted in the Great Plains. Overall in the Great Plains, the greatest implementation of NT is in the northern Great Plains, followed by the central Great Plains and lastly the southern Great Plains with less than 5% of farmers in 2004, in part due to higher potential evapotranspiration (PET; Hansen et al., 2012) which can account for almost a 50% difference in water use per unit biomass from North Dakota to Texas (Robinson and Nielsen, 2015). The drastic change in PET from the northern to southern Great Plains explains how rotations with cover crops can more easily be implemented in certain regions of the country while in others fallow periods are needed in dryland production for soil water storage (Robinson and Nielsen, 2015; Unger and Vigil, 1998).

Although fallow management is still practiced in the southern Great Plains, conservation agriculture practices should be implemented to maintain viable agricultural production lands. Current farming practices remove large portions of crop biomass during harvest without return of residues and nutrients back to the soil for maintenance of organic matter. Paired with a fallow period and environmental conditions ideal for residue mineralization, this production system leads to degradation of soil organic matter (Stewart et al., 2006) and resulting negative impacts on soil aggregation. Additionally, fallow practices can negatively impact soil aggregation (Baumhardt et al., 2015) soil water infiltration, microbial populations, and nutrient buildup. A look at how practices such as no-till and cover crops impact soil properties in place of a fallow period will help farmers and researchers weigh the pros and cons of adopting conservation agriculture practices in the Texas Rolling Plains.

## 1.2. Soil Health

The term soil health originated from the concept of soil quality, first introduced in the late 1970s by Warkentin and Fletcher (1977) at the International Seminar on Soil Environment and Fertilizer Management in Intensive Agriculture. In response to the 1930's Dust Bowl, which affected 400,000 $km^2$ of land (Lal, 2009) and caused the subsequent loss of topsoil from erosion, the United States government established the Soil Erosion Service to evaluate and provide solutions to erosion issues (Karlen et al., 2010). Shortly after, the division was renamed to the Soil Conservation Service, known today as the Natural Resources Conservation Service (NRCS) encompassing its purpose to sustain soil and water resources throughout public and private lands (Karlen et al., 2010). As a result, scientists created tools and resources for soil erosion and management practices. In the latter part of the twentieth century, it was realized there was more to be done to improve soil than mitigate erosion and the concept evolved into what is referred today as soil health (Karlen et al., 2003).

From these ideas, methods to evaluate the change in soil quality needed to be developed. Multiple tests were established to contain a suite of biological, physical and chemical tests. Recently, emphasis has been put on inclusion of biological indicators in these tests as microbial activity plays a vital role in nutrient supply and soil processes (Franzluebbers, 2016; Lehman et al., 2015; Stewart et al., 2018). It is important to mention that over the years of developing tests, it has been well noted that they should include not only measurements for dynamic soil properties that change over short periods of time due to such things as management practices, but must also consider the inherent soil properties that exist in certain soils (Karlen et al., 2003; Roper et al., 2017). From these tests, researchers and farmers get a sense of how management practices might improve, degrade or maintain soil health (Karlen et al., 2011).

As defined by the United States Department of Agriculture Natural Resources Conservation Service (USDA-NRCS), soil health is the "continued capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans" (NRCS, 2019a). In recent years, there has been an increasing soil health movement, with initiation of organizations such as the Soil Health Institute and creation of soil health tests including the Cornell Assessment of Soil Health (CASH) and the Haney Test for Soil Health (HTSH). Along with a resurging interest in the subject, many research programs across the country are evaluating soil health. Ongoing research is being done to understand and optimize methods and interpretations of soil health indicators across laboratories and in various cropping and climate systems (Franzluebbers, 2016; Hurisso et al., 2018; Roper et al., 2017; Stewart et al., 2018). Researchers are using a variety of soil health indicators in their studies; however, there is no consensus as to which indicators should be used in a more uniform process that can be compared across laboratories and regions (Stewart et al., 2018).

Along with the NRCS definition, the agency provides four management practices to encourage the maintenance and improvement of soil health including: less disturbance to the soil, vary crop selection to diversify organisms in the soil, maintain living roots, and maintain soil coverage (NRCS, 2019b). Cover cropping and a reduction in tillage are just two of the management practices used to promote soil health.

## 1.3. Soil Moisture

Though no-till and cover crops have many benefits, they can impact soil moisture- a critical issue in semi-arid locations and still one of the primary reasons producers remain hesitant to adopt cover crops in these areas. With shifts in climate and droughts impacting parts of the southern United States, producers are being mindful of the influence to water supplies and irrigation costs. Particularly, Texas production is mainly under dryland, relying on seasonal precipitation for crop growth, and use irrigation on less than 13% of cropland in the state (NASS, 2013). Additionally, the Texas Rolling Plains area receives an average annual rainfall of 500-710 mm (NOAA, 2018), and has experienced exceptional drought and highly variable climactic conditions over the past 7-8 years. Therefore, soil moisture preservation is of significant importance, as farmers must consider the conservation of soil water for optimal cash crop production.

Tillage practices can impact the efficiency of fallow on soil water storage, where elimination of tillage can result in substantial cash crop residues on the soil surface to mitigate evaporation from the soil and encourage infiltration (Unger, 1978). However, in a warm semi-arid environment, rapid degradation of crop residues may leave the soil with minimum coverage. By incorporating cover crops into rotation, additional residue can prolong protection of the soil while also keeping hold of cash crop residues on the surface, compounding the benefits of surface cover (Kaspar and Singer, 2011). Recent research has shown that reduced fallow and the implementation of conservation tillage or no-till saw greater precipitation storage during the fallow period in dryland farming (Peterson et al., 2012; Stewart et al., 2010; Unger et al., 2012; Unger et al., 2006).

While cover crops are present, soil moisture will be used by that crop and stored moisture can be depleted from the soil profile prior to cash crop planting. However, if there is adequate precipitation prior to cash crop planting, recharge of moisture can occur and may not negatively impact the subsequent crop supply. In the region of interest, most precipitation events occur in late summer and early fall months, between cover crop termination and winter wheat planting; therefore, the rotation may be ideal in regards to soil water recharge from precipitation events preceding wheat production.

Multiple studies have cautioned against use of cover crops in place of fallow in semi-arid regions, as they will deplete soil moisture from subsequent cash crop yields (Nielsen et al., 2016; Unger and Vigil, 1998). Though the authors discouraged use of cover crops in a semi-arid region, Unger and Vigil (1998) encouraged use of conservation tillage where cash crop residues remain on the surface. These residues provide only a portion of cover crop benefits, and exclude the ability to deter nutrient leaching or contribute additional nutrients for soil organic matter (SOM) buildup (Unger and Vigil, 1998).

Nielsen et al. (2016) noted recent studies throughout the Great Plains with various results of cover cropping impacts on crop yields, where studies in the southern region generally saw negative impacts on cash crop yields. In this same study, incorporation of cover crops resulted in greater percentages of residue cover after cover crop termination and after wheat planting when compared to the wheat-fallow controls. As a result, precipitation storage efficiency was also greater for cover crop treatments. In terms of yield, only half of the treatments showed significantly lower yields compared to the wheat-fallow control (Nielsen et al., 2016). Additional studies have shown that under conservation tillage and cover crops, there were no significant differences in cotton lint yield compared to conventional tillage or those practices without cover crops in semi-arid regions of Texas (DeLaune et al., 2012; Sij et al., 2003). Hesitations surrounding cover crop use and soil moisture may be due in part to how long the cover crop is in place and using moisture.

### 1.4. Tillage Practices

Historically, tillage had been a way for producers to control weeds, prepare seedbeds for planting and accelerate nutrient mineralization (Reicosky et al., 2011). As previously mentioned, deep tilling was encouraged in the Great Plains as a means to increase water storage (Shaw, 1911) and further inventions such as the subsurface packer (Stewart et al., 2010) were adopted by an influx of settlers to the area. As a result of the 1930s Dust Bowl, tillage practices were reevaluated to include the importance of crop residues for maintaining soil protection, thus, the development of stubble-mulch tillage (Unger et al., 2010).

After its development, multiple studies in the Great Plains cited water conservation and other benefits associated with stubble-mulch tillage (Greb et al., 1979; Johnson et al., 1974) which can be due in part to residues of the cash crops in systems like continuous cropping or winter wheat-fallow-grain sorghum-fallow (Unger and Vigil, 1998). However, although this practice is less intense than conventional till, it still disturbs the soil surface due to tillage (Claassen et al., 2018), known to cause destruction of aggregates in soil, increased carbon and nutrient release (Reicosky and Allmaras, 2003) and greater evaporation of soil water stores when turning over the soil (Hatfield, 2011). Because of these impacts, rising fuel costs (Stewart et al., 2010) and pressures from consumers for more sustainable practices, there has been a recent shift towards conservation agriculture (CA) practices such as no-tillage. Conservation tillage is a globally broadly defined term (Reicosky and Allmaras, 2003), however, the USDA defines conservation tillage as "a broad range of soil tillage systems that leave residue cover on the soil surface, substantially reducing the effects of soil erosion from wind and water" (NAL, 2007), where at least 30% of residues remain after harvest (ERS, 2017; Reicosky and Allmaras, 2003). These practices result in a buildup of soil carbon and soil organic matter (Reicosky et al., 2011; Robinson and Nielsen, 2015) and increase precipitation storage efficiency (Nielsen and Vigil, 2010).

Buildup of organic matter and nutrient inputs to soils can promote carbon (C) sequestration and inorganic forms of nitrogen (N), critical elements for soil fertility and crop production. Use of conventional tillage has long degraded soils, exposing carbon stores protected in aggregates while increasing mineralization and release of $CO_2$ from microbial respiration (Lal and Kimble, 1997; Unger and Blanco-Canqui, 2012). No-tillage practices not only reduce aggregate disturbance, but maintain cash-crop post-harvest residue on the surface, keeping soil cooler and slowing decomposition of organic matter (OM) by microorganisms. However, in regions with lower precipitation leading to fewer residues after harvest, buildup of soil C may take significantly longer compared to agroecosystems in more mesic climates (Unger and Blanco-Canqui, 2012). Therefore, other short-term benefits must be realized to encourage initiation of this practice by farmers for long-term outcomes in order to deter soil degradation (Stewart et al., 2006).

Concerns with adoption of NT can span from the need of tillage to suppress weed populations in crop fields to breaking up compacted soils. In response to these concerns, components of CA, such as NT, should not be used alone, but rather in conjunction with other conservation management practices (Huggins and Reganold, 2008; Reicosky et al., 2011). It is noted that when NT is combined with cover crop use, there are benefits to soil physical properties, soil organic carbon (SOC) storage (Blanco-Canqui et al., 2011) and soil biological activity (Mbuthia et al., 2015).

## 1.5. Cover Crops

The use of cover crops (CC) is not new to agriculture, as they have been a means to maintain soil fertility since the establishment of agriculture and civilization (Lal, 2015). These crops can minimize the fallow period after cash crop harvest and serve to protect soils from wind and water erosion, increase soil health, suppress weeds and minimize compaction of soils (USDA, 2013). Additionally, they can increase water infiltration (Blanco-Canqui et al., 2011), build up organic matter (Balkcom et al., 2015), and soil organic carbon and nitrogen (Blanco-Canqui et al., 2012).

Since the 1990s, cover crop use has not been encouraged in the semi-arid Great Plains regions, particularly the southern areas due to concerns of declines in soil moisture and therefore adverse impacts on crop yields (Unger and Vigil, 1998). These same authors stated that functions of cover crops such as prevention of nutrient leaching from the soil profile or organic matter inputs will not be realized from conservation practices where surface residues are left post-harvest from the cash crops, although soil moisture can increase.

With rotations integrating cover crop residue, additional organic matter is incorporated to offset residue loss from harvesting and help SOC building. As previously mentioned, limitations of soil C accumulation occur due to sparse residue build up and may also be due to low amounts of available N to provide an optimal C:N ratio for C sequestration (Lal, 2015; Unger and Blanco-Canqui, 2012). Certain crops like legumes with nitrogen-fixing capabilities may provide additional inorganic N sources to the soil as plant-available ammonium and nitrate while simultaneously contributing to carbon sequestration.

Recently, multi-species mixtures have been recommended for use as cover crops. Mixtures may be admirable for their ability to generate more biomass (Wortman et al., 2012), increase soil organic C more than single species (Faé et al., 2009), and enhance functional diversity (i.e., qualities provided by grasses or legumes). Additionally, Nielsen et al. (2015) found that mixtures did not use water differently than single-species cover in a semi-arid environment, but did use more water than fallow treatments. The authors noted future research to evaluate additional benefits of cover crop mixtures to the soil environment is needed. Though cover crops are widely encouraged in production systems, the above-mentioned benefits can vary depending on cover crop type, location and management (Blanco-Canqui et al., 2012), including termination timing. Moreover, variances and mixed perceptions of the practice may cause producers to be hesitant to use cover crops (Reimer et al., 2012), where only 4% of farmers in the US were reported to use cover crops between 2010 and 2011 (Wade et al., 2015).

The USDA NRCS recommends terminating cover crops 15 days or earlier prior to cash crop planting for Zone 2, which includes the Texas Rolling Plains. According to the NRCS, these recommendations are made to attain cover crop benefits without reducing cash crop yield due to soil water uptake (USDA, 2014). The region of significance in this study practices low-input production with low returns; thus there is interest in growing the cover crop out to crop maturity and harvesting it as a double crop (DC), with opportunity to provide additional income to producers. However, both cover and double cropping may raise hesitations for adoption from producers. For cover crops in general, seed and management-associated expenses and recovery of these costs are limiting factors dissuading farmers from adopting or continuing the practice (Dunn et al., 2016). Dryland double cropping does not qualify for crop insurance under the Farm Service Agency (FSA) program and requires further investigation to see if it is not only profitable, but has the possibility to enhance soil health benefits while not detrimentally impacting cash crop yield.

The concept of reduced fallow has been encouraged in the United States including areas of the Great Plains (Baumhardt et al., 2015) and recent publications have provided information on termination timing of cover crops in other areas of the country (Balkcom et al., 2015; Blanco-Canqui et al., 2012). Yet no studies have looked specifically at termination timing of cover crops and the opportunity for double cropping in the Texas Rolling Plains environment.

## 1.6. Objectives and Hypotheses

Cover crops can be implemented into winter wheat systems as a means to improve soil health following wheat-fallow production. However, these crops will use soil moisture leading to increased concern for cash crop failure as a result of potential inadequate soil moisture. This has led to producer hesitation in adopting this practice, especially in dryland production systems of semi-arid regions. By investigating the implementation of cover crops into a dryland NT system, this research will analyze their impact on soil health parameters and moisture during the fallow period and subsequent winter wheat growth. Cover crops may not provide immediate economic benefit to producers; therefore, a look at how double crops- an economic option to grow cover crops to maturity and be harvested for grain- affect soil health and moisture will be evaluated. Specifically, cowpea and mung bean will be evaluated as double crops as these are two legumes of interest in the area. Mixtures will be assessed as a cover crop based on recommendations from the NRCS as part of their cost-share program. Mixtures will also be evaluated as a double crop (to be harvested as hay) in order to evaluate its benefits as a cover crop and its opportunity as an additional income source to producers. Analysis of soil moisture changes with a cover crop will provide further insight into better management practices in intensified cropping systems, particularly in areas with marginal moisture. Understanding termination time to optimize benefits from cover crops or double crops can help with future recommendations for farmers applying these practices in the Texas Rolling Plains.

The objectives of this study were to: (1) determine the impact of cover cropping vs. double cropping and cover crop termination timing on soil biological parameters, (2) analyze cover and double cropping impacts on carbon sequestration and dynamics in conservation agriculture practices, and (3) assess the use of cover crops and double crops on changes in soil moisture and soil physical properties. This will inform producers about the impact of growing crops during summer fallow in a semi-arid environment to assist them in making decisions about conservation agriculture practices.

The following hypotheses will be tested:

1. With a longer presence of a living plant, we predict an improvement in soil health, as seen by changes in mineralizable carbon and microbial biomass.
2. By intensifying a wheat-fallow system, additional summer crop biomass will have a positive impact on carbon abundance and nitrogen availability.

3. With a later termination time of the summer crop soil moisture will be utilized longer by that crop, however, there will be an enhancement in soil structure and ability of soil moisture to recharge upon rainfall.

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# 2. ENHANCING SOIL HEALTH WITH INTENSIVE FALLOW MANAGEMENT IN WINTER WHEAT SYSTEMS

## 2.1. Introduction

Below the Earth's surface lies a vast diversity of soil microbial life contributing to: nutrient cycling for plant availability, physical integrity of soil and habitat for macrofauna, and carbon sequestration along with a multitude of other processes (Lehman et al., 2015). These microbes provide many benefits through their role in soils, however, anthropogenic activities such as fertilization (Franzluebbers, 2016), soil disturbance (i.e., tillage), or habitat and nutrient degradation due to fallow periods (Lehman et al., 2015) can negatively impact these physically small, yet vital, microbial populations.

As the idea of soil quality began to emerge, the importance of including biology (Lehman et al., 2015) within that framework was recognized. In addition to a focus on chemical and physical soil components, it became clear the need to include biological tests within the soil health structure (Franzluebbers, 2016). Microbial populations are at the core of soil health; therefore, their roles in soil processes should not be ignored, nor should agricultural management and the impact these practices have on biological components of soil health (Franzluebbers, 2016; Stewart et al., 2018).

For centuries, tillage has been an integral part of agriculture, as a way to turn soil beds and bring up nutrients for new seeds. Yet tillage can cause issues in regards to soil structure (Brady and Weil, 2010) and loss of nutrients (Lal et al., 2007). Tillage also disrupts arbuscular mycorrhizae fungi (AMF) networks and the benefits they provide such as carbon storage, soil aggregation and provision of nutrients to host plants. By moving from intensive to more conservative tillage practices such as no till, in combination with crop rotations and cover cropping, increases in microbial populations and activity have been noted (Acosta-Martínez et al., 2007; Mbuthia et al., 2015). In semi-arid regions with dryland production, intensifying monocropping systems may be more important to increasing soil microbial biomass (Acosta-Martínez et al., 2011).

The AMF may be of particular importance when evaluating the role of cover crops in carbon sequestration as well as soil structure. AMF are ubiquitous in soils and colonize roots of most plants. In this symbiotic relationship, nutrients beyond the rooting zone of the plant are taken up by an extended AMF network and provided to the host plant in exchange for carbon sources. Because of this, AMF can be considered a large store for carbon in soils. Additionally, their branching hyphae play a role in the formation of soil aggregates by acting as temporary binding agents to stabilize macroaggregates and therefore, microaggregates (Miller and Jastrow, 2000).

It is widely known that tillage causes soil disturbances, including the disruption of mycorrhizal hyphae networks. However, bare fallow periods (typically a year or more) can also impact AMF by reducing AM propagules in soil and therefore colonization. To address this problem, past studies suggested practices that maintained AMF populations be implemented (Thompson, 1990, 1994). One practice as suggested by the NRCS for improvement in soil health, would be the continual growing of roots in the soil- such as cover crops. A question of interest is if the timing of cover crop termination and therefore the period of fallow between crops in rotation might affect the abundance and activity of AMF and other soil microorganisms in soils and how this might further impact their presence during wheat growth.

Cover crops not only benefit AMF, but soil microbes in general by providing nutrients such as carbon and nitrogen through above and belowground resources (Lehman et al., 2015). It has also been noted that by having greater plant diversity, these nutrient provisions increase, therefore increasing microbial activity and their role in carbon sequestration (Lange et al., 2015). Soil health tests such as the one proposed by Franzluebbers et al. (2000) are rapid and easily implemented to quantify the amount of microbial respiration and indicate the microbial activity in soils.

In the Texas Rolling Plains, NT practices have slowly increased and alongside that, interest in the use of cover crops in these systems. Additionally, double cropping would be an alternative to cover cropping with potential economic gain for producers. However, little is known about how cover cropping or double cropping will affect soil microbial populations and activity, therefore influencing soil health.

The first objective of this study was to evaluate how cover cropping and double cropping, therefore the duration of rooting systems and buildup of crop residue, impact soil microbial biomass. Not only will biomass indicate the microbial populations in soil, but also measuring microbial activity will indicate their potential in soil processes. Our second objective was to measure soil microbial activity by mineralizable carbon. We hypothesize that through intensification of a wheat-fallow system, microbial activity and abundance will increase over time benefiting soil health conditions.

## 2.2. Materials and Methods

### *2.2.1. Field description and soil sampling*

This research study was initiated in 2015 and conducted at the Texas A&M AgriLife Research and Extension Center at Vernon (34.09°N, 99.37°W). The surrounding region is referred to as the Texas Rolling Plains and considered a semi-arid environment with a 30-year average annual rainfall of 710 mm, with most precipitation occurring between May and June (Figure 2-1). The soil in this study is classified as a Miles fine sandy loam (fine-loamy, mixed, superactive, thermic Typic Paleustalf).

In 2001, production of monocropped Hard Red winter wheat (wheat-fallow) began under dryland, rainfed conditions. In 2015, the following summer crops were incorporated into rotation: cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata*), and a mixture of grasses (foxtail millet (*Setaria italica*); pearl millet (*Pennisetum glaucum*); hegari sorghum (*Sorghum bicolor*)) and legumes (cowpea; mung bean; guar (*Cyamopsis tetragonoloba*)). The mixture consisted of 60% legumes and 40% grasses based on seed weight (Table 2-1). Legume species were chosen based on their adaptability to the region. Mixtures were chosen based on the NRCS recommendation to build soil health. The experimental design was a randomized complete block design with eight treatments in four replicate plots (n = 32). Plot dimensions were 1.71 m x 6.71 m. Mung bean treatments were seeded at 22 kg ha$^{-1}$ while cowpea and mixture treatments were seeded at 28 kg ha$^{-1}$.

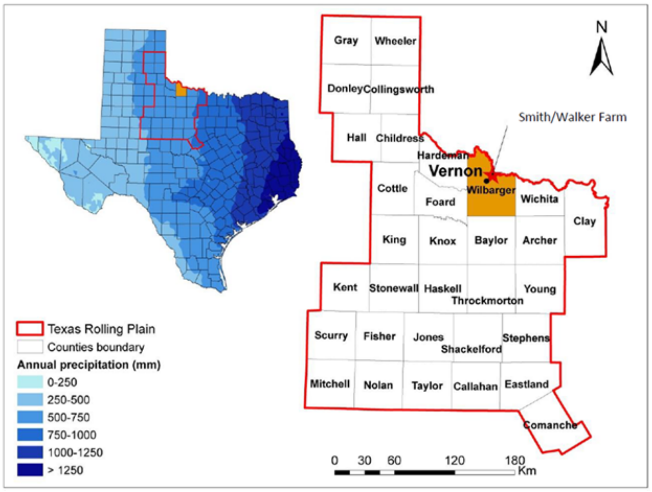

**Figure 2-1 Map of Texas outlining the area of interest, Texas Rolling Plains, in red and depicting annual precipitation gradient across the state. Image courtesy of Dr. Ale, Geospatial Hydrology, Texas A&M AgriLife Research and Extension Center, Vernon, TX.**

Cover crops were planted in early June each year, shortly after wheat harvest. Each single species was managed as a cover crop terminated 55-75 days after planting (DAP) and as a double crop harvested for grain (75-120 DAP). The mixture was managed as a cover crop terminated early (55-75 DAP), late (75-90 DAP) or harvested as a hay crop (75-120 DAP, Table 2-2). These treatments were compared to a wheat-fallow control. All cover crops as well as the late terminated mixture were chemically terminated using the herbicide glyphosate or dicamba. Single species double crops were harvested based on their time to maturity. The hay mixture treatment was harvested as a double crop with all biomass removed and only stubble remaining. Winter wheat was planted in early November each year and drill- seeded at a rate of 67 kg ha$^{-1}$. In 2016,

**Table 2-1 Mixture cover crop species and seeding rates.**

| Cover crop species | Scientific Name | Seeding rate (kg ha$^{-1}$) |
|---|---|---|
| Cowpea | *Vigna unguiculata* | 7.28 |
| Mung bean | *Vigna radiata* | 5.60 |
| Guar | *Cyamopsis tetragonoloba* | 3.92 |
| Foxtail millet | *Setaria italica* | 2.24 |
| Pearl millet | *Pennisetum glaucum* | 3.36 |
| Hegari sorghum | *Sorghum bicolor* | 5.60 |

**Table 2-2 Summer cover crop treatments, seeding rates and respective termination times for 2017 and 2018 seasons.**

| Summer Crop | Seeding Rate (kg ha$^{-1}$) | Termination/Harvest Date (2017, 2018) |
|---|---|---|
| Fallow (W-F) | -- | -- |
| Cowpea Cover (CP-C) | 28 | August 10, 2017, August 24, 2018 |
| Cowpea Double (CP-D) | 28 | October 30, 2017, October 10, 2018 |
| Mung bean Cover (MB-C) | 25 | August 10, 2017, August 24, 2018 |
| Mung bean Double (MB-D) | 25 | September 14, 2017, September 19, 2018 |
| Mixture Early Termination (M-E) | 25 | August 10, 2017, August 24, 2018 |
| Mixture Late Termination (M-L) | 25 | September 12, 2017, October 5, 2018 |
| Mixture Hay (M-H) | 25 | September 14, 2017, September 28, 2018 |

nitrogen fertilizer (UAN, 28-0-0) was broadcast at a rate of 36.4 kg N ha$^{-1}$. In 2017 no fertilizer was applied in order to evaluate the effects of legume and mixture covers on soil nitrogen values. Broadleaf weeds were controlled throughout the year with XtendiMax (dicamba) herbicide at 0.38 kg ha$^{-1}$.

Soil samples were collected around termination times of cover crops and critical stages of wheat growth. In 2017, one soil sampling occurred prior to wheat planting. In

2018 samples were collected around early and late termination of cover crops and mixtures, and once two weeks after wheat was planted. In 2019, soils were collected during the reproductive state of wheat growth. Soils were collected from each treatment plot using a 5-cm diameter hand auger. Samples were randomly collected within each plot at 0-10 and 10-20 cm depths and composited for analysis. For phospholipid fatty acid analysis (PLFA), soils were placed in bags and stored on ice in the field until samples were shipped for analysis later that day. The remainder of soil was oven dried at 60°C for at least three days.

### *2.2.2. Routine soil analysis*

Soil samples were collected at the October 2017 sampling date from 0-10 and 10-20 cm depths and composited before drying for 72 hours at 60°C. Afterwards, a subsample from each plot was passed through a 2-mm sieve and sent to the Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory in College Station, TX. A Mehlich III extraction (Mehlich, 1984) was performed for P, K, Ca, Mg, S and Na analysis using Inductively Coupled Plasma. Soil water pH was evaluated using the method from (Schofield and Taylor, 1955) where soils were placed in a 1:2 soil:water solution for 30 minutes. Afterwards, pH was read using a hydrogen selective electrode. Soil electrical conductivity (EC) was determined from the method by (Rhoades, 1982) where the previously mentioned solution was used and samples were measured as µmho/cm by a conductivity probe. Nitrate-nitrogen ($NO_3^-$-N) was determined using the methods of Keeney and Nelson (1982) and Kachurina, et al.

(2000). At least 2 grams of soil was reacted in a 10:1 KCl: soil solution where 1 N KCl is used. The soil solution was then shaken for 5 minutes at 200 oscillations per minute (opm) and filtered using a Whatman No. 2 filter. The amount of nitrate was determined via the reduction of nitrate to nitrite using a cadmium column and measured using spectrophotometry (Keeney and Nelson, 1982). All above-mentioned measurements were reported a dry soil basis.

### *2.2.3. Phospholipid fatty acid analysis*

At each sampling date, soils from the 0-10 cm depth at each plot were collected and homogenized and a roughly 100 g subsample was bagged, transported on ice and sent to Ward Laboratories, Inc. (Kearney, NE) for phospholipid fatty acid analysis (PLFA). Upon arrival, soils were kept frozen until analysis. A detailed outline of the methodology is provided by Teague et al. (2019). Briefly, 4 g of soil was reacted with a dichloromethane (DCM):methanol (MeOH):citrate buffer solution followed by a DCM and NaCl solution to extract total lipids. The organic fraction was separated and further reacted with DCM:MeOH before being dissolved in DCM and stored. In a silica gel column containing DCM, total lipids were then separated into classes by washing samples with DCM, acetone and methanol to remove neutral, glycolipid and phospholipid classes, respectively.

Via methanolysis, fatty acid methyl esters (FAMEs) were created from the neutral and phospholipid fractions. Samples were transferred to a gas chromatograph (GC) vial and analyzed on a Varian 3900GC using a CP-8400 autosampler and flame ionization detector (FID). Signature biomarker peaks were identified against known standards and abundance was determined from the area under peaks, as compared to a 19:0 FAME standard (Teague et al., 2019).

PLFA biomarkers were categorized by the following microbial classes used in determination of microbial population biomass present in each soil sample: total microbial, total bacterial, total fungal (to include arbuscular mycorrhizal fungi) and arbuscular mycorrhizal fungi. Microbial biomass data was reported in ng $g^{-1}$ soil.

### *2.2.4. Mineralizable carbon*

Mineralizable carbon values were obtained using a modified version of the flush of carbon dioxide from soils after rewetting from Franzluebbers et al. (2000) as outlined in Franzluebbers (2016). Soil samples from 0-10 and 10-20 cm depths at each sampling date were oven dried at 60°C for at least 72 hours. Samples were then passed through a 4.75-mm sieve where large pieces of debris were removed. From there, approximately 100 g of soil was weighed into a volumetric glass jar and the volume of soil was recorded. Values for weight and volume were then used to calculate a 50% water-filled pore space (WFPS) for each soil sample.

For the microcosm setup, 1 L glass mason jars were used to hold the volumetric jar containing soil, alkali trap and humidity vial. For the alkali trap, 10 mL of a 1 M NaOH solution was pipetted into a glass jar and a vial containing 10 mL of deionized water was placed in the microcosm in order to sustain humidity during the incubation period (Franzluebbers et al., 2000). Prior to incubation, each soil sample was rewetted up to 50% WFPS using deionized water. A layering method was used to apply water in thirds throughout the soil to assure complete capillary rewetting of the samples. From here, the rewetted soils were placed in the microcosm along with the alkali trap and humidity vial, the jar was sealed tight and all jars were placed in an incubator at 25°C for 72 hours.

After the incubation period, alkali traps were removed from the microcosms and capped until subsequent titration. The alkali solution was transferred to a 50 mL beaker, with 2 mL of 1.5 M $BaCl_2$ and 1 drop of phenolphthalein indicator. A small stir bar was placed in the beaker to allow adequate mixing during titration. Amended from the method described by Franzluebbers (2016): a 0.5 N HCl solution was used to titrate the alkali solution instead of a 1 N HCl solution as the 0.5 N provided greater sensitivity in detection of differences for these samples (unpublished data). Titrations were performed using a digital burette for acid volumes recorded to two decimal places. Titrations ended once the pink color was no longer visible (however the solution may still be white), indicating the end point was reached. The amount of acid used to titrate each sample was recorded and used in the following equation to calculate the carbon dioxide evolved:

$$CO_2\text{-C (mg kg}^{-1}\text{ soil)} = (\text{mL}_{(\text{blank})} - \text{mL}_{(\text{sample})}) \times \text{N} \times \text{M/S}$$

N being the normality of the acid solution (in this experiment, 0.5 N), M being the conversion of carbon mass from $\text{cmolc}_c$ to g (6000), and S being the weight of soil in grams (~100 g).

### *2.2.5. Statistical analysis*

Analysis of variance (ANOVA) was used to determine treatment, depth and date effects of a randomized complete block design (PROC GLIMMIX, SAS 9.4, 2013). For PLFA, only treatment and date effects were evaluated in an ANOVA as samples were only collected at the 0-10 cm depth. For all tested parameters, treatment was the main effect while replication was the random variable. Means differences were determined using Fisher's least significant differences (LSD) with an alpha level of 0.05.

## 2.3. Results

### *2.3.1. Soil chemical analyses*

After three years of cover crops in rotation, a one-time soil chemical analysis for samples taken October 2017 prior to wheat planting showed no treatment effects for soil nutrients at both sampled depths, with the exception of $NO_3^-$-N at the surface (Table 2-3, Figure 2-3**)**. For most properties, there was a depth effect but no depth by treatment interaction. Averages across all treatments are presented by depth in Table 2-4. Soil pH

**Table 2-3 ANOVA p-value results testing significance of treatment and depth on soil physiochemical properties at October 2017 sampling. (*) Indicate differences at $p < 0.05$ while (**) indicate differences at $p < 0.01$.**

| Model Effects | pH | EC | $NO_3^-$-N | P | K | Ca | Mg | S | Na |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | 0.752 | 0.312 | 0.181 | 0.843 | 0.623 | 0.403 | 0.396 | 0.519 | 0.689 |
| Depth | 0.016 * | 0.145 | 0.038 * | < 0.001 ** | 0.275 | < 0.001 ** | < 0.001 ** | 0.025 * | < 0.001 ** |
| Treatment*Depth | 0.288 | 0.823 | 0.010** | 0.218 | 0.762 | 0.053 | 0.443 | 0.332 | 0.511 |

was slightly acidic ranging from 5.79 to 5.95, with higher values in the subsurface depth. Higher concentrations of Melich P were seen in the 0-10 cm depth, while Ca, Mg and Na values were greater at lower depths.

No treatment interaction occurred for the macronutrient P (Table 2-3), however there was a depth effect (Figure 2-2). Most treatments had significantly greater Melich P values at the surface depth while others, with the exception of M-L, had numerically greater P values.

**Table 2-4 Average values of soil physiochemical properties from October 2017 reported by depth. Letters indicate differences between depths. Values with (*) indicate significant differences ($p < 0.05$) seen between treatments at the corresponding depth.**

| Depth | pH | EC | $NO_3^-$-N | P | K | Ca | Mg | S | Na |
|---|---|---|---|---|---|---|---|---|---|
| cm | | µmhos cm$^{-1}$ | ---------- | ---------- | ---------- | mg kg$^{-1}$ | ---------- | ---------- | ---------- |
| 0-10 | 5.79 b | 78.54 | 7.10* a | 30.37 a | 145.32 | 340.56 b | 147.31 b | 6.20 a | 0.51 b |
| 10-20 | 5.95 a | 70.83 | 5.70 b | 18.53 b | 151.27 | 664.27 a | 201.57 a | 5.78 b | 4.19 a |

Nitrate was the only nutrient presenting a treatment by depth interaction during the October 2017 sampling (Table 2-3). Differences between depths occurred for W-F ($p = 0.036$) and CP-D ($p = 0.034$). Statistical differences across treatments were only seen at the 0-10 cm depth ($p = 0.024$). Treatment $NO_3^-$-N concentrations at the surface depth ranged from 3.60 to 10.81 mg kg$^{-1}$ soil. Here, CP-C, CP-D, and M-E had significantly greater $NO_3^-$-N concentrations than mix hay (M-H) and numerically greater values than all other treatments (Figure 2-3). Nitrate for W-F fell in the middle of treatment values at 6.36 mg kg$^{-1}$ $NO_3^-$-N.

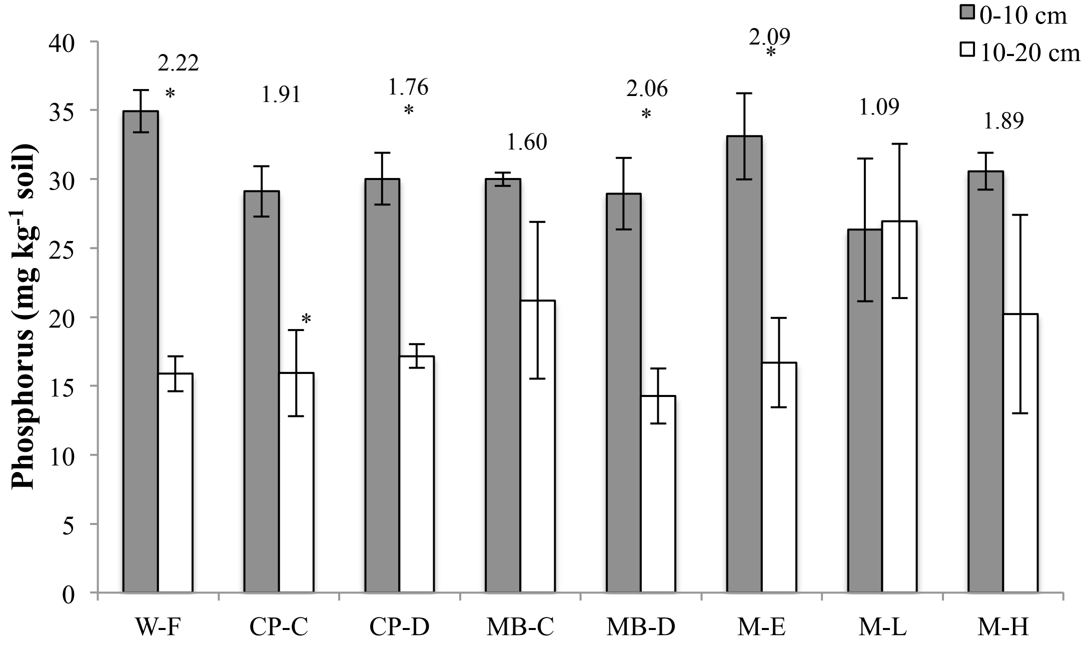

**Figure 2-2 Phosphorus concentrations by treatment at 0-10 and 10-20 cm sampling depths from October 2017. (*) Indicate significant difference ($p < 0.05$) of values between depths. Numerical values indicate the ratio of phosphorus at 0-10 to 10-20 cm. Error bars represent standard error of mean.**

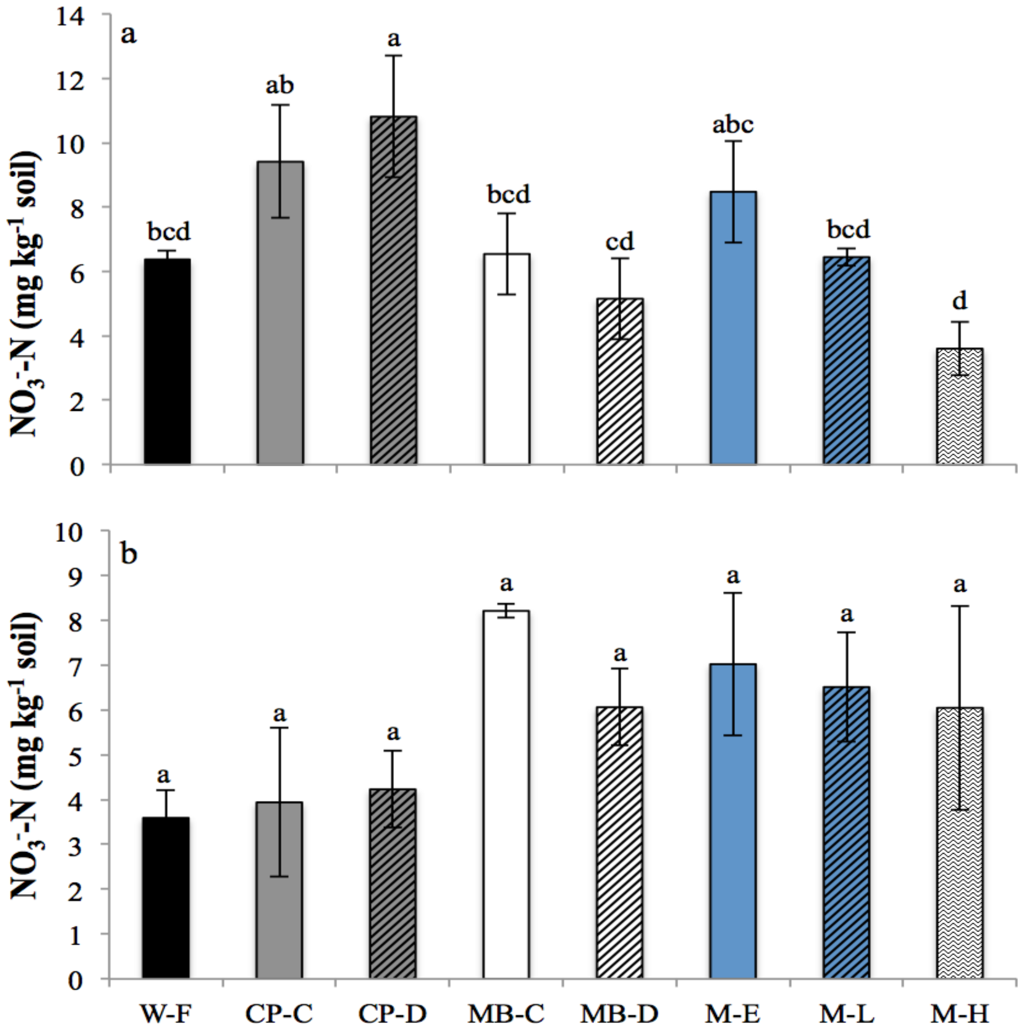

**Figure 2-3 Nitrate-nitrogen concentrations from October 2017 as impacted by cover crop treatments at 0-10 (a) and 10-20 (b) cm depths. Different lower case letters indicate significance ($p < 0.05$) between treatments. Note difference in scale between depths. Error bars represent standard error of mean.**

### *2.3.2. Phospholipid fatty acid analyses*

Phospholipid fatty acid analysis shows cover crops had an impact on microbial biomass at critical points throughout the year (Table 2-5). Overall, cover and double crops resulted in numerically greater total soil microbial biomass compared to W-F.

Significant findings were seen during August 2018 (p = 0.026), when temperatures and lack of precipitation resulted in stressful environmental conditions. Here, all cover and double crop treatments with the exception of M-H had significantly greater microbial biomass than W-F. Legume double crop treatments had 2.5 to 3.1 times greater microbial biomass and the later terminated mixture had 4.1 times more biomass compared to W-F. It is also important to note that double crops trended higher compared to cover crop treatments. Although no treatment effects were seen through the remainder of sampling times for total microbial biomass, intensified treatments generally trended higher compared to W-F. Similar findings were seen with the bacterial biomass analysis.

Although the fungal biomass analysis did not present statistical differences at the August 2018 sampling, trends were noted. Here, legume as well as mixture cover and double crop treatments saw 5.4 to almost 26 times higher fungal biomass values compared to W-F during the summer sampling period. Statistical differences were noted at the November 2018 sampling shortly after wheat planting where all intensified treatments, with the exception of CP-C, displayed 1.2 to 3.8 times more fungal biomass compared to W-F (p = 0.016).

When looking at specific fungal groups, cover crops affected arbuscular mycorrhizal fungi shortly after wheat planting. Here, single species double crops and cover crop mixtures had 6.2 to 8.0 times significantly greater AMF biomass compared to W-F (p = 0.008). Early terminated legume cover crops and M-H trended higher than W-F.

**Table 2-5 Effect of treatment on select soil microbial biomass as determined by phospholipid fatty acid analysis at all sampling dates. Lower case letters indicate significant differences ($p < 0.05$) at the corresponding sampling date. All biomass values are reported on a ng $g^{-1}$ basis.**

| | Total | | | | | Bacteria | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Oct 2017 | Aug 2018 | Oct 2018 | Nov 2018 | Apr 2019 | Oct 2017 | Aug 2018 | Oct 2018 | Nov 2018 | Apr 2019 |
| W-F | 679 | **662 d** | 661 | 1660 | 1499 | 319 | **197 d** | 220 | 546 | 592 |
| CP-C | 984 | **1412 cb** | 776 | 1513 | 2032 | 449 | **440 bc** | 266 | 627 | 682 |
| CP-D | 1504 | **1652 abc** | 643 | 2309 | 2128 | 620 | **455 bc** | 216 | 971 | 925 |
| MB-C | 790 | **1371 bc** | 1098 | 1628 | 1845 | 399 | **441 bc** | 414 | 746 | 688 |
| MB-D | 1046 | **2068 ab** | 1323 | 2334 | 1729 | 519 | **602 abc** | 474 | 976 | 713 |
| M-E | 804 | **2467 ab** | 1066 | 2927 | 1926 | 398 | **824 ab** | 434 | 1375 | 820 |
| M-L | 1605 | **2722 a** | 1217 | 2120 | 2037 | 696 | **922 a** | 509 | 1013 | 758 |
| M-H | 1337 | **1072 cd** | 1865 | 1908 | 1743 | 633 | **327 cd** | 586 | 858 | 669 |
| p value | 0.476 | **0.026*** | 0.283 | 0.260 | 0.960 | 0.595 | **0.043*** | 0.304 | 0.079 | 0.910 |

| | Fungi | | | | | AMF | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Oct 2017 | Aug 2018 | Oct 2018 | Nov 2018 | Apr 2019 | Oct 2017 | Aug 2018 | Oct 2018 | Nov 2018 | Apr 2019 |
| W-F | 68 | 12 | 22 | **108** | 169 | 10 | 0 | 2 | **10 d** | 37 |
| CP-C | 131 | 165 | 40 | **64** | 166 | 23 | 108 | 13 | **18 cd** | 30 |
| CP-D | 189 | 115 | 17 | **147** | 251 | 32 | 35 | 3 | **60 abc** | 69 |
| MB-C | 106 | 127 | 83 | **129** | 121 | 22 | 40 | 16 | **28 d** | 30 |
| MB-D | 137 | 217 | 111 | **212** | 99 | 25 | 35 | 19 | **70 ab** | 22 |
| M-E | 101 | 235 | 50 | **409** | 170 | 23 | 48 | 6 | **123 a** | 37 |
| M-L | 313 | 317 | 100 | **243** | 130 | 48 | 62 | 24 | **78 ab** | 47 |
| M-H | 162 | 66 | 210 | **170** | 133 | 36 | 15 | 29 | **51 bcd** | 34 |
| p value | 0.637 | 0.187 | 0.336 | **0.016*** | 0.853 | 0.705 | 0.599 | 0.704 | **0.008*** | 0.740 |

### *2.3.3. Mineralizable carbon*

At the first sampling date prior to wheat planting in 2017, summer crops had no impact on mineralizable C at either sampling depth (Figure 2-4). For the continuation of sampling periods, this remained at the 0-10 cm depth. Throughout the summer and winter growing seasons there was an overall increase in mineralizable C values, possibly due to availability of cover crop residues and plant roots from wheat growth. At this depth, rotations that included a double crop or later terminated cover crops (M-L) generally trended higher in mineralizable C compared to wheat-fallow. In the subsurface samples, lower mineralizable C values were seen throughout sampling periods, likely due to less rooting density and nutrients for microbial activity. Treatment effects were seen in August 2018 and November 2018. In August 2018, mixtures cover crop treatments had significantly greater mineralizable C values ($p = 0.014$) than wheat-fallow. Cowpea and mungbean and M-H treatments had numerically greater mineralizable C values compared to W-F. Similar trends can be seen at the 0-10 cm depth for this sampling period. At this point in the year, intervals of low precipitation and high temperatures can lead to periods of stress for plants and microbes. Significant differences were also seen at the November 2018 sampling ($p = 0.005$), a few weeks after wheat planting. Here, most treatments receiving a summer cover had significantly greater mineralizable C values compared to W-F and the remaining treatments trended higher than W-F. Mixtures and double crops seemed to result in more mineralizable C.

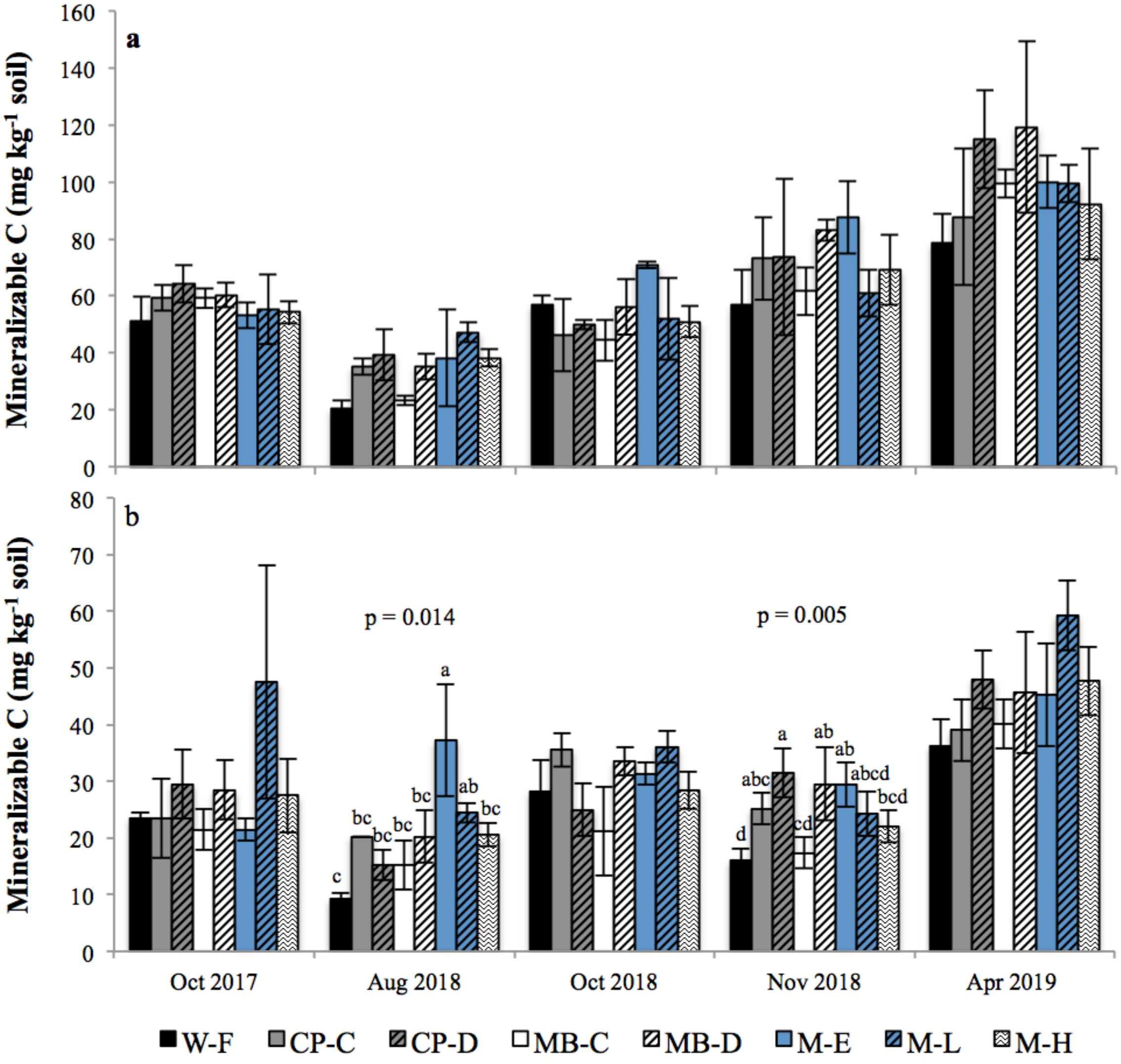

**Figure 2-4 Mineralizable C values as affected by cover and double crop treatments at 0-10 (a) and 10-20 (b) cm depths at all sampling dates. Lowercase letters indicate significant differences ($p < 0.05$) between treatments at that sampling date. Corresponding p-values are reported. Note difference in scales between depths. Error bars represent standard error of mean.**

## 2.4. Discussion

### *2.4.1. Soil chemical properties*

In general, implementing cover and double crops into rotation had no effect on key soil nutrients. This can be expected as it may take several years to decades for concentrations of these nutrients to accumulate to levels that are significantly greater than those in the control treatment. Phosphorus values were higher in the surface depth, likely due to greater presence of microbes, particularly mycorrhizal fungal associations, which are known to increase plant available phosphorus. In contrast, greater values were seen for Ca, Mg, and Na at the subsurface depth, potentially due to leaching through the soil profile over time.

As the adoption of no-till becomes more widespread, there are growing concerns of P stratification with higher concentrations near the soil surface, which increases the potential for P losses to surface runoff and subsequent downstream impacts. Ratio values are presented to look at the concentration of P in the surface sampling depth compared to the sub-surface depth. Though no statistical differences arise for the ratios between treatments, there is a trend of lower ratio values for cover and double-cropped treatments compared to the wheat-fallow control, which sees over two times more P in the upper depth. This may indicate that early cover and double cropping in a no-till system can alleviate P stratification throughout the soil profile.

Nitrate in W-F plots may be greater than some cover and double crop treatments due to natural cover during the fallow period and mineralization of this and wheat residue over time. Lower values for M-L and MB-D may be attributed to the type of crop (i.e., cereal versus legume; Rosecrance et al., 2000) and timing of crop termination and therefore release of nitrogen to the soil (Sievers and Cook, 2017). With a later termination of these crops, mineralization and release of $NO_3^-$ to the soil will be delayed compared to the previously terminated cover crops and M-E. M-H values were lowest right before wheat planting, likely due to removal of biomass and therefore low residue buildup and N supply.

### *2.4.2. Phospholipid fatty acid analysis*

Intensification of a wheat-fallow system in a dryland environment did have a positive impact on soil microbial populations by increasing the microbial biomass during critical periods throughout the year. By including a cover or double crop into rotation, the fallow period was eliminated throughout the summer and microbial populations were maintained compared to a system where fallow saw a decrease in microbial biomass. Similar findings have been seen across the country where cover crop incorporation into production systems increases soil microbial biomass (Rankoth et al., 2018; Finney et al., 2017). This could be attributed to cover and double crops providing fresh inputs from existing roots versus decomposed residues found in a fallow system (Calderón et al., 2016). In general, there seemed to be no statistical differences between the choice of a single species legume or a mixture crop, however, mixtures trended higher in microbial biomass. This may be attributed to higher herbage values of mixture treatments (Figure A-1) compared to legumes, allowing for additional resources available to microbes. More importantly, double cropping seemed to result in greater microbial biomass throughout sampling dates, indicating the importance of extending the growing time of the summer crop to provide continued resources to soil microbes while reducing the fallow period.

Additionally, it was seen that AMF biomass was typically higher in intensified treatments compared to a fallow system. Thompson (1990, 1994) cited a ‘long-fallow disorder’ where mycorrhizal propagules can decrease in the presence of fallow ultimately impacting mycorrhizal colonization and benefits to the roots of subsequent crops. Here, there is an indication that maintaining a fallow period for multiple years results in cumulative declines in AMF biomass, as seen by results for August 2018, where all cover and double crops were in the same growth stage and in November 2018 where the only difference between treatments is the length of fallow between wheat crops. By the time wheat was planted, double crop and mixture cover crop treatments presented greater AMF biomass by at least 80% compared to W-F. Similar findings were noted by Somenahally et al. (2018), where AMF biomass was 30-70% higher during wheat growth in plots with a cowpea cover crop treatment as compared to plots with a summer fallow. Legume cover cropping and haying of biomass in the M-H plots did show higher values than W-F but were lower compared to double crops and mixture cover crops. Eliminating ground cover early on or removing residues from returning to the soil can negatively impact microbial communities during the summer fallow months. Increasing crop diversity in cover crop mixtures has been shown to produce greater herbage (Wortman et al., 2012) and may balance early termination of these treatments.

Maintaining fungal communities and biomass in agricultural soils can in turn favor C sequestration and formation of soil aggregates (Jastrow et al., 2007).

No differences were seen between treatments in any of the microbial biomass categories toward the end of wheat growth in April 2019. Similar findings were observed after one year of cover crops in rotation by Calderón et al. (2016), who concluded there might be short-term effects due to cover crop planting. Although there may seem to be short-term effects over a growing season, our observations indicate cumulative effects are present especially at the August 2018 sampling, when all cover and double crops are the same age. Keeping a living cover crop during the traditional fallow period may provide continued resources for soil microbes (Kaspar and Singer, 2011), which in turn can benefit nutrient cycling for subsequent crops (Bowles et al., 2017; Acosta-Martinez et al., 2007; Mbuthia et al., 2015).

### *2.4.3. Mineralizable carbon*

Over time in the top surface sampling, mineralizable C values generally increased across treatments. This may be attributed to the increase in cover crop residues (Ghimire et al., 2019) and wheat root production providing carbon resources to soil microorganisms (Franzluebbers et al., 1994). Although there were no significant differences between treatments at each sampling date, those with double crop or later terminated cover crops (M-L) tended to have higher mineralizable C values compared to W-F. This is also observed at the 10-20 cm depths. As noted before, at the August 2018 sampling date, all crops were the same age so any differences noted are likely due to a cumulative effect from early years of intensification. MB-C values were generally similar to or below those of W-F at the 0-10 cm depth. This may be attributed to mung bean being a short season legume with a lower C:N ratio compared to cowpea. Therefore upon termination, the crop is readily mineralized and residues may not be available as long compared to those from cowpea.

Culman et al. (2013) noted that crop rotation most influenced C mineralization which agrees with Hurriso et al. (2016) who reported that cover cropping has more influence on C mineralization compared to permanganate oxidizable carbon (POxC). This supports findings in the current study where more significant results and general trends of intensified treatments were present for mineralizable C compared to POxC (section 3.3.1). It was unexpected to have no significant findings between intensified treatments and W-F in the 0-10 cm depths, where most microbial activity takes place and changes due to management practices are typically seen. Franzluebbers et al. (2000) proposed that semi-arid climates with sporadic precipitation events could result in lower flushes of $CO_2$ in shorter versus longer incubation periods due to more rapid decomposition of residues.

Significant differences for mineralizable C were found in the 10-20 cm depth at two critical time periods: August 2018 when production systems were stressed under hot and dry environmental conditions and November 2018 when wheat planting occurred. During August 2018, mixture cover crop plots were significantly greater than W-F and cowpea and mung bean plots trended higher. Again, at this point in time, all intensified treatments had crops in the same growth stage, therefore differences arising between mixtures and single species as well as the fallow control can be a cumulative effect from years of intensification. As Somenahally et al. (2018) observed legacy effects of microbial biomass from the incorporation of cover crops into a wheat-fallow system; here we demonstrate there may also be legacy effects in microbial activity and therefore nutrient cycling. Additionally, at the 0-10 cm depth double crops had numerically greater C mineralization than cover crops, which had greater rates than W-F plots. This indicates that during phases of stress, these plots display additional microbial activity possibly helping soil microbes be more resilient compared to those with fallow periods. At both depths, the fallow treatment displayed low mineralizable C values showing that during fallow little to no C inputs detrimentally impact microbial activity (Ghimire et al., 2019).

In November 2018, most treatments had numerically greater mineralizable C values at the 10-20 cm depth than W-F with cowpea and cover crop mixtures trending highest. Again, this implies that cover or double cropping can provide consistent resources to microbes whereas the presence of fallow periods impact cycling of nutrients and soil health during critical stages of wheat development. By reducing fallow in a wheat monocropping system, microbial biomass C and therefore mineralizable C increase (Franzluebbers et al., 1994)- both benefits to building soil health. Additionally, it is shown that double cropping can provide similar soil health benefits as cover cropping.

## 2.5. Conclusion

The establishment of a summer crop in a winter wheat-fallow monocropping production system results in continuous resource inputs to soil microbes via plant residues and roots, resulting in increased microbial biomass and activity. These changes in turn can benefit nutrient cycling processes carried out by soil microbial populations. Specifically, these increases were noted during periods of stressful environmental conditions, indicating that intensification of summer fallow can sustain soil microbial populations until the following cash crop. Additionally, maintaining a cover crop for a longer period of time can result in a double crop, which not only provides an economic opportunity for producers but also added benefits to soil health. Cover cropping and particularly double cropping should be encouraged in NT semi-arid production systems in the Texas Rolling Plains region as an early means to maintain soil microbial dynamics and build soil health.

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# 3. SOIL CARBON AND NITROGEN DYNAMICS IN RECENTLY INTENSIFIED DRYLAND WINTER WHEAT SYSTEMS

## 3.1. Introduction

Soils are the largest terrestrial pool of C, with an estimated 4,000 Pg C down to a 2 m depth (Lal, 2015). Although soils are major reservoirs of organic C, many land use practices can result in significant transfers of soil C to the atmosphere and hydrosphere. To minimize the potential for soil C losses, tillage and other agriculture practices that cause soil disturbance throughout the year are being reconsidered and alternatives that include less soil turnover are being recommended. When minimizing tillage and plowing in agroecosystems, soil aggregation is increased (Six et al., 2000) thereby physically protecting organic C from mineralization and release as $CO_2$. These practices also keep crop residues on the surface, resulting in greater C and N incorporation into soils when tillage is not used (Chen et al., 2014). In semi-arid regions where biomass production and fast mineralization create impediments, the greater issue in regards to carbon sequestration is not necessarily the quality of the residues (in regards to C, N and other nutrients), but the quantity of plant inputs to continue the process of sequestration (Lal, 2004).

The use of fallow plays a role in soil degradation as bare soil without plant cover leads to loss of topsoil and nutrients to wind and water erosion. In place of fallow, cover crops provide additional plant and root biomass favoring the persistence and potentially the accrual of organic C in soils (Lal, 2015; Lehman et al., 2015). It is estimated that when using cover crops or a combination of both no tillage and cover cropping, rates of soil C sequestration can be 0.32 Mg C $ha^{-1}$ $yr^{-1}$ and 0.4-0.8 Pg C $yr^{-1}$ for global arable lands, respectively (Lal, 2015; Poeplau and Don, 2015). Since dryland soils cover approximately 47% of the terrestrial surface (Lal, 2004) and store 646 Pg of organic C to 2 m (equivalent to 32% of the global soil organic C pool) (Plaza et al., 2018), it is important to develop and apply agricultural land use practices that will contribute to the persistence and sustainability of this key C reservoir.

Various analytical procedures have been developed to characterize and quantify the components of the soil C pool, such as total carbon (TC), soil organic carbon (SOC), particulate organic carbon (POC), and microbial biomass carbon (MBC). However, these fractions can pose limitations. MBC can be expensive (Culman et al., 2012) and time consuming to measure. Though SOC is among the most important indicators of soil health (Lehman et al., 2015), its fractions are slow to build up and change over time due to management (Weil et al., 2003); they may not be ideal predictors of how new changes in management practices are affecting C dynamics in agricultural systems. Of soil health indicators, mineralizable C and permanganate oxidizable C (POxC) can be used as complementary tests for the relationship of C dynamics in a cover cropped system (Hurisso et al., 2016). These tests also correspond to biological activity in soils and therefore can infer how changes in management impact soil microbes (Franzluebbers, 2016; Weil et al., 2003) and soil health.

In the 1990s a method was proposed by Blair et al. (1995) and later modified by Weil et al. (2003) to quantify the “active” C fraction of soils via permanganate oxidizable carbon. A previous study by Culman et al. (2012) concluded that POxC was most related to SOC, however when examining particulate organic C (POC) pools, POxC was more related to those decomposed POC fractions. This in turn can indicate to scientists the potential for carbon sequestration of treatments based on POxC results (Culman et al., 2012). Additionally, the authors concluded that POxC was proportionally the most responsive C fraction to changes in management, such as cover cropping- a useful indicator for C changes in early intensification of wheat systems.

Numerous studies have been done using the POxC indicator to quantify active C fractions in agricultural systems (Culman et al., 2012; Hurisso et al., 2016; Jagadamma et al., 2019; Roper et al., 2017), yet most of these are in regions outside of the southern United States. Morrow et al. (2016) scored POxC highest out of other indicators and encouraged its use in soil health assessment in the Pacific Northwest. However, few studies using POxC have recently been underway near the region of interest in the southern Great Plains (Burke et al., 2019; Thapa et al., 2018) for the purpose of evaluating soil health.

Soil aggregation is not only key in soil physical structure; maintenance of aggregates plays an important role in C sequestration. As proposed by Six et al. (2000), reduced tillage lessens disturbance of macroaggregates and allows for the formation of microaggregates where mechanisms for C stabilization and sequestration occur. However, changes in tillage alone may not promote C sequestration and the use of cover crops is recommended (Blanco-Canqui et al., 2011; Conceição et al., 2013; Veenstra et al., 2007). Particularly in dryland agriculture, reducing the fallow period and accumulating residues at the soil surface has been related to increases in macroaggregation and C buildup (Shaver et al., 2003).

In addition, a reduction in tillage can increase the extent and continuity of mycorrhizal hyphae networks, which play a crucial role in aggregate formation through their fine hyphae networks and secretions allowing binding of soil matter (Jastrow et al., 2007). Cover cropping can also improve aggregation through rooting, secretion of exudates, and impacts to soil microbial communities which also play a role in aggregation (Lal, 2015).

Many studies concerning soil C examine only the top 30 cm of the soil profile although most soil carbon is stored below this depth (Syswerda et al., 2011). Of the 67 experiments examined in a meta-analysis by West and Post (2002), only two studies sampled soils below 30 cm. This lack of deep soil sampling may be due to the assumption that most C and nutrient dynamics are in the top portions of the soil profile (Richter and Billings, 2015) even though these lower portions of soils may also change with management practices (Fontaine et al., 2007) and over longer periods of time. Studies should therefore look deeper than 20 or 30 cm to gain a better understanding of C cycling in the deeper subsoil layers and the potential these depths hold for carbon storage.

Though the C cycle can be seen as its own entity and cycling scheme, N has an intimate role in C sequestration in soils. For example, legumes are N-fixing plants and add plant and microbe available N to the soil. These crops can be incorporated into cropping systems to mitigate the imbalance of C and N supplies necessary for C sequestration (Lal, 2004; Lal, 2015; Stockmann et al., 2013; Unger and Blanco-Canqui, 2012). In a global analysis, it was found that integration of grasses and cereals held the greatest potential for C sequestration in soils (Mathew et al., 2017). However, in mixtures, the role of legumes may prove beneficial by providing N for grasses to take up and immobilize (Blanco-Canqui et al., 2015; Lehman et al., 2015), while balancing the C:N ratio. It has also been noted that summer legumes in particular produce high biomass, an important point for creating residue on soil surfaces (Mansoer et al., 1997; Blanco-Canqui et al., 2011), although their lower C:N ratios (Kaspar and Singer, 2011) along with increased summer temperatures may lead to fast decomposition of residue, potentially negatively impacting inputs to the soil. Therefore, a look at how legumes and mixtures containing grasses will affect C and N abundances will give insight into potential summer cover crops for use in the Texas Rolling Plains.

The objectives of this study were to: (i) investigate the use of the recent soil health indicator, POxC, in a semi-arid dryland system to measure changes in soil active carbon levels due to cover crop incorporation, (ii) quantify the change in C and N concentrations in soils shortly after intensification of a wheat-fallow system and (iii) determine the impact of cover and double cropping on aggregate associated C and N concentrations. An analysis of C and N concentrations within aggregates can give insight to how a NT and cover crop management system in a semi-arid environment may influence C sequestration. We hypothesized that POxC would be a sensitive indicator to changes in active C due to cover and double cropping and that intensification of a wheat-fallow system would increase active C values as well as soil organic C and total N throughout the soil profile.

## 3.2. Materials and Methods

### *3.2.1. Field description and soil sampling*

A two-year study was conducted at the Texas A&M AgriLife Research and Extension Center at Vernon (34.09N, 99.37W) in a dryland winter wheat experimental plot. The system had been under NT wheat-fallow management since 2001. The study was initiated during the summer of 2015 when single species and mixture cover crops were incorporated into rotation: cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata*), and a mixture consisting of grasses (foxtail millet (*Setaria italica*); pearl millet (*Pennisetum glaucum*); hegari sorghum (*Sorghum bicolor*)) and legumes (cowpea; mung bean; guar (*Cyamopsis tetragonoloba*)). The area receives 30-year average annual rainfall of 710 mm and the soil is classified as a Miles fine sandy loam (fine-loamy, mixed, superactive, thermic Typic Paleustalf). The experiment was set as a randomized complete block design of eight treatments with four replicates per treatment.

Shortly after wheat harvest, cover crops were planted in June where single species were managed as a cover crop and as a double crop harvested for grain. Mixtures were managed as a cover crop terminated early or late and as a double crop harvested as a hay crop. All seven treatments were compared to a wheat-fallow control (Table 2-2). Cover crops were terminated using glyphosate or dicamba. Xtendimax (dicamba) was used for broadleaf weed control between crops. Winter wheat was seeded at 67 kg $ha^{-1}$ in early November of each year. Nitrogen (UAN, 28-0-0) was applied 36.4 kg N $ha^{-1}$ in 2016 prior to wheat planting, however nitrogen was not applied in 2017 to study the impact of legumes versus mixtures on soil nitrogen values.

At each sampling date, soils were randomly taken at 0-10 and 10-20 cm depths from each treatment plot and composited for analysis. A subsample from each composite was taken and air-dried for active C (POxC) analysis. The remaining soil was oven dried at 60°C for at least three days. During sampling in November 2018, a hydraulic auger with a 5-cm diameter was used to retrieve soil samples down to 90 cm. Four cores were extracted from each plot and sectioned in five depth increments (0-10, 10-20, 20-30, 30-60, and 60-90 cm) used for chemical and physical analysis.

### *3.2.2. Permanganate oxidizable carbon*

The method for active carbon or permanganate oxidizable carbon (POxC) originally from Blair et al. (1995) was modified by Weil et al. (2003) and outlined in Culman et al. (2017). First, a 0.2 M potassium permanganate ($KMnO_4$) stock solution was made and adjusted to a pH of 7.2 using 0.1 M NaOH. The solution was mixed and brought to volume with calcium chloride ($CaCl_2$) to enhance flocculation of particulate matter in solution and avoid interference in spectrophotometer readings. Four standards (0.005, 0.01, 0.015, 0.02 M) were prepared in 10 mL volumes from the stock solution. Standards were prepared fresh each day samples were run to ensure the most accurate standard curve readings. Air-dried soils were weighed to 2.50 (± 0.02) g into a 50-mL centrifuge tube. To the centrifuge tube containing soil, 18 mL of deionized water and 2 mL of 0.2 M $KMnO_4$ were added, tubes were quickly capped and immediately set on a shaker at about 240 oscillations per minute for exactly two minutes.

As recommended by Culman et al. (2017), batches of no more than 10 samples were run at one time to ensure shaking and settling times were followed. Following two minutes of shaking, samples were gently inverted by hand to remove soil particles from the side of each tube. They were uncapped and placed in the dark for 10 minutes to allow settling. After exactly 10 minutes- so further reaction time was avoided- 0.5 mL of reaction sample was pipetted into another 50 mL centrifuge tube filled with 49.5 mL of deionized water.

From here, 200 μL of each sample dilution was loaded onto a clear 96-well plate. In addition, four blanks of deionized water and triplicates of each standard were included. To ensure accurate readings, three replicate plates were run for each sample. Samples were analyzed at 550 nm on an Epoch Microplate Spectrophotometer (BioTek, VT, USA) using the BioTek Gen5 software (BioTek, VT, USA). Standard curves were constructed for each plate and the amount of active C in each sample was calculated using the following equation:

$$\text{POxC (mg kg}^{-1}\text{ soil)} = [0.02\text{ mol L}^{-1} - (a + b \times \text{Abs})] \times (9000\text{ mg C mol}^{-1}) \times (0.02\text{ L solution Wt}^{-1})$$

Where 0.02 mol $L^{-1}$ is the solution concentration during the reaction, a is the intercept and b is the slope of the standard curve, Abs is absorbance, 9000 mg C $mol^{-1}$ is the amount of C oxidized when converting 1 mole of $KMnO_4$ from $Mn^{+7}$ to $Mn^{+2}$, 0.02 L is the amount of solution reacted with Wt, the weight of the soil.

### *3.2.3. Inorganic nitrogen*

In addition to the routine soil chemical analysis performed in October 2017, inorganic forms of N were measured at each sampling date. The methods by Keeney and Nelson (1982) and Dorich and Nelson (1983) were adapted for extraction of $NO_3^-$ and $NH_4^+$, respectively. Briefly, soils were oven dried at 60°C for 72 hours and passed through a 2-mm sieve where large organic debris was removed. Then, 2 g of homogenized sieved soil was weighed and placed in a 50 mL centrifuge tube. To this, 20 mL of a 1 M KCl solution was added. The centrifuge tubes were capped and placed on a reciprocal shaker (Eberbach Corp., Belleville, MI, USA) and shaken for one hour at 160 oscillations per minute. Tubes were removed and gently shaken by hand to remove any soil particles from sides of tubes. The solution was then poured onto a Whatman No. 42 filter paper; the extract was collected and transferred to 20 mL plastic scintillation vial for storage until analysis. Samples were run on a Skalar San$^{++}$ Continuous Flow Analyzer (Skalar Analytical B.V., Netherlands).

### *3.2.4. Organic carbon and total nitrogen sampling and analysis*

During November 2018, soil samples were collected down to 90 cm using a hydraulic auger (Giddings Machine Co., Inc., Windsor, CO, USA) and separated into the following increments: 0-10, 10-20, 20-30, 30-60, and 60-90 cm. Two cores were collected for each plot, separated by depth and composited. Soils were dried, passed through a 2-mm sieve and homogenized. Subsamples from each were pulverized using a ring and puck mill (Angstrom, Belleville, MI, USA). From these, 30-40 mg was weighed into silver foil capsules and treated for removal of carbonates by acid fumigation following the procedure outlined in Harris et al. (2001). Analysis for percent total organic carbon (%TOC) was performed on a Vario TOC Select Cube (Elementar Analysensystem GmbH, Langenselbold, Germany). An additional 500 mg was weighed for percent total nitrogen (%TN) and was detected using an Elementar VarioMax TC/TN combustion analyzer (Elementar Analysensystem GmbH, Langenselbold, Germany). Two additional cores were taken from each plot, separated by depth and oven dried at 105°C. Average weights of each depth from the two cores were used for bulk density calculations. Bulk density and %TOC measurements were used to calculate SOC stocks (Mg $ha^{-1}$; Wright and Hons, 2005a). TN stocks were calculated using bulk density and %TN.

Additionally, aggregates from 0-10 and 10-20 cm depths were separated into four class sizes: > 2000 μm (large macroaggregates), 2000-250 μm (small macroaggregates), 250-53 μm (microaggregates), and < 53 μm (silt + clay) using a Ro-Tap Sieve Shaker (WS Tyler, Mentor, OH). Each fraction was then analyzed for %TOC as described above.

### *3.2.5. Statistical Analysis*

Treatment effects were evaluated for each test using PROC GLIMMIX (version 9.4, 2013, SAS Institute Inc., Cary, NC). A two-way ANOVA evaluated the effects of treatment and depth on SOC and TN throughout the soil profile for the November 2018 sampling. A three-way ANOVA determined the effects of treatment, date and depth on POxC and inorganic nitrogen values. Means differences were determined using Fisher's LSD at an alpha level of 0.05. Regression analyses were performed for POxC with mineralizable carbon and SOC at both the 0-10 and 10-20 cm depths to determine the relationship between various tests for carbon.

## **3.3. Results**

### *3.3.1. Permanganate oxidizable carbon*

For the test of active carbon using the method of permanganate oxidizable carbon (POxC), treatment, date and depth main effects were observed as well as a date by depth interaction (Table 3-1). Significant differences were not present between treatments at the 0-10 cm depth. In Oct 2017 prior to wheat planting, C-DC and mixture cover crop treatments exhibited greater POxC values compared to W-F by 46.63 to 56.16 mg kg$^{-1}$ soil (Figure 3-1). However, mung bean values remained similar to W-F. Mung bean is a short-season crop harvested at maturity, about 60 DAP and therefore provides a shorter periods of living roots compared to cowpea which matures around 120 DAP. and therefore provides a longer period of living roots. The following year during August 2018, double crop and later terminated mixture plots trended higher in POxC values by

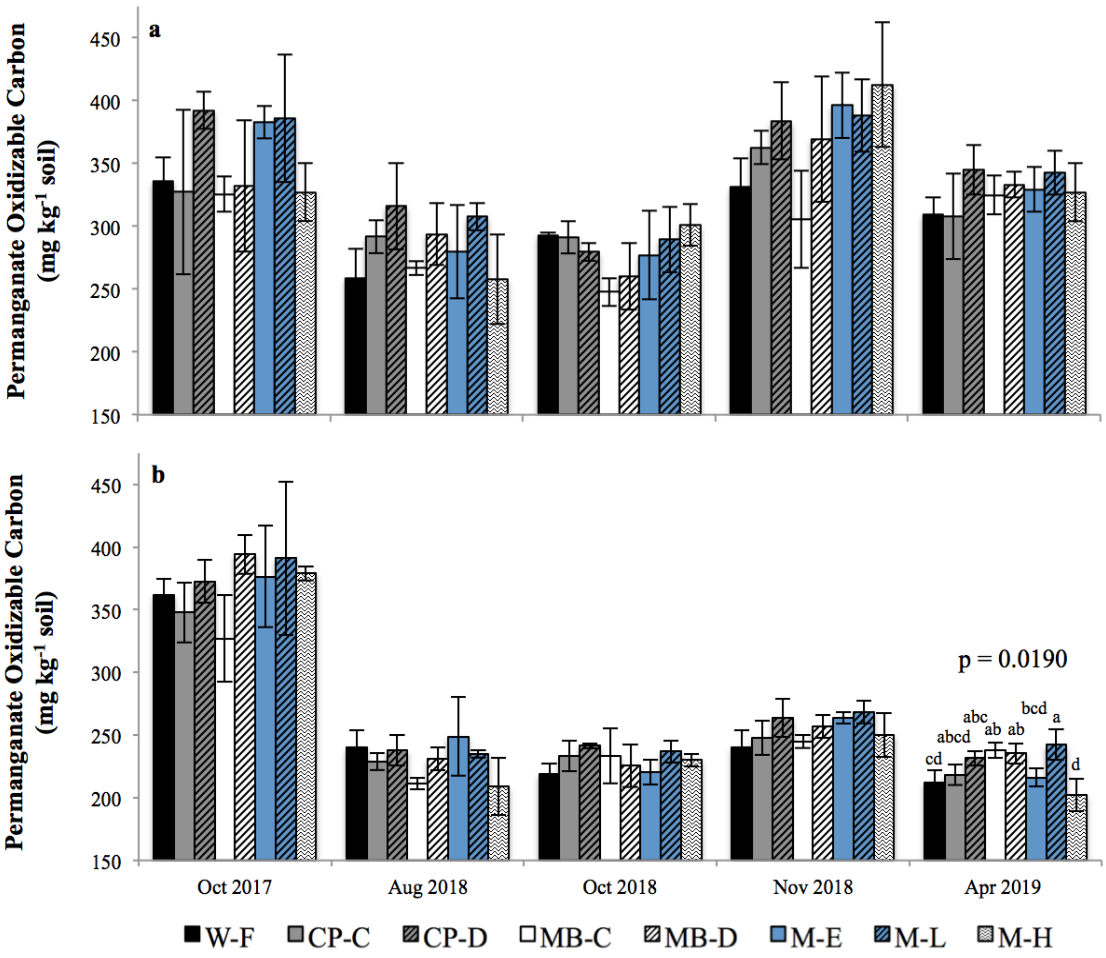

**Figure 3-1 Permanganate oxidizable carbon at 0-10 (a) and 10-20 (b) cm depths as affected by cover crop treatment at all sampling dates. Lower case letters indicate significant differences at $p < 0.05$. Error bars represent standard error of mean.**

35.06 to 57.2 mg kg$^{-1}$ soil compared to W-F. Similar to October 2017, in August 2018 and October 2018, M-H values were comparable to that of W-F. Frequent rainfalls during fall of 2018 may have resulted in less microbial activity and therefore less mineralization of fresh carbon inputs and active carbon values at the surface depth. Shortly after wheat planting most treatments (with the exception of MB-C) trended greater in POxC values compared to W-F by 31.36 to 81.42 mg $kg^{-1}$ soil. Double crop and mixture trends continued throughout the remainder of wheat growth at this depth.

At the subsurface depth, double crops and mixtures trended higher than W-F for the October 2017 sampling. After this sampling, POxC values were lower at all other dates. This may be attributed to differences in summer crop biomass (Figure A-1) and therefore residue and root accumulation in 2017 versus 2018. After wheat reemergence in April 2019, statistical differences between treatments were seen at the 10-20 cm depth. Here, M-L and mung bean treatments had significantly greater POxC values compared to the W-F control by 23.39 to 30.21 mg $kg^{-1}$ soil. All other treatments trended higher than W-F except M-H although these treatments were not statistically different from each other.

**Table 3-1. ANOVA p-value results for POxC, inorganic N, SOC and TN testing for significance of treatment, date and depth.**

| Model Effect | POxC | $NO_3^-$ | $NH_4^+$ | SOC | TN |
|---|---|---|---|---|---|
| Treatment | 0.010 | <0.001 | 0.001 | 0.437 | 0.094 |
| Date | <0.001 | <0.001 | <0.001 | | |
| Depth | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Treatment*Date | 0.955 | <0.001 | 0.459 | | |
| Treatment*Depth | 0.781 | 0.637 | 0.010 | 0.263 | 0.545 |
| Date*Depth | <0.001 | 0.229 | <0.001 | | |
| Trt*Date*Depth | 0.995 | 0.991 | 0.235 | | |

### *3.3.2. Inorganic nitrogen*

Inorganic nitrogen pools, nitrate ($NO_3^-$) and ammonium ($NH_4^+$), were evaluated separately for the effect of summer intensification on these soil values. Both pools saw significant treatment, date and depth main effects (Table 3-1). $NO_3^-$ had a treatment by date interaction while $NH_4^+$ saw significant treatment by depth and date by depth interactions. $NO_3^-$ and $NH_4^+$ concentrations are presented by date and depth in Figure 3-2 and Figure 3-3, respectively. Cover crops significantly influenced $NO_3^-$ concentrations in the top and subsoil depths at two critical periods throughout the year- August and November. At 0-10 cm during August 2018 ($p = 0.034$), the W-F treatment contained significantly greater $NO_3^-$ concentrations compared to CP-C by 18.4 mg $kg^{-1}$ and all mixture treatments by 17.1 up to 23.9 mg $kg^{-1}$, while numerically greater than CP-D and mung bean plots by 3.8 to 13.1 mg $kg^{-1}$. After cover crops were terminated and wheat was planted, W-F plots saw the lowest $NO_3^-$ concentrations at both depths compared to those treatments receiving a summer crop. At 0-10 cm ($p = 0.010$), plots with legume double crops and mixture cover crops contained 1.8 to 2.9 mg $kg^{-1}$ significantly more $NO_3^-$ compared to 1.7 mg $kg^{-1}$ for W-F. In the subsurface sample, similar findings were noted at the November sampling ($p = 0.001$).

When evaluating $NH_4^+$ concentrations, statistically significant differences due to cover or double cropping were seen only at the October 2017 sampling. Here, MB-D comprised significantly greater $NH_4^+$ concentrations than CP-C, MB-C, M-E, M-H and W-F. $NH_4^+$ concentrations at the 10-20 cm depth were overall higher in the October 2017 sampling compared to all other sampling dates. Intensified plots tended to see greater $NH_4^+$ concentrations compared to W-F at the August 2018 sampling. Double crops and mixtures tended to contain more $NH_4^+$ at each sampling date than W-F and legume cover crop treatments.

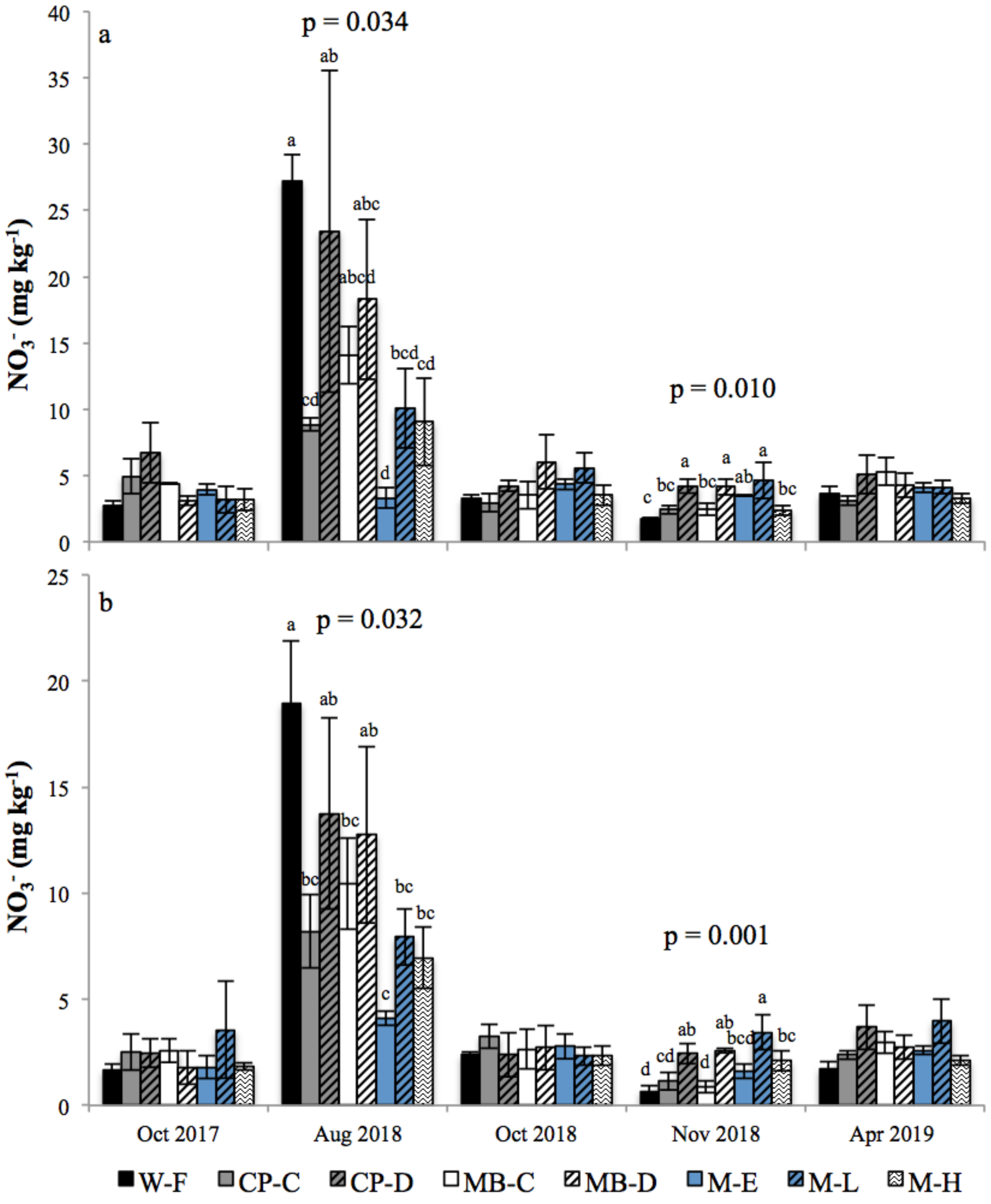

**Figure 3-2** NO$_3^-$ **concentration at 0-10 (a) and 10-20 (b) cm depths as affected by cover crop treatment across all sampling dates. Lower case letters indicate significant differences at** $p < 0.05$**. Note difference in scale between depths. Error bars represent standard error of mean.**

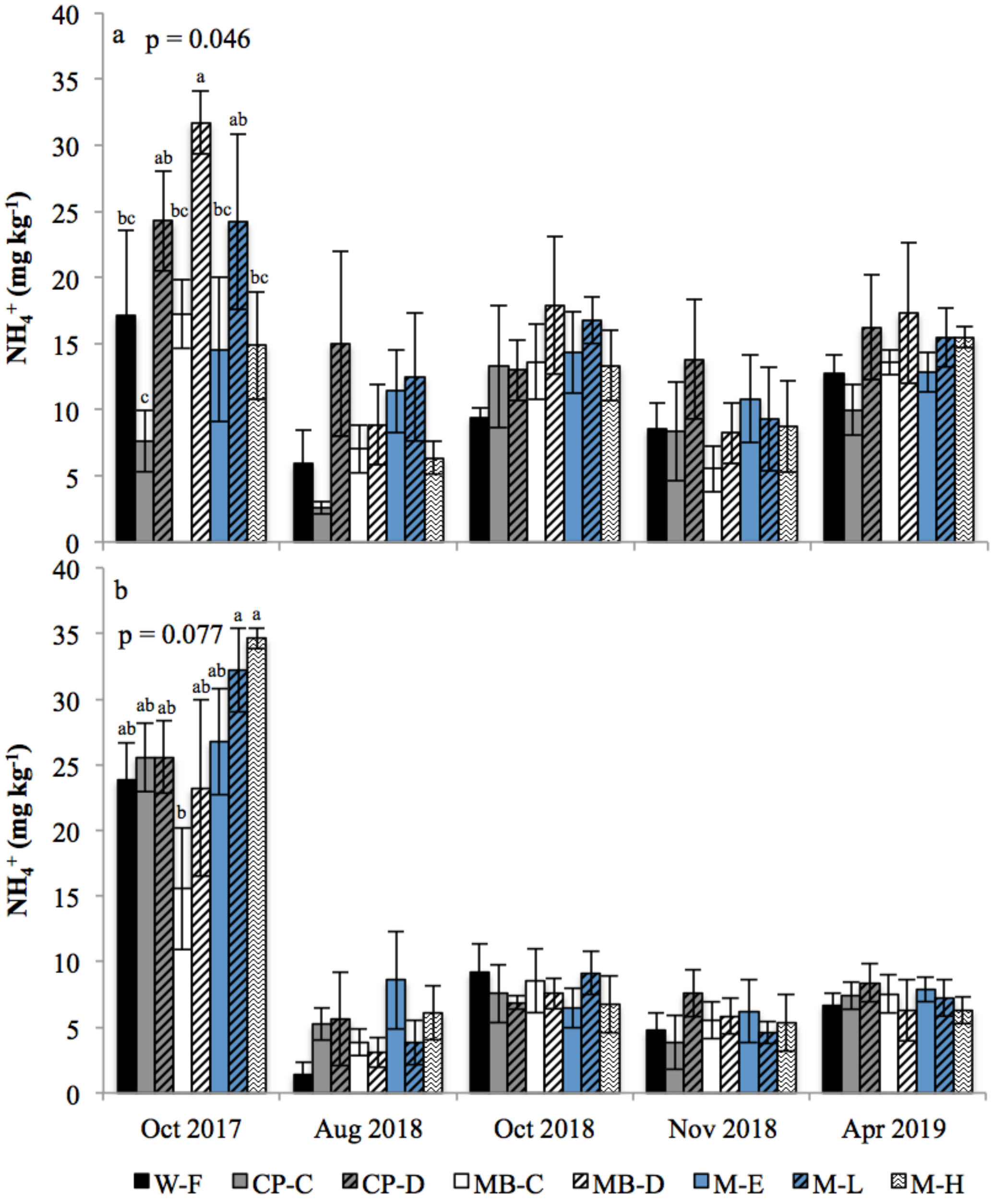

**Figure 3-3 $NH_4^+$ concentration at 0-10 (a) and 10-20 (b) cm depths as affected by cover crop treatment across all sampling dates. Lower case letters indicate significant differences at $p < 0.05$. Note difference in scale between depths. Error bars represent standard error of mean.**

### *3.3.3. Organic carbon and total nitrogen by depth*

Soil organic carbon stocks (Mg $ha^{-1}$) were evaluated down to 90 cm two weeks following wheat planting in November 2018. Upon performing an ANOVA evaluation, treatment was not a significant main effect, although depth was (Table 3-1). Significant differences were seen between treatments at the 20-30 and 60-90 cm increments (Figure 3-4). There was no treatment effect on SOC stocks at all depths when evaluating treatments categorized as either cover cropping or double cropping compared to fallow ($p > 0.212$). Although no statistical differences were found at the 0-10 and 10-20 depths, plots receiving a summer cover trended higher in SOC stocks by 0.15 to 1.63 Mg $ha^{-1}$ at the 0-10 cm depth compared to W-F. Double cropped single species and the late terminated mixture treatments trended higher in SOC stocks compared to wheat fallow at the 10-20 cm depth.

Overall, increases in SOC stocks were seen at the 20-30 cm depth. Here, differences were seen between treatments with MB-D and M-E having significantly greater values by 0.42 and 0.52 Mg $ha^{-1}$, respectively, compared to W-F. The remaining mixture and cowpea treatments trended higher than W-F by 0.14 to 0.40 Mg $ha^{-1}$. Interestingly, significant differences were seen at the lowest sampling depth, 60-90 cm. Here, M-E, M-H, CP-D and W-F are statistically greater than M-L. All values were numerically lower than W-F with the exception of the M-E treatment.

There was no treatment effect for total N (Table 3-1) however, there was a significant depth effect. Results are presented by depth increment in Figure 3-5. No treatment differences were detected at any of the five depths, although p-values were lower for the surface ($p = 0.217$) and subsurface ($p = 0.135$) sampling. At the 0-10 cm

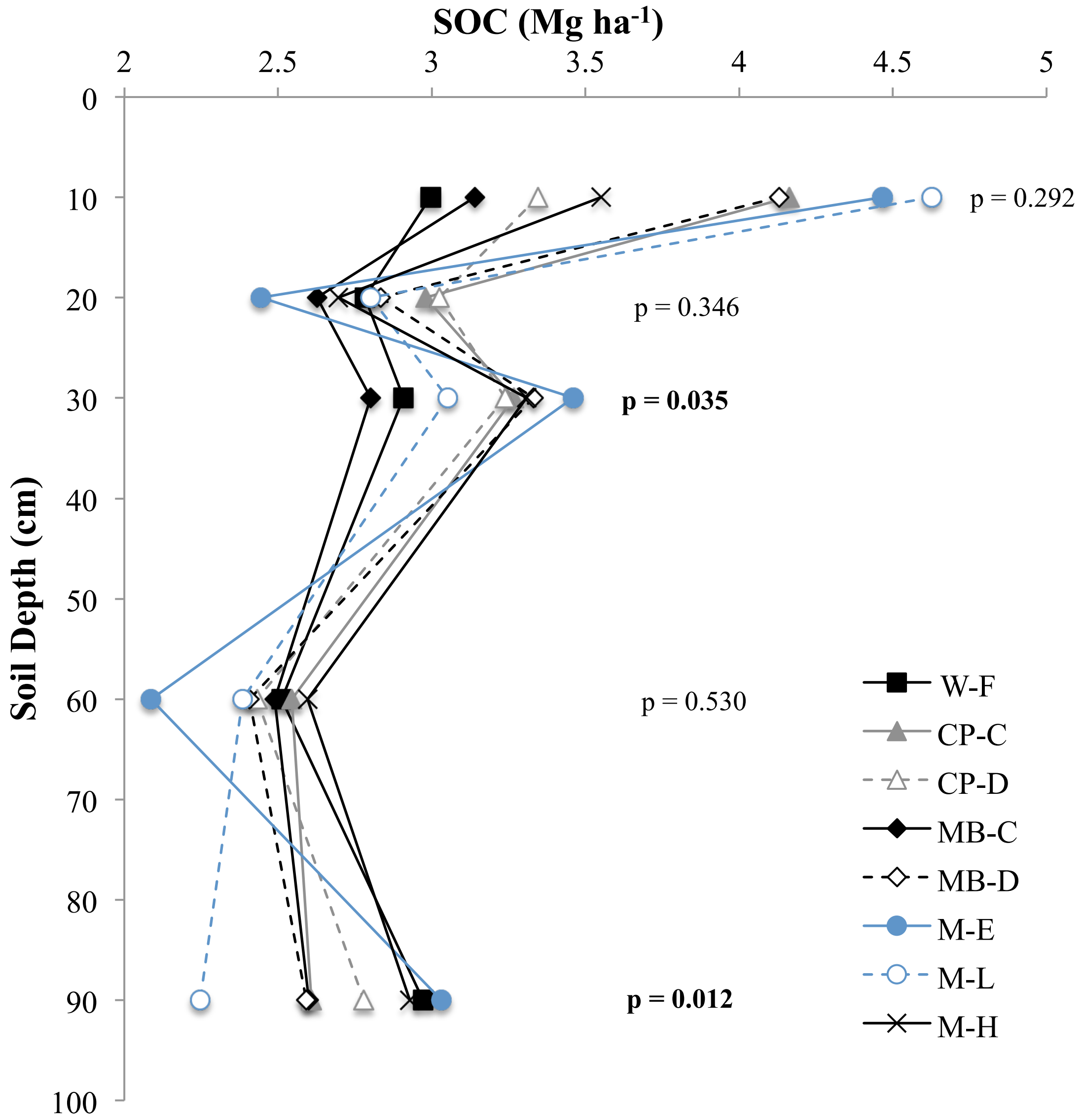

**Figure 3-4 Effect of intensified treatments on SOC stocks down to 90 cm sampled during November 2018. Bold p-values indicate significant differences between treatments at $p < 0.05$.**

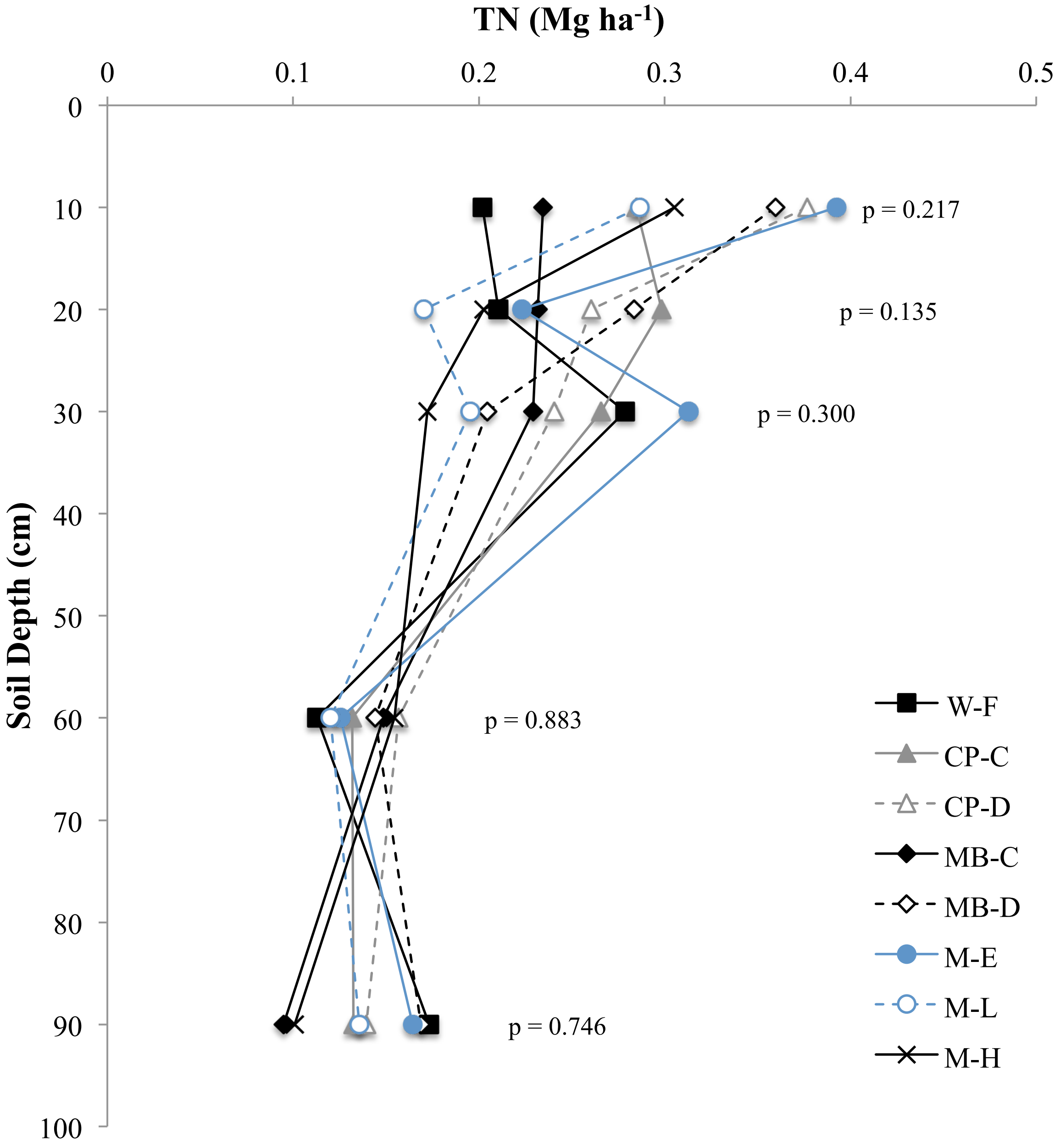

**Figure 3-5 Effect of intensified treatments on soil TN stocks down to 90 cm sampled during November 2018. Bold p-values indicate significant differences between treatments at $p < 0.05$.**

increment, single species double crops, M-H and M-E had numerically more TN compared to legume cover crops and W-F. Here, all intensified treatments had greater TN stocks than W-F by 0.03 to 0.19 Mg ha$^{-1}$. Both cowpea treatments continued to trend higher down to the 30cm depth while TN stocks for MB-D and M-H steadily declined to this depth. In contrast, W-F TN stocks increased from 0.20 to 0.28 Mg ha$^{-1}$ from the surface down to the 30 cm depth. In general, the M-L treatment had numerically lower TN stocks compared to most other treatments throughout all depths.

Soil organic carbon stocks were further evaluated in four aggregate fractions at the 0-10 and 10-20 cm depths (Figure 3-6). A three-way ANOVA found significant aggregate fraction and depth main effects as well as an aggregate fraction by depth interaction (Table 3-2). Overall, greater SOC stocks were found in the aggregate fractions of the surface sampling. In the surface aggregates, significantly more SOC was found in the small macroaggregate class followed by the microaggregate class. Similarly, the greatest SOC stocks in the subsurface depth were in the small macroaggregate fraction, however, followed by the large macroaggregate fraction. Although no significant differences were found among treatments in any aggregate class at both depths, trends can be noted.

When looking at the aggregate classes in the surface sampling, treatments receiving a summer crop generally had greater SOC stocks compared to wheat-fallow. At the subsurface sampling, lower stocks are seen for intensified treatments in the large macroaggregate fraction, however this trend is opposite in the small macroaggregate fraction; when combined, all intensified treatments had higher SOC stocks in the macroaggregate fractions (> 250 μm) in this depth.

Regression analyses (Figures 3-7 and 3-8) showed significant relationships between POxC and both mineralizable carbon and soil organic carbon. Across all sampled dates a linear relationship occurs between mineralizable carbon and POxC where the $R^2$ value is 0.207 (Figure 3-7). A correlation exists between POxC and SOC where $R^2$ is equal to 0.346 (Figure 3-8). This relationship is based on one sampling date as SOC was only collected and analyzed after wheat planting in November 2018.

**Table 3-2 ANOVA p-value results of SOC of aggregate fractions sampled during November 2018 testing for significance of treatment, aggregate fraction and depth.**

| Model Effect | SOC |
|---|---|
| Treatment | 0.026 |
| Aggregate Fraction | <0.001 |
| Depth | <0.001 |
| Treatment*Aggregate Fraction | 0.250 |
| Treatment*Depth | 0.129 |
| Aggregate Fraction*Depth | <0.001 |
| Treatment*Aggregate Fraction*Depth | 0.201 |

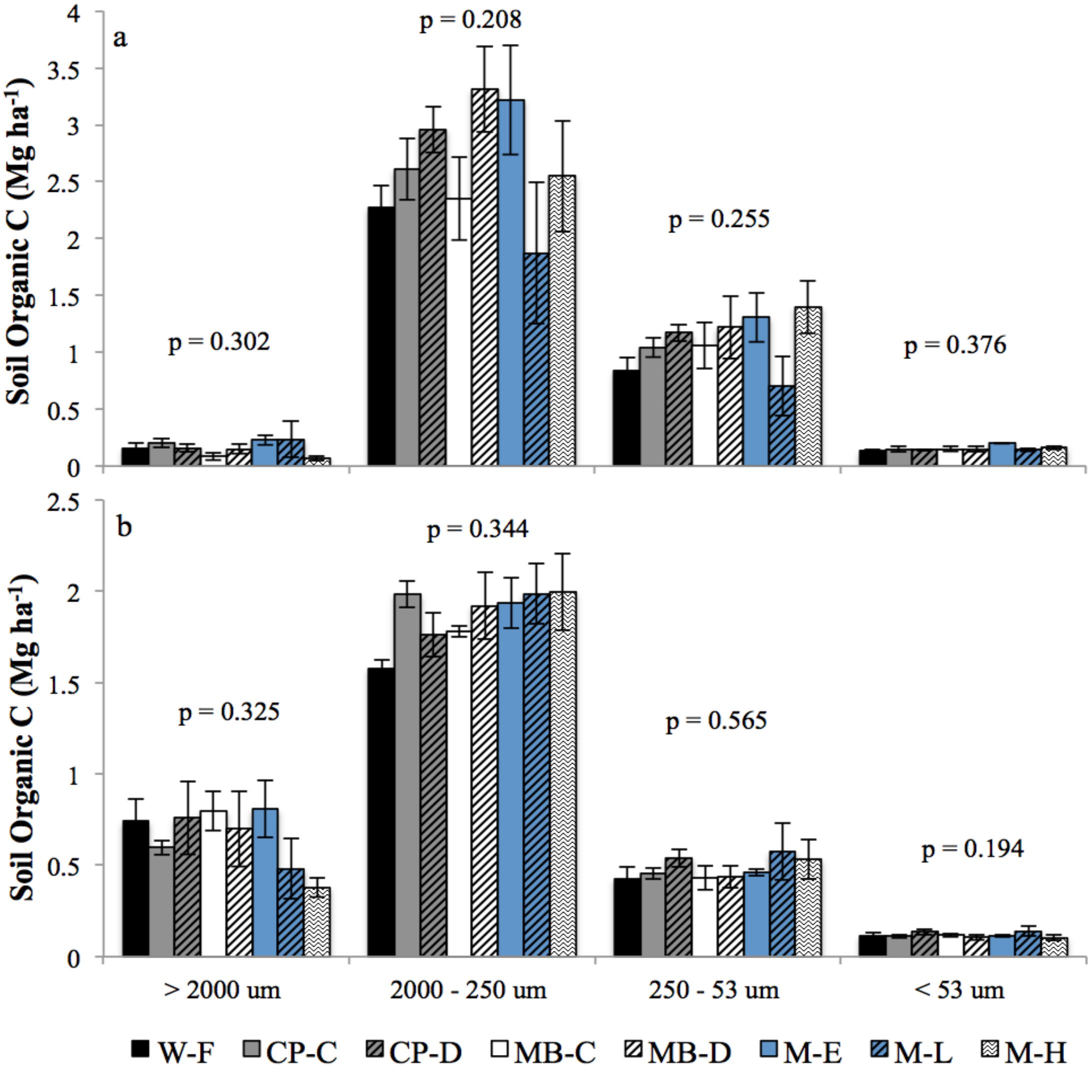

**Figure 3-6 Aggregate SOC stocks sampled during November 2018. Values are presented by aggregate fraction and summer crop treatment at 0-10 (a) and 10-20 (b) cm. Note difference in scale between depths. Error bars represent standard error of mean.**

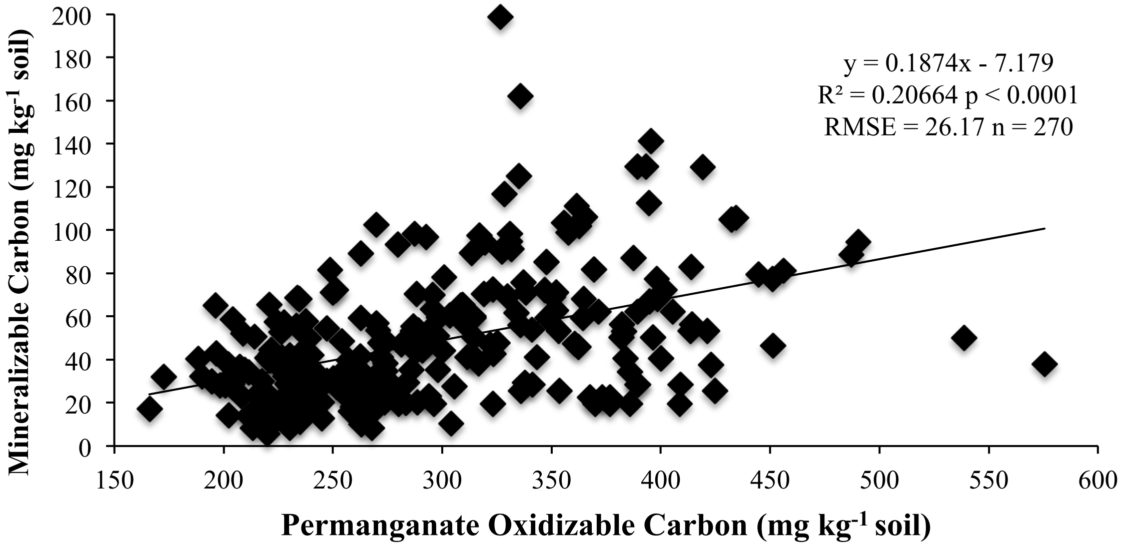

**Figure 3-7 Regression analysis of mineralizable C and permanganate oxidizable carbon across all sampling dates. A linear relationship exists with a coefficient of determination of 0.21. n = 270.**

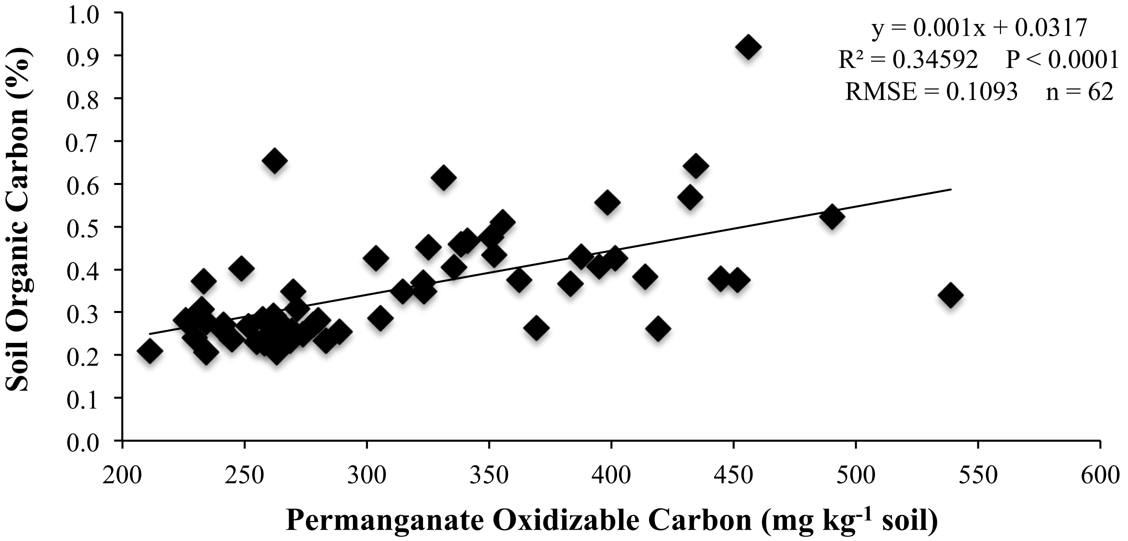

**Figure 3-8 Regression analysis of SOC and permanganate oxidizable carbon from November 2018 samples shortly after wheat planting. A linear relationship exists with a coefficient of determination of 0.35. n = 62.**

## 3.4. Discussion

### *3.4.1. Permanganate oxidizable carbon*

Increased interest into the rapid and easily carried out soil health test for active carbon has led to its use throughout the United States. In this study, POxC was evaluated after both three and four years of cover crops including throughout one season of cover crop and winter wheat growth. No differences between cover crop treatments were seen during any of the five sampling times in the soil surface depth and only once, at wheat reemergence, in the subsurface samples. Overall, higher POxC values were observed across treatments after termination of cover crops in October 2017, likely due to fresh residue returning to the soil. When significant differences were observed in the 10-20 cm depth during active wheat growth, M-L was greater than W-F and M-H. This may indicate that removal of biomass and fallow results in less carbon inputs during wheat production.

The lack of significant differences across treatments as well as sampling dates was surprising since the test for active carbon measures the more labile and sensitive fraction of carbon and has been shown to be influenced by management practices (Weil et al., 2003). However, in a meta-analysis of multiple research studies using POxC, Hurisso et al. (2016) found that tillage practice had more influence on changes in POxC values whereas mineralizable carbon showed more of a response to cover cropping. This may explain why POxC values did not differ significantly between treatments – all under no-till management – throughout the growing seasons but why more differences and consistent trends were seen in mineralizable carbon results.

A study in a semi-arid region of New Mexico found no differences in POxC values between management practices down to 40 cm, including native pasture and tilled cropland (Thapa et al., 2018). Another study in a long-term cotton system in the Texas High Plains found changes in POxC values for treatments throughout the growing season (Burke et al., 2019). Similar results were seen by Ghimire et al. (2019) and in the current study. Additionally, differences were seen between conventionally tilled and no-till plots; however, differences were not seen between the two no-till treatments with various cover crops (mixed species and rye). Both of these studies (Burke et al., 2019; Thapa et al., 2018) were done in the southern part of the United States in semi-arid regions where high temperatures can favor higher mineralization rates of residue. It can take a longer time to see results for SOM buildup between management practices in these environments (Acosta-Martinez et al., 2010; Sainju et al., 2009) and this may also be the case for accumulation of active carbon fractions in newly intensified management systems in semi-arid environments.

When comparing POxC to other measured carbon fractions in this study, a linear relationship was observed between mineralizable carbon and POxC ($R^2$= 0.207). A study evaluating management practices and POxC in Tennessee (Jagadamma et al., 2019) found a strong linear relationship between POxC and SOC ($R^2$ = 0.93) which agrees with findings from Burke et al. (2019) with an $R^2$= 0.75. In the current study a linear relationship was seen between POxC and SOC, however, with a lower $R^2$= 0.35. This lower regression coefficient may be due to these data only being from one sampling date during the study, at the beginning of wheat growth. This does show that POXC can be an indicator for soil health and carbon changes in semi-arid regions, however, may not be able to predict soil management changes early on in implementation of these practices.

### *3.4.2. Inorganic nitrogen*

Cover and double cropping did have a positive effect on inorganic N values throughout the summer fallow and winter growing seasons. Overall higher $NO_3^-$ concentrations seen during August 2018 were likely a result of active N fixation from the presence of legumes either as a single species crop or incorporated into mixtures. Fallow plots during this time saw the greatest $NO_3^-$ concentrations at both depths. At this point soil microbial populations can decompose any wheat residues post-harvest where the $NO_3^-$ will accumulate in the fallow soils while present cover and double crops can take up the available nitrogen. The practice of cover cropping has shown to be beneficial by reducing N losses, particularly through the use of non-legumes (Tonitto et al., 2006; Gabriel et al., 2012). As the season progressed, overall $NO_3^-$ concentrations decreased where it may have been taken up by the maturing cover and double crops (Rosecrance et al., 2000) or lost to the soil and atmosphere, which can occur in the case of fallow periods (Kaspar and Singer, 2011).

Shortly after winter wheat planting, legume double crop and mixture cover crop treatments saw significantly more $NO_3^-$ in the soil compared to the control. Sievers and Cook (2018) found that most N was lost by the legume cover crop hairy vetch within the first few weeks after termination while N from a cereal rye cover was slower to be released. They conclude that when using the legume cover crop, a delay in termination may prove beneficial to reduce leaching of the easily mineralized N of the plant residues. When considering cover crop termination, delaying termination can increase N accumulation in cover crops (Sainju and Singh, 2001; Clark et al., 1997), which can be made available later on for the cash crop. The current study provides support where the delay in removal of the legume double crop resulted in greater soil $NO_3^-$ concentrations by the time winter wheat was planted. In addition, those mixtures with non-legumes saw higher values, likely due to incorporation of a slower decomposing residue (grasses) that help in the immobilization of inorganic nitrogen (Rosecrance et al., 2000; Sievers and Cook, 2018).

$NH_4^+$ concentrations were noticeably greater than those for $NO_3^-$ for almost all sampling dates, particularly in the surface sampling. Although $NH_4^+$ is typically converted to $NO_3^-$ quickly after its production (Mengel et al., 2001), this buildup of $NH_4^+$ in the soil could have occurred due to multiple environmental conditions. The process of nitrification resulting in the production of $NO_3^-$ from $NH_4^+$ can be hindered due to acidic pH of soil, dry soil and high temperature conditions (Mengel et al., 2001), all of which are common in this semi-arid region. By incorporation of a summer crop, particularly a double crop or mixture, adequate nitrogen fixation and residue is delivered to the soil where more $NH_4^+$ is supplied than in a fallow period. Under optimal conditions this source of N provided by a summer crop can be converted via nitrification for cash crop availability as seen during the November 2018 sampling period.

One concern that is often mentioned is in the case of later termination of cover crops, or in this case double cropping; how the availability of N to the cash crop is impacted by the uptake and immobilization in the previous crop. Multiple sources cite concerns that later termination of a cover crop results in untimely immobilization of N from the following cash crop (Dabney et al., 2001; Alonso-Ayuso et al., 2014). The current study otherwise shows that $NO_3^-$, and in general $NH_4^+$ concentrations were greater after wheat planting in treatments that include a legume double crop compared to the wheat-fallow control. In regards to $NO_3^-$, the mixture treated as a double crop (M-H) was not statistically different from W-F at the 0-10 cm depth however, was greater at 10-20 cm potentially due to intensive biomass removal in this treatment. This increase in inorganic N may be due to adequate time between double crop harvest and wheat planting – about 4 to 6 weeks for CP-D and MB-D, respectively- where residues were able to decompose.

### *3.4.3. Organic carbon and total nitrogen dynamics throughout the soil profile*

SOC results indicate that cover cropping can increase C stocks not only at shallower soil depths but also throughout the soil profile. Lal (2015) cited multiple studies throughout the United States where the use of no till and cover cropping increased soil organic C in the upper 20-30 cm of the soil profile. An overall increase in SOC at the 20-30 cm increment may be due to a transition between the A and Bt horizons where an increase in bulk density may impact values. Although statistical differences were found in the 20-30 cm depth increment in the current study, a lack of significant differences above this depth may be due to the duration of cover crop intensification and climate (Chu et al., 2017; Blanco-Canqui et al., 2015). In semi-arid regions, the amount of biomass C can hinder SOC sequestration (Lal, 2004), which can explain a slower change in this C pool due to the typically lower biomass values in a region like the TRP. Yet results are indicative that SOC buildup is occurring as a consequence of intensification of a wheat-fallow system.

Lal (2004) mentions SOC values can increase from legume incorporation because of their contribution of N through biological nitrogen fixation (BNF). This would satisfy the C and N values necessary for adequate C sequestration and can explain why most SOC values of single species legume treatments- cover and double cropped- trended higher than that of wheat-fallow in the two upper depth increments. In both depths, the mung bean cover crop treatment values are similar to or lower than wheat-fallow. The lower C:N ratio of mung bean (Figure A-1) in conjunction with typically lower biomass may result in fast decomposition of the residues and therefore less chance for incorporation into the more stabilized soil fractions. Mixtures on the other hand contain grasses, which have been shown to sequester more C compared to legumes (Mathew et al., 2017; Blanco-Canqui et al., 2013). Wright and Hons (2005b) highlight the importance of C:N quality where crops of higher C:N ratios in their study favored SOC accumulation. A variation in C:N ratios and therefore decomposition rates may explain why mixture cover crop treatments showed higher SOC densities in the upper 10 cm depth.

Notably, significant differences due to cover crop treatments were seen at the 60-90 cm increment ($p = 0.012$). Although most changes in C are expected in the upper most portion of the soil profile (Grandy and Robertson, 2007), the possibility of root inputs at a depth contributing to microbial mineralization of older carbon stores (Stockmann et al., 2013) may explain why most cropped treatments had significantly or numerically lower values compared to the W-F plot. However; root additions, particularly from plant species with deep rooting, can encourage increases in carbon at depths in NT systems (Stavi et al., 2012), yet it is unclear if these inputs could offset the rate of mineralization (Stockmann et al., 2013).

Similar findings can be seen with total N stocks throughout the soil profile where the intensification of a wheat-fallow system with summer crops led to improved N stocks in the upper portions of the profile. Particularly, legume double crops showed greater TN at the topsoil, likely due to a longer duration for N fixation in conjunction with N rich residue (Blanco-Canqui et al., 2012). Figure 3-5 shows a gradual decline in N stocks moving down to the 30 cm depth, noting MB-D declines faster than other treatments. As mung bean is a short-season crop, harvest of this treatment was done before other double crops, which may have lasting effects to adding N back to the soil. Interestingly, W-F has the second highest TN value at the 20-30 cm increment. A study by Wright and Hons (2005b) showed similar SOC and soil organic nitrogen (SON) storage for W-F systems as intensified systems due to N immobilization in wheat residues and a reduction in decomposition. These same experimental sites saw increased microbial biomass and mineralizable C in intensified treatments (Franzluebbers et al., 1994), which can indicate increased activity and mineralization of residues. Although these findings were in the upper portion of the soil, this may explain the shift in TN seen in the current study at the 20-30 cm depth.

When looking more closely at the C pool sizes of aggregate classes, cover and double cropping resulted in greater SOC stocks throughout multiple aggregate classes at both depths. Most SOC is held in the macroaggregate fractions (> 250 um), where majority of C can be sequestered when moving towards a less intensive system, such as NT (Grandy and Robertson, 2007). Macroaggregation also plays an important role in development of stable microaggregates within the macroaggregate fraction (Six et al., 2000). Carbon within the microaggregate fraction has been found to be more stable, an important role in C sequestration (Jastrow et al., 1996). In the current study, most treatments with cover or double crops had numerically greater SOC stocks in the microaggregate fraction, at both sampling depths. Although no significant differences were seen at this point in time, it is likely due to the slow accrual of C through management changes, therefore more time may be needed to see a more positive impact from cover cropping on aggregate C sequestration in this environment. Findings support the hypothesis that incorporating a summer crop will increase C sequestration.

## 3.5. Conclusion

After four years of incorporating cover and double crops into a dryland wheat monocropping system, changes were observed across SOC and total and inorganic N pool sizes. During summer months, cover and double crops were able to take up inorganic forms of N to be made available later for the cash crop; leaving fields fallow may contribute to N loss and a decrease in availability at wheat planting. Not only does summer cropping have an implication for N made available to the cash crop, but environmentally by reducing N loss from the system. SOC and TN showed increasing pool sizes in the upper portions of the soil profile after five years of intensification. Specifically, SOC stocks increased in macroaggregate fractions, indicating a positive effect of cover crops on C sequestration in this dryland practice. Although POxC showed significant relationships with mineralizable C and SOC, longer periods of cover and double cropping may be needed before seeing changes from this indicator in these specific environments. Incorporating mixtures as cover crops or double cropping with single species legumes can be a means to increase SOC and total and inorganic N pools while double cropping can serve as an option for an additional economic opportunity for producers in this region.

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# 4. IMPACTS OF COVER AND DOUBLE CROPPING ON SOIL PHYSICAL FACTORS AND SOIL MOISTURE

## 4.1. Introduction

Semi-arid regions are known for markedly lower and more sporadic precipitation events, therefore making conservation of water resources a top priority for agricultural producers. During the early years of cultivation in the United States Great Plains, tillage was a means to prepare the land for crops while conserving soil water stores (Shaw, 1911). However, it is now known that tillage plays a role in the destruction of aggregates leading to poorer soil structure and erosion while also increasing water loss through via surface evaporation and reduction in infiltration (Reicosky et al., 2011; Hatfield, 2011). While moving away from intensive tillage practices, other conservation agriculture practices have been encouraged in recent years, including the use of cover crops in production rotations.

Cover crops are known to have impacts both above and below the soil surface that result in a positive impact on soil physical factors and water relations. The aggregation process uses organic matter to adhere to soil particles, where the organic matter decomposition slows (Six et al., 2000). During this process under NT, microaggregates will form within macroaggregates, where microaggregates will become stable with slower SOC turnover (Jastrow, 1996; Six et al., 2000) favoring C sequestration. Cover cropping directly and indirectly plays a role in aggregation by providing organic material via the incorporation of aboveground residues, roots and root exudates, while providing habitat for fungal hyphae, soil macro and microfauna which produce binding agents such as polysaccharides (Jastrow, 1996; Lal, 2015). However, mixed responses to incorporation and duration of cover crops on changes in aggregate size distribution have been observed in recent studies (Blanco-Canqui et al., 2011; Lal, 2015). This poses a need to better understand the role cover crops play in soil structure changes across various production and climatic conditions.

A long-time concern about intensifying traditional wheat monocropping systems in the Great Plains stems from the fact that including more plants means using more resources, particularly soil water. Research looking from north to south throughout the Great Plains reveals adoption of these practices can be limited by climatic conditions that either encourage soil water storage or loss from evaporation (Robinson and Nielsen, 2015). As the chance of loss of water stores increases as producers move southward, fallow periods have been recommended to conserve what soil water is present for the subsequent cash crop (Unger and Vigil, 1998; Nielsen et al., 2015). However, studies have shown that residues from cover cropping facilitate soil water storage (Clark et al., 1997; Blanco-Canqui et al., 2011) while cover crops themselves have not impacted soil water for the following crop (Barker et al., 2018).

In the Texas Rolling Plains, as interest in NT increases, so does that of using cover crops in production systems, as these practices can be adopted together with compounding benefits (Blanco-Canqui et al., 2011). The question is whether adopting intensified cropping practices in a NT dryland system will impact soil water content in a way that poses detriments to cash crop yields. The purpose of this study was to evaluate short-term changes in soil physical characteristics, particularly dry aggregate size distribution and water infiltration, as well as water content throughout the cover crop growing season into the early phases of cash crop production. We hypothesize that by intensifying a wheat monocropping system and reducing the fallow period, soil structure and water infiltration will improve. We also hypothesize cover crop treatments will therefore see an increase in soil water content compared to a traditional wheat-fallow system.

## 4.2. Materials and Methods

### *4.2.1. Field description and soil sampling*

In 2001, NT management began on a dryland winter wheat-fallow experimental plot at the Texas A&M AgriLife Research and Extension Center at Vernon (34.09N, 99.37W). The soil type is a Miles fine sandy loam (fine-loamy, mixed, superactive, thermic Typic Paleustalf) and the area has a 30-year average annual rainfall of 710 mm. The study was initiated in 2015 where single species and mixture cover crops were incorporated into rotation: cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata*), and a mixture consisting of 60% grasses (foxtail millet (*Setaria italica*); pearl millet (*Pennisetum glaucum*); hegari sorghum (*Sorghum bicolor*)) and 40% legumes (cowpea; mung bean; guar (*Cyamopsis tetragonoloba*)). Four replicates per treatment were set in a randomized complete block design for a total of 32 plots.

Cover crops were planted shortly after wheat harvest each year and were managed as a cover crop and as a double crop harvested for grain. Mixtures were managed as a cover crop terminated early or late. Additionally, mixtures were evaluated as a double crop harvested as a hay crop. A wheat-fallow control was compared against there seven treatments (Table 2-2). Termination of cover crops was done chemically using glyphosate or dicamba. Broadleaf weeds were controlled between each crop using XtendiMax (dicamba) at 0.38 kg ha$^{-1}$. In early November of each year, winter wheat was seeded at 67 kg ha$^{-1}$. In 2016, N fertilizer (UAN, 28-0-0) was applied at a rate of 36.4 kg N ha$^{-1}$. In 2017, N fertilizer was not applied in order to quantify the effects of legumes and mixtures on soil inorganic N.

From composited soil samples taken during November 2018, subsamples were gently taken and air-dried for mean weight diameter of aggregates (MWDA) and further C and N analyses.

### *4.2.2. Mean weight diameter of aggregates*

During November 2018 sampling, a portion of each composited plot sample was removed and air-dried for at least 96 hours for measurement of MWDA. Aggregates were gently crushed to pass through a 4-mm sieve after which approximately 100 g was weighed out. From here, samples were shaken on a Ro-Tap Sieve Shaker (WS Tyler, Mentor, OH) at precisely 278 oscillations per minute and 150 taps per minute for 30 seconds where they were partitioned into the following aggregate fraction (class) sizes: > 2000 μm (macroaggregates), 2000-250 μm (large microaggregates), 250-53 μm (small microaggregates), and < 53 μm (silt + clay). Soil collected on each sieve was then weighed and recorded. MWDA was calculated using the following equation described by Youker and McGuinness (1957):

$$\sum_{i=1}^{n} \bar{x}_i w_i$$

where $\boldsymbol{\bar{x}_i}$ is the average diameter of the sieve size and $\boldsymbol{w_i}$ is the weight of soil collected on that sieve. Each calculation was then summed to give a single MWDA value for 0-10 and 10-20 cm depths per plot. For the sieve size of > 2000 μm, the average diameter was determined by doubling the size of the sieve diameter for the upper boundary.

### *4.2.3. Water infiltration*

Water infiltration was measured in the field during April 2019 during the reproductive state of wheat using the Cornell Sprinkle Infiltrometer and corresponding method as described in *Field Procedures and Data Analysis for the Cornell Sprinkle Infiltrometer* (van Es and Schindelbeck, 2003). At each plot, a single metal ring was inserted into the soil until the drainage hole was flush to the surface and adjusted so the ring was level to the ground. Wheat was clipped and any large debris was removed within the ring to avoid interference with water flow from the infiltrometer. Adjacent to the ring, a hole was dug large enough for a plastic beaker to be placed for runoff collection. The runoff tube was connected to the ring and positioned in a downward slope to the beaker. Next, the sprinkler was filled to the top with deionized water and capped to maintain the water level.

The sprinkler was placed on the metal ring and once ready, it was uncapped and the initial water height was recorded at the start of the timer. Once runoff occurred the time and water height was again recorded. Thereafter, water height and volume were recorded every 5 minutes for around 45 minutes or until the water flow subsided.

### *4.2.4. Soil volumetric water content*

In December 2017, 5TM soil moisture and temperature sensors with EM50 data loggers (METER Group, Inc., WA, USA) were installed in each plot at 10 and 20 cm depths to measure soil moisture by volumetric water content (VWC; $m^3m^{-3}$). Data collection was set to every 12 hours for two daily readings, which were averaged. Data analysis focused on the period from before cover crop termination in July 2018 until after wheat planting- a critical time period for changes in soil moisture and potential impacts on subsequent crop. For the purpose of this study, only weekly readings will be presented in the results.

### *4.2.5. Statistical analysis*

Data were analyzed using the PROC GLIMMIX procedure in SAS (version 9.4, 2013, SAS Institute Inc., Cary, NC). A two-way ANOVA analyzed the effects of treatment and depth on MWDA. Additionally, a three-way ANOVA was used to determine the effects of treatment, depth and aggregate class on the percent aggregate distribution. Water infiltration was analyzed for treatment differences at each date at the alpha = 0.05 level. Treatment differences for percent aggregate distribution and volumetric water content are discussed at a significance of p = 0.05 and p = 0.10.

## 4.3. Results

### *4.3.1. Mean weight diameter of aggregates and aggregate distribution*

Mean Weight Diameter of Aggregates (MWDA) was measured at the November 2018 sampling period to see how intensifying summer fallow could impact soil structure over time. When treatments were pooled together across depths, there was a treatment effect where all treatment MWDA was significantly greater than M-H. When analyzed for significance among treatments at both the surface and subsurface depths, no differences were seen between treatments (Figure 4-1). There was not a treatment by depth interaction (Table 4-1), however there was a depth effect where MWDA was significantly greater in 10-20 cm compared to 0-10 cm samples, with the exception of M-L where MWDA at either depth was not significantly different from each other. CP-C and mixture cover crop treatments had a numerically greater MWDA compared to W-F in the surface sampling depth. In the subsurface depth, CP-D, Mung bean treatments and M-E had a numerically greater MWDA than W-F. However, no clear trend exists among treatments.

**Table 4-1 ANOVA p-value results for MWDA from November 2018 testing for significance of treatment and depth.**

| Model Effect | MWDA |
|---|---|
| Treatment | 0.025 |
| Depth | <0.001 |
| Treatment*Depth | 0.348 |

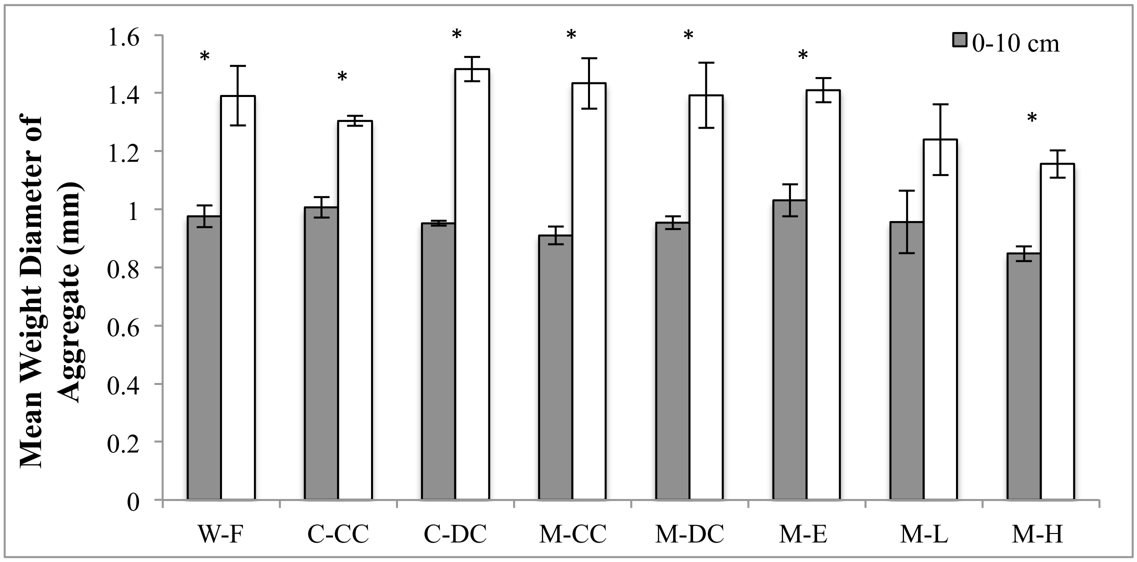

**Figure 4-1 Effect of cover and double cropping on Mean Weight Diameter of Aggregates (MWDA) for 0-10 and 10-20 cm depths sampled during November 2018. No significant differences ($p < 0.05$) were determined between treatments at either depth. (*) Indicate a significant difference ($p < 0.05$) in MWDA values between depths. Error bars represent standard error of mean.**

Aggregates were further evaluated by their percent distribution within the soil sample. When evaluating a three-way ANOVA, there was an aggregate fraction main effect. Treatment by class and depth by class interactions were observed as well as a three-way interaction between treatment, depth and class (Table 4-2). At the 0-10 cm sampling, the small macroaggregate class saw a significantly greater aggregate distribution, followed by the microaggregate, large macroaggregate and silt + clay fractions. The 10-20 cm depth as well saw the significant majority of aggregates in the small macroaggregate class, however the next greatest distribution was in the large macroaggregates.

**Table 4-2 ANOVA p-value results for aggregate distribution from November 2018 testing for significance of treatment, depth and aggregate fraction (class).**

| Effect | Aggregate distribution |
|---|---|
| Treatment | 0.9998 |
| Depth | 0.8200 |
| Aggregate fraction | <0.0001 |
| Treatment*Depth | 0.9998 |
| Treatment*Aggregate fraction | <0.0001 |
| Depth*Aggregate fraction | <0.0001 |
| Treatment*Depth*Aggregate fraction | 0.0018 |

Percent distribution of each aggregate class for both the 0-10 and 10-20 cm depths are presented in Table 4-3. Significant differences at $p < 0.05$ were seen for the small macroaggregate class in the 10-20 cm depth ($p = 0.026$). Here, M-H had significantly greater aggregate distribution than CP-D, MB-C, M-E and W-F, with 7.8 to 13.3% more small macroaggregates. CP-C, MB-D, M-L and M-H had 1.6 to 7.8% more aggregates in this fraction than the wheat-fallow control.

Differences were also seen in fractions of both sampling depths at $p < 0.10$. For the large macroaggregate class in the 10-20 cm depth ($p = 0.058$), somewhat opposite differences from the small macroaggregate class were seen across treatments where CP-D, MB-C, and M-E had significantly greater distribution in the class compared to M-H by 11.8 to 16.0%. In the 0-10 cm depth, significant findings were observed in the large macroaggregate and microaggregate classes. In the large macroaggregate class ($p = 0.055$), M-E and CP-C had significantly greater distribution compared to M-H. In the microaggregate fraction ($p = 0.059$), M-H had a numerically greater percentage compared to all other treatments, with the exception of MB-C.

**Table 4-3 Distributions of soil weight by aggregate fraction (class) and MWDA values from November 2018 at the 0-10 and 10-20 cm depths. Values in bold are significant at $p < 0.05$ and different letters indicate differences among treatments. Italicized values are significant at $p < 0.10$.**

| 0-10 cm Treatment | Large Macroaggregate | Small Macroaggregate | Microaggregate | Silt + Clay | MWDA mm |
|---|---|---|---|---|---|
| | Aggregate Distribution (%) | | | | |
| W-F | *4.794 abc* | 71.000 | *21.821 b* | 2.385 | 0.976 |
| CP-C | *6.484 ab* | 69.135 | *21.948 b* | 2.433 | 1.006 |
| CP-D | *4.598 abc* | 69.084 | *23.782 b* | 2.535 | 0.952 |
| MB-C | *3.041 bc* | 69.381 | *24.956 ab* | 2.622 | 0.910 |
| MB-D | *3.775 bc* | 71.715 | *22.169 b* | 2.341 | 0.954 |
| M-E | *7.932 a* | 67.420 | *21.842 b* | 2.805 | 1.030 |
| M-L | *3.820 bc* | 70.311 | *20.085 b* | 2.157 | 1.045 |
| M-H | *1.909 c* | 66.230 | *29.252 a* | 2.610 | 0.847 |
| p-value | *0.055* | 0.393 | *0.059* | 0.604 | 0.081 |

| 10-20 cm Treatment | Large Macroaggregate | Small Macroaggregate | Microaggregate | Silt + Clay | MWDA mm |
|---|---|---|---|---|---|
| | Aggregate Distribution (%) | | | | |
| W-F | *22.732 ab* | **60.998 bc** | 14.318 | 1.951 | 1.390 |
| CP-C | *17.970 abc* | **66.113 ab** | 14.060 | 1.856 | 1.305 |
| CP-D | *27.839 a* | **55.483 c** | 14.640 | 2.038 | 1.482 |
| MB-C | *24.609 ab* | **59.866 bc** | 13.785 | 1.741 | 1.433 |
| MB-D | *22.248 ab* | **62.580 abc** | 13.393 | 1.778 | 1.392 |
| M-E | *23.653 ab* | **60.360 bc** | 13.992 | 1.996 | 1.410 |
| M-L | *16.013 bc* | **65.163 ab** | 16.639 | 2.185 | 1.239 |
| M-H | *11.821 c* | **68.768 a** | 17.528 | 1.883 | 1.155 |
| p-value | *0.058* | **0.026** | 0.630 | 0.731 | 0.121 |

### *4.3.2. Water infiltration*

In early April 2019, during the reproductive state of wheat, water infiltration rate was measured for all treatments (Figure 4-2). No statistical differences were detected between treatments ($p = 0.251$), however, general trends were found. At this point in time, there were few precipitation events at the beginning of the month and no precipitation in the latter half of March. Therefore, fields saw dry soil conditions at the time of sampling. Overall, all intensified treatments saw greater infiltration rates compared to the wheat-fallow control. Mainly mixture treatments, regardless of being treated as a cover or double crop, saw higher rates by 0.07 to 0.10 cm $min^{-1}$ (70.4 to 103.5%) over wheat-fallow management.

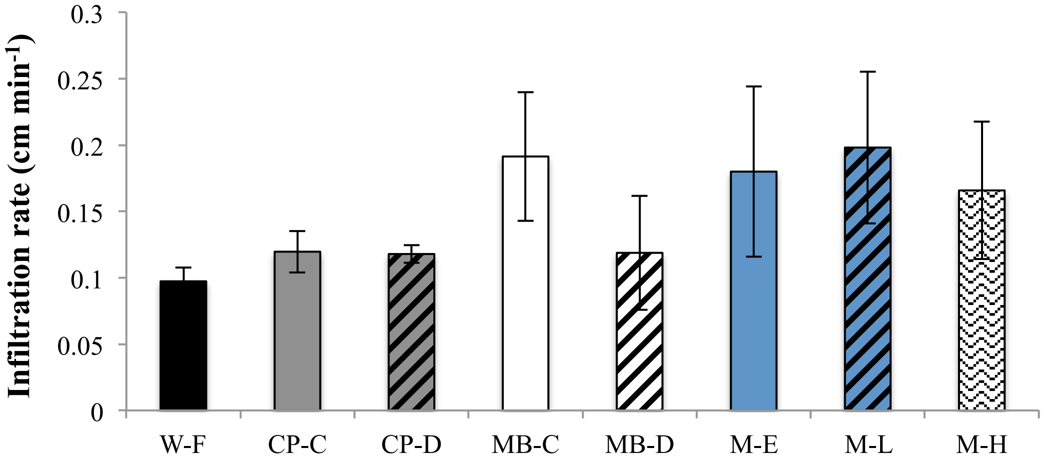

**Figure 4-2 Water infiltration rates during April 2019 as affected by treatment. No statistical differences ($p < 0.05$) were detected among treatments. Error bars represent standard error of mean.**

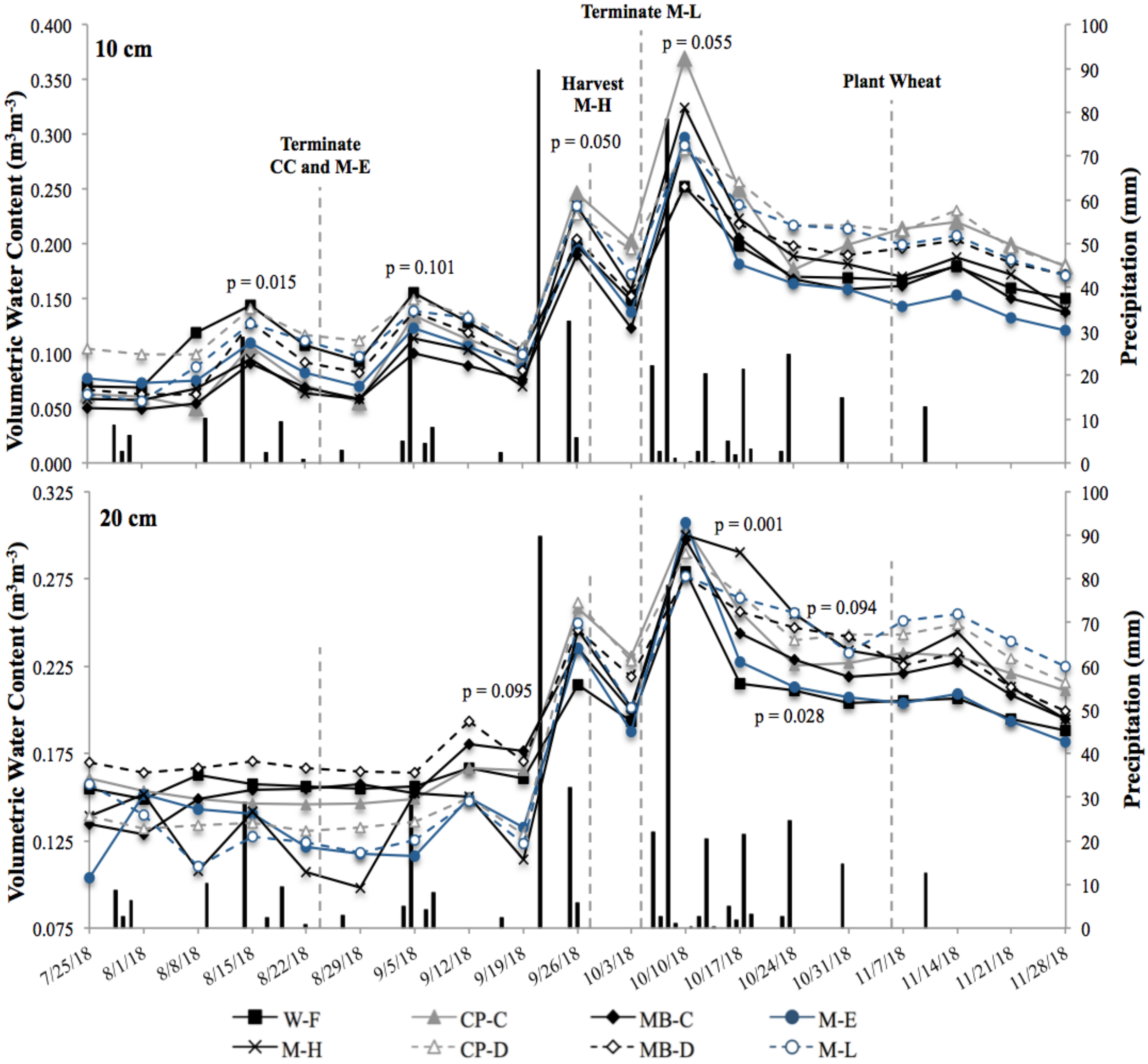

**Figure 4-3 Soil volumetric water content (VWC) shortly after cover and double cropping planting through wheat planting during 2018. Significant differences found at $p < 0.10$ are depicted above the corresponding date values were recorded. VWC is shown for both the 10 cm (top) and 20 cm (bottom) soil depths.**

### *4.3.3. Volumetric water content*

After cover crops were planted in early June 2018, a decrease in volumetric water content was seen for those treatments receiving a summer cover crop or double crop (Figure 4-3). VWC of fallow treatments remained greater after about two months of cover crop growth, due to use of soil moisture by the crop. Seventy days after planting (August 15th), the fallow treatment had significantly greater VWC ($p = 0.015$) compared to CP-C, MB-C, M-E and M-H however was not different from the double crop or mix late treatments. At this point in time, all summer crops are the same age; therefore these differences may be cumulative effects from the previous rotations.

Throughout August, fallow and double crops trended higher in VWC. After another larger precipitation event on September 5th, similar significant differences ($p = 0.101$) can be seen where fallow, double crop and late terminated mixture and CP-C were statistically greater than M-E, M-H and MB-C. Cover crop treatments continued to have lower VWC values after their termination on August 24th. However, after the M-H plots were terminated and harvested for hay on September 28th and following large precipitation events, VWC in this treatment quickly increased and became significantly greater than W-F. During the month of October frequent precipitation events resulted in overall larger VWC values. At October 10th ($p = 0.055$) in contrast to summer months, cover crop, M-E and M-H treatments were in general numerically greater than double crop and later terminated plots. Prior to wheat planting, double crop treatments trended greater in VWC compared to cover crops. W-F remained lower than most treatments and there were no statistical differences in VWC leading up to and following wheat planting.

Similar findings were seen at the 20 cm subsurface readings. During cover crop growth, W-F treatments had numerically higher VWC values than most intensified treatments. Once M-H was terminated and harvested, VWC values for this treatment became greater than W-F. Once all summer crops were terminated or harvested and rain events occurred prior to wheat planting, a shift in VWC was seen similar to that at the 10 cm depth where W-F had lower VWC compared to most all other treatments. Between when the last harvest occurred on October 5th and planting on November 5th, multiple precipitation events resulted in significant differences (October 17th, 24th, 31st) where double crop and M-L and M-H generally being significantly greater than the W-F control and numerically greater than cover crops and M-E plots. Similar to VWC at the 10 cm depth, double crops trended higher than cover crops before and following wheat planting.

## 4.4. Discussion

### *4.4.1. Impacts on soil physical structure*

After four years of intensifying a wheat-fallow system, significant findings were observed between treatments in multiple aggregate classes at both sampling depths. At the soil surface (0-10 cm), the cowpea cover crop treatment and early-terminated mixture cover crop saw a numerically greater distribution of large macroaggregates than the wheat-fallow treatment. This supports the idea that including covers builds soil aggregation. However, double-cropped treatments saw a lower percentage of large macroaggregates than W-F. This may be due to biomass removal and the lack of residue return – a key to maintaining aggregate stability and protection from dispersion due to environmental conditions (Blanco-Canqui and Lal, 2009). In the subsurface soil, M-H continued to have the least aggregate distribution in the large macroaggregate class. M-H also saw the lowest MWDA values at both depths, again indicating the short-term impacts of removing substantial herbage from double cropped systems on aggregate fractionation (Hammerbeck et al., 2012) and formation.

When looking at the microaggregate fraction, MB-C and M-H present the higher distribution compared to other treatments. Both treatments also see the lowest macroaggregate percent distribution; a lack of macroaggregation in these treatments may shift aggregate distribution to the smaller fraction and indicates less stable aggregation in these treatments in the upper soil profile. Overall, there was a general increase in microaggregate distribution for intensified treatments at the surface. Tisdall and Oades (1982) along with other researchers over the years proposed a mechanism in which stable microaggregates developed within macroaggregates, where upon natural breakdown of these macroaggregates release stable microaggregates to return to the aggregate forming process (Jastrow 1996; Six et al., 2000). This mechanism may explain the increase in microaggregates of intensified treatments at the surface, however future work would need to examine the micro- within-macroaggregates (Kong et al., 2005).

Reicosky et al. (2011) emphasize that no-till systems may need several years to see a change in aggregate stability. Sainju et al. (2003) saw an increase in aggregation after about five years due to the incorporation of cover crops in a fine sandy loam system. Similarly, Blanco-Canqui et al. (2013) observed an increase in MWDA after five years of cover cropping in a semi-arid environment compared to a fallow control. Although significant differences are seen within aggregate classes, MWDA values remain the same between treatments at both depths. With lower organic carbon and organic matter values, it may take longer to see a large change in aggregation in this semi-arid environment however there is indication that cover and double cropping is beginning to impact soil aggregation of a wheat monocropping system.

A depth interaction for the MWDA may be due to textural differences, although the USDA soil series classification considers both depths to be in the same horizon (down to 25 cm). Soil texture classification between the two depths has yet to be analyzed to confirm why the MWDA is greater in the subsurface samples. Wind and other erosion factors may play a role in the smaller aggregate sizes present in the surface samples as compared to the subsurface.

### *4.4.2. Soil water infiltration and water content*

Mixtures may have seen higher infiltration rates during the wheat reproductive phase due to varying biomass production as well as decomposition rates between treatments. Mixtures had numerically greater biomass over the past three years as well as greater C:N ratios compared to single species legumes (Figure A-1). Lower C:N ratios of single species legumes result in faster rates of decomposition and therefore may have little amount of surface residue from legumes at this time compared to the grass/legume mixture. However, this does not explain the similar infiltration rate seen for MB-C as the mixture treatments. One possible explanation may lie beneath the soil surface.

Cover crop residues play a key role in infiltration by protecting the soil surface and decreasing runoff, however, roots also influence infiltration by providing pathways for water via root channels (Brady and Weil, 2010). Mung bean is known to have a widespread root system that may provide greater opportunity for water to move through the soil profile and explain the greater infiltration rate (Baligar and Fageria, 2007). As mentioned before, double crop biomass removal may result in less surface residue to help with water infiltration, explaining the lower infiltration rates of legume double cropped treatments. Although results show early positive impacts of cover cropping on water infiltration, more information about how infiltration is impacted during cover crop growth and prior to wheat planting may help explain findings in regards to soil moisture content. Therefore, timing of infiltration measurements should be considered to include multiple dates across the wheat cash crop growing period.

During summer months, cover crops were actively growing and using soil water resources. As expected, the VWC of those plots receiving a summer cover was lower than W-F at this time. Although a few statistical differences did arise throughout the cover crop period, they disappeared by the time wheat was planted and the weeks following. In regards to cover selection, when cover and double crops were analyzed for differences amongst each other, in some instances, mixtures used water differently than single species. However, in these instances, there were also differences among cover and double crops and no consistent trend between mixtures versus other covers. This is in agreement with Nielsen et al. (2015) who also noted changes in VWC were not different between a mixture treatment and single species cover crops. This study, like the current one, did also see a decrease in VWC for cover crop treatments compared to fallow. However, data in Nielsen et al. (2015) was only collected between cover crop planting and termination, when it is expected for crops to use water. By expanding data collection through cover crop termination and past wheat planting, our findings showed after termination of the cover and adequate rainfall events, soil VWC is recharged.

A long-term study in Hesston, KS, plots with cover crops saw 35% greater water content compared to a rotation with no cover (Blanco-Canqui et al., 2011). Data was collected at one time point in the spring, presumably during wheat growth. Although this study did not present VWC data throughout wheat growth, it supports the current findings that cover crops maintain greater VWC compared to W-F after cash crop planting. In a semi-arid study located in Spain, VWC was recorded throughout and after the cover crop season (Alonso-Ayuso et al., 2014). When evaluating the 0-20 cm data, fallow plots had larger VWC values compared to the first and second cover crop termination times. However, after termination in both years, the plots with cover had similar or greater VWC throughout the following months. Again, these results support the findings in this study.

Much concern remains about using cover crops in semi-arid and dryland production systems for reasons concerning soil moisture (Unger and Vigil, 1998) and impacts on yields and costs (Nielsen et al., 2015; Robinson and Nielsen, 2015; Holman et al., 2018). Although it is shown that cover crops do in fact use soil moisture during growth, terminating a cover or double crop with adequate timing before planting can allow for precipitation events to recharge soil moisture levels. As there are no variances among MWDA, water content differences over this period of time may be due to cover crop residue rather than improvement in soil structure. In a no-till system, residues from a cover crop can influence evaporative loss from the soil surface (Blanco-Canqui et al., 2011) especially during hotter points in the summer. Blanco-Canqui et al. (2015) mentioned that early termination recommendations such as those proposed by Unger and Vigil (1998) of cover crops to conserve moisture might not be viable in semi-arid regions. The findings in this current study provide evidence showing that early termination of a cover crop is a possibility, but as well as later termination and harvest of a double crop, in a year of adequate precipitation without resulting in a soil moisture deficit prior to wheat planting.

In 2018, below average precipitation values were reported for the summer months (especially at cover crop planting where June values were over 75 mm lower than the 30-year average). However, September and October saw over twice the average precipitation, contributing enough precipitation for recharge of soil water prior to planting. This is not always the case in the Texas Rolling Plains, as the region often experiences long droughts. Because of this, continual observation of soil moisture in years of below average or sporadic precipitation and drought are needed for a better understanding of how cover crops in dryland production may impact soil moisture.

## 4.5. Conclusion

Findings from the current study indicate that including cover or double crops in a wheat-fallow monocropping system can positively impact soil physical properties over the short-term. Although no significant differences were seen from treatments for the numerical mean weight diameter of aggregates, a closer look at the soil aggregate fractions provides evidence of a summer crop beginning to increase aggregation. Residues from summer crops can persist throughout the wheat season and increase water infiltration rates during the critical reproductive phase. Summer cropping does deplete soil moisture, however, we show that in a year of adequate precipitation between cover termination and cash crop planting, including cover or double crops into rotation does not negatively impact soil water content and therefore would not be the factor impacting cash crop yields. More time may be needed to see significant changes in soil physical properties however there are early indications of positive effects due to intensified management of a semi-arid dryland system.

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# 5. CONCLUSIONS

After four years of cover and double cropping in a traditional dryland winter wheat monocropping system, soil biological, chemical and physical properties were improved. The incorporation of a cover or double crop during the summer months provided additional root and residue sources to soil microbes, thereby increasing microbial biomass and activity, which in turn affects nutrient cycling throughout the soil profile. During periods of stress, particularly in the summer months, it was found that microbial biomass was greater in covered treatments compared to wheat-fallow, indicating the stability these alternative cropping systems provide. In this same period, the presence of an additional crop resulted in lower nitrate values. However by the time of wheat planting, cover and double cropping provided more nitrate to the soil compared to wheat-fallow indicating that cover and double cropping can reduce nitrate leaching during a traditional fallow period and provide this inorganic nitrogen back to the soil for the following cash crop. By the time winter wheat was planted, total nitrogen values were typically greater for intensified treatment than wheat-fallow in the upper portions of the profile due to return of cover crop residues.

Carbon sequestration is critical to maintaining and building soil organic matter while providing nutrients to soil microbial populations. In this study SOC was improved by the presence of cover and double crops in the upper portions of the soil profile. Additionally, when evaluating carbon within aggregate fractions, values in the macro- and micro-aggregate fractions trended higher compared to wheat-fallow. This provides early indication that intensified systems are able to sequester carbon, however continual studies are needed to see how these values change over time. Soil water was used during summer months by cover and double crops, yet adequate time between cover crop termination or double crop harvest and wheat planting allowed for a recharge in soil moisture due to precipitation. Additionally, volumetric water content was greater in double cropped and later terminated treatments and generally greater in cover cropped treatments compared to wheat-fallow plots at the time wheat was planted.

Certain soil parameters, such as POxC, water infiltration and MWDA, generally did not show significant differences due to treatment. This crop rotation is still relatively new and more time may be needed to determine the long-term effects and changes in this production system.

Cover or double cropping is recommended to producers in the Texas Rolling Plains region as a means to not only protect their soil from erosion but to build soil health and physical properties. Although costs of cover and double cropping as well as soil moisture preservation cause concern for adoption, this study demonstrates the positive impacts cover or double cropping can have on soil parameters without depleting soil water content before cash crop planting. Producers may consider double cropping as it is shown to improve certain biological and chemical properties over cover cropping while not negatively impacting soil moisture values for the following cash crop. Double cropping may provide additional income to the producer in an adequate year of precipitation or be a viable crop option in other years.

A longer term investigation can provide more information on how soil health parameters and moisture are affected by summer intensification. This information can also provide insight into the economics of adopting cover cropping practices in the Texas Rolling Plains.

# APPENDIX A

## APPENDIX FIGURES AND TABLES

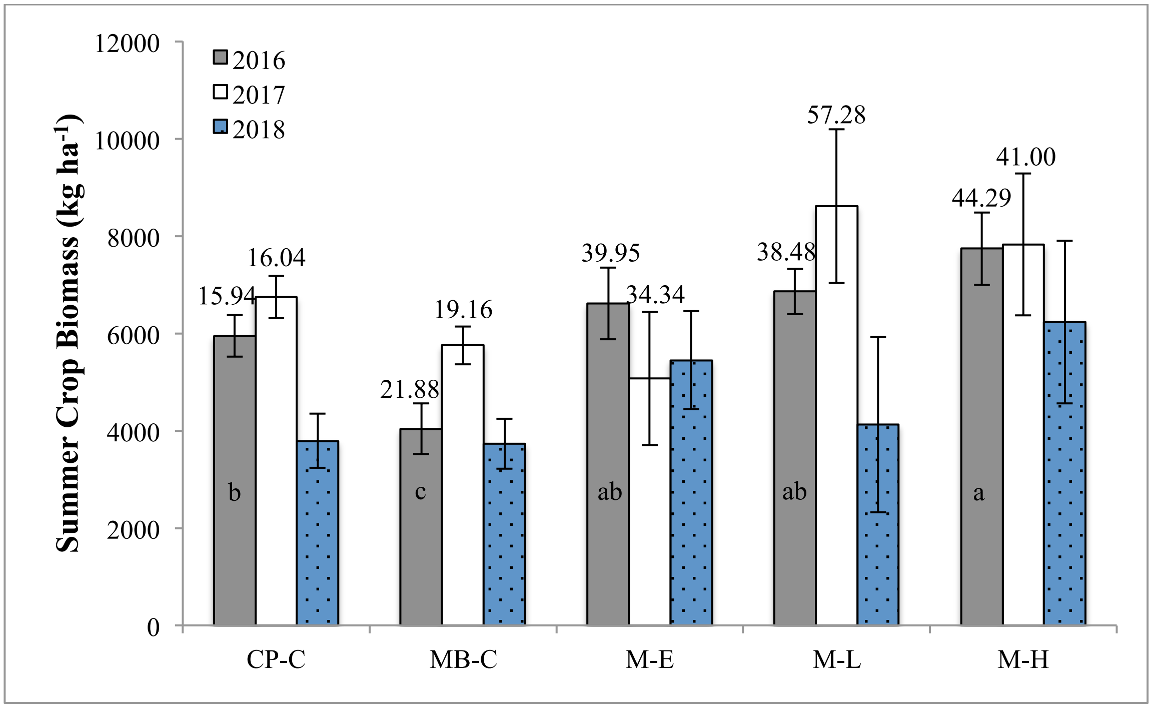

**Figure A-1 Summer crop biomass for cover crop and mixture treatments from 2016 to 2018. Values above bars indicate the C:N ratio for the summer treatment. Lower case letters within bars indicate significant differences ($p < 0.05$) between treatments in that year. Error bars represent standard error of mean.**