INFLUENCE OF COVER CROP SELECTION ON MICROBIAL ACTIVITY DURING A TRANSITION TO ORGANIC AGRICULTURE

A Thesis

by

LEAH MARIE ELLMAN-STORTZ

Submitted to the Office of Graduate and Professional School of
Texas A&M University
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Katie Lewis
Co-Chair,	Terry Gentry
Committee Members,	Paul De Laune
	Elizabeth Pierson
Head of Department,	Wayne Smith

August 2021

Major Subject: Soil Science

ABSTRACT

This study sought to investigate the impact of organic management and cover crop use, selection, and seeding rate on biological parameters of soil health after one year of transitioning to organic management in a semi-arid region. Protecting soil health is an essential task for modern agriculture, and it is largely controlled by microbial communities which decompose plant residues and cycle nutrients.

Only a limited amount of arable land exists to support human populations, but much of this is already subject to degradation through intensive agriculture. In recent years, consumer awareness relating to sustainability has led to an elevated demand for organic products. Farmers looking to pursue organic management are required by the USDA to undergo a three-year transition period before they can sell their products as certified organic. One practice which is encouraged in organic management is cover cropping, which is typically used to limit wind erosion. This practice also benefits soil health through stimulating microbial activity and encouraging both nutrient cycling and organic matter buildup.

Because organic management is associated with depressed yields – particularly during the transitional years - studies should focus on management practices that are best related to rapid improvements in soil health, such as cover crop selection.

This study focused on indicators of soil biological activity under a cotton-peanut rotation in West Texas during the first year of transition to organic management. Results from this research indicated that the selected cover crop seeding rates appeared to have little influence on microbial activity under plots transitioning to organic management. However, there were indications of elevated microbial activity under cover cropped treatments as compared to fallow after only one cover crop rotation, and both mineralizable carbon (CMIN) and N-acetyl-β-D-glucosaminidase (NAG) enzyme activities were elevated under rye/vetch treatments as compared to rye alone at limited points in the season.

DEDICATION

This work is dedicated to Evan, my loving husband who came with me on this adventure, and to Pa, who always kept a beautiful garden.

ACKNOWLEDGEMENTS

I would like to extend a heartfelt thanks to all those who supported me on this journey. To my parents, Marty and Lisa, for instilling in me a love of education; to my brothers, for their excited curiosity about everything that was happening in the lab; to my Aunt Sheila and Uncle Nick, for sparking my curiosity about the natural world; and to my husband, Evan, who was here with me every step of the way.

I would also like to thank Dr. Joseph Storlien, who had first introduced me to soil science during my undergraduate years.

Thanks especially to my committee, without whom none of this would have been possible. Dr. Katie Lewis, you provided me with a wonderful project and advice whenever things did not go my way. Dr. Terry Gentry, you accepted me into this program and patiently guided me through my research. Thank you for believing in me! Dr. Paul DeLaune provided me with an excellent second study site, and Dr. Elizabeth Pierson constantly pushed me to further explore my ideas.

Finally, I would like to thank Mark McDonald, Brian Hux, and Joseph Burke for their support and friendship, and our research techs for assisting with data collection. This study absolutely would not have been possible without you.

CONTRIBUTORS AND FUNDING SOURCES

This work was made possible by grant funding through the United States Department of Agriculture's (USDA) Southern SARE Program and an Excellence Fellowship from the Texas A&M University College of Agriculture and Life Sciences.

NOMENCLATURE

- AMF: arbuscular mycorrhizal fungi
- BG: β-glucosidase
- CMIN: mineralizable carbon
- CO_2: carbon dioxide
- G+ : Gram positive
- G-: Gram negative
- FAME: Fatty acid methyl esters
- GHG: greenhouse gas
- MBC: microbial biomass carbon
- NAG: N-acetyl-β-D-glucosaminidase
- NMIN: mineralizable nitrogen
- N_2O: nitrous oxide
- PLFA: phospholipid fatty acid
- SOC: soil organic carbon
- SOM: soil organic matter

TABLE OF CONTENTS

Page

I. LITERATURE REVIEW

II. INFLUENCE OF COVER CROP SELECTION ON SOIL BIOLOGICAL PARAMETERS DURING A TRANSITION TO ORGANIC AGRICULTURE

III. FUTURE DIRECTIONS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

LITERATURE REVIEW

West Texas: Background

The Southern High Plains ecoregion of Texas represents a semi-arid region of economic value due to its role in peanut (*Arachis hypogea*) and dryland cotton (*Gossypium hirsutum*) production. In particular, although Texas is not known for organic management, it was responsible for approximately 95% of all organic peanut production and nearly 90% of organic cotton production in the US as of 2011 (USDA, 2013). However, although both cotton and peanut are drought-resistant crops (Acosta-Martinez et al., 2004), conditions associated with semi-arid climates may still be yield-limiting.

The High Plains and Rolling Plains ecoregions of Texas receive only 381-508 and 508-711 mm annual rainfall, respectively (Texas Ecoregions, 2020). Soils in semi-arid climates also tend to be low in soil organic matter (SOM), which can result in poor nutrient availability and water retention, thereby presenting issues with yield (Nautiyal et al., 2010). Additionally, wind erosion is common in these regions, which further exacerbates loss of organic matter. Such pressures can aggravate difficulties associated with implementing organic management, especially since low yields are expected during the initial years of management transitions. These factors, coupled with the need for water conservation due to drought and excess irrigation pressures on the Ogallala Aquifer, necessitate studies on management practices in this region to improve soil health (Cano et al., 2018).

Soil Health

While “soil health” may be considered akin to “soil quality” (Franzluebbers, 2015; Graber, n.d.; NRCS, 2019), studies associated with this term tend to focus on identifying and quantifying biological indicators of a soil’s ability to function as a living ecosystem, as opposed to physical attributes alone (Franzluebbers, 2015; Graber, n.d.). Soil has been described as an “ecosystem foundation,” making the study of its health important in agriculture, as it pertains to influences on yield outcomes (Schoonover and Crim, 2015). According to the NRCS, there are five essential functions that healthy soils perform. These include: water regulation, sustaining plant and animal life, pollutant buffering, structural support, and nutrient cycling (NRCS, 2019). These functions have been corroborated by a number of authors. For example, Watts et al. (2001) describes soils with good physical health as “strong when wet and weak when dry” so as to maintain structure while still allowing for root penetration. Stockdale et al. (2006) also discusses healthy, productive soils in terms of SOM and its role in nutrient cycling. Schoonover and Crim (2015) expands upon this, explaining that the quality and productivity of soils often rely upon physical properties such as texture, water holding capacity, and organic matter content. They further explain the role of SOM in terms of soil water holding capacity and the benefits that this property provides to plants and soil biota. Although a soil’s health is largely tied to its ability to be productive, Schoonover and Crim (2015) caution that this should be understood in the context of “how well a soil functions within its specific ecosystem.”

Microbial communities contribute to soil functions through a variety of activities. For example, Dick (2011) notes the importance of enzymes produced by soil microbes as “the drivers for biogeochemical cycling in soils.” Numerous agricultural studies have linked soil enzyme activities and microbial community structures to fertility and yield (Verstraete and

Voets, 1977; Mbuthia et al., 2015). Soil microbes produce extracellular enzymes to catalyze reactions required for the decomposition of organic residues, which then release simple sugars and other small molecules for microbes to use as energy, build SOM, and allow soils to support plant life through nutrient cycling (Schoonover and Crim, 2015; Acosta-Martinez et al., 2004).

Although biological indicators involving microbial community structure and function play an important role in measuring soil health, much work is yet to be done to advance these factors. For example, Stewart et al. (2018) notes that as of 2018, many biological indicators of soil health, such as enzyme activities, were only considered Tier 2 indicators by the Soil Health Institute. Gianfreda et al. (2005) explains that this is, in part, because methodologies “are often not applicable to soils that are so different from each other that it is difficult to handle them in the same way.” Therefore, agricultural studies focusing on soil health may be considered novel and important in their roles in advancing agriculture. However, care should be taken in selecting analytical procedures – particularly when studying soils across different regions.

Historically, there has been an incomplete understanding of soil health, resulting in management practices – such as high chemical inputs, reduced organic inputs, and excessive tillage – which may not properly support the biological function of soils (Franzluebbers, 2015). Such practices, performed intensively and over a long period of time, may result in soil degradation, which in agriculture is understood to be a loss in quality and productivity (Lal et al., 1989). Excessive use of tillage and pesticides are two examples of practices which may lead to degradation, and indications of degradation include (but are not limited to): erosion, salinization, biological degradation, and loss of soil structure (Lal et al., 1989).

Soil Organic Matter: A Vital Component of Soil Health

Soil organic matter plays an important role in maintaining function. It can be defined as “the fraction of the soil that consists of plant or animal tissue in various stages of breakdown” (Cornell University, 2008) and can be expected to make up 3-5% of many agricultural soils (Weil and Brady, 2019). However, SOM tends to be quite low in semi-arid regions which are characterized by hot, dry climates (Nautiyal et al., 2010).

Of special interest is the involvement of SOM in soil biogeochemical cycling, soil aggregation, and its role in the global carbon cycle as a C sink (Weil and Brady, 2019). SOM tends to be difficult to characterize, as organic matter exists in various stages of decomposition. However, it is generally considered to be equivalent to soil organic carbon (SOC) (Santos et al., 2012). Change in SOC based on a given treatment is not always feasible to measure in the short term, but there are labile fractions of SOC which are sensitive to changes in management, and these in turn can predict how SOM – and by proxy, soil health – may react over time.

One such labile fraction is mineralizable carbon (CMIN). This is commonly correlated to microbial biomass carbon (MBC) and represents microbial necromass and decaying organic substrates (Hurisso et al., 2016). It is sensitive to changes in management and is considered to be a useful indicator of soil health due to its associate with decomposition of organic matter and “short-term soil nutrient availability” (Hurisso et al., 2016). However, it is not strongly related to C accumulation. Previous studies have also found this fraction of labile carbon to be associated with nitrogen mineralization (NMIN), which is another important component of soil health (Franzluebbers et al., 2000).

Enzyme activities, although not strictly labile carbon fractions, are still associated with SOM because they drive nutrient cycling by organic matter. Specifically, activities of carbohydrate associated enzymes, or glycosidases, aid in the release of simple sugars from organic substrates and "are important in providing labile C and energy sources to support microbial life in soil" (Dick, 2011). This is especially true as soil microbial activity tends to be limited by carbon-based energy sources (Ekenler and Tabatabai, 2002). Two such enzymes which are sensitive measures of soil health include β-glucosidase (BG) and N-acetyl-β-D-glucosaminidase (NAG). Both are involved in carbon cycling and are similarly structured. However, while they both catalyze the release of simple sugars from substrates for microbes to use as energy, they are distinct in that BG catalyzes the release of glucose as the final step in cellulose breakdown while NAG is involved in catalyzing the release of amino sugars from chitin residues (Dick, 2011). Notably, the byproducts of chitin breakdown, amino sugars, are thought to comprise 5-10% of organic nitrogen in soils (Ekenler and Tabatabai, 2002), making NAG an important marker of nitrogen availability. Since chitin is associated with the cell walls of insects, fungi, and to some extent bacteria, and cellulose is an incredibly common plant polymer, these represent two distinct and important substrate sources for enzymatic activity.

Soil Health: Challenges in Modern Agriculture

One concern with soil health in agriculture is soil degradation. This is typically thought of as the loss of soil structure and SOM associated with it (Stockdale, 2006). For example, heavy tillage exposes protected minerals and organic matter to the elements, and this practice may result in elevated erosion, loss of SOC, and poor structural stability if done intensively (Schoonover and Crim, 2015; Franzluebbers et al., 2005). Degraded soils with poor structural stability may then crust or compact easily, and the loss of pore structure may contribute to poor water holding capacity, limiting the ability of a soil to remain productive (Schoonover and Crim, 2015).

On the biological side, applications of chemical fertilizers, pesticides, and herbicides may also have degrading effects on soils if overused. For example, Wilson et al. (2009) found that the hyphal abundance of AM fungi – which associate with most plants and aid in phosphorus acquisition – had a strong positive linear relationship to soil aggregation, and that when fungicide was continuously applied to plots for a number of years, both hyphal abundance and soil macroaggregate size decreased. Furthermore, excess chemical nitrogen application has been found to impact microbial activity (Zhang et al., 2012), and nitrogen fertilizer may have long-term impacts for groundwater contamination, as between 12% and 17% of the initial application may continue to be released into the hydrosphere over a 100-year period (Sebilo et al., 2013).

Organic Management: A Brief History and Explanation

Prior to the 1940s, all agriculture was what we would today consider "organic." Methods for developing genetically modified (GMO) crops had not yet been developed, nor had most modern pesticides and herbicides been invented. Most importantly, however, nitrogen-based chemical fertilizers did not become commonplace until after WWII, although some early chemical fertilizers for P and K were in use (Russel and Williams, 1977). Prior to this time, farmers mainly relied on organic inputs from manure, compost, and legume rotations. The discovery of the Haber-Bosch process in the early 1900s would eventually spark the global shift towards high intensity, high productivity agriculture with the end of WWII in the 1940s, when unused munitions factories switched to producing readily affordable nitrogen fertilizers (Heckman, 2006; Russel and Williams, 1977; Smil, 1999). The result was a worldwide population boom as agricultural production efficiency soared. However, this was not without consequence, as high intensity agriculture led to inevitable soil degradation.

Popularity of organic products did not begin to take off until the 1960s, with the publication of *Silent Spring* (Klonsky and Tourte, 1998). By the 1970s, consumer environmental concerns led to a demand for organic agriculture (SARE Outreach, 2003). National organic standards were enacted in the 1990s with the Organic Foods Production Act (OFPA) and the formation of the National Organic Substances Board which provides oversight for allowed substances in organic farming (SARE Outreach, 2003). Rules regarding these were finalized in 2002. Since then, demand has continued to grow, driven by growing consumer concerns over personal health, climate change, and sustainable agriculture. According to the USDA, at least 4% of national food sales are organic, with “the value of sales in the US more than doubling between 2011 and 2016 to $7.6 billion.” (n.d.; 2019). Once established, certified organic production can be a highly lucrative management practice. In 2019, for example, one West Texas farming operation saw returns of $1,200 per ton of organically farmed peanut compared to $400 per ton of conventional peanut with comparable yields for each (Huguley, 2019).

According to the National Organic Program (2021), the current definition of organic production is “a production system that is managed in accordance with the Act and regulations in this part to respond to site-specific conditions by integrating cultural, biological and mechanical practices that foster cycling of resources, promote ecological balance, and conserve biodiversity.” The main goal of organic management is to promote soil biological health by avoiding synthetic inputs in favor of organic amendments and mechanical weed management

(Kristiansen et al., 2006). This commonly involves practices to build SOM. However, the process of building SOM and the microbial community involved in nutrient cycling can take years, and success is often driven by climate and soil type (Santos et al., 2012). Although there is no universal method of organic management, the USDA has published a national set of criteria required for organic certification. For products to be sold as organic in the US, organic management is required for a minimum of three consecutive years (USDA, 2015; SARE Outreach, 2003). This also coincides with the timeframe after which improvements in SOM and microbial biomass can be documented in temperate regions (Santos et al., 2012). Synthetic fertilizers and GMO crops are not permitted, and there are strict limits on pesticide and herbicide options (USDA, 2015; SARE Outreach, 2003).

Notable yield reductions are expected during the transitional years to organic agriculture as chemical, physical, and biological soil properties adjust to the new management, and some studies have indicated that organic yields may be lower than conventional by up to 26% (Skinner et al., 2014). However, careful management strategies may eventually result in yields comparable to those obtained by conventional methods once adequate time has passed (Zinati, 2002). Evidence cited to support such claims typically originates from studies which were conducted in temperate regions though, and may not reflect the experiences of farmers who work in semi-arid climates (Peters, 1991; Clark et al., 1998).

Yield limits do not mean that organic management is without benefit, however. For example, long-term studies comparing organic and conventionally managed systems have found higher soil pH levels and improvements in soil carbon stocks under organic systems (Santos et al., 2012; Clark et al., 1998), and this was “largely explained by differences in inputs” (Clark et al., 1998). These inputs may refer to organic fertilizers such as compost and manure, cover crop residues, and N-fixation from legume rotations. Improvements to soil carbon stocks are important, as adequate levels of organic carbon are required for supporting microbial communities and related nutrient cycling activities. Notably, the Santos study was conducted in a tropical, semi-arid region and documented improvements in microbial biomass after only 2 years (2012).

Cover Crops: Roles in Maintaining Soil Health

One management practice recommended for organic growers is cover cropping, which can protect against wind erosion in bare fallow fields (Matson et al., 1997). Importantly, cover crops have also been found to promote soil health by encouraging microbial activity, as can be observed by elevated soil respiration levels when compared to a winter fallow (Daryanto et al., 2018; Kim et al., 2020). Cover cropping is associated with SOM accumulation “across multiple different climates” (Daryanto et al., 2018), and root contributions by cover crop rotations have also been found to increase SOC storage (Franzluebbers, 2005). Common species used as winter cover crops in the Southwestern US include: cereal rye (*Secale cereal*), forage radish (*Raphanus sativus*), and hairy vetch (*Vicia villosa*). Each are associated with distinct benefits for the soil. The relatively high levels of lignin in rye residues are associated with slow decomposition rates and a greater ability to increase SOC as compared to readily decomposable legume and radish cover crops (Sainju et al., 2002). Legume cover crops – for example, vetch – have been found to increase levels of soil nitrogen through associations with nitrogen fixing bacteria (Latos, 2009 p. 62), and the large taproot diameter of radish, coupled with their rapid decomposition rates, may assist with water infiltration and soil oxygen penetration by alleviating compaction (Daryanto, 2018; Chen and Weil, 2010).

Although Texas was among the top 10 states in terms of total number of farm operations using cover crops as of 2017, this had only increased by 3.6% since 2012, and cover cropping still accounts for a mere 6.3% of available cropland (LaRose and Meyers, 2019). There are a number of challenges related to the adoption of cover crops across the US. Roesch-McNally et al. (2017) and Bergtold et al. (2012) cite the cost and extra work associated with cover cropping as contributing reasons for the slow adoption of this practice throughout the US. For example, the USDA indicates that seed for radish and rye cover crops can cost anywhere between $13 to $22 per acre, depending on seeding method and seeding rate, while hairy vetch can cost from $30 up to $40 per acre (2014). This does not take into account additional costs of using organic seed for cover crops in an organic farming system. Nitrogen fertilizer savings from using a legume cover crop may also be lost if farmers fail to "adjust N fertilization rates to the cash crop immediately following the cover crop due to the potentially limited availability of N provided by the cover crop or risk-averse behavior" (Bergtold et al., 2012). This, coupled with few obviously consistent benefits to yield can turn farmers away from implementing cover crops (Bergtold et al., 2012). In semi-arid regions, conventional wisdom dictates that cover crops will over-use precious water resources, thus adding another barrier to cover crop adoption. However, studies out of both Texas and Iowa have begun to challenge these assumptions. For example, although cover cropping has indeed been shown to have an impact on available soil water at the beginning of the growing season, multiple studies have found that cover cropping often does not negatively impact available soil moisture at the middle of the growing season, when crops have the greatest demand for water (Burke et al., 2021; Bergtold et al., 2012). Furthermore, these studies indicate that cover cropping may in fact promote soil water infiltration and storage, thus providing an added benefit to farmers in semi-arid regions (Burke et al., 2021; Bergtold et al., 2012).

Peanut: A Globally Important Legume Crop

Peanut (*Arachis hypogaea)*, also known as groundnut, is a globally important legume crop prized for its oilseed production and its many uses in both cooking and biodiesel (Russell, 2019). This plant is thought to have originated in South America, and is well adjusted to warm climates (American Peanut Council, 2020; Baughman et al., 2019). Countries that dominate global peanut production include: China, India, Nigeria, and the southern US (National Peanut Board, 2021). Within the US, peanut "is the second most important legume oil crop" (Acosta-Martinez, 2007). Nationally, the dollar value of peanut production was $1.2 billion as of 2020, an increase from $7.9 million in 2009 (USDA NASS, 2021). Texas and Georgia are among the top producers in the US, and an estimated average of 3,100 lb/ac was harvested in Texas in 2018 (Russell, 2019).

As legumes, peanuts are reliant on effective nodulation by rhizobia in the genus *Bradyrhizobium* or with bradyrhizobia symbionts to fix nitrogen, and they are commonly rotated with cotton in Texas to replenish soil nitrogen levels and reduce the need for chemical fertilizers (Baughman et al., 2019). One study from West Texas also found that continuous peanut and inclusion of a peanut rotation with dryland cotton could result in greater levels of organic carbon, MBC, and enzyme activities, potentially due to "larger organic residue accumulation, lower OM turnover, and continuous rhizosphere substrates from peanut, involved in N fixation and AMF associations" (Acosta-Martinez, 2007).

Peanuts are well suited to the climate regions of Texas, as they require adequate heat for germination. For example, 18.3 C at 4 inches soil depth is generally accepted as the preferred germination temperature (Baughman et al., 2019). The Southern Rolling Plains, specifically the High Plains of West Texas, are especially conducive to peanut production because the semi-arid climate favors low disease pressure from common fungal pathogens (Baughman et al., 2019). Nodulation is also less likely to be suppressed by drought in peanuts as compared to other legumes such as soybeans (Tajima et al., 2008). This crop is therefore an excellent choice for semi-arid regions such as West Texas, although excessive heat at planting does have the potential to kill off bradyrhizobia symbionts prior to nodulation (Baughman et al., 2019).

Study Objectives

Ecoregions in West Texas are valuable agricultural centers of cotton and peanut production, and organic production of these crops is growing in importance. However, regional challenges necessitate further studies on organic management practices to improve soil health. Although multiple studies have investigated soil health in organic systems, many of these have occurred in temperate regions with soils high in SOM, and only a limited number have occurred within the first three years of management implementation. This is largely due to the expectation that no significant change in SOC will be seen until after three years have passed, and that resources would be better allocated towards studying more long-term effects of organic management. However, some studies have observed improvements in parameters associated with SOM – such as microbial biomass – after only two years of organic management (Santos et al., 2012). Early changes in soil health as management shifts to organic production are of special interest to farmers in these regions who are looking to balance the economic risk with the reward of successful organic production. Abundance of SOM, which is crucial for effective organic farming, is far lower in semi-arid regions than in temperate ones, and the climate of semi-arid regions does not easily lend itself towards improvements in soil health such as building SOM. Therefore, early changes in soil health when transitioning management practices and organic practices which are best suited to providing early improvements – such as cover crop selection – should be identified.

The objectives of this study are therefore threefold. A) To identify and quantify biological markers of change in soil health after one year of organic management in a cotton-peanut rotation, B) To determine the influence of cover crop use and selection on these biological markers, and C) To determine if and how microbial activity may be influenced by cover crop seeding rates.

CHAPTER 2

INFLUENCE OF COVER CROP SELECTION ON SOIL BIOLOGICAL PARAMETERS DURING A TRANSITION TO ORGANIC AGRICULTURE

Abstract

This study sought to measure the impact of organic management and cover crop selection on soil health after one year of transitioning to an organic management cotton-peanut rotation. Soil health is largely controlled by microbial communities which produce the enzymes required to decompose plant residue and cycle nutrients, and is therefore essential for modern agriculture to protect.

Although Texas is not widely known for organic management, it is responsible for approximately 95% of organic peanut production. There is a growing consumer demand for organic products, and when managed effectively, this can be a lucrative practice for farmers. However, farmers looking to pursue organic management are required to undergo a three-year transition period to obtain USDA certification. This transitional period is often associated with depressed yields, so studies focusing on management practices best designed for rapid improvements in soil health are of benefit to farmers.

Growers in organic management are encouraged to utilize cover cropping practices, which may improve soil health through stimulating microbial activity. Other benefits associated with cover cropping include reduced wind erosion and improved soil water storage and nutrient cycling.

This study sought to identify cover crop selections and seeding rates best suited to improving soil health within the first year of transition to organic management under a cotton-

peanut rotation in West Texas. Results of this study suggest that cover crop seeding rates appeared to have little influence on soil health indicators. However, plots cover-cropped to a rye/vetch mix experienced elevated CMIN and NAG activities, signaling the early contribution of the rye/vetch treatment to organic N in the system. Use of a cover crop also resulted in greater enzyme activities and *in situ* respiration as compared to the fallow after only one cover crop rotation, and these results represented early elevated biological activities under plots treated with a cover crop. Although these results were limited, they may foreshadow more distinct differences in soil health that could become apparent over time.

Introduction

This study sought to identify a cover crop treatment that could best improve soil health in the early stages of a cotton-peanut rotation transitioning to organic management in West Texas. Peanut (*Arachis hypogaea* L.) is an important oil crop in the US (Acosta-Martinez, 2007) and is known for its versatile uses in both cooking and biodiesel (Russell, 2019). The US ranks among the top 4 peanut-producing countries in the world, with a national production value of $1.2 billion as of 2020 (USDA NASS, 2021), and southern states are responsible for most peanut farming operations (Russell, 2019). In particular, Texas is responsible for 95% of organic peanut production in the US (USDA, 2013).

In its most basic form, organic management refers to practices which favor organic amendments such as compost, manure, N-fixation by legumes, and cover crop residues over chemical N-fertilizers (Kristiansen et al, 2006). A more official definition, as per the National Organic Program, refers to organic production as "a production system that is managed in accordance with the Act and regulations in this part to respond to site-specific conditions by integrating cultural, biological and mechanical practices that foster cycling of resources, promote ecological balance, and conserve biodiversity" (2021). Popularity of organic products has increased over the past few decades as consumers shy away from conventional practices out of concern for health and environmental sustainability (Fraiser et al., 1988), and demand has only continued to grow in the US (USDA, 2019). If properly established, organic management can be a profitable practice (Huguley, 2019). However, for crops to be sold as USDA-certified organic, plots must have experienced a minimum of three years of continuous organic management, and amendments must adhere to OMRI standards (USDA, 2015). Numerous meta-analyses have found that yields from organic agriculture experience high temporal variability and are typically

5% to 34% lower than conventional (Knapp et al., 2018; Seufert et al., 2012), which may require greater land use for farmers to turn a profit (Skinner et al., 2019; Skinner et al., 2014). However, it is unclear if such yield losses are notable for organic legume crops such as peanut.

Soil under organic management commonly experiences greater microbial activities and soil C levels than under conventional management, and this tends to be due to organic inputs such as compost or manure (Santos et al., 2012; Nautiyal et al., 2010). Use of manure can lead to higher MBC and reduced G+/G- ratios as compared to soils treated with chemical N fertilizer alone (Zhang et al., 2012). Although one goal of organic management is to build SOM and the microbial community involved in nutrient cycling, it can take years for this to occur, and success is often driven by climate and soil texture (Santos et al., 2012).

The effectiveness of organic management at improving soil health varies with regional climate, soil characteristics, and the management itself, but there are certain practices associated with organic agriculture which have been shown to improve soil health. Rotations with legume crops such as peanut can improve soil OC, MBC, enzyme activities, and plant available N (Acosta-Martinez, 2007; Kristiansen, 2006), and the USDA recommends that organic growers make use of cover cropping, which protects against wind erosion (Matson et al., 1997).

Importantly, cover cropping has been found to promote soil health by encouraging microbial activity. This practice often increases soil CO_2 fluxes through microbial respiration, and global warming potentials from this tend to be offset by improvements to yield and carbon sequestration (Daryanto et al., 2018). Cover crop use has been shown to result in greater levels of microbial abundance when compared to being left fallow (Kim et al., 2020). Cover cropping is also associated with improved soil C storage and SOM accumulation (Mbuthia et al., 2015; Daryanto et al.). Common species used as winter cover crops in the Southwestern US include:

cereal rye (*Secale cereal*), forage radish (*Raphanus sativus*), and hairy vetch (*Vicia villosa*) – each of which has implications for soil health in terms of microbial community composition, activity, and nutrient cycling. For example, Mbuthia et al. (2015) found elevated G+ FAME biomarkers under plots treated with vetch. Lower levels of AMF were also found under these plots, potentially because N-fixation by rhizobia associated with vetch provided the cash crop with enough resources to allocate most photosynthate to its shoots as opposed to its roots. Georgieva et al. (2005) found a low C:N ratio under vetch cover crop litterbags as compared to rye, and there were differences in bacterial and fungal biomasses with higher bacterial biomass under vetch and greater fungal biomasses under rye. Rye is associated with SOC buildup due to its relatively high lignin levels and slow decomposition (Sainju et al., 2002), and radish may assist with water infiltration and soil oxygen penetration by alleviating compaction (Daryanto, 2018; Chen and Weil, 2010).

Cover cropping only accounts for 6.3% of available cropland in Texas (LaRose and Meyers, 2019), and this state has been fairly slow to adopt this practice. Cover crops are typically used to mitigate wind erosion in West Texas, which is a common issue in that region. Water conservation is also necessary due to the dry climate and pressures on the Ogallala Aquifer (Cano et al., 2018). There are concerns about excess water usage by cover crops, which has discouraged more widespread use in West Texas. However, studies out of Iowa and Texas indicate that certain cover crops may improve water infiltration and storage, and that their impact on soil water during the middle of the growing season may not be detrimental to cash crops (Burke et al., 2021; Bergtold et al., 2012). Additional barriers to cover crop adoption include unpredictable benefits, additional effort, and varying seed costs (Roesch-McNally et al., 2017; Bergtold et al., 2012). For example, although non-organic rye and radish cover crop seed can cost \$30 ha^{-1} at minimum, prices can range up to \$50 ha^{-1}, and hairy vetch can cost up to \$89 ha^{-1} (USDA, 2014). Prices vary based on seeding method, seeding rate, and whether or not organic cover crop seed is required. High cover crop biomass is associated with elevated soil health benefits when compared to fallow or low cover crop biomass (Thapa, 2021). Elevated seeding rates may be useful to achieve high cover crop biomasses, but previous research has investigated the impact of cover crop seeding rates with varying results. One 2019 study investigated both cereal rye and winter wheat cover crops at 2 different seeding rates, and found that the higher seeding rate resulted in greater biomass only for winter wheat at during the 2nd year of the study (Haramoto, 2019). Another study reported more consistent increases in cover crop biomasses under elevated seeding rates, but that percent emergence of cover crops decreased under high seeding rates (Brennan and Boyd, 2012).

Current literature suggests that differences between organic and conventional management do not become apparent until adequate time has passed for the soil microbial community to adjust to the new management. Typically, these differences are expected to be observed after 3 years of continuous organic management, but this may vary by region (Santos et al., 2012). Different cover crop selections have also been shown to vary in the benefits that they provide to soil health (Mbuthia et al., 2015; Georgieva et al., 2005), and this may also translate to variations in the timeline under which soil health benefits may be observed.

This study investigated plots under conventional and recently transitioned organic management with four cover crop treatments. These treatments included the following: rye, radish, rye/vetch, and rye/vetch/radish. Cover crop use was expected to result in greater microbial activity than winter fallow, and cover crop mixes were expected to result in greater microbial activity than single cover crop selections. Additionally, although organic management was expected to result in elevated microbial activity, it will likely be too early in the study for any yield benefit to be observed. Given that organic management is an economic risk due to the timeframe to achieve certification and limits on pesticides and amendments, studies on early changes to soil health under different cover crop selections will benefit farmers looking to pursue this management practice.

The objectives of this study were therefore threefold:

A) To identify and quantify biological markers of change in soil health after one year of a cotton-peanut rotation under organic management in West Texas.

B) To determine the influence of cover crop use, selection, and organic management on these biological markers.

C) To determine if and how biological markers of soil health may be influenced by cover crop seeding rates in organic agriculture.

Materials and Methods

Site History

This study was conducted at the AgriLife Research Centers at Lubbock (33.693, -101.828) and Vernon (34.091, -99.365), TX during the summer of 2020 (Fig. 1). These locations represent the Southern High Plains and Southern Rolling Plains ecoregions of Texas, both of which are semi-arid environments with an average annual rainfall of 381-508 and 508-711 mm, respectively (Texas Ecoregions, 2020). Soils were characterized as an Olton clay loam at the Lubbock site, and a Miles loamy fine sand at the Vernon site (Web Soil Survey, 2020).

Figure 1. Site locations.

Prior to 2019, plots for this study at the Lubbock location had been under a cotton/peanut rotation for two years, preceded by continuous cotton in 2016 and grain sorghum in 2014 and 2015. Vernon plots had previously been planted to winter wheat for 10 years. Locations used for this study were planted to cotton in 2019 and peanut in 2020.

Lubbock and Vernon plots were planted to peanut variety ACI 236 on May 7, 2020 and May 11, 2020, respectively. A June re-plant was required for Lubbock on June 10 due to poor initial growth. Lubbock plots were fertilized with urea ammonium nitrate at 100 kg ha^{-1} (conventional plots) and compost at 9 metric tons ha^{-1} in 2019 during the previous cotton rotation and did not receive fertilizer in 2020. This is a common practice when growing organic peanuts (Baughman et. al., 2019). The Lubbock site also utilized flood irrigation and strip tillage, but tillage was minimized under organic plots to attempt to replicate conservation tillage. The Vernon organic plots received 9.9 tons ha^{-1} composted manure application on April 27 after cover crop termination, and the conventional plots received 167 kg ha^{-1} ammonium polyphosphate (10-34-0) fertilizer knifed in on May 6. This site utilized pivot sprinkler irrigation and conventional tillage across all treatments. Two additional tillage passes were done in-season to minimize weed pressures, and residues from cover crops and composted manure were incorporated into the soil to a greater extent than at Lubbock.

Study Design

The study consisted of two randomized complete blocks (RCB) for organic (ORG) and conventional (CON) management, and each included four cover crop treatments. For crops to be certified organic by the USDA, chemical drift from conventional management is not permitted – hence the 2 RCBs. Organic plots with multiple seeding rates per cover crop treatment were also included to investigate whether seeding rate could reasonably be expected to impact soil health and weed control (data not presented) within the first year of management. Fallow control plots were included under conventional management only. This was done both for practicality reasons of maintaining an organic winter fallow, and because the study sought to compare organic management with a cover crop (most intensive) against the conventional with a cover crop (moderate) and conventional with winter fallow (baseline control). Cover crop treatments included rye (*Secale cereale*), radish (*Raphanus sativus*), rye/vetch mix (rv), and rye/radish/vetch (rvr) mixes (Table 1). Each treatment included three replicates and four rows per plot. Plot area sizes were 10.7 m x 4.1 m, with each row being 1 m wide.

Table 1. Cover crop treatments.

Management	**Cover Crop**	**Seeding rate ($kg\ ha^{-1}$)**
Organic	Rye	33.63
	Radish	11.21
	Rye/Vetch	28.02/5.6
	Rye/Vetch/Radish	28.02/3.36/2.24
Conventional	Fallow	n/a
	Rye	33.63
	Radish	11.21
	Rye/Vetch	28.02/5.6
	Rye/Vetch/Radish	28.02/3.36/2.24

Organic management was implemented by using mechanical weed control and mechanical cover crop termination via roller/crimper or stalk cutter, and composted manure was used in place of chemical fertilizer. Conventional management received chemical fertilizer, along with chemical weed control and cover crop termination. Cover crops were terminated in late April of 2020. Termination for conventional cover crops at Lubbock required an application of 2,4-D amine on March 31 and two applications of Roundup on April 27 and May 5. Cover crops under organic management at Lubbock were terminated by stalk cutter on April 29. Vernon cover crops were all terminated on April 17, with 4 pt/ac Enlist Duo herbicide applied to conventionally managed cover crops, and sweep plow for organic.

Data Collection

Cover crop herbage mass was collected prior to termination at Lubbock and Vernon from two 2.7 m^2 areas of each plot on April 2 and 16, respectively. The herbage mass was weighed after drying to a constant weight in a forced air oven.

Soil sampling and gas flux collections were conducted during the last two weeks of each month between April and November. See Table 2 for collection dates per location and corresponding peanut growth stages according to Boote (1982). Pre-plant collections occurred shortly before cover crop termination at Lubbock and shortly after termination at Vernon. Soil from these collections were analyzed for enzyme activities and mineralizable C.

Table 2. Sampling dates and peanut growth stages by location.

Lubbock		**Vernon**	
Sampling Date	**Growth Stage**	**Sampling Date**	**Growth Stage**
4/20/2020 – 4/22/2020	Pre-plant	4/23/2020 – 4/24/2020	Pre-plant
5/17/2020-5/19/2020	V1	5/28/2020-5/29/2020	V1
6/27/2020-6/29/2020	V1 (re-plant)	6/30/2020-7/2/2020	R1
7/14/2020-7/16/2020	R2	7/17/2020-7/19/2020	R3
8/13/2020-8/15/2020	R3	8/16/2020-8/18/2020	R4
9/23/2020	R4-R5	9/29/2020	R6-R7
11/18/2020-11/20/2020	Post-harvest	11/5/2020-11/6/2020	Post-harvest

Soil samples were collected along the nontraffic rows at 0-10 and 10-20 cm depths. Six cores were collected and divided by depth before compositing at Lubbock using a 2.5 cm diameter soil probe, and 3 composite cores were collected at Vernon using a 7.6 cm diameter soil auger. Soils were air-dried and sieved to 2 mm.

Gas flux readings were collected with a portable FTIR gas analyzer (Gasmet DX4040 FTIR, Gasmet Technologies, Helsinki, Finland). Polyvinylchloride (PVC) soil collars (20 cm outer diameter) were set in the field for 24 hrs, and 5 height measurements were taken to calculate soil collar volume. Collections were taken by vacuum sealing a LiCor chamber (Li-8100-103, Li-Cor Biosciences, Lincoln, NE USA; 4.8 L volume) to soil collars so that emissions could cycle through the gas analyzer. Readings for CO_2, N_2O, and CH_4 were collected for 8 minutes followed by a 5-minute interval between readings for the gas analyzer to return to atmospheric CO_2. A filtration system was used to prevent moisture and dust buildup inside the gas analyzer. Additional collections for soil surface moisture percentage and temperature were collected with a field hydraprobe (Stevens Field POGO Turf and Soil Insight Tool and Hydraprobe II RS485, Stevens Water Monitoring Systems, Inc, OR USA) in tandem with gas analyzer readings, as these factors contribute to soil greenhouse gas (GHG) fluxes (Wagai et al., 1998).

The CO_2 flux slopes with an r^2 value of 0.7 or greater were considered significant enough for use in analysis (McDonald et al., 2019). These were then converted to mg CO_2-C m^{-2} hr^{-1} with the ideal gas law equation:

$$PV = nRT$$

This took into account the survey chamber and soil collar volumes, atmospheric pressure at each location, and air temperature at the time of each reading. Cumulative fluxes were then calculated by averaging fluxes at two given collection dates, multiplying by 24 hrs and the number of days between collection dates, and adding this to the previous cumulative flux to date.

In August, when peak nodulation was expected (Baughman et al., 2019), roots and aboveground peanut biomass were collected from plot rows 1 and 4 (0.18 m^2 area) of the organic and conventional rye and rye/vetch treatments, along with the fallow. Due to a radish cover crop failure, which occurred solely at Lubbock, radish and rye/vetch/radish treatments were excluded. Nodule counts were then recorded according to size category (0-2 mm, 2-4 mm, > 4 mm diameter) and placement on root system (taproot, lateral root). Percent active nodulation was determined by slicing nodules in half to determine if they exhibited a pink to bright red color, which would indicate the presence of active leghemoglobin and nitrogenase activity (Dupont et al., 2012). Only nodules from the 2-4 mm and > 4 mm categories were opened, as it would have been impractical to open all nodules 0-2 mm in diameter, and nodule counts from the 0-2 mm category were excluded when calculating percent active nodulation. Taproot length and three taproot diameters were also collected per plant to allow results to be normalized according to taproot surface area (cm^2).

Soil Biological Parameters

Mineralizable Carbon

Mineralizable C was determined using a 3-day CO_2 incubation as described by Franzluebbers (2015). Briefly, 40 g of air-dried soil was introduced to a 50 mL centrifuge tube and rewet to 60% water filled pore space (Hurisso, 2016); 10 mL of 1 N NaOH was added to a second 50 mL centrifuge tube.

Upon introducing water and NaOH, both tubes were placed in a 1 L glass jar which contained 10 mL of water to maintain humidity. This setup was immediately sealed and incubated at 25 C for three days (72 hrs). After this timeframe had passed, the NaOH tube was removed from the jar and titrated with 0.5 N HCl. For titrations, 2 drops of phenolphthalein indicator and 1 mL of 1.5 M $BaCl_2$ was added to each NaOH trap to precipitate out barium carbonate. Evolved CO_2 was then calculated from the volume of HCl used to neutralize unreacted NaOH according to the following equation from Franzluebbers (2015).

$$CO_2 - C(mg\ kg^{-1} soil) = \left(ml_{[blank]} - ml_{[sample]}\right) * N * M/S$$

Enzyme Activity

Standard procedures for measuring soil enzyme activities were based off of work by Eivazi and Tabatabai (1988), Deng and Popova (2011), and Parham and Deng (2000). Activities of β-glucosidase (BG) and N-acetyl-β-D-glucosaminidase (NAG) were measured by microplate assay to allow for high-throughput testing. The assay was a scaled down version of the recommended NRCS colorimetric benchtop method (Stott, 2019), with reagents concentrated so that the reaction could occur in 2.2 mL 96-deepwell plates. This method involved incubating replicates of air-dried soil (0.5 g) with a substrate and start buffer (MUB at pH 6 for β-glucosidase or acetic acid at pH 5.5 for N-acetyl-β-D-glucosaminidase) plus at least one negative sample control with buffer and no substrate for one hour at 37 C. This was followed by addition of $CaCl_2$ to prevent OM dispersion (Deng and Popova, 2011, p. 198), a stop buffer (THAM for β-glucosidase or NaOH for N-acetyl-β-D-glucosaminidase) to end the reaction, and the addition of substrate to the negative sample control to account for background readings. (Stott, 2019) Reagents were concentrated to the following: 0.0625 mol β-glucosidase substrate, 0.5 mol THAM stop buffer, 0.0125 mol N-acetyl-β-D-glucosaminidase substrate, 0.25 mol sodium acetate pH 5.5 start buffer, 2.5 mol NaOH stop buffer, 2.5 mol $CaCl_2$.

Published microplate methods by Popova and Deng (2010) and Jackson et al. (2013) recommend NaOH for the β-glucosidase assay to avoid high background readings, but Acosta-Martinez (2019) and Eivazi and Tabatabai (1994, p. 825) caution against this, as NaOH may hydrolyze β-glucosidase substrate bonds and interfere with results. Therefore, THAM was used as the stop buffer for β-glucosidase, as per the NRCS methods (Stott, 2019). Microplate methods by Popova and Deng (2010) and Deng et al. (2013) also recommend additional sample replicates when working with concentrated reagents, so 5 sample replicates were used (3 positive, 2 negative controls). The remainder of the reaction was carried out according to NRCS methods (Stott, 2019). Plates were shaken for 5 minutes at the end of the experiment to ensure that the reaction was fully terminated, and these were then centrifuged for 5 minutes at 500 rpm to separate the solution from soil solids. Solution samples were then placed on a 96-well plate to be read at 405 nm, and were diluted if necessary. Calibration curves were prepared according to NRCS methods, and enzyme activity was calculated as µg p-nitrophenol per gram of soil per hour.

PLFAs

A portion of each composited sample from the April collection (0-10 cm and 10-20 cm depths) was freeze-dried and sent to the University of Missouri Soil Health Assessment Center in Columbia, MO for PLFA testing. Testing was carried out according to the Buyer/Sasser extraction method using the MIDI Sherlock GC software (Microbial Identification Inc., Newark, DE).

Statistical Analysis

Results were analyzed using PROC GLIMMIX in SAS (Version 9.4, SAS Institute Inc. Cary, NC). Unless otherwise noted, alpha = 0.05.

The experimental design only contained replicates for fallow plots under conventional management. This is because the project sought to avoid tillage when possible, and it would have been difficult to maintain an organic no-till winter fallow without the use of herbicide. To account for this, data for the main portion of this study were initially analyzed without the fallow to determine if any management by cover crop interactions existed. Most results had no such interaction, so results for management and cover crop selection were then re-analyzed separately with the fallow included.

Results and Discussion

Yield

Results of this study revealed that although both locations saw significant differences in cover crop herbage mass according to management, there were no differences in final peanut yield based on management or cover crop selection (Table 3).

In situ Respiration

At Lubbock, greater cumulative CO_2 fluxes were observed under conventional (CON) management as compared to the organic (ORG) or fallow treatments whereas at Vernon, there were no differences in cumulative ORG or CON CO_2 fluxes, but use of a cover crop ultimately resulted in greater fluxes than the fallow (Table 3, Figure 2). Management by event interactions occurred at both locations, and due to management by cover crop interactions at Lubbock, cumulative CO_2 data were graphed over time according to both management and cover crop treatments (Table 4, Figure 2).

At Lubbock, soil surface moisture percentage tended to be higher under CON than ORG (Table 4). This was also occasionally the case at Vernon, but results varied by event (Table 4). Correlation analyses indicated that CO_2 fluxes within each given month were significantly related to soil surface moisture readings at both locations, and that fluxes at the Lubbock location were also strongly correlated to soil surface temperature with weaker correlations to cover crop biomass (Table 5). *In situ* CO_2 fluxes are a measure of microbial respiration and, by extension, activity. Fluxes are commonly understood to be positively influenced – up to a point – by soil temperature and water content (Wagai et al., 1998). Additionally, C and N inputs from cover crops act as substrates for microbes to use as energy and are associated with elevated GHG fluxes in the form of CO_2 and N_2O (Daryanto et al., 2018).

Table 3. Cover crop biomass, peanut yield, and cumulative CO_2 flux results.

		Lubbock			**Vernon**		
Manage	**Cover Crop**	**Cover Crop Biomass (kg ha^{-1})**	**Peanut Yield (kg ha^{-1})**	**Cumulative CO_2 (kg ha^{-1})**	**Cover Crop Biomass (kg ha^{-1})**	**Peanut Yield (kg ha^{-1})**	**Cumulative CO_2 (kg ha^{-1})**
ORG	Rye	1481^{b}	4589	1648	2595^{ab}	4132	4257
	Radish	n/a	n/a	n/a	833^{c}	3387	3921
	Rye/Vetch	2020^{a}	4591	2198	1345^{bc}	3595	4432
	Rye/Vetch/Radish	1303^{b}	4589	1647	1935^{abc}	4150	3747
CON	Rye	1437^{b}	4590	2584	3396^{a}	3137	4402
	Radish	n/a	n/a	n/a	3093^{a}	2759	3664
	Rye/Vetch	1185^{b}	4587	2344	2956^{ab}	3705	4147
	Rye/Vetch/Radish	1019^{b}	4587	2242	3479^{a}	3247	5775
p-values	Management	**0.017**	0.322	**0.001**	**0.001**	0.192	0.465
	Cover Crop	0.062	0.891	0.164	0.277	0.725	0.667
	Interaction	0.095	0.391	0.082	0.658	0.812	0.412

Significant p-values in bold.

Letters that are the same in each column indicate that Fisher's LSD are not significantly different at p = 0.05.

CO_2 results represent cumulative flux at the end of the season.

Fallow excluded to allow for interactions.

Table 4. ANOVA results for CO_2 flux, % soil moisture, and soil surface temperature (C). All interactions. Lubbock and Vernon analyzed separately. Fallow excluded for interactions to be calculated.

	Lubbock			**Vernon**		
	CO_2	**% Moist**	**Soil Temp**	**CO_2**	**% Moist**	**Soil Temp**
Effect	*p-values*					
cover	0.092	0.87	0.377	0.665	0.851	0.953
Manage	**0.0003**	**<0.0001**	0.122	0.554	0.962	**0.049**
cover*manage	**0.04**	0.091	0.954	0.378	0.198	0.755
Event	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
cover*event	0.916	0.68	0.597	0.919	0.162	0.998
manage*event	**<0.0001**	**<0.0001**	0.284	**0.016**	**0.0004**	**0.001**
cover*manage*event	0.525	0.85	0.891	0.959	0.809	0.997

Significant p-values in bold

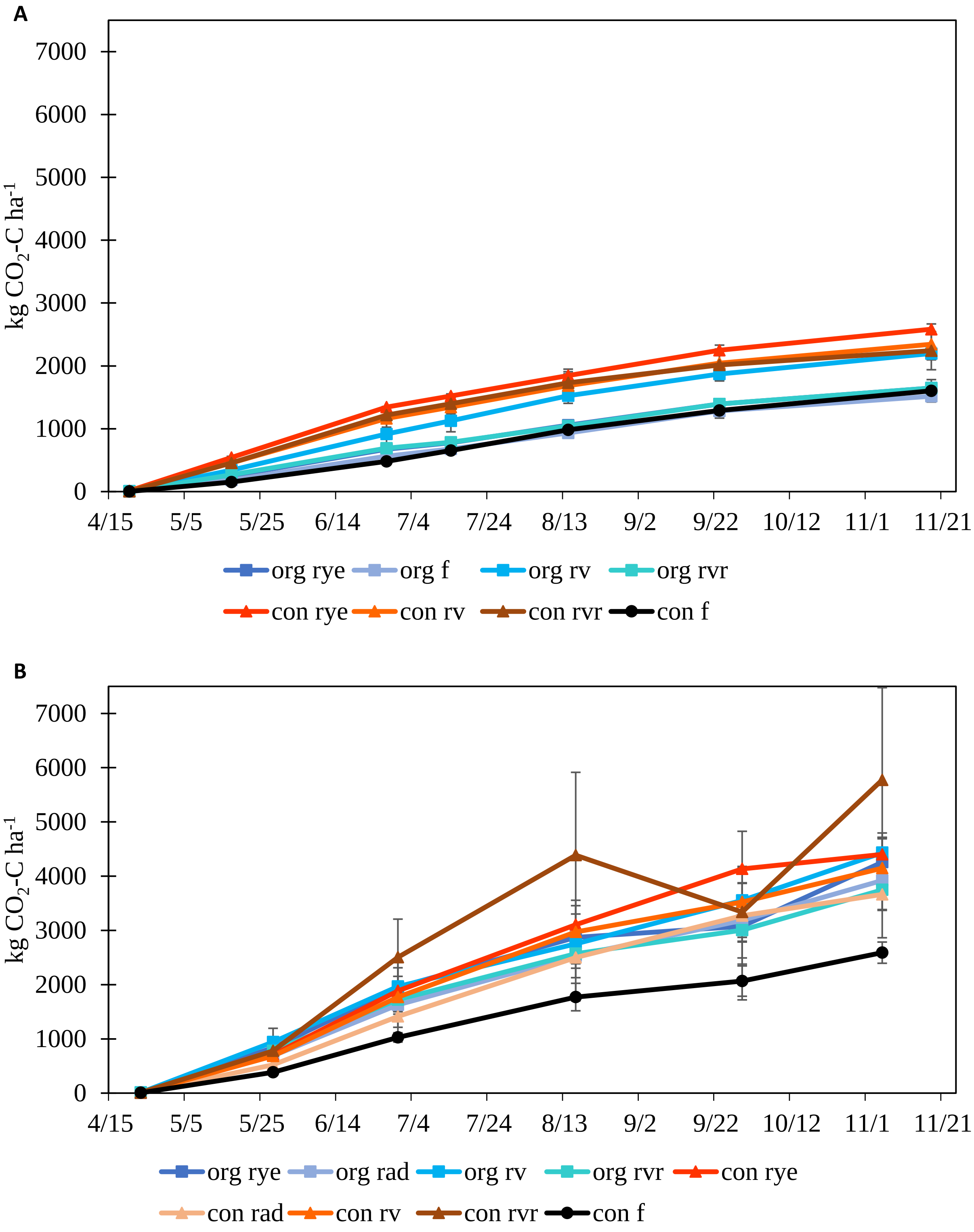


Figure 2. Cumulative CO_2 fluxes at Lubbock (A) and Vernon (B) over time. Samples collected monthly (n = 3). Bars indicate standard error of the mean.

Table 5. Pearson’s correlations at Lubbock and Vernon

	Pierson's Correlations										
	% Moist	**Soil Temp**	**CO_2**	**Cover biomass**	**Yield**	**CMIN 0-10**	**CMIN 10-20**	**BG 0-10**	**BG 10-20**	**NAG 0-10**	**NAG 10-20**
						Lubbock					
% Moist	-------										
Soil Temp	0.079	-------									
CO_2	0.641***	0.339***	-------								
Cover biomass	0.04	0.091	0.145**	-------							
Yield	0.016	-0.055	-0.018	0.163***	-------						
CMIN 0-10	0.208***	-0.292***	0.071	0.173***	0.063	-------					
CMIN 10-20	0.157***	-0.162***	0.156***	0.009	-0.008	0.463***	-------				
BG 0-10	0.263***	-0.089	-0.18**	0.229**	-0.024	0.036	-0.056	-------			
BG 10-20	0.362***	0.283***	0.14	0.122	0.021	-0.28***	-0.22***	0.398***	-------		
NAG 0-10	0.068	-0.259***	-0.176*	0.106	-0.002	0.41***	0.327***	0.165*	-0.202**	-------	
NAG 10-20	-0.022	0.098	0.051	0.144	-0.017	0.048	0.237***	0.158*	0.218**	0.261***	-------
						Vernon					
% Moist	-------										
Soil Temp	-0.25***	-------									
CO_2	0.395***	0.028	-------								
Cover biomass	-0.03	0.041	0.065	-------							
Yield	0.008	-0.051	0.009	-0.106*	-------						
CMIN 0-10	-0.034	-0.025	0.024	-0.027	0.128*	-------					
CMIN 10-20	-0.2**	0.102	-0.068	-0.011	0.03	0.483***	-------				
BG 0-10	0.117	0.206**	0.198*	-0.102	0.286***	0.412***	-0.005	-------			
BG 10-20	0.118	0.047	0.145	-0.003	0.166*	0.331***	0.301***	0.46***	-------		
NAG 0-10	-0.166*	0.086	-0.374***	-0.032	0.121	0.358***	0.254***	0.333***	0.195**	-------	
NAG 10-20	-0.167*	0.102	-0.389***	0.008	0.054	0.303***	0.414***	0.115	0.403***	0.504	-------

* Pearson’s Correlation is significant at p = 0.1

** Pearson’s Correlation is significant at p = 0.05

*** Pearson’s Correlation is significant at p = 0.01

0-10 and 10-20 refer to collection depth in cm

Numerous studies have indicated that cover crop usage results in elevated microbial activities (Kim et al., 2020; Daryanto et al., 2018). In particular, one meta-analysis by Kim et al. (2020) found positive global effect size means for β-glucosidase (BG) and CO_2 respiration under cover crop treatments, indicating that elevated microbial activities could be expected when such treatments are applied. Likewise, cover crop use from this study typically resulted in greater cumulative respiration than the fallow both in terms of selection and management, although this was only significant at the Lubbock location (Figure 2a).

Wagai et al. (1998) also suggests that the presence of cover crop residues can lower soil surface temperatures, which may lead to lower CO_2 fluxes. This was not apparent in the results, although significantly greater levels of cover crop biomass were harvested under organic management as compared to conventional (Table 3). Instead, higher moisture levels under CON likely stimulated microbial activity in the Lubbock plots, resulting in greater *in situ* respiration (Figure 4). Cumulative CO_2 flux results for fallow from this location were similar to those observed under organic management (Table 3, Figure 2b), and this may have resulted from low levels organic inputs. At Vernon, there was a management by event interaction where CON was greater than ORG during one collection event. However, end-of-season cumulative fluxes for this location were not different between ORG and CON, likely due to the use of composted manure and fertilizer at the beginning of the season because these may stimulate microbial activity and respiration (Santos et al., 2012; Nautiyal et al., 2010).

Enzyme Activity

Enzyme activities for β-glucosidase (BG) and N-acetyl-β-D-glucosaminidase (NAG) were quantified at 3 points throughout the growing season. These included pre-plant (April), mid-season (July, August), and post-harvest (November). The Vernon mid-season collection occurred in July while Lubbock's occurred in August due to replanting at the Lubbock location.

Enzyme data for management and cover crop selection were analyzed separately and pooled to include both depths as there were no depth or management by cover interactions (Table 6).

Table 6. ANOVA results for CMIN, BG, and NAG. All interactions. Lubbock and Vernon analyzed separately. Fallow excluded for interactions to be calculated.

	Lubbock			**Vernon**		
	CMIN	**BG**	**NAG**	**CMIN**	**BG**	**NAG**
Effect	*p-values*					
Cover	0.803	0.216	0.408	0.400	0.860	0.082
Management	**0.033**	0.432	0.595	**0.008**	**<0.0001**	0.929
Event	**<0.0001**	**<0.0001**	0.158	**<0.0001**	**0.047**	**0.009**
Management*cover	0.783	0.764	0.301	0.117	0.429	0.270
Cover*depth	0.703	0.891	0.492	0.222	0.503	0.511
Management*depth	0.975	0.518	0.206	**0.008**	0.098	0.918
Management*cover*depth	0.490	0.713	0.584	0.274	0.553	0.581
Cover*event	0.570	0.685	**0.019**	**0.040**	0.572	0.127
Management*event	**0.017**	0.954	**0.011**	**0.0003**	0.057	**0.0002**
Management*cover*event	1	0.170	0.850	0.070	0.582	0.950
Cover*event*depth	0.678	0.346	0.387	0.889	0.597	0.848
Management*event*depth	0.937	0.642	0.382	0.057	0.679	0.413
Management*cover*event*depth	0.806	0.638	0.777	0.307	0.950	0.943

Significant p-values in bold (p = 0.05)

Results for BG found differences at the Vernon location alone. BG activities according to cover crop selection did not differ from each other (Table 7), but cover cropping with rye and mixes did result in greater BG activities than the fallow (Table 8, Figure 3a). BG activity for management at Vernon was greater for ORG than CON or fallow during both the pre-plant and mid-season collections (Figure 3b). Lubbock results showed no difference in BG activity, although management appeared to follow a similar pattern (Figure 4).

Table 7. ANOVA results for enzyme activities by collection event. Fallow excluded to calculate interactions.

	Lubbock						**Vernon**					
	BG			**NAG**			**BG**			**NAG**		
	Manage	**Cover**	**Interact**	**Manage**	**Cover**	**Interact**	**Manage**	**Cover**	**Interact**	**Manage**	**Cover**	**Interact**
Month	*p-values*											
Pre-plant	0.741	0.728	0.497	0.159	0.697	0.709	**0.024**	0.233	0.824	0.186	**0.017**	0.359
Mid-season	0.897	0.845	0.929	0.134	0.181	0.261	**<0.0001**	0.797	0.469	**0.0009**	0.156	0.641
Post-harvest	0.836	0.705	0.914	0.094	**0.031**	0.975	0.318	0.795	0.603	0.0097	0.13	0.356

Significance at p = 0.05

Significant p-values in bold

Table 8. ANOVA results for enzyme activities by collection event. Fallow included.

	Lubbock				**Vernon**			
	BG		**NAG**		**BG**		**NAG**	
	Manage	**Cover**	**Manage**	**Cover**	**Manage**	**Cover**	**Manage**	**Cover**
Month	*p-values*							
Pre-plant	0.451	0.542	0.168	0.581	**0.004**	0.049	0.192	**0.022**
Mid-season	0.659	0.749	0.21	0.241	**<0.0001**	0.214	**0.0007**	0.113
Post-harvest	0.593	0.69	0.282	**0.03**	0.152	0.448	**0.027**	0.219

Significance at p = 0.05

Significant p-values in bold

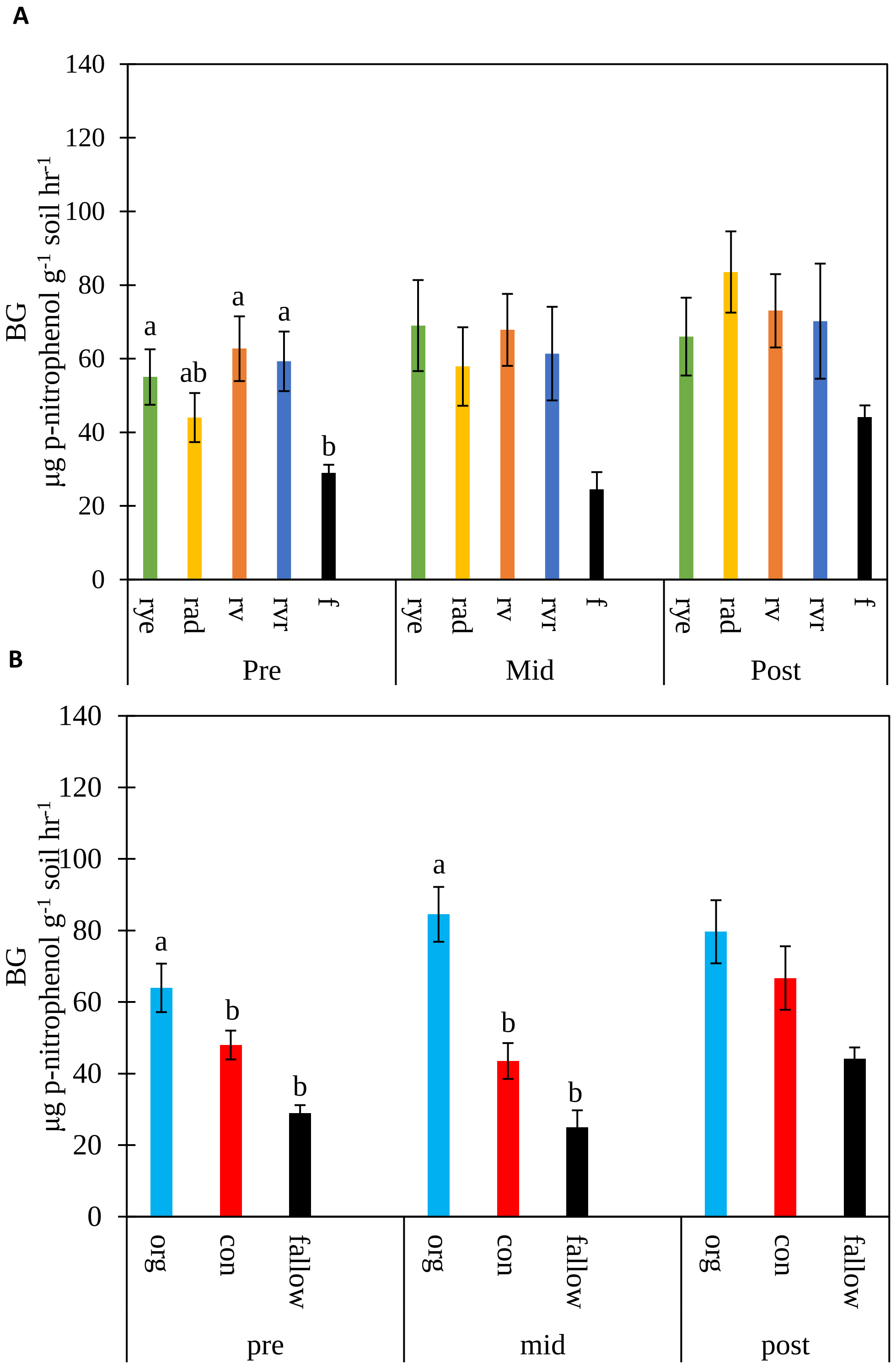


Figure 3. β-glucosidase enzyme activity at Vernon according to cover crop selection (A, n = 12) and management (B, n = 24). Fallow n = 6. Depths 0-10 cm and 10-20 cm pooled due to no depth interactions. Results analyzed within each collection event. Bars with the same letters indicate that Fisher's LSD are not different at p = 0.05. Bars indicate standard error of the mean.

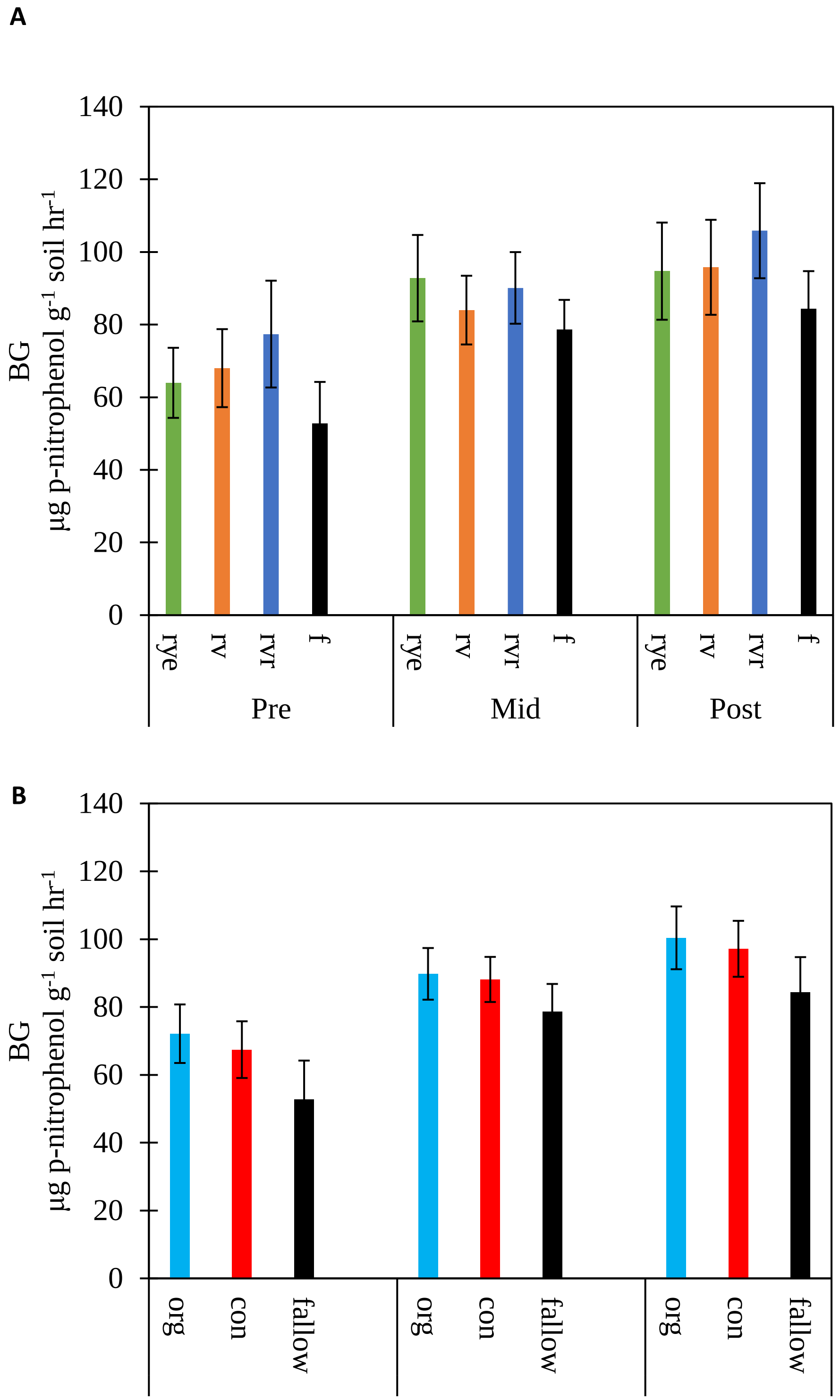


Figure 4. β-glucosidase enzyme activity at Lubbock according to cover crop selection (A, n = 12) and management (B, n = 18). Fallow n = 6. Depths 0-10 cm and 10-20 cm pooled due to no depth interactions. CON rad removed and ORG rad treated as organic fallow. Results analyzed within each month. Bars with the same letters indicate that Fisher's LSD are not different at $p = 0.05$. Bars indicate standard error of the mean.

Higher BG activity under ORG compared to CON at Vernon was likely driven by the application of fertilizer and composted manure at the beginning of the growing season, as the composted manure would have provided additional organic substrates for BG enzymes to degrade (Gianfreda, 2005; Zhang et al., 2015a). Additionally, incorporation of cover crop residues and composted manure through tillage likely contributed to the more notable results at Vernon compared to Lubbock. Results from Vernon are also in keeping with those from Zhang et al. (2015b), which found that BG enzyme activities had a strong positive response to organic amendments such as manure as compared to synthetic fertilizer inputs. Rye was expected to result in greater BG activities later in the season because of slower decomposition from its high lignin content, but this did not hold true for either location.

NAG results according to cover crop selection are of particular interest. At Lubbock, NAG results for the rye/vetch treatment were elevated at the post-harvest collection (Table 8, Figure 5a). Likewise at the Vernon pre-plant collection, NAG activities under the rye/vetch treatment were elevated (Table 8, Figure 6a). The Lubbock location experienced no differences in NAG activity according to management while Vernon did at both the mid and post-harvest collections (Figure 5b, Figure 6b).

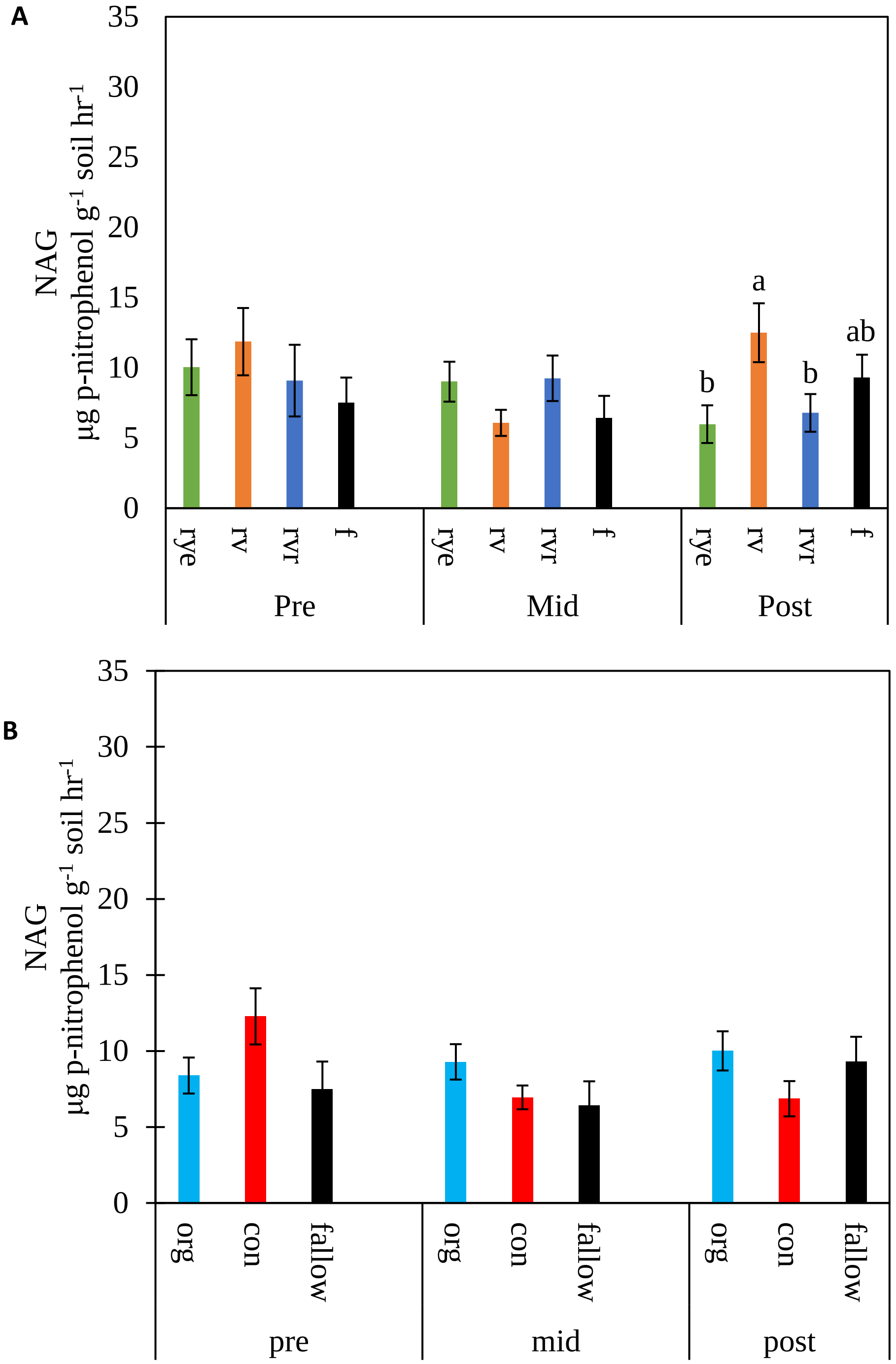


Figure 5. N-acetyl-β-D-glucosaminidase enzyme activity at Lubbock according to cover crop selection (A, n = 12) and management (B, n = 18). Depths 0-10 cm and 10-20 cm pooled due to no depth interactions. CON rad removed, and ORG rad used as organic fallow due to radish failure. Results analyzed within each collection event. Bars with the same letters indicate that Fisher's LSD are not different at p = 0.05. Bars indicate standard error of the mean.

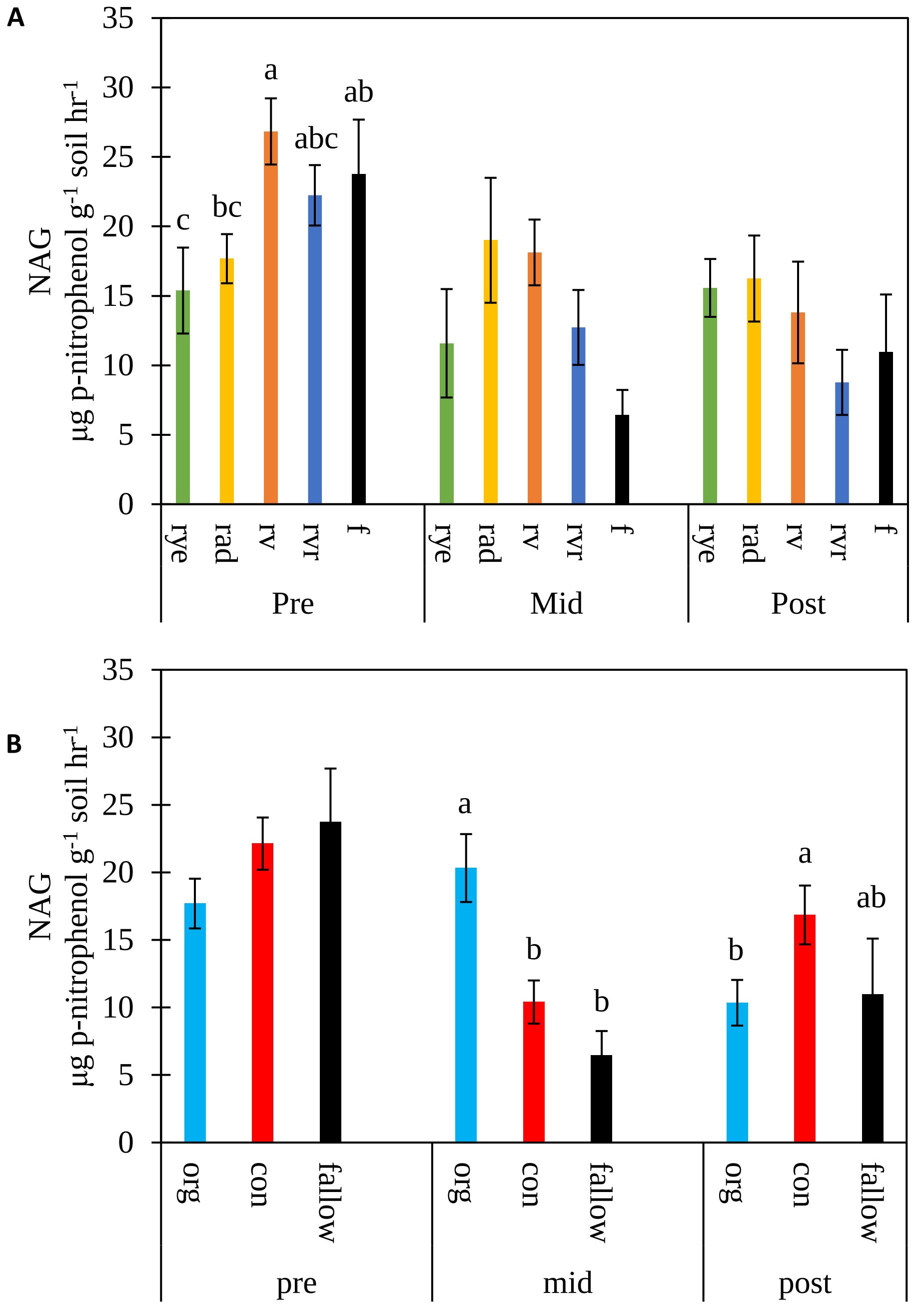


Figure 6. N-acetyl-β-D-glucosaminidase enzyme activity at Vernon according to cover crop selection (A, n = 12) and management (B, n = 24). Depths 0-10 cm and 10-20 cm pooled due to no depth interactions. Results analyzed within each collection event. Bars with the same letters indicate that Fisher's LSD are not different at p = 0.05. Bars indicate standard error of the mean.

Vetch is a legume which undergoes nodulation, and for nodulation to occur, rhizobia produce Nod factors which are comprised of lipo-chitin oligosaccharides (Dupont et al., 2012). Previous studies have reported degradation of Nod factors by chitinases (Schultze, et al., 2001; Perret et al., 2000). The contribution of Nod factors to organic N is unclear and remains to be tested, but NAG results may reflect the degradation of residual Nod factors from the rye/vetch treatment, although Nod factors would not be expected to be present at high concentrations in soil.

Our results are similar to those of Georgieva et al, 2005, who found differences in soil health properties between rye and vetch cover crops – for example, lower C:N ratios in soils previously treated with a vetch cover crop presumably due to N-fixation by rhizobia associated with this legume. Jian (2020) also reported similar findings but suggests that significant differences in soil health properties are more likely to be observed between single cover crop use of rye and vetch as compared to rye versus a rye/vetch mix. Therefore, while it is significant that our study noted a difference between the rye and rye/vetch treatment in terms of CMIN and NAG, it is unsurprising that these results were limited to one collection event per location. However, results likely represent an early indication of contributions to available N in systems under the rye/vetch treatment when compared to rye alone. Rye/vetch/radish treatments used lower seeding rates of vetch than rye/vetch, which may explain why NAG results were either lower or not different than rye/vetch at both locations.

Mineralizable Carbon

Mineralizable carbon (CMIN) had no management by cover crop interactions (Table 5), thus allowing data according to management and cover crop selection to be graphed independently. Collections for CMIN occurred monthly, and there was both a depth and month interaction at Vernon. Therefore, Vernon results were analyzed by event at both the 0-10 and 10-20 cm depths.

At the Vernon location, there were no differences in CMIN according to cover crop selection (Table 6, Figure 7), but there were differences according to management in 3 of the events analyzed when the fallow was included (Table 9, Figure 8). During the April collection prior to composted manure application, Vernon CMIN from conventional (CON) management was greater than organic (ORG) and fallow at the 10-20 cm depth ($p = 0.025$). This likely reflects the contribution of high cover crop herbage mass under the CON treatment to CMIN (Table 3). However, the opposite was true in July, post-composted manure application, when CMIN from ORG greater than CON and fallow at both depths (0-10 cm: $p < 0.0001$, 10-20 cm: $p = 0.013$) and September, when CMIN from ORG and CON were greater than the fallow at the 0-10 cm depth ($p = 0.089$). Results from the July collection reflect the contribution of composted manure, incorporated through tillage, to the system since ORG was greater than CON at both depths. CMIN tends to correlate well to MBC (Franzluebbers, 2015) which responds more positively to organic amendments as compared to chemical fertilizers (Zhang et al., 2012). Therefore, these results offer further support for conclusions derived from BG and CO_2 data at Vernon; application of composted manure quickly results in elevated soil biological activities.

Table 9. ANOVA results for CMIN by collection event. Fallow excluded for interactions to be calculated.

		Lubbock			**Vernon**		
		Manage	**Cover Crop**	**Interact**	**Manage**	**Cover Crop**	**Interact**
Month	**Depth (cm)**			*p-values*			
April	0-10	0.208	0.26	0.911	0.22	0.138	**0.083**
	10-20	**0.046**	0.681	0.719	0.221	0.486	0.68
May	0-10	0.159	0.153	0.4	n/a	n/a	n/a
	10-20	0.126	0.901	0.828	n/a	n/a	n/a
June	0-10	0.394	0.799	0.914	n/a	n/a	n/a
	10-20	0.854	0.286	0.303	n/a	n/a	n/a
July	0-10	0.289	0.918	0.736	< **0.0001**	0.108	0.83
	10-20	0.152	0.33	0.954	**0.045**	0.735	0.988
August	0-10	0.356	0.849	0.356	0.898	0.996	0.702
	10-20	0.177	0.762	0.529	0.129	0.559	0.776
September	0-10	0.396	0.312	0.644	0.53	0.8	0.107
	10-20	0.238	0.329	0.599	0.208	0.513	0.468
November	0-10	**0.077**	0.152	0.492	0.89	0.888	0.484
	10-20	0.41	0.3	0.49	0.781	0.343	0.54

Significance at p = 0.1

Significant p-values in bold

Table 10. ANOVA results for CMIN by collection event. Fallow included.

		Lubbock		Vernon	
		Management	**Cover Crop**	**Management**	**Cover Crop**
Month	**Depth (cm)**		*p-values*		
April	0-10	**0.027**	**0.051**	0.598	0.321
	10-20	0.116	0.923	**0.025**	0.215
May	0-10	0.249	0.16	n/a	n/a
	10-20	0.168	0.968	n/a	n/a
June	0-10	0.588	0.896	n/a	n/a
	10-20	0.857	0.195	n/a	n/a
July	0-10	0.3	0.842	**<0.0001**	0.42
	10-20	0.418	0.625	**0.013**	0.359
August	0-10	0.516	0.901	0.988	0.999
	10-20	0.206	0.776	0.265	0.725
September	0-10	**0.07**	**0.056**	**0.089**	0.272
	10-20	0.411	0.436	0.191	0.423
November	0-10	0.176	0.258	0.214	0.461
	10-20	0.435	0.242	0.932	0.425

Significance at p = 0.1

Significant p-values in bold

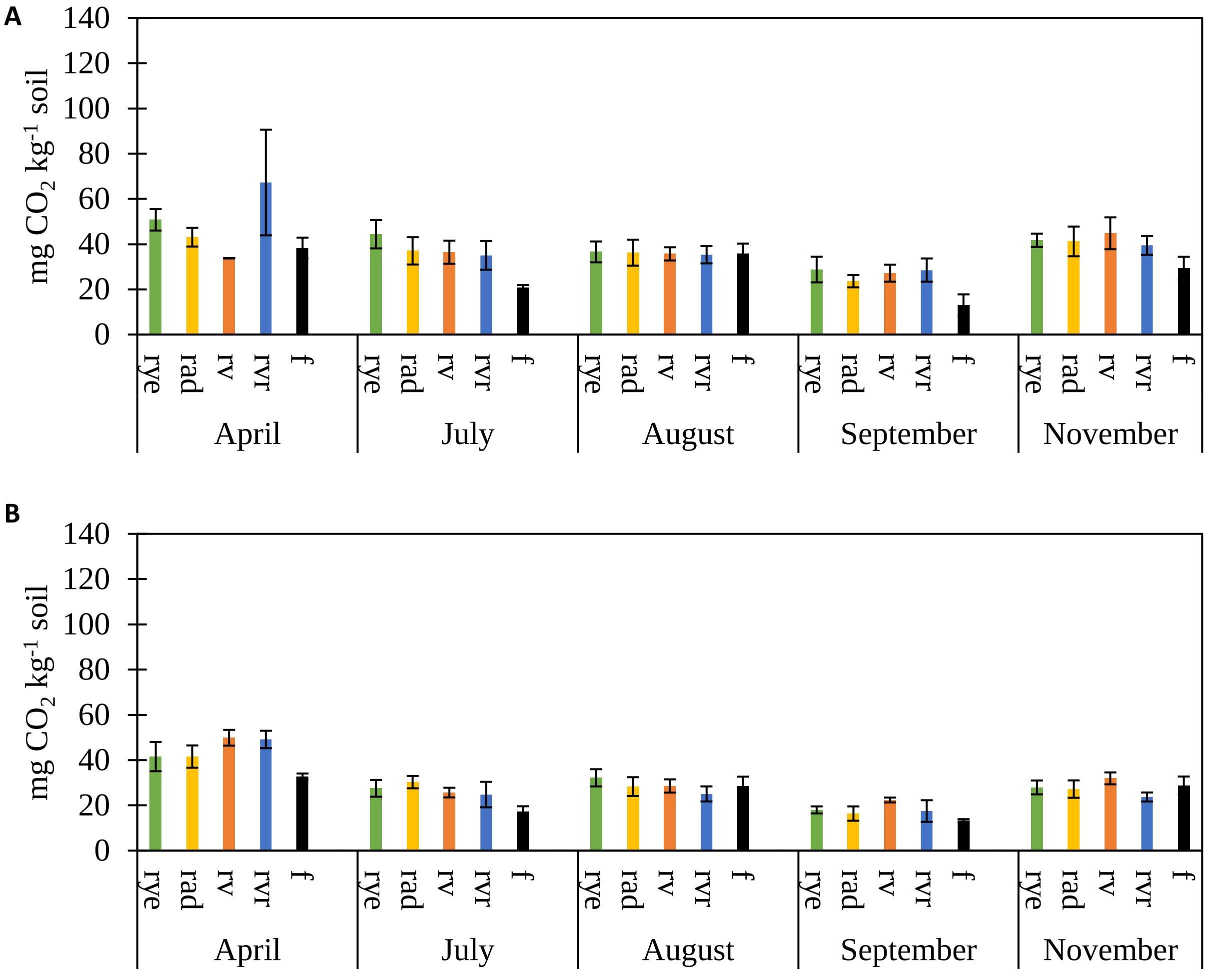


Figure 7. CMIN at Vernon according to cover crop selection (n = 6). Results analyzed within each collection event and by depth (A = 0-10 cm, B = 10-20 cm). May and June results in supplemental. Bars with the same letter indicate that Fisher's LSD are not different at p = 0.1. Bars indicate standard error of the mean.

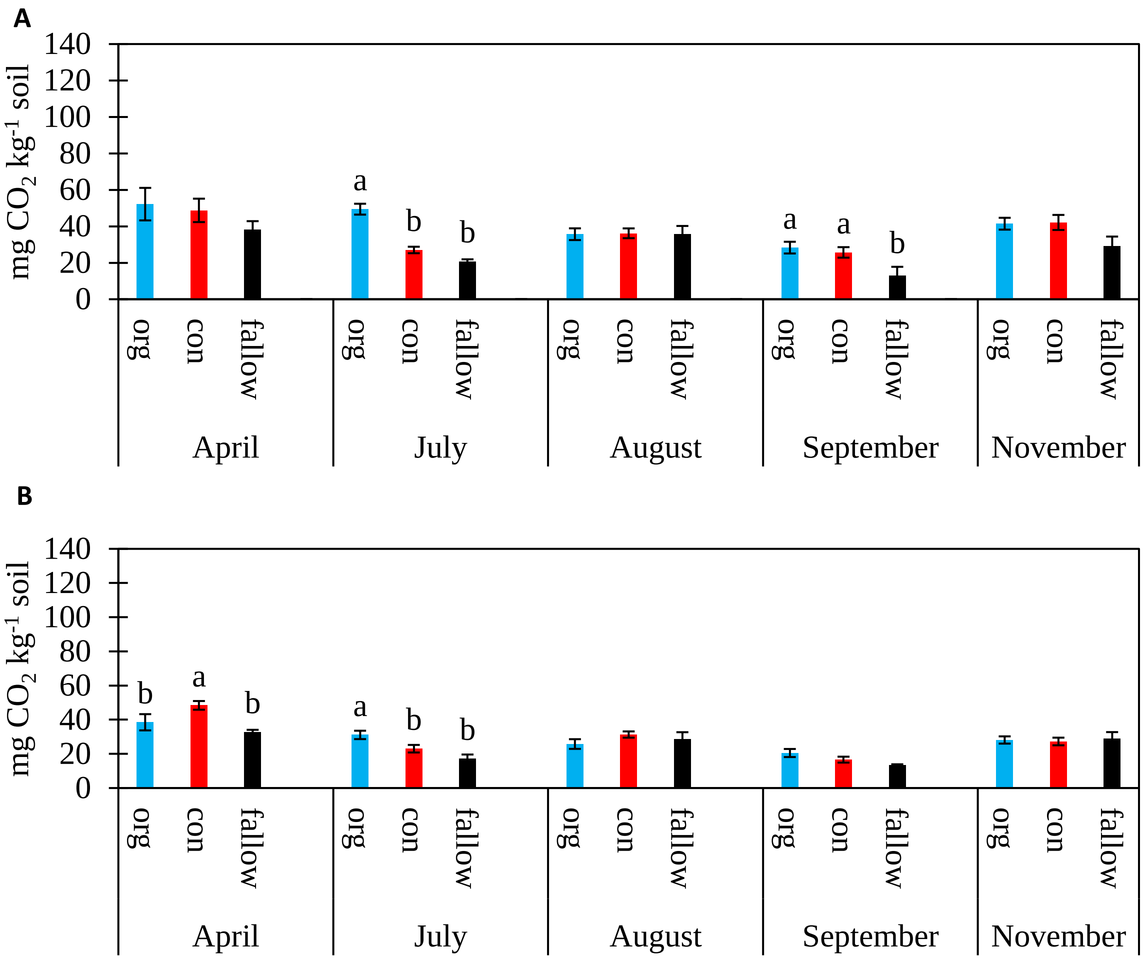
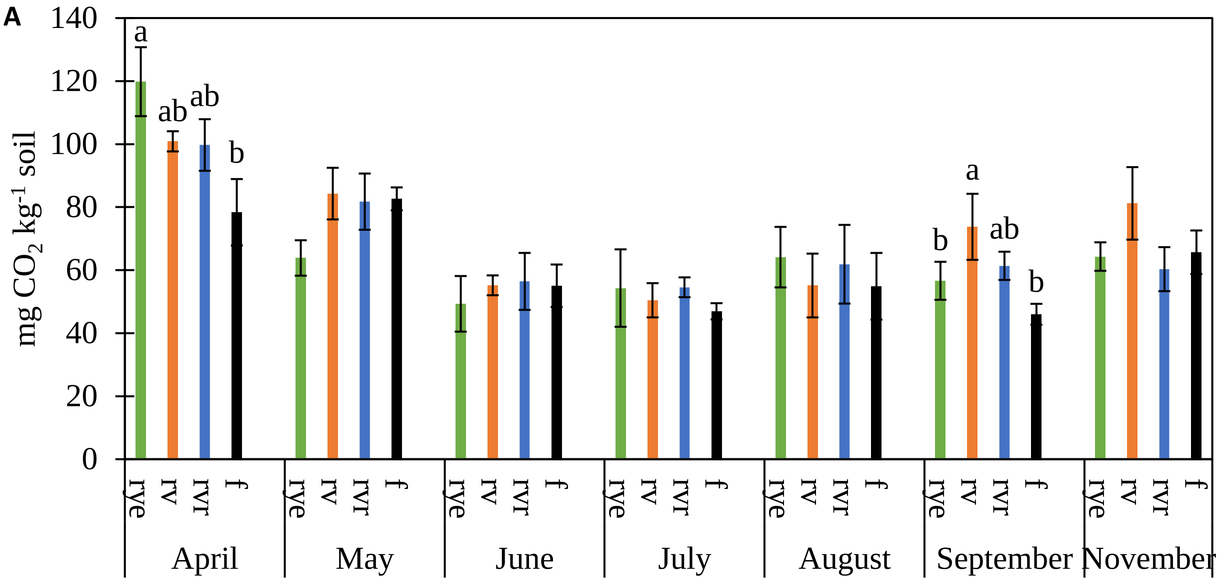


Figure 8. Mineralizable carbon at Vernon according to management (n = 12). Results analyzed within each collection event and by depth (A = 0-10 cm, B = 10-20 cm). Fallow n = 3. May and June results in supplemental. Bars with the same letter indicate that Fisher's LSD are not different at p = 0.1. Bars indicate standard error of the mean.

At the Lubbock location, there was no depth interaction (Table 6), but CMIN values were still analyzed according to depth to remain consistent with the Vernon analysis. Additionally, due to the radish cover crop failure, CON radish was removed from analysis, and ORG radish was treated as an organic fallow. Results were initially analyzed with radish included, and the use of ORG radish as a fallow did not change the outcome.

Unlike Vernon, the Lubbock location did see two instances of differences according to cover crop selection when alpha = 0.1 (Table 10). The April 0-10 cm collection indicated that

CMIN values for rye were greater than the fallow (p = 0.051). Additionally, the September 0-10 cm collection found that CMIN from the rye/vetch treatment was greater than the rye and fallow treatments (p = 0.056, Table 10, Figure 9). CMIN values for ORG and CON managements were not different at this collection, and ORG was greater than the fallow (Table 10, Figure 10). September CMIN results for cover crop selection mimic post-harvest (November) NAG results at Lubbock. CMIN at both locations was also moderately to strongly correlated to NAG at both respective depths (Table 5). Measures of CMIN have long been considered related to NMIN (Franzluebbers, 2020). NAG likewise may be considered related to NMIN, as this enzyme catalyzes the mineralization of organic N compounds in the form of amino sugars. CMIN results from Lubbock therefore support conclusions drawn from NAG results in terms of early contributions to organic N by the rye/vetch treatment.



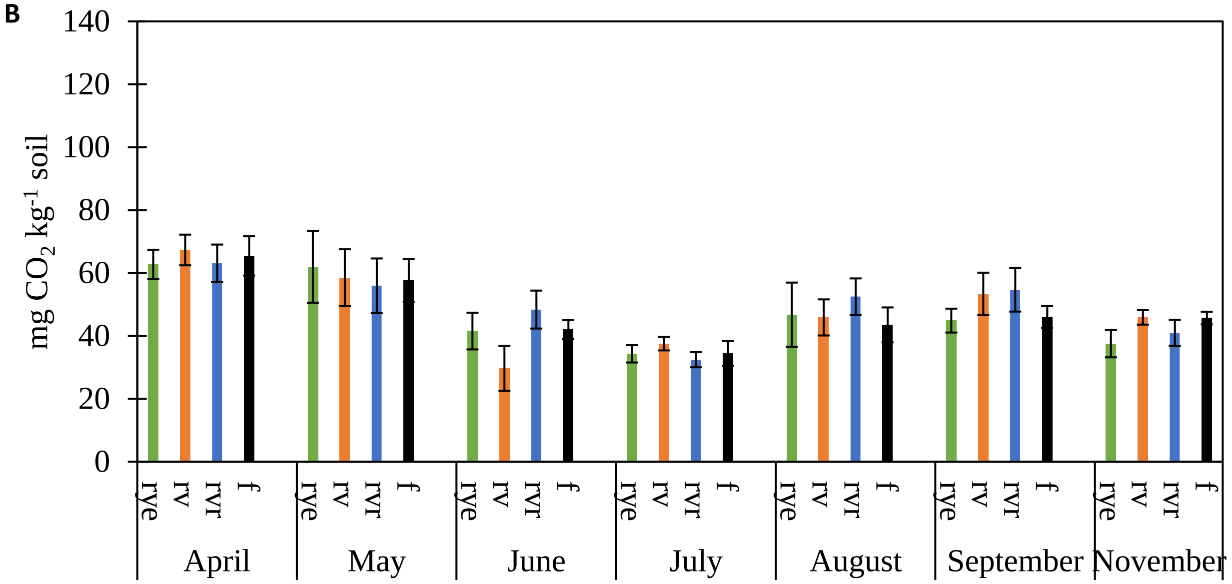


Figure 9. Mineralizable carbon at Lubbock according to cover crop selection (n = 6). Results analyzed within each collection date and by depth (A = 0-10 cm, B = 10-20 cm). CON rad excluded, and ORG rad treated as fallow. Bars with the same letter indicate that Fisher's LSD are not different at $p = 0.1$. Bars indicate standard error of the mean.

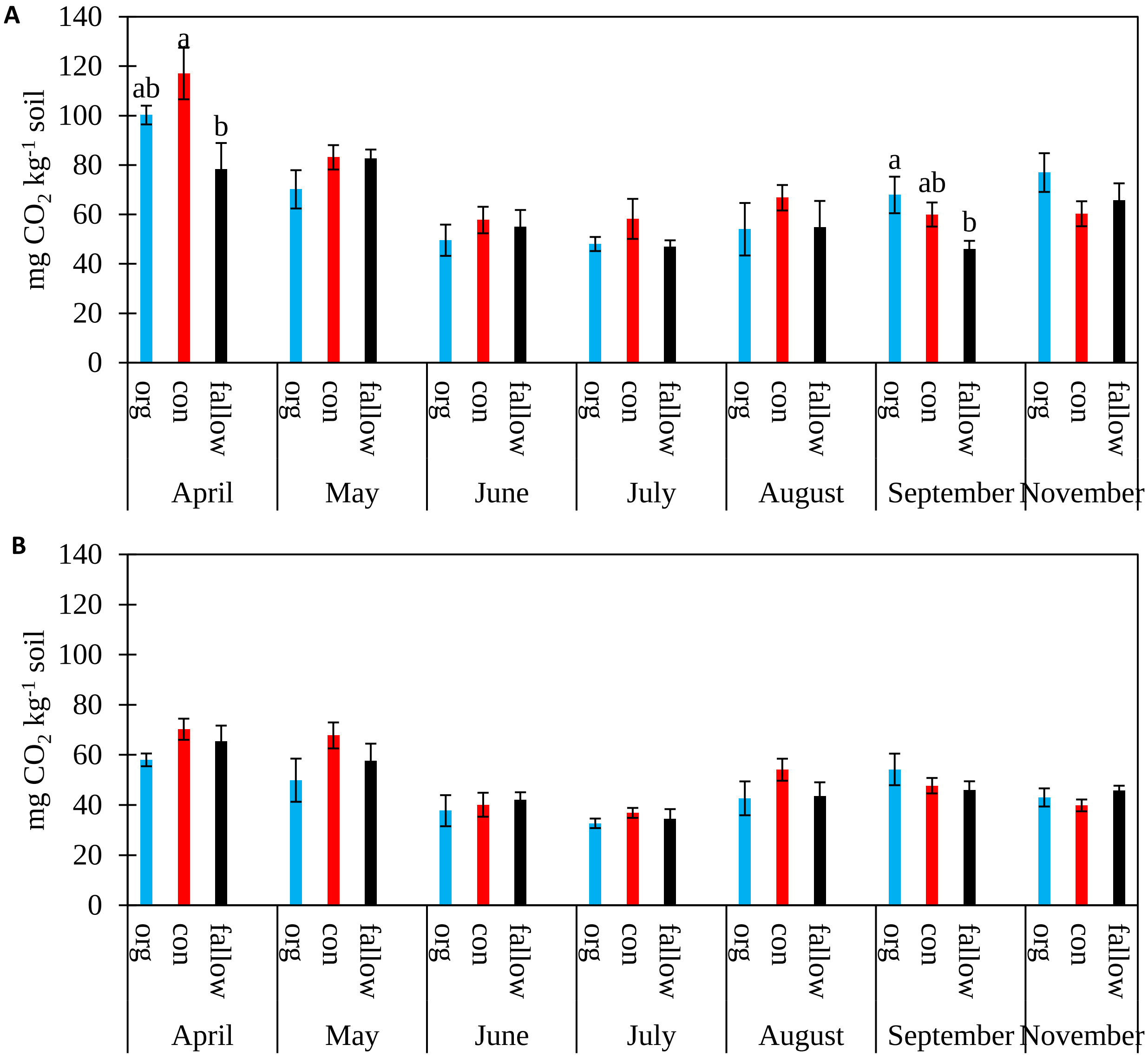


Figure 10. Mineralizable carbon at Lubbock according to management (n = 9). Fallow n = 6. Results analyzed within each collection event and by depth (A = 0-10 cm, B = 10-20 cm). CON rad excluded, and ORG rad treated as fallow. Bars with the same letter indicate that Fisher's LSD are not different at p = 0.1. Bars indicate standard error of the mean.

Nodulation

Nodulation patterns varied between both locations. At Vernon, nodule counts were higher, but percent active nodulation was lower than what was observed for Lubbock (Table 11). Peanuts at the Lubbock location were one month behind those at Vernon due to the June replant, potentially contributing to the count differences between locations. All peanuts at Lubbock and CON peanuts at Vernon were inoculated with *Bradyrhizobium* at the beginning of the season while ORG peanuts at Vernon were not and instead relied on native soil rhizobia. Additionally, irrigation water nitrate levels at both locations were monitored and recorded as 8 ppm and 20 ppm at Lubbock and Vernon, respectively. Irrigation water nitrate levels and the application of chemical N fertilizer and composted manure at Vernon could have contributed to the high-count-low-active-nodulation results at this location since nodulation is negatively related to available N (Baughman et al., 2019; Weaver, 1974; Castro et al., 1999), and legumes can sanction resources to their nodules if adequate N is present (Kiers et al., 2003). Additionally, since peanuts are capable of forming nodules with multiple different species of rhizobia, they are considered a fairly promiscuous legume (Castro et al., 1999), and previous studies indicated that many rhizobia species native to Texas soils may form ineffective nodules on peanuts (Weaver, 1974). Therefore, the Vernon results relating to high nodule counts and low percent active may also have been driven by ineffective native rhizobia species, especially since there was not a recent history of peanut rotations at this location. Rhizobia species at this location would first need to be characterized to confirm this.

Table 11. Values and p-values of nodule counts and % active nodulation at Lubbock and Vernon. Fallow excluded to allow for interactions.

		Lubbock		**Vernon**	
Manage	**Cover Crop**	**Nodule Counts (count cm^{-2})**	**% Active Nodulation**	**Nodule Counts (count cm^{-2})**	**% Active Nodulation**
ORG	Rye	1.42^{bc}	64^{b}	13.56^{a}	18^{a}
	Rye/Vetch	1.17^{c}	61^{b}	11.24^{ab}	8^{b}
CON	Rye	1.87^{ab}	82^{a}	8.23^{b}	5^{b}
	Rye/Vetch	2.00^{a}	81^{a}	10.19^{b}	3^{b}
p-values	Management	**0.0003**	**0.002**	**0.005**	**0.0004**
	Cover Crop	0.730	0.693	0.867	**0.015**
	Interaction	0.26	0.842	0.055	0.115

Significant p-values in bold

Fisher's LSD for values in each column with the same letter are not significantly different at $p = 0.05$

Pearson's correlations of nodulation data against CMIN over the course of the season indicated that nodulation data taken in August had multiple strong positive correlations to CMIN at both locations, particularly against early and mid-season collections when nodules would have been forming (Table 12).

Table 12. Pearson's correlation coefficients of nodule data against monthly CMIN results.

Pearson's Correlation Coefficients						
			Lubbock		Vernon	
	Event	Depth	Nodule Counts	% Nodulation	Nodule Counts	% Nodulation
CMIN	April	0-4"	0.638*	0.248	0.341	-0.144
		4-8"	0.32	0.373	0.372	-0.348
	May	0-4"	0.437	0.634*	n/a	n/a
		4-8"	0.551*	0.528*	n/a	n/a
	June	0-4"	0.413	0.254	n/a	n/a
		4-8"	0.124	0.401	n/a	n/a
	July	0-4"	0.632*	0.393	0.557*	0.049
		4-8"	0.123	0.371	0.546*	-0.148
	August	0-4"	0.323	-0.003	0.215	0.349
		4-8"	-0.266	0.253	0.163	0.035
	September	0-4"	-0.327	-0.319	0.519*	-0.19
		4-8"	-0.423	-0.427	0.158	-0.577*
	November	0-4"	-0.51	-0.785*	0.422	-0.418
		4-8"	-0.518*	-0.078	0.269	-0.246

* Pearson's correlation is significant at $p = 0.05$.

At the Lubbock location, nodulation did not appear to be depressed in winter fallow plots. Nor was there a significant difference in nodulation according to cover crop selection. However, plots under organic management appeared to experience lower nodule counts and percent active nodulation than those under conventional or fallow treatment (Figure 11). Few studies investigating legume nodule count responses to different inputs exist, and these are mainly focused on soybean nodulation. Even so, Lubbock peanut results are in agreement with previous research which reported that the application of manure alone either depressed or did not improve nodule counts in soybean crops when compared to recommended fertilizer dosages (Devi et al., 2013; Amadou et al., 2021).

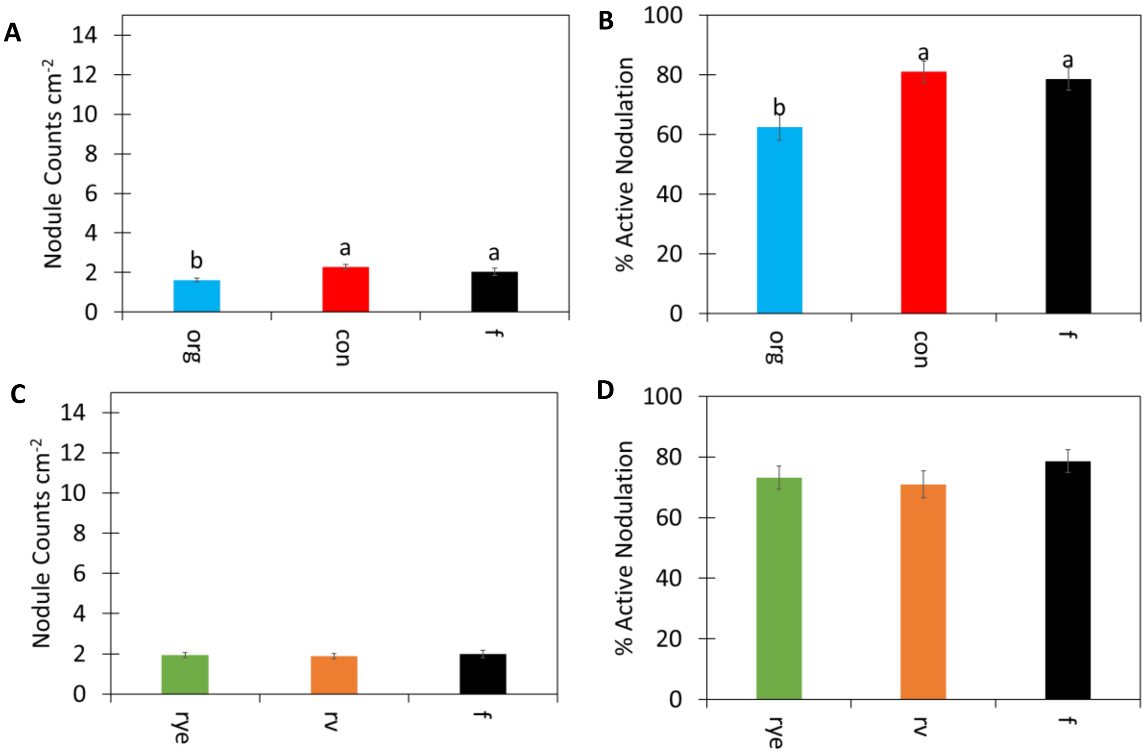


Figure 11. Nodule data from Lubbock location. Counts cm^{-2} according to management (A) and cover crop selection (B), and % active nodulation according to management (B) and cover crop selection (D). Bars with the same letter indicate that Fisher's LSD is not significantly different at p = 0.05. Bars indicate standard error of the mean.

At Vernon, however, plots treated with a winter fallow experienced lower nodule counts than cover crop treatments. Additionally, plots under organic management experienced significantly greater nodule counts than conventional or fallow treatments (Figure 12).

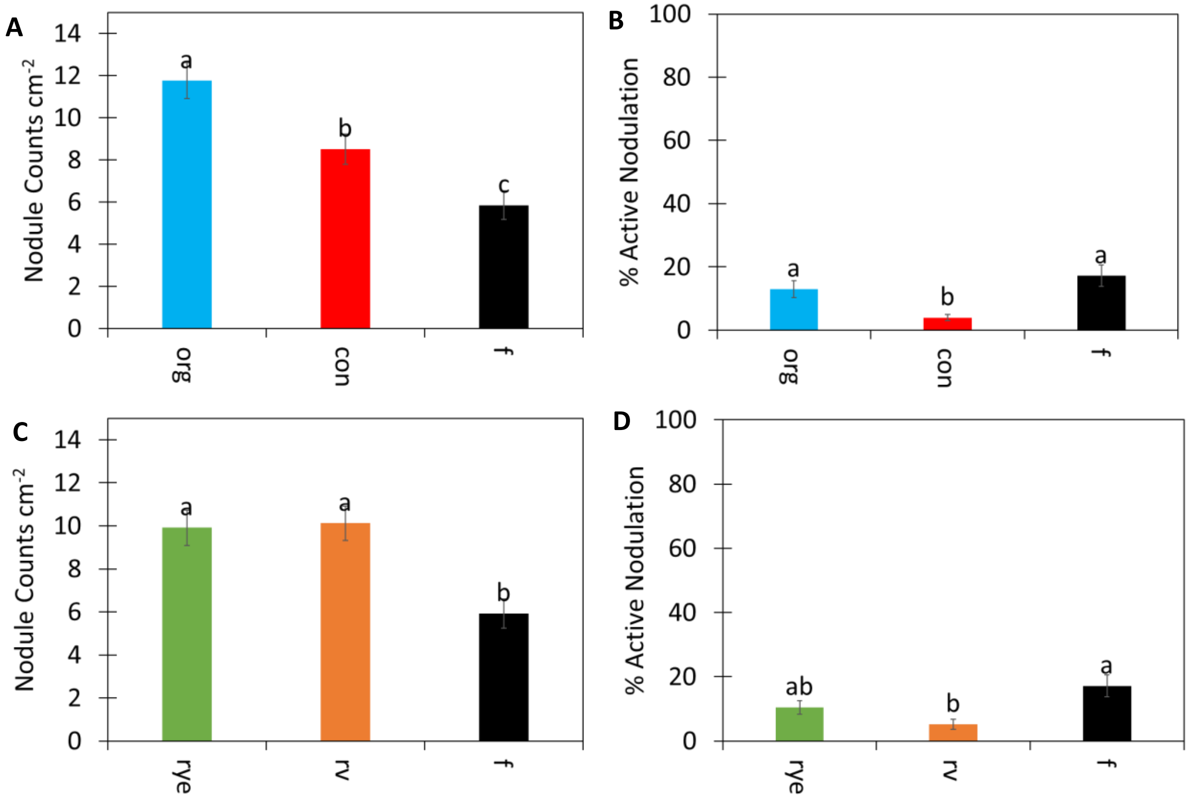


Figure 12. Nodule data from Vernon location. Counts cm^{-2} according to management (A) and cover crop selection (B), and % active nodulation according to management (B) and cover crop selection (D). Bars with the same letter indicate that Fisher's LSD is not significantly different at p = 0.05. Bars indicate standard error of the mean.

Vernon plots that had the rye or rye/vetch cover crop treatment did not differ from each other in terms of nodule counts, but nodule counts from both were significantly greater than the fallow. However, conventional management and rye/vetch cover crop treatments both resulted in significantly lower percent active nodulation than the fallow, and there was an interaction where organic rye/vetch experienced significantly lower percent active nodulation than organic rye (Table 11). Organic peanuts at Vernon were not inoculated with *Bradyrhizobium*, so nodulation was more likely to be influenced by soil environmental factors. Results support previous conclusions drawn from the Vernon NAG data that the rye/vetch cover crop treatment is beginning to improve plant available N, as this could explain why percent active nodulation under the organic rye/vetch was lower than the fallow and organic rye at Vernon.

Organic Seeding Rates

Additional organic plots were included in this study to investigate whether cover crop seeding rate would result in significantly different soil health outcomes for each cover crop selection. Rye, radish, rye/vetch, and rye/vetch/radish each included two different seeding rates (Table 13). Differences in cover crop herbage mass were only noted at the Lubbock location (Table 14), and there was a cover crop by rate interaction where greater herbage mass was available under the high rye/vetch/radish treatment compared to the low rye/vetch/radish treatment ($p = 0.025$). There were commonly rate by event or cover crop interaction for indicators of biological activities, but biological activities under high seeding rates were not consistently greater than low seeding rates (Table 14). No other differences in herbage mass were reported according to seeding rate from any of the other treatments. Peanut yield differed according to cover crop seeding rate only at Lubbock ($p = 0.038$), and yields from plots treated with a lower cover crop seeding rate were greater than those from plots treated with a higher cover crop seeding rate, but the difference was quite small (4095 kg ha^{-1} compared to 4092 kg ha^{-1}). Previous research indicated that soil biological activities under cover cropped systems may be positively influenced by cover crop residue biomass (Thapa, 2021). Although this current study did find elevated cover crop herbage mass under high seeding rates for rye/vetch/radish treatment at Lubbock, no other consistent benefit to cover crop herbage mass or biological activity was reported for high seeding rates, as has previously been the case with similar studies (Haramoto, 2019).

Table 13. Organic seeding rates.

Organic Cover Crop	**Seeding rate (kg ha^{-1})**
Rye	33.63
*Rye	100.88
Radish	11.21
*Radish	33.63
Rye/Vetch	28.02/5.6
*Rye/Vetch	84.06/16.81
Rye/Vetch/Radish	28.02/3.36/2.24
*Rye/Vetch/Radish	84.06/10.09/6.73

* Indicates high seeding rates. These were only included in the organic seeding rate investigation, when they were compared against the standard, lower seeding rate

Table 14. ANOVA results comparing recommended organic cover crop seeding rates to elevated seeding rates at Lubbock and Vernon

	Lubbock								**Vernon**							
	yield	**cover biomass**	**% moist**	**temp**	**CO_2**	**CMIN**	**BG**	**NAG**	**yield**	**cover biomass**	**% moist**	**temp**	**CO_2**	**CMIN**	**BG**	**NAG**
Effect	*p-values*															
Rate	**0.038**	0.088	0.055	0.464	0.074	0.536	0.373	0.305	0.663	0.348	0.510	0.847	0.463	0.798	0.975	0.594
Cover	0.293	**0.004**	0.409	0.983	0.327	0.672	0.377	0.559	0.208	0.084	0.978	0.902	0.821	0.004	**0.031**	0.347
Cover* Rate	0.121	**0.025**	0.334	0.557	**0.037**	0.676	0.769	0.590	0.771	0.188	0.220	0.787	0.740	0.755	0.759	**0.007**
Event	n/a	n/a	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.099	n/a	n/a	<0.0001	<0.0001	<0.0001	<0.0001	0.031	0.001
Rate* Event	n/a	n/a	0.327	0.787	0.079	0.231	0.232	**0.008**	n/a	n/a	0.653	0.382	0.953	**0.004**	0.214	0.349
Cover* Event	n/a	n/a	0.997	0.802	0.894	0.733	0.510	**0.010**	n/a	n/a	0.822	1	1	0.148	0.141	0.391
Cover* Rate* Event	n/a	n/a	0.954	0.802	0.966	0.893	0.981	0.223	n/a	n/a	0.901	0.946	0.992	**0.029**	0.743	0.761
Depth	n/a	n/a	n/a	n/a	n/a	<0.0001	<0.0001	<0.0001	n/a	n/a	n/a	n/a	n/a	<0.0001	<0.0001	0.736
Rate* Depth	n/a	n/a	n/a	n/a	n/a	0.672	0.285	0.404	n/a	n/a	n/a	n/a	n/a	0.318	0.608	0.745
Cover* Depth	n/a	n/a	n/a	n/a	n/a	0.653	0.683	0.149	n/a	n/a	n/a	n/a	n/a	**0.016**	0.689	0.887
Cover* Rate* Depth	n/a	n/a	n/a	n/a	n/a	0.319	0.552	0.686	n/a	n/a	n/a	n/a	n/a	0.475	0.486	0.828
Cover* Event* Depth	n/a	n/a	n/a	n/a	n/a	0.209	0.833	0.550	n/a	n/a	n/a	n/a	n/a	0.393	0.597	0.950
Rate* Event* Depth	n/a	n/a	n/a	n/a	n/a	0.965	0.254	0.409	n/a	n/a	n/a	n/a	n/a	0.083	0.841	0.448
Cove* Rate* Even* Depth	n/a	n/a	n/a	n/a	n/a	0.995	0.514	0.945	n/a	n/a	n/a	n/a	n/a	0.698	0.974	0.969

Significance at p = 0.05

Significant p-values in bold

See Table 1 for seeding rates

PLFAs

At Vernon, no depth interaction was apparent, so both depths were analyzed together (Table 15). Data for Lubbock were again pooled for both depths, but there were depth interactions for parameters such as Fungi:Bacteria ratios, so these were graphed according to depth (Table 16).

Both sites saw generally greater levels of PLFA markers under ORG, along with interactions which typically indicated that PLFA quantities for single cover crop selections were lower under CON compared to ORG (Table 17, Table 18). Elevated total PLFAs typically correspond to microbial biomass and can therefore be used as microbial biomass estimates (Willers et al., 2015; Zelles et al., 1995). Since microbial communities often respond positively to manure applications and other organic inputs, results are in accordance with both Zhang et al. (2012) and Hammesfahr et al (2008). The Lubbock location had not received fertilizer or composted manure since 2019, so elevated microbial biomass estimates from PLFA data may be indicative of long-term effects of manure on microbial communities which may be less apparent under other parameters such as CMIN or enzyme activities.

Table 15. ANOVA results for Vernon PLFAs. Fallow excluded to allow for interactions.

Effect	**AMF**	**G-**	**Fungi**	**G+**	**Actino**	**Total PLFA**	**Fungi/ Bacteria**	**G+/G-**
	pmol g^{-1} soil							
	p-values							
Cover	0.187	0.149	0.155	0.545	0.828	0.213	0.337	0.114
manage	**0.015**	0.156	0.874	**0.0001**	**0.0004**	**0.024**	0.163	**<.0001**
cover*manage	**0.032**	0.073	0.672	**0.004**	**0.026**	**0.024**	0.987	**0.044**
manage*depth	0.591	0.720	0.593	0.258	0.451	0.836	0.747	0.320
cover*depth	0.704	0.294	0.176	0.450	0.253	0.239	0.367	0.247
cover*manage*depth	0.710	0.414	0.804	0.640	0.676	0.697	0.945	0.067

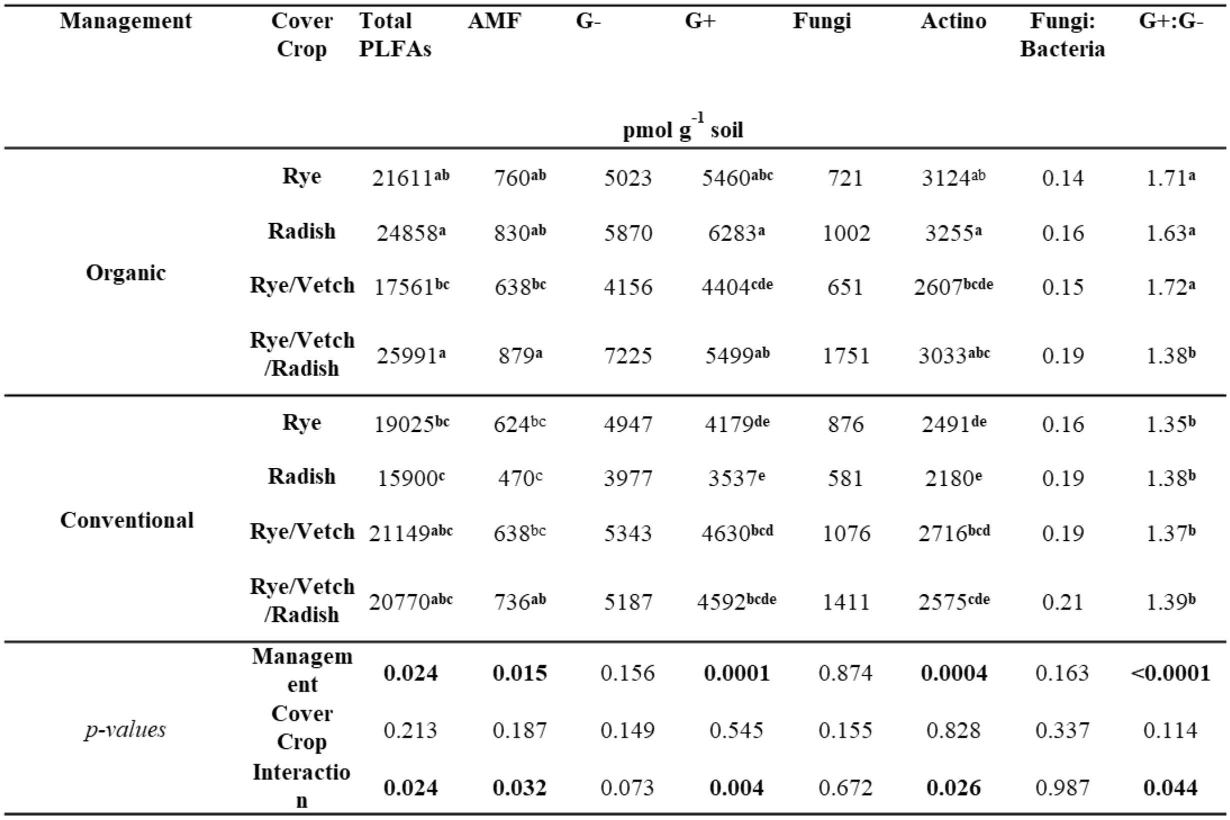
Table 16. ANOVA results for Lubbock PLFAs. Fallow excluded to allow for interactions.

Effect	**AMF**	**G-**	**Fungi**	**G+**	**Actino**	**Total PLFA**	**Fungi/ Bacteria**	**G+/G-**
	pmol g^{-1} soil							
	p-values							
cover	0.076	**0.034**	0.468	0.401	0.274	**0.039**	0.649	0.744
manage	**<.0001**	**0.0005**	0.054	**<.0001**	**<0.0001**	**<.0001**	**0.001**	0.341
cover*manage	**0.020**	**0.027**	0.223	0.662	0.512	0.206	0.302	0.214
manage*depth	**0.006**	0.378	0.061	0.163	**0.002**	0.158	**0.008**	0.195
cover*depth	**0.045**	0.097	0.863	0.323	0.261	0.243	0.533	0.978
cover*manage*depth	0.399	0.331	**0.014**	0.906	0.268	0.463	**0.003**	0.282

At the Vernon location, a management by cover interaction occurred for total PLFAs and PLFA indicators associated with AMF, G+ bacteria, and actinomycetes (Table 17). Results indicate that organic radish and rye/vetch/radish plots contained significantly greater microbial biomass estimates in terms of pmol of total PLFAs than organic rye/vetch, conventional rye, and conventional radish treatments. Additionally, PLFA markers for actinomycetes were greater under ORG radish, ORG rye, and ORG rye/vetch/radish compared to CON rye and CON radish (p = 0.026). PLFA markers for G+ and AMF were also greater under ORG rye, ORG radish, and ORG rye/vetch/radish compared to CON rye and radish (p = 0.004 and 0.032, respectively). There were no differences in fungi, G-, or Fungi:Bacteria ratio estimates. However, the Vernon location did see elevated G+/G- under ORG, which was unexpected and opposite to conclusions of previous reports (Zhang et al., 2012). Changes in G+/G- ratios tend to indicate change in environmental stress, although there is debate over whether high or low ratios indicate greater stress (Gukert et al., 1986; Keynan and Sandler, 1983; Pennanen et al., 1996). Ratios could also be indicative of carbon sources that are most prominent in the system. Fanin et al. (2019)

suggests that high G+:G- ratios indicate complex carbon sources while low ratios indicate simple, cellulose-derived sources. The Vernon location typically had low G+:G- ratios under CON, and these plots also experienced high levels of cover crop herbage mass. Since composted manure was not applied until after PLFA collections, results likely reflect high herbage mass availability under CON at Vernon.

Table 17. PLFA results at Vernon.

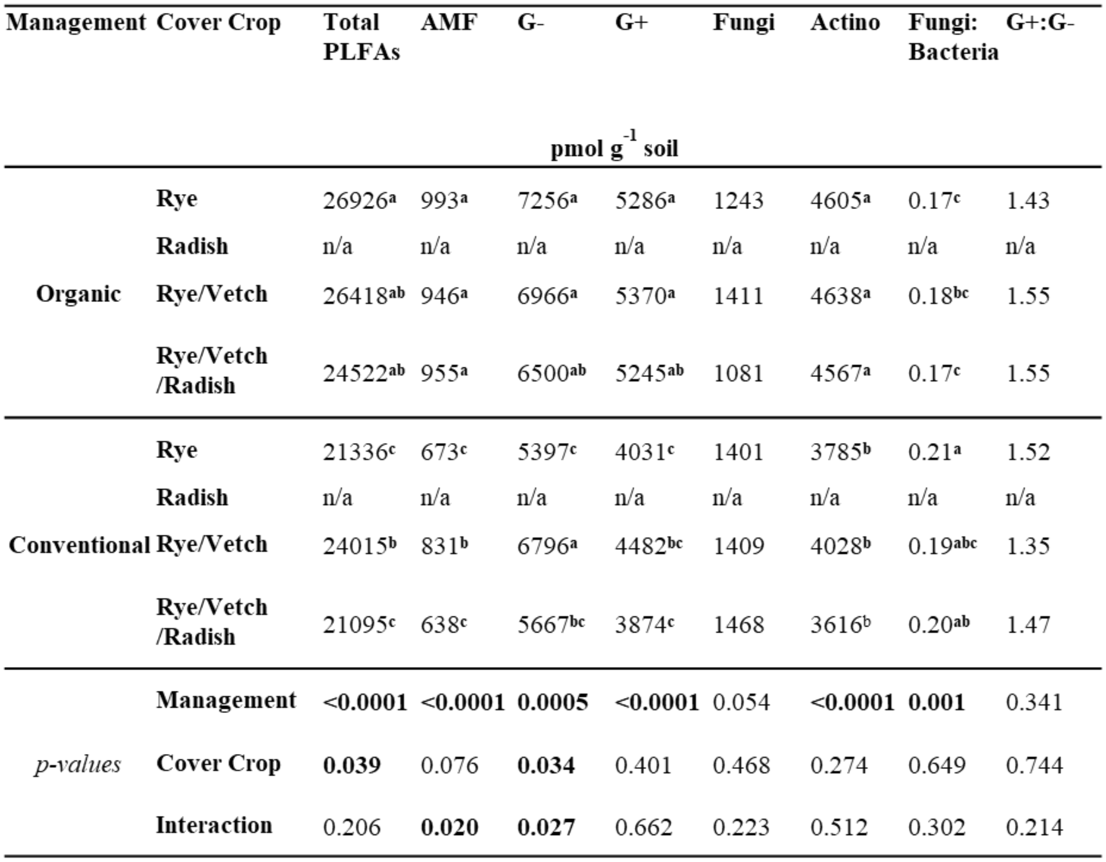


Management	**Cover Crop**	**Total PLFAs**	**AMF**	**G-**	**G+**	**Fungi**	**Actino**	**Fungi: Bacteria**	**G+:G-**
					pmol g^{-1} soil				
Organic	**Rye**	21611^{ab}	760^{ab}	5023	5460^{abc}	721	3124^{ab}	0.14	1.71^{a}
	Radish	24858^{a}	830^{ab}	5870	6283^{a}	1002	3255^{a}	0.16	1.63^{a}
	Rye/Vetch	17561^{bc}	638^{bc}	4156	4404^{cde}	651	2607^{bcde}	0.15	1.72^{a}
	Rye/Vetch /Radish	25991^{a}	879^{a}	7225	5499^{ab}	1751	3033^{abc}	0.19	1.38^{b}
Conventional	**Rye**	19025^{bc}	624^{bc}	4947	4179^{de}	876	2491^{de}	0.16	1.35^{b}
	Radish	15900^{c}	470^{c}	3977	3537^{e}	581	2180^{e}	0.19	1.38^{b}
	Rye/Vetch	21149^{abc}	638^{bc}	5343	4630^{bcd}	1076	2716^{bcd}	0.19	1.37^{b}
	Rye/Vetch /Radish	20770^{abc}	736^{ab}	5187	4592^{bcde}	1411	2575^{cde}	0.21	1.39^{b}
p-values	**Management**	**0.024**	**0.015**	0.156	**0.0001**	0.874	**0.0004**	0.163	**<0.0001**
	Cover Crop	0.213	0.187	0.149	0.545	0.155	0.828	0.337	0.114
	Interaction	**0.024**	**0.032**	0.073	**0.004**	0.672	**0.026**	0.987	**0.044**

Lubbock results indicated that greater microbial biomass estimates for total PLFAs, G+ and actinomycete indicators were available under ORG compared to CON (Table 18). This was similarly the case for AMF and G- PLFAs, but there were management by cover interactions as well – specifically for the CON rye/vetch treatment, which saw greater PLFA levels for these parameters than other CON cover crop treatments.

Unlike Vernon, there were no differences in G+:G- ratios at Lubbock. However, there were lower Fungi:Bacteria ratios under ORG management and the CON rye/vetch treatment at the 0-10 cm depth, indicating a community shift to greater bacteria prevalence (Table 18, Figure 13), opposite to previous reports (Hammesfahr et al, 2008).

Table 18. PLFA results at Lubbock.



Management	Cover Crop	Total PLFAs	AMF	G-	G+	Fungi	Actino	Fungi: Bacteria	G+:G-
		pmol g^{-1} soil							
Organic	**Rye**	26926^{a}	993^{a}	7256^{a}	5286^{a}	1243	4605^{a}	0.17^{c}	1.43
	Radish	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	Rye/Vetch	26418^{ab}	946^{a}	6966^{a}	5370^{a}	1411	4638^{a}	0.18^{bc}	1.55
	Rye/Vetch /Radish	24522^{ab}	955^{a}	6500^{ab}	5245^{ab}	1081	4567^{a}	0.17^{c}	1.55
Conventional	**Rye**	21336^{c}	673^{c}	5397^{c}	4031^{c}	1401	3785^{b}	0.21^{a}	1.52
	Radish	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	Rye/Vetch	24015^{b}	831^{b}	6796^{a}	4482^{bc}	1409	4028^{b}	0.19^{abc}	1.35
	Rye/Vetch /Radish	21095^{c}	638^{c}	5667^{bc}	3874^{c}	1468	3616^{b}	0.20^{ab}	1.47
p-values	**Management**	**<0.0001**	**<0.0001**	**0.0005**	**<0.0001**	0.054	**<0.0001**	**0.001**	0.341
	Cover Crop	**0.039**	0.076	**0.034**	0.401	0.468	0.274	0.649	0.744
	Interaction	0.206	**0.020**	**0.027**	0.662	0.223	0.512	0.302	0.214

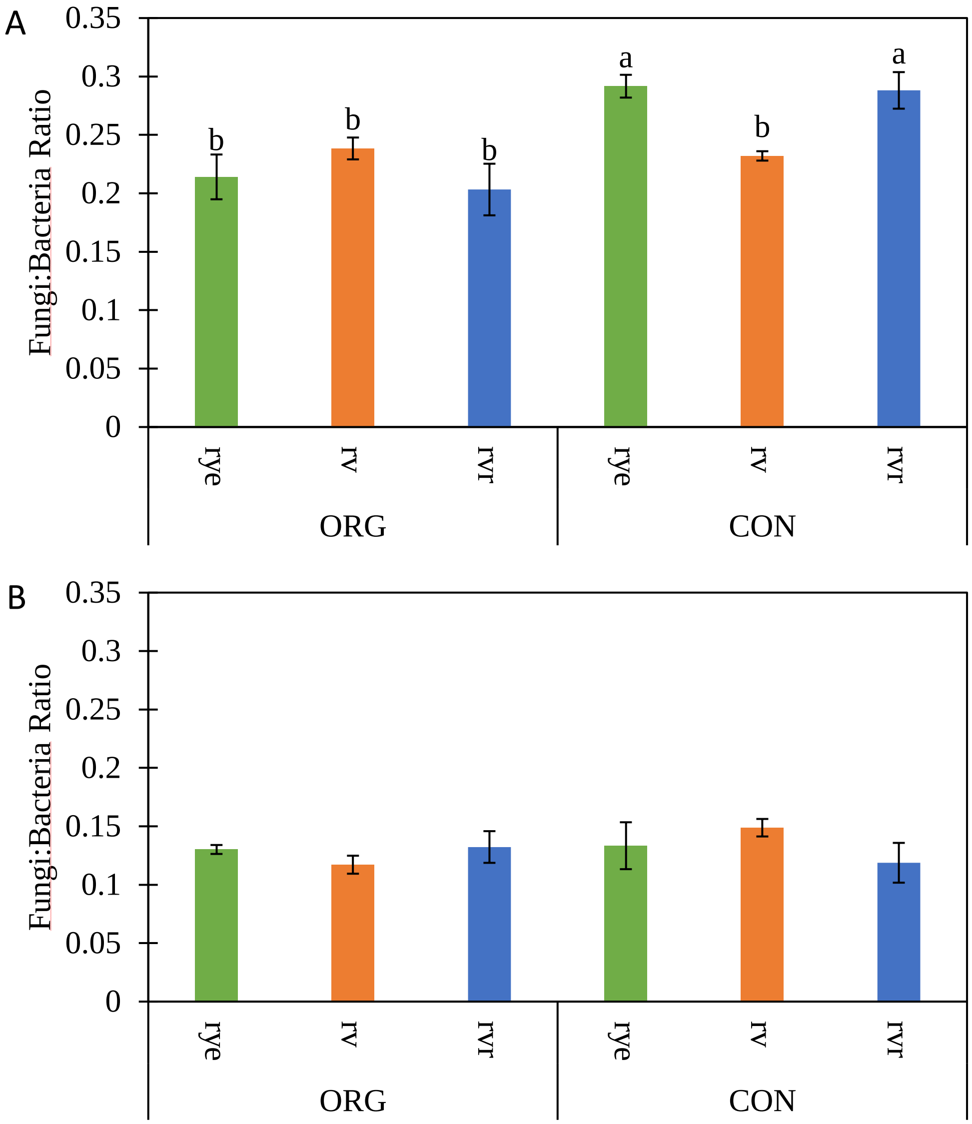


Figure 13. Fungi:Bacteria PLFA ratios Lubbock according to management and cover crop selection (n = 3). Depths 0-10 cm (A) and 10-20 cm (B) analyzed separately. Radish removed due to cover crop failure. Bars with the same letters indicate that Fisher's LSD are not different at p = 0.05. Bars indicate standard error of the mean.

Conclusions

Results focus on the first year of transition to organic management and identify emerging trends in soil health that may become more prominent with time.

Markers of change in soil health were best examined as a whole to identify trends. *In situ* respiration, CMIN, and enzyme activities all indicated some form of difference in soil health based on management, cover crop use, or both. Soil respiration appeared to be sensitive to organic inputs, regardless of the source, but results were also influenced by soil moisture and temperature. BG and CMIN appeared to respond positively to recent composted manure applications – as evidenced by elevated ORG results at Vernon when compared to CON and fallow. NAG appeared to be sensitive to changes in the system based on cover crop selection, and results were supported to an extent by both CMIN and nodulation data.

Differences in biological activities between organic and conventional management systems are driven primarily by inputs (Santos et al., 2012; Nautiyal et al., 2010; Clark, 1998). Previous studies have reported that inclusion of organic amendments such as manure stimulates BG enzyme activity (Gianfreda, 2005; Zhang et al., 2015a; Zhang et al. 2015b). However, since Lubbock – which had received composted manure in 2019 – did not experience such trends according to management, these results may be somewhat ephemeral. Given that it is notoriously difficult to build SOM in semi-arid regions due to the hot, dry nature of the climate, it is all the more likely that differences between management systems may be less apparent in years when composted manure is not applied, but future monitoring is needed to confirm this.

This study also builds on previous reports of elevated biological activities under plots with a cover crop when compared to winter fallow (Daryanto et al. 2018; Kim et al. 2020) and suggests that this may begin as early as one rotation after cover crop implementation in semi-arid regions. Results are supported primarily by *in situ* respiration, BG, and to a more limited extent, NAG and CMIN. These are indicative of microbial activities, and since microbes are drivers of nutrient cycling (Schoonover and Crim, 2015; Acosta-Martinez, 2004), results have long-term implications for nutrient availability.

There were limited differences in biological activities according to cover crop selection. Results from NAG activities at both sites may indicate that the rye/vetch treatment has begun to contribute to NMIN in the system. When legumes – such as vetch – begin to recruit rhizobia for nodulation, rhizobia produce Nod factors composed of lipo-chitin oligosaccharides, which contain chitin compounds (Dupont et al., 2012). NAG may therefore have acted upon residues associated with these, but this remains to be tested. The September collection also saw elevated CMIN under rye/vetch at Lubbock (0-10 cm). Since CMIN is associated with NMIN (Franzluebbers, 2020), this offers further supporting evidence that rye/vetch may have begun to contribute to N in the system. Finally, at Vernon, percent active nodulation was depressed under ORG rye/vetch compared to ORG rye, which may have been due to legume sanctions if the rye/vetch treatment was contributing to organic N (Kiers et al., 2003). The exact drivers of observed nodulation patterns at Lubbock and Vernon are still somewhat unclear, but available N levels – including N-availability from rye/vetch treatment – could have influenced results.

Finally, this study sought to investigate whether cover crop seeding rate could result in higher cover crop biomasses and biological activities. Previous research has indicated that cover cropped systems with high cover crop biomasses experience more soil biological activity than those with lower levels of biomass residue (Thapa, 2021). However, there was little evidence that elevated seeding rate resulted in greater biomass than recommended seeding rate, and differences according to biological activities based on seeding rates were limited. Results suggest that increased spending on higher cover crop seeding rates is likely unnecessary.

This study reports preliminary data from what will be a long-term organic study, and biological indicators provide evidence that C and N in this particular system have already begun to respond to inputs from composted manure and cover crop residues, but elevated seeding rates – specifically those used in this study – are unlikely to provide the biomass required to initiate greater biological responses. Future studies may wish to investigate cover crop biomasses and soil health responses under even lower seeding rates to determine if further cost savings from cover crop seed application are feasible while still maintaining soil health benefits. Finally, yield losses did not occur under organic peanut compared to conventional peanut during the first year of transitioning to organic management. Therefore, this study provides farmers in semi-arid regions with valuable data on what to expect from soil health and yield when transitioning to organic management.

CHAPTER 3

FUTURE DIRECTIONS

Results reported here represent preliminary data from what will be a long-term study of soil health with regards to organic management and cover crop selection in a semi-arid region. Thus, further monitoring of sites will occur.

Additional parameters not mentioned in this study will be of special interest. For example, data on weed pressures, nitrate/ammonia, soil moisture at multiple depths, and PLFAs were collected but not presented. Cover crop selections have been noted to provide varying benefits based on each of these parameters, and together, they will provide further context to the results of this study. Over time, data from presented parameters and additional parameters will allow farmers to make informed choices on ideal cover crop selections when transitioning to organic management.

Results suggest that the rye/vetch treatment has begun to contribute to organic N in the system – possibly due to the breakdown of rhizobia Nod factors by chitinases. However, confirming this was beyond the scope of this study, and although previous experiments have observed the breakdown of Nod factors by various chitinases (Schultze, et al., 2001; Perret et al., 2000), it is unclear to what extent this may contribute to organic N. Therefore, future studies should be conducted to quantify this. Nod factors could be a little-discussed source of organic N. Alternatively, if the breakdown of Nod factors has a minute contribution to organic N, caution should be taken in interpreting these results.

Finally, there was little evidence that elevated seeding rates in organic plots corresponded to elevated cover crop herbage mass and biological activities. However, previous studies have noted that seeding rates may influence herbage mass and soil biological activity, and that this can vary year to year (Thapa, 2021, Brennan and Boyd, 2012, Haramoto, 2019). Further monitoring will be needed to confirm the value of elevated cover crop seeding rates at improving soil health in this region. Future monitoring may also wish to investigate soil health contributions of lower seeding rates in the event that an economic benefit may be derived from these.

REFERENCES

Acosta-Martinez, V., D. Rowland, R.B. Sorensen, K.M. Yeater. 2007. Microbial Community Structure and Functionality Under Peanut-Based Cropping Systems in a Sandy Soil. *Biology and Fertility of Soils.* 44:681-692

Acosta-Martínez, V., D.R. Upchurch, A.M. Schubert, *et al.* 2004. Early Impacts of Cotton and Peanut Cropping Systems on Selected Soil Chemical, Physical, Microbiological and Biochemical Properties. *Biology and Fertility of Soils.* 40:44–54

Acosta-Martinez, V., L. Perez-Guzman, J. Johnson. 2019. Simultaneous determination of B-glucosidase, B-glucosaminidase, acid phosphomonoesterase, and arylsulfatase activities in a soil sample for a biogeochemical cycling index. *Applied Soil Ecology.* 142: 72-80.

Acosta-Jurado, S., D. Rodriguez-Navarro, Y. Kawaharada, J.F. Perea, A. Gil-Serrano, H. Jin, Q. An, M.A. Rodriguez-Caravajal, S.U. Anderson, N. Sandal, J. Stougaard, J. Vinardell, J.E. Ruiz-Sainz. 2016. *Sinorhizobium fredii* HH103 invades *Lotus burttii* by crack entry in a nod factor – and surface polysaccharide – dependent manner. *The American Phytopathological Society.* 29(12): 925-937.

AgDaily Staff. 2019. The List of Organic Pesticides Approved by the USDA. *AgDaily.* Retrieved from: https://www.agdaily.com/technology/the-list-of-pesticides-approved-for-organic-production/

Amadou, A., X. Song, S. Huang, A. Song, Z. Tang, W. Dong, S. Zhao, B. Zhang, K. Yi, F. Fan. 2021. Effects of long-term organic amendment on the fertility of soil, nodulation, yield, and seed quality of soybean in a soybean-wheat rotating system. *Journal of Soils and Sediments.* 21: 1385-1394

Baughman, T., P. Dotray, M. Black, et al. 2019. Texas Peanut Production Guide. *Texas Cooperative Extension: The Texas A&M University System.* Retrieved from: http://texaspeanutboard.com/wp-content/uploads/peanutproductionguide.pdf

Bergtold, J.S., P.A. Duffy, D. Hite, R.L. Raper. 2012. Demographic and Management Factors Affecting the Adoption and Perceived Yield Benefit of Winter Cover Crops in the Southeast. *Journal of Agricultural and Applied Economics.* 44(1)99-116

Boote, K.J. 1982. Growth stages of peanut (*Arachis hypogaea* L.). *Peanut Science.* 9(1): 35-40

Brennan, E.B., N.S. Boyd. 2012. Winter cover crop seeding rate and variety affects during eight years of organic vegetables: I. cover crop biomass production. *Agronomy Journal.* 104:684-698.

Burke, J.A., K.L. Lewis, G.L. Ritchie, P.B. DeLaune, J.W. Keeling, V. Acosta-Martinez, J.M. More, T. McLendon. 2021. Net Positive Soil Water Content Following Cover Crops with no Tillage in Irrigated Semi-Arid Cotton Production. *Soil and Tillage Research.* 208:104869

Cano, A., A. Nunez, V. Acosta-Martinez, M. Schipanski, R. Ghimire, C. Rice, C. West. 2018. Current knowledge and future research directions to link soil health and water conservation in the Ogallala Aquifer region. *Geoderma*. 328: 109-118.

Castro, S. M. Permigiani, M. Vinocur, A. Fara. 1999. Nodulation in peanut (*Arachis hypogaea* L.) roots in the presence of native and inoculated rhizobia strains. *Applied Soil Ecology.* 13: 39-44.

Cheng, L, et al. 2012. Arbuscular Mycorrhizal Fungi Increase Organic Carbon Decomposition Under Elevated CO_2. *Science.* 337:1084-1087.

Chen, G., R.R. Weil. 2010. Penetration of Cover Crop Roots Through Compacted Soils. *Plant Soil.* 331:31-43

Clark, M.S., W.R. Horwath, C. Shennan, K.M. Scow. 1998. Changes in Soil Chemical Properties Resulting from Organic and Low-Input Farming Practices. *Agronomy Journal*. 90(5):662-671

Cover Crop Basics for Southeast Missouri. 2014. *United States Department of Agriculture (USDA).* Retrieved from: https://www.nrcs.usda.gov/Internet/FSE_PLANTMATERIALS/publications/mopmsbr12100.pdf

Daryanto, S., B. Fu, L. Wang, P. Jacinthe, W. Zhao. 2018. Quantitative synthesis on the ecosystem services of cover crops. *Earth-Science Reviews.* 185:357-373.

Deng, S., I. Popova, L. Dick, R. Dick, 2013. Bench scale and microplate format assay of soil enzyme activities using spectroscopic and fluorometric approaches. *Applied Soil Ecology*. 64: 84-90.

Deng, S., and I. Popova. 2011. Carbohydrate Hydrolases. In Dick, R (Ed.), Methods of Soil Enzymology. (pgs. 185-209). Madison, WI: *Soil Science Society of America*.

Devi, K.N., T.B. Sing, H.S. Athokpam, N.B. Singh, D. Samurailatpam. 2013. Influence of organic, biological and organic manures on nodulation and yield of soybean (*Glycine max* Merril L.) and soil properties. *Australian Journal of Crop Science.* 7(9): 1407-1415

Dick, R. 2011. Methods of Soil Enzymology. *SSSA Book Series.* SSSA, Madison, WI. doi:10.2136/sssabookser9.frontmatter

Dupont, L., G. Alloing, O. Pierre, S. El Msehli, J. Hopkins, D. Herouart, P. Frendo. 2012. The legume root nodule: from symbiotic nitrogen fixation to senescence. *IntechOpen.*

Eivazi, F., M.A. Tabatabai. 1998. B-D-Glucosidase. In Dick, R (Ed.), Methods of Soil Enzymology. (pgs. 196-198). Madison, WI: *Soil Science Society of America.*

Ekenler, M., M.A. Tabatabai. 2002. B-Glucosaminidase Activity of Soils: Effect of Cropping Systems and its Relationship to Nitrogen Mineralization. *Biology and Fertility of Soils*. 36:367-376

Fanin, N., P. Kardol, M. Farrell, M. Nilsson, M.J. Gundale, D.A. Wardle. 2019. The ratio of gram-positive to gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. *Soil Biology and Biochemistry.* 128:111-114.

Franzluebbers, A. 2015. Should Soil Testing Services Measure Soil Biological Activity? *Agricultural and Environmental Letters.*

Franzluebbers, A. 2005. Soil organic carbon sequestration and agricultural greenhouse gas emissions in the southeastern USA. *Soil & Tillage Research*. 83:120-147.

Franzluebbers, A., R.L. Haney, C.W. Honeycutt, H.H. Schomberg, F.M. Hons. 2000. Flush of Carbon Dioxide Following Rewetting of Dried Soil Relates to Active Organic Pools. *Soil Science Society of America Journal*. 64:613-623

Franzluebbers, A. 2020. Soil carbon and nitrogen mineralization after the initial flush of CO_2. *Agricultural and Environmental Letters.*

Fraser, D.G., J.W. Doran, W.W. Sahs, and G.W. Lesoing. 1988. Soil Microbial Populations and Activities Under Conventional and Organic Management. *Journal of Environmental Quality.* 17:585-590

Georgieva, S., S. Christensen, H. Petersen, P. Gjelstrup, K. Thorup-Kristensen. 2005. Early decomposer assemblages of soil organisms in litterbags with vetch and rye roots. *Soil Biology and Biochemistry.* 37(6): 1145-1155

Gianfreda, L., M.A. Rao, A. Piotrowska, G. Palumbo, C. Colombo. 2005. Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution. *Science of The Total Environment.* 342(1-3): 265-279

Graber, S. n.d. Soil Quality and Soil Health. *Natural Resource Conservation Service Kansas.*

Retrieved from: https://www.nrcs.usda.gov/wps/portal/nrcs/detail/ks/newsroom/features/?cid=nrcs142p2_033494

Guckert, J.B., M.A. Hood, D.C. White. 1986. Phospholipid ester-linked fatty acid profile changes dueing nutrient deprivation of Vibrio cholerae: Increases in the trans/cis ratio and proportions of cyclopropyl fatty acids. *Applied Environmental Microbiology*. 52(4), 794-801.

Hammesfahr, U., H. Heuer, B. Manzke, K. Smalla, S. Thiele-Bruhn. 2008. Impact of the antibiotic sulfadiazine and pig manure on the microbial community structure in agricultural soils. *Soil Biology and Biochemistry.* 40(7):1583-1591.

Haramoto, E. 2019. Species, Seeding Rate, and Planting Method Influence Cover Crop Services Prior to Soybean. *Agronomy Journal.* 111:1068-1078

Heckman, J. 2006. A History of Organic Farming: Transitions from Sir Albert Howard's "War in the Soil" to USDA National Organic Program. *Renewable Agriculture and Food Systems*. 21(3):143-150

Housman, M., S. Tallman, C. Jones, P. Miller, C. Zabinski. 2021. Soil biological response to multi-species cover crops in the Northern Great Plains. *Agriculture, Ecosystems & Environment.* 313: 107373

Huguley, S. 2019. Floyds' Competitive Spirits Fuel Diversification and Growth. *Southwest FarmPress.* Retrieved from: https://www.farmprogress.com/peanuts/floyds-competitive-spirits-fuel-diversification-and-growth

Hurisso, T, et al. 2016. Comparison of Permanganate-Oxidizable Carbon and Mineralizable Carbon for Assessment of Organic Matter Stabilization and Mineralization. *Soil Science Society of America Journal.* 80:1352-1364.

IPCC, 2019: Summary for Policymakers. In: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems [P.R. Shukla, J. Skea, E. Calvo Buendia, V. Masson-Delmotte, H.- O. Pörtner, D. C. Roberts, P. Zhai, R. Slade, S. Connors, R. van Diemen, M. Ferrat, E. Haughey, S. Luz, S. Neogi, M. Pathak, J. Petzold, J. Portugal Pereira, P. Vyas, E. Huntley, K. Kissick, M. Belkacemi, J. Malley, (eds.)]. In press.

Jackson, C., H. Tyler, J. Millar. 2013. Determination of Microbial Extracellular Enzyme

Activity in Waters, Soils, and Sediments using High Throughput Microplate Assays. *Journal of Visualized Experiments.* (80): 50399

Jian, J., B.J. Lester, X. Du, M.S. Reiter, R.D. Stewart. 2020. A calculator to quantify cover crop effects on soil health and productivity. *Soil and Tillage Research.* 199: 104575

Keynan, A., N. Sandler. 1983. Spore research in historical perspective. In: A. Hurst, G.W. Gould (Eds.), The Bacterial Spore, Vol. 2. Academic Press, New York, NY, pp. 1-48.

Kheira, A.A. 2009. Macromanagement of Deficit-Irrigated Peanut with Sprinkler Irrigation. *Agricultural Water Management.* 96(10):1409-1420

Kiers, E.T., R.A. Rousseau, S.A. West, R.F. Denison. 2003. Host sanctions and the legume-rhizobium mutualism. *Nature*. 425: 78-81

Kim, N., M.C. Zabaloy, K. Guan, M.B. Villamil. 2020. Do cover crops benefit soil microbiome? A meta-analysis of current research. *Soil Biology and Biochemistry.* 142:107701

Klonsky, K., and L. Tourte. 1998. Organic Agricultural Production in the United States: Debates and Directions. *American Journal of Agricultural Economics.* 80(5):1119-1124

Kristiansen, P., A. Taji, J. Reganold. 2006. Organic Agriculture: A Global Perspective. *Cornstock Publishing Associates.* Ithaca, New York.

Knapp, S., M.G.A. van der Heijden. 2018. A global meta-analysis of yield stability in organic and conventional agriculture. *Nature Communications.* 9:3632.

Lal, R. 2012. Climate Change and Soil Degradation Mitigation by Sustainable Management of Soils and Other Natural Resources. *Agricultural Resources*. 1(3):199-212

Lal, R., G.F. Hall, and F.P. Miller. 1989. Soil Degradation: I. Basic Processes. *Land Degradation & Rehabilitation*. 1:51-69

LaRose, J., R. Myers. 2019. Progress Report: Adoption of Soil Health Systems Based on Data from the 2017 U.S. Census of Agriculture. *Soil Health Institute.* Retrieved from: https://soilhealthinstitute.org/wp-content/uploads/2019/07/Soil-Health-Census-Report.pdf

Latos, Tomas H. 2009. *Cover Crops and Crop Yields*. Nova Science Publishers.

Mbuthia, L.W., V. Acosta-Martinez, J. DeBruyn, S. Schaeffer, D. Tyler, E. Odoi, M. Mpheshea,

F. Walker, J. Eash. 2015. Long Term Tillage, Cover Crop, and Fertilization Effects on Microbial Community Structure, Activity: Implications for Soil Quality. *Soil Biology and Biochemistry*. 89:24-34

McDonald M.D., K.L. Lewis, G.L. Ritchie, P.B. DeLaune, K.D. Casey, L.C. Slaughter. 2019. Carbon dioxide mitigation potential of conservation agriculture in a semi-arid agricultural region. *AIMS Agriculture and Food.* 4(1): 206-222.

National Organic Program, 7B C.F.R. §205.2 (2021)

Nautiyal, C.S., P.S. Chauhan, C.R. Bhatia. 2010. Changes in soil physio-chemical properties and microbial functional diversity due to 14 years of conversion of grassland to organic agriculture in semi-arid agroecosystem. *Soil & Tillage Research*. 109:55-60.

NRCS (Natural Resources Conservation Service). 2019. Healthy Soils for Life. *Natural Resources Conservation Service.* Retrieved from: https://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/health/

Parham, J., S. Deng. 2000. Detection, quantification and characterization of B-glucosaminidase activity in soil. *Soil Biology & Biochemistry.* 32:1183-1190

Peanuts: A Brief History. 2020. *American Peanut Council.* Retrieved from: https://www.peanutsusa.com/2-about-peanuts/7-peanuts-a-brief history.html#:~:text=The%20peanut%2C%20while%20grown%20in,Europe%2C%20peanuts%20went%20with%20them.

Peanut Country, U.S.A. 2021. *National Peanut Board.* Retrieved from: https://www.nationalpeanutboard.org/peanut-info/peanut-country-usa.htm

Pennanen, T., A. Frostegard, H. Fritze, E. Baath. 1996. Phospholipid fatty acid Composition and heavy metal tolerance of soil microbial communities along two heavy metal-polluted gradients in coniferous forests. *Applied Environmental Microbiology.* 62(2), 420-428.

Perret, X., C. Staehelin, W.J. Broughton. 2000. Molecular basis of symbiotic promiscuity. *Microbiology and Molecular Biology Reviews.* 64(1): 180-201.

Peters, S. 1991. Organic and Conventional Beyond Transition. *Rodale Research Center.* Retrieved from: http://www.reap-canada.com/online_library/Magazine%20Articles/Crops/44%20Organic%20and.pdf

Popova, I., S. Deng. 2010. A high-throughput microplate assay for simultaneous colorimetric quantification of multiple enzyme activities in soil. *Applied Soil Ecology.* 45: 315-318.

Roesch-McNally, G.E., A.D. Basche, J.G. Arbuckle, J.C. Tyndall, F.E. Miguez, T. Bowman, R. Clay. 2017. The trouble with cover crops: farmers' experiences with overcoming barriers to adoption. *Renewable Agriculture and Food Systems.* 1-12

Rowland, D.L, W.H. Faircloth, P. Payton, D.T. Tissue, J.A. Ferrell, R.B. Sorenson, C.L. Butts. 2012. Primed Acclimation of Cultivated Peanut (*Arachis hyppgaea* L.) Through the Use of Deficit Irrigation Timed to Crop Developmental Periods. *Agricultural Water Management.* 113:85-95

Russell, A. 2019. Texas Crop and Weather Report – Sept. 11, 2019. *AgriLife Today*. Retrieved from: https://agrilifetoday.tamu.edu/2019/09/11/texas-crop-and-weather-report-sept-11-2019-peanut-harvest/

Russel, D.A., G.G. Williams. 1977. History of Chemical Fertilizer Development. *Soil Science Society of America Journal.* 41(2):260-265

Sainju, U.M., Singh, B.P., Whitehead, W.F. 2002. Long-term effects of tillage, cover crops, and nitrogen fertilization on organic carbon and nitrogen concentration in sandy loam soils in Georgia, USA. *Soil & Tillage Research.* 63:3-4.

SARE Outreach, 2003. Transitioning to Organic Production. *The Sustainable Agriculture Network (SAN).* Retrieved from: https://www.sare.org/wp-content/uploads/Transitioning-to-Organic-Production.pdf

Santos, V, et al. 2012. Soil microbial biomass and organic matter fractions during transition from conventional to organic farming systems. *Geoderma.* 170:227-231.

Schoonover, J., J. Crim. 2015. An Introduction to Soil Concepts and the Role of Soils in Watershed Management. *Journal of Contemporary Water Research & Education.* 154:21-47

Schultze, M., C. Staehelin, F. Brunner, I. Genetet, M. Legrand, B. Fritig, E. Kondorosi, A. Kondorosi. 2001. *The Plant Journal.* 16(5): 571-580.

Sebilo, M., Mayer, B., Nicolardot, B., et al. 2013. Long-term fate of nitrate fertilizer in agricultural soils. *Proceedings of the National Academy of Sciences.* 110(45).

Seufert, V., N. Ramankutty, J.A. Foley. 2012. Comparing the yields of organic and conventional agriculture. *Nature.* 485: 229-234.

Skinner, C., Gattinger, A., Muller, A., et al. 2014. Greenhouse gas fluxes from agricultural soils under organic and non-organic management – A global meta-analysis. *Science of the Total Environment*. 468-469 (2014) 553-563.

Skinner, C, et al. 2019. The impact of long-term organic farming on soil-derived greenhouse gas emissions. *Nature Scientific Reports*. 9:1702.

Smil, V. 1999. Detonator of the Population Explosion. *Nature*. 400, 415

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. *Web Soil Survey.* Available online at the following link: http://websoilsurvey.sc.egov.usda.gov/. Accessed [1/23/2021].

Steward, R.D., J. Jian, A.J. Gyawali, W.E. Thomason, B.D. Badgley, M.S. Reiter, and M.S. Strickland. 2018. What We Talk about When We Talk about Soil Health. *Agricultural & Environmental Letters.*

Stockdale, E.A., M.A. Shepherd, S. Fortune, and S.P. Cuttle. 2002. Soil Fertility in Organic Farming Systems – Fundamentally Different? *Soil Use and Management.* 18:301-308

Stott, D.E. 2019. Recommended Soil Health Indicators and Associated Laboratory Procedures. Soil Health Technical Note No. 450-03. *U.S. Department of Agriculture, Natural Resources Conservation Service.*

Tajima, R., J. Abe, O.N. Lee, S. Morita, and A. Lux. 2008. Developmental Changes in Peanut Root Structure During Root Growth and Root-Structure Modification by Nodulation. *Annals of Botany.* 101(4):491-499

Tajima, R., O.N. Lee, J. Abe, A. Lux, and S. Morita. 2007. Nitrogen-Fixing Activity of Root Nodules in Relation To their Size in Peanut (*Arachis hypogaea* L.). *Plant Production Science.* 10(4):423-429.

Texas Ecoregions. 2020. Texas Parks & Wildlife. Retrieved from: https://tpwd.texas.gov/education/hunter-education/online-course/wildlife-conservation/texas-ecoregions#:~:text=The%20High%20Plains%20region%2C%20together,4%2C500%20feet%20above%20sea%20level.

Thapa, V.R., R. Ghimire, V. Acosta-Martinez, M.A. Marsalis, M.E. Schipanski. 2021. Cover crop biomass and species composition affect soil microbial community structure and enzyme activities in semiarid cropping systems. *Applied Soil Ecology.* 157:103735

USDA. 2013. Organic Production. *USDA Economic Research Service.* Retrieved from: *https://www.ers.usda.gov/data-products/organic-production/*

USDA (United States Department of Agriculture). 2015. Introduction to Organic Practices. *USDA Agricultural Marketing Services.* Retrieved from: https://www.ams.usda.gov/sites/default/files/media/Organic%20Practices%20Factsheet.pdf

USDA (United States Department of Agriculture). N.d. Organic Market Summary and Trends. *USDA Economic Research Service.* Retrieved from: https://www.ers.usda.gov/topics/natural-resources-environment/organic-agriculture/organic-market-summary-and-trends/

USDA (United States Department of Agriculture). 2019. Organic Agriculture: Title I (Commodities), Title II (Conservation), Title III (Trade), Title V (Credit), Title VII (Research), Title X (Horticulture), and Title XI (Crop Insurance). *USDA Economic Research Service.* Retrieved from: https://www.ers.usda.gov/agriculture-improvement-act-of-2018-highlights-and-implications/organic-agriculture/

USDA. 2014. Cover crop basics for Southeast Missouri. *USDA Natural Resources Conservation Service.* Retrieved from: https://www.nrcs.usda.gov/Internet/FSE_PLANTMATERIALS/publications/mopmsbr12100.pdf

USDA NASS. 2021. Peanuts. *USDA NASS QuickStats.* Retrieved from. https://quickstats.nass.usda.gov/results/D551EB2D-6DEC-38F3-9AD4-E085CC39F14C?pivot=short_desc

Verstraete, W., J.P. Voets. 1977. Soil Microbial and Biochemical Characteristics in Relation to Soil Management and Fertility. *Soil Biology and Biochemistry.* 9(4):253-258

Wagai, R., K.R. Brye, S.T. Gower, J.M. Norman, L.G. Bundy. 1998. Land use and environmental factors influencing soil surface CO2 flux and microbial biomass in natural and managed ecosystems in southern Wisconsin. *Soil Biology and Biochemistry.* 30(12):1501-1509

Watts, C.W., W.R. Whalley, D.J. Longstaff, R.P. White, P.C. Brooke, and A.P. Whitmore. 2001. Aggregation of a Soil with Different Cropping Histories Following the Addition of Organic Materials. *Soil Use and Management.* 17:263-268

Weaver, R.W. 1974. Effectiveness of Rhizobia forming nodules on Texas grown peanuts. *Peanut*

Science. 1 (1):23-25.

Weil, R.R., N.C. Brady. 2019. Elements of Nature and Properties of Soils, 4th Edition. *Pearson.*

Willers, C. P.J. Jansen van Rensburg, S. Claassens. 2015. Phospholipid fatty acid profiling of microbial communities – a review of interpretations and recent applications. *Journal of Applied Microbiology.* 119:1207-1218.

Wilson, G.W.T., C.W. Rice, M.C. Rillig, A. Springer, and D.C. Hartnett. 2009. Soil Aggregation and Carbon Sequestration are Tightly Correlated with the Abundance of Arbuscular Mycorrhizal Fungi: Results from Long-Term Field Experiments. *Ecology Letters.* 12:452-461

Zelles, L., Q.Y. Bai, R. Rackwitz, D. Chadwick, F. Beese. 1995. Determination of phospholipid- and lipopolysaccharide-derived fatty acids as an estimate of microbial biomass and community structures in soils. *Biology and Fertility of Soils.* 19(2-3), 115-123.

Zhang, Q., I. Shamsi, D. Xu, G. Wang, X. Lin. et al. 2012. Chemical fertilizer and organic manure inputs in soil exhibit a vice versa pattern of microbial community structure. *Applied Soil Ecology.* 57:1-8.

Zhang, Q., W. Zhou, G., Liang, J. Sun, X. Wang, P. He. 2015a. Distribution of soil nutrients, extracellular enzyme activities and microbial communities across particle-size fractions in a long-term fertilizer experiment. *Applied Soil Ecology.* 94. 59-71

Zhang, X., W. Dong, X. Dai, S. Schaffer, F. Yang, M. Radosevich, L. Xu, X. Liu, X. Sun. 2015b. Responses of absolute and specific soil enzyme activities to long term additions of organic and mineral fertilizer. *Science of The Total Environment.* 536:59-67

Zinati, G.M. 2002. Transition from Conventional to Organic Farming Systems: I. Challenges, Recommendations, and Guidelines for Pest Management. *HortTechnology.* 12(4):606-610