COVER CROP AND ROTATION INTENSITY EFFECTS ON SOIL HEALTH AND YIELD IN CORN-SOYBEAN CROPPING SYSTEMS

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JACOB T. YOUNG

Dr. William Wiebold, Dissertation Supervisor

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The undersigned, appointed by the dean of the Graduate School,

have examined the dissertation entitled

COVER CROP AND ROTATION INTENSITY EFFECTS ON SOIL HEALTH AND YIELD IN CORN-SOYBEAN CROPPING SYSTEMS

presented by Jacob T. Young,

a candidate for the degree of

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and hereby certify that, in their opinion, it is worthy of acceptance.

Dr. William Wiebold

Dr. Kaitlyn Bissonnette

Dr. Felix Fritschi

Dr. William Stevens

Dr. Raymond Massey

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract

In the Midwest, corn-soybean [*Glycine max* (L). Merrill] is the dominant biennial cropping system, which covers approximately 75% of the arable land surface (Hatfield et al., 2007; Plourde et al., 2013). The growing demand for corn (*Zea mays* L.) and its financial competitiveness as a cash crop over the past two decades has led to an increased use of more corn-intense cropping systems. This increase in corn intensity within corn-soybean rotations in the Midwest has caused concern for maintaining soil health and cash crop yields for the long-term. The implementation of cover crops and crop rotation are widely promoted management strategies that have been shown to enhance soil health in agricultural systems, and may lead to increases in cash crop yields.

The objectives of Chapter II of this dissertation were to examine the influence of cover crops, crop rotation, year, and their combination on several soil health indicators and cash crop yield. The soil health indicators of bulk density, water stable aggregates, soil moisture, total organic carbon, active carbon, potentially mineralizable nitrogen (PMN), and soil microbial community composition via a phospholipid fatty acid (PLFA) analysis were measured in 2017 and 2018 in Columbia, MO under no-till conditions. Grain yields of corn and soybean were recorded from 2016-2019. Crop rotation treatments significantly improved water stable aggregates and corn yield. Cover crop treatments led to significant improvements in several soil health indicators (water stable aggregates, soil moisture, PMN, AMF, gram negative bacteria, and the gram positive / gram negative ratio) while maintaining yield in soybean and decreasing yield in corn.

The objectives of chapter III of this dissertation were to evaluate the long-term effects of increased corn frequency within a corn-soybean rotation on several soil health quality indicators and evaluate the long-term corn and soybean yield responses to ten different corn-soybean rotations. In order to better represent the long-term impacts of increased corn rotation intensity within rotations on soil health, corn rotation frequency (CRF) ratings were assigned to each rotation treatment based on the percentage of corn within each rotation. Utilizing these ratings when evaluating the soil data allows for effects of increased corn within rotations to be more easily identified. Soil measurements were taken in 2014 and included several indicators of soil physical, chemical, and biological health to provide a snapshot of conditions as a result of nine years of the ten rotation treatments being in place. Yield data was collected from 2007 – 2019 to evaluate the long-term effects of various corn intensities within corn-soybean rotations. Overall, corn yields were significantly improved in the first year after soybean, and with fewer consecutive years of corn in the rotation cycles. Soybean yields were most significantly improved after following two years of corn, and when avoiding consecutive years of soybean. Although the two-year corn-soybean rotation yields were statistically similar to soybean following two years of corn in 9 of 11 years in this study. For soil measurements, significant improvements from increased corn rotation intensity were seen in bulk density, total nitrogen, PMN, TOC, active carbon, SOM, β-glucosidase, overall microbial biomass and diversity, AMF, gram negative bacteria, gram positive bacteria, and actinobacteria. These results provide valuable information to producers aiming to improve soil physical, chemical, and biological function while also maintaining the highest yield potential in corn-soybean rotations.

CHAPTER I

LITERATURE REVIEW

Soybean Production and Economic Impact

Soybean [*Glycine max* (L). Merrill] is the leading oilseed crop produced and consumed in the world today (Wilcox, 2004), and it is one of the most economically important crops produced in the United States. The three major producing countries are: the United States (120.5 million metric tons), Brazil (117.0 million metric tons), and Argentina (55.3 million metric tons) (USDA-FAS, 2020). These three countries account for over 80% of world production. Soybean is also the most economically important agricultural export of the United States, with $21.6 billion worth of soybean being exported in 2017 (USDA-FAS, 2017).

In the United States, soybean and corn (*Zea mays* L.) are the top cash crops grown. In 2019, corn was grown on 37.1 million hectares, and soybean grown on 32.4 million hectares (USDA-NASS, 2019). In 2018, Missouri ranked sixth among all U.S. states for soybean hectares planted (2.4 million) and seventh in total production (7.1 million metric tons) (USDA-NASS, 2019).

Soybeans are grown for numerous end uses, including oil products, whole-bean products, and meal products (Smith and Huyser, 1987). In 2018, soybean accounted for 61% of the world's oilseed production (361 million metric tons) and 70% of the world's protein meal consumption (235 million metric tons). In the U.S., soybean meal is a vital component of livestock feed, primarily for poultry and swine, with 18.0 and 8.2 million metric tons being consumed by each, respectively. Soybean meal is a leading livestock feed component due in part to its high protein content (~40%), and its amino acid balance (ASA, 2019).

Soybean History

Cultivated soybean is thought to have been domesticated from its wild relative [*Glycine soja* (L). Merrill] in northeastern China in the 11th century B.C (Carter Jr. et al., 2004). The exact origin location of soybean is unknown, though many regions have been identified as candidate regions, including: northeastern China, southern China, the Yellow River valley of central China, and various other regions in Korea and Japan (Carter Jr. et al., 2004). Additionally, Chinese literature suggests that soybean was first cultivated during the Shang dynasty from 1,700 to 1,100 BC (Wilson, 2008). During this time, soybean was primarily grown for the seeds to be used as fresh, fermented, and dried food products (Gibson and Benson, 2005).

It was not until the late 1700's that soybean was introduced into the United States. Samuel Bowen, a seaman with the British East India Company, brought soybean to Savannah, Georgia from China and requested that Surveyor General Henry Yonge cultivate them on his farm. By 1766, Bowen also began growing soybean at his nearby plantation in Thunderbolt, Georgia (Hymowitz and Shurtleff, 2005).

During the mid-19th century, soybean was introduced to the current Midwestern United States region. Throughout most of the 1800's, soybean was primarily grown as a forage crop to be used for hay, or plowed under as a green manure crop as a means to improve soil structure and fertility (Hymowitz, 1990).

Prior to World War II, the United States imported over 40% of its edible fats and oils, mostly from southeast Asia (Gibson and Benson, 2005). During the war, domestically produced soybean oil was used as the primary replacement of the imported fats and oils, and it was additionally used to manufacture glycerin, an ingredient used in explosives (Hymowitz, 1990). The meal by-product from oil extraction was used to increase livestock production in the United States and as a vegetable protein meat extender in Europe during this time. By the mid-20th century, the United States soybean crop had increased to over 2 million hectares, and the United States had become a large exporter of soybean and soybean byproducts to international markets.

Today, the popularity of soybean and soybean byproducts is increasing for both consumer and industry uses. A multitude of products can be made from soybean, including: livestock feed, biofuels, carpet and upholstery, industrial solvents and lubricants, foams, candles, crayons, and printer ink (NCSPA, 2019). Currently, soybean is grown in 31 U.S. states, and is the second largest cash crop grown in the United States, behind corn (USDA-NASS, 2019).

Soybean has an additional characteristic that makes it a popular choice of U.S. farmers, its unique fertility requirement (Drinkwater et al., 2000). Soybean is a member of the Fabaceae (or Leguminosae) family. As a legume, it is able to fix its own nitrogen (N) from the atmosphere (N_2) into the plant-available form ammonia (NH_3), through a symbiotic relationship between the soybean plant and *Bradyrhizobium japonicum*, a rhizobacteria. Due to this ability to provide its own nitrogen, soybean has vastly different N fertilization requirements than the other common cash crops in the region, including corn and wheat (*Triticum aestivum* L.).

Research focusing on improving soybean yield through breeding and genetics is crucial to meeting the production needs in the U.S., research investigating the influence of management practices on soybean yield and soil health is also important. Such research can provide significant insights into ways to maximize production and profitability while also and reducing the potential negative environmental impacts. Investigating the potential benefits of cover crops and crop rotation in soybean and corn cropping systems and their effects on soil health is a research topic that warrants more attention.

Cropping Systems

A cropping system refers to the crop species grown and the production practices used. In the Midwest, corn-soybean is the dominant cropping system, which covers approximately 75% of the arable land surface (Hatfield et al., 2007; Plourde et al., 2013). The simplest cropping system used in the Midwest is continuous cropping. Continuous cropping involves planting the same crop on the same land for two or more years. The amount of land area in the Midwest devoted to continuous cropping is not known, as the USDA-NASS no longer provides that information. However, the use of continuous cropping can be inferred from the hectarages of corn and soybean. This is possible because corn and soybean are by far the most common crops in most Midwestern states (USDA-NASS, 2019). For example, in 2019 the nationwide hectarages of corn was 37.1 million and soybean 32.4 million. The ratio of corn to soybean hectarages is 1.15. With this ratio being larger than 1.0 there is more land area planted to corn than to soybean, so continuous corn is a likely result. In Missouri in 2019, the hectarages of corn was 1.3 million and soybean 2.1 million. The ratio of corn to soybean hectarages is 0.62. Missouri’s ratio is much less than 1.0, and this indicates that continuous soybean is common.

The intensification of continuous cropping in the Midwest has caused concern for maintaining soil health for the long-term (Bennett et al., 2012), and concerns about sustainability and environmental degradation. (Foley et al., 2011). Continuous cropping systems have been shown to be unsustainable for long-term yield and soil health (Ashworth et al., 2017). Continuous cropping of corn and/or soybean have led to increases in weed pressure (Higgs et al., 1990) and pests (Chen et al., 2001), along with decreases in yield (Chen et al., 2001), soil quality (Karlen et al., 2006), and microbial biomass (McDaniel et al., 2014). As outlined in this section, continuous cropping damages soil health and leads to reductions in yield.

Soil Health

Soil health is a key driver in achieving sustainable food production in order to feed the human population. Soil health can be broadly defined as the "continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans." (Lehman et al., 2015). Weil (2004) states that healthy soil functions include: (1) producing healthy plants, (2) cycling and retention of nutrients, (3) providing habitat for soil microorganisms, (4) supplying plant and soil microorganisms with air and water for subsistence, (5) maintaining water quality and protecting it from contamination, (6) providing physical support for vegetation, (7) protecting against toxin accumulation and transport of natural and synthetic compounds, and (8) filtering elements to protect animals, plants, and the environment from adverse exposure. These functions can be influenced by agricultural management strategies, including diversified cropping rotations and cover cropping.

According to the NRCS (2012), “soil erosion involves the breakdown, detachment, transport, and redistribution of soil particles by forces of water, wind, or gravity.” Soil erosion of croplands is of particular concern to agriculturalists because of the impacts on soil quality and overall crop productivity by removing topsoil, reducing soil organic matter, and diminishing the physical integrity of soils (Lal, 1998). Additionally, soil erosion also has the potential for off-site impacts affecting water quality, air quality, and biological activity (Lal, 1998; NRCS, 2012). Additionally, erosion leads to decreases in other functions of soil health. Soil erosion decreases water infiltration rates, water holding capacity, nutrient availability, and microbial diversity (Pimentel and Burgess, 2013).

Water infiltration is the process in which water from the soil surface enters the soil profile, and is one of the most influential processes that occurs on the earth’s surface (Liu et al., 2018). Water infiltration into the rhizosphere is essential for proper plant growth and development due to the transport of solutes and nutrients (Jarvis, 2007). Increased water infiltration rates also influences the amount of runoff water and soil erosion that occurs due to rain or irrigation (De Roo et al., 1992; Karlen et al., 1997). Increasing the amount of soil surface residue present can slow the rate and speed of water travelling across the soil surface, making soil erosion less likely (Godwin, 1990).

Bulk density is the dry weight of soil divided by its volume, and is used as an indicator of soil compaction (NRCS, 2008). Soil compaction can be caused by prolonged use of agricultural tillage machinery, and poor soil management practices (Hamza and Anderson, 2005). This compaction can lead to plant growth impediments and reduced uptake of nutrients and water, ultimately leading to reduced cash crop yields (Amuri et al., 2008). Larger bulk density values can affect the development of plants by increasing resistance to root penetration and decreasing water infiltration (Nazário et al., 2009). Bulk density is dependent upon a soil's pore space, and higher porosity in a soil is correlated with lower bulk density. According to a study by Udawatta et al. (2006), bulk density values greater than 1.8 g/cm^3 completely inhibit root development, values less than 1.5 g/cm^3 were less restrictive, and values less than 1.3 g/cm^3 did not show any root growth restriction.

Soil aggregates consist of soil particles bound together as a result of the interaction between soil microorganisms, plants, and their products with soil mineral components (Allen et al., 2011). Aggregate stability refers to the ability of soil particles to resist disturbance when external forces are applied (NRCS, 1996). Aggregate stability is influenced by soil chemical and biological properties. Additionally, soil structure and soil management practices have been shown to have significant impacts on aggregate stability (Dalal and Moloney, 2000; Moebius et al., 2007). The formation of soil aggregates has been shown to reduce the negative effects of erosion events, compaction, and evaporation (Kladivko, 2001).

Soil organic matter is regarded as the most important indicator of soil health due to the interaction it has with the biological, chemical, and physical environment (Karlen et al., 2003), and is crucial to improving soil structure, soil fertility, and other biological, chemical, and physical functions (Skjemstad et al., 1998). It contains decomposing (active) organic matter, stabilized organic matter (humus), fresh residues, and small living organisms (NRCS, 2014). Soil organic matter will generally be highest in areas of minimal disturbance, where biomass accumulation is higher, and where organic material is added to the soil surface (NRCS, 2014).

Soil microorganisms contribute to overall soil health in many ways, including: soil structure, decomposition, nutrient cycling, and disease prevention (Garbeva et al., 2004). Beneficial soil microorganisms share a symbiotic relationship with plants, where the microorganisms rely on root exudates from the plants. In return, the microorganisms can sequester nutrients from the soil solution, and later return those nutrients to the soil for plant use as the microorganisms decompose (Garbeva et al., 2004). Mycorrhizae, plant symbiotic fungi, benefit plants by aiding roots in absorbing more water and nutrients via extraradical hyphae. These functions of the soil microbial community drive sustainable soil health, and can lead to improvements in crop productivity.

Phospholipid fatty acids (PLFA) are an indicator of soil health that provides information on abundance and diversity of the soil microbial populations. PLFA analysis can measure shifts in microbial community makeup as a result of management practices such as cover crops and crop rotation (Bååth et al., 1995; Frostegård et al., 1993). PLFA analysis utilizes membrane lipids within microorganisms as biomarkers for specific constituencies of organisms, which creates an overall profile of the soil microbial community structure (Frostegård et al., 2011; Steenwerth et al., 2002). PLFA analyses are more sensitive compared to other methods, and are widely used as a means to estimate shifts in populations within the soil microbial community.

Crop Rotation

Crop rotation refers to a series of dissimilar crops being planted in the same field, typically following a defined order (e.g. corn/soybean/wheat), in order to better manage soil quality, soil fertility, water, weeds, and diseases within an agroecosystem (Lehman et al., 2015). In the Midwest, corn-soybean is the dominant cropping system, which covers approximately 75% of the arable land surface (Hatfield et al., 2007; Plourde et al., 2013).

Crop rotation has multiple advantages over continuous cropping, including: increased yield, improved soil fertility and soil structure, improved water retention, and a reduced risk of crop failure (Bullock, 1992; Sauerborn et al., 2000). It is also hypothesized that improving aboveground biodiversity will lead to improvements in belowground biodiversity, which can lead to significant effects on several soil health and crop productivity factors.

Soil Health

Diversifying crop rotations can lead to improvements in overall soil health as compared to continuous cropping (Bullock, 1992). Prior research has shown soil organic matter to be affected by different crop rotations. Although the rate of carbon input and loss largely depends on soil type, cropping system, and environmental conditions; soil organic matter can be influenced by crop rotation by impacting soil carbon dynamics. Wegner et al. (2015) found that combining no-tillage practices with cash crops grown in a rotation led to significant increases in soil organic matter. A long-term study conducted by Van Eerd et al. (2014) investigated the effects of tillage and soybean-corn-winter wheat crop rotations on soil organic matter and total nitrogen over 11 and 15 years. Their research showed that both soil organic matter and total nitrogen were improved with the combination of no-tillage and cash crops grown in a rotation. A 15-year study conducted by Zuber et al. (2015) in Illinois showed that a corn-soybean-wheat rotation significantly increased soil organic matter compared to both the continuous corn and continuous soybean treatments. Conversely, results from a 49-year study in Ohio by Kumar et al. (2012) found no significant differences in soil organic matter between the continuous corn and corn-soybean rotations.

Utilizing crop rotations, especially under no-tillage conditions, has been shown to improve bulk density, soil water retention, and water infiltration (Kemper et al., 2011; Kumar et al., 2012). A study by Pikul et al. (2008) showed significant decreases in bulk density by 2% under a diverse four-crop rotation (corn-soybean-wheat-alfalfa) than under a two-crop rotation (corn-soybean). Additionally, a more diverse cropping system coupled with no-tillage practices led to significant decreases in bulk density as compared to less diverse crop rotations and mono-cropping (Riedell et al., 2013). Utilizing management strategies such as no-tillage and crop rotation can lead to increases in soil organic matter, which can also lead to improved water infiltration rates (Martens and Frankenberger Jr, 1992). Hangen et al. (2002) reported higher water infiltration rates under diversified crop rotations and no-tillage treatments due to the increases in macropores present in the soil as compared to other treatments. Diversifying crop rotations have also been shown to increase earthworm populations, which ultimately led to increased water infiltration rates (Francis and Knight, 1993).

Microbial Community

Soil microbial communities are an integrative parameter of soil quality due to their involvement in many agroecosystem processes, including: nutrient cycling and organic matter turnover (Yao et al., 2000; Yao et al., 2006). There is evidence in the literature that diversified cropping systems are associated with impacts on soil microbial community dynamics, although the effect on individual members and their contribution to cash crop yields is not fully understood (Yin et al., 2004).

The likely reason soil microbial communities are influenced by diversified crop rotations is due to the higher input of organic matter and carbon into the soil profile (Miller and Dick, 1995; Moore et al., 2000). A meta-analysis by Venter et al. (2016) showed diversifying crop rotations may increase, decrease, or have no significant effects on soil microbial communities. A study by Zhang et al. (2014) showed no significant increases in microbial biomass between a corn-soybean rotation and a continuous corn rotation, but the relative abundance of certain microbial groups increased in the rotation treatment. Sun et al. (2016) found significant increases in saprophytic fungi, arbuscular mycorrhizal fungi (AMF), and overall fungi in the no-tillage, corn-soybean rotation treatment as compared to the continuous corn treatment. Conversely, Liu et al. (2017) reported no significant differences for microbial communities between treatments with crop rotations and treatments with mono-cropping. This review of the relevant literature surrounding soil microbial communities highlights a need for additional research investigating the long-term effects of management practices such as crop rotation and cover cropping on the abundance and diversity of the soil microbial community.

Yield

As discussed above, it is recognized that crop rotation aids in improving soil fertility and soil health, which may lead to increases in cash crop yields. Crops that are in a rotation often experience what is referred to as the rotation effect, which is the phenomenon of yield increases seen in crops grown for the first time on a piece of land, or when grown in a rotation (Bennett et al., 2012; Foley et al., 2011; Hilton et al., 2013). The underlying mechanisms behind the rotation effect are not fully understood, however, that increased nutrient cycling, improved soil health, and the breakup of weed and disease cycles may be contributors (Hilton et al., 2013).

For soybean, the phenomenon of the rotation effect has been well documented, and soybean grown in a monocrop generally leads to yield decreases (Karlen et al., 2013; Wilhelm and Wortmann, 2004). Because corn produces substantially more residue than soybean, soybean grown in a rotation with corn can lead to increases in the relative amount of microbial biomass and diversity. This can ultimately lead to increases in nitrogen availability for the succeeding cash crop grown (Moore et al., 2000).

An Indiana study spanning 20 years conducted by West et al. (1996) found that soybean in a rotation with corn showed significant increases in yield as compared to continuous soybean, regardless of tillage practice. This research was confirmed by another long-term study from Kelley et al. (2003), in which soybean rotated with either grain sorghum [*Sorghum bicolor* (L.) Moench] or wheat showed approximately a 15% yield increase compared to the continuous soybean treatment. Another comprehensive long-term study investigating yield and yield variability among several crop rotations found that rotations of two or more species led to increased crop yields as compared to monocrop treatments (Grover et al., 2009). Additionally, there is evidence that crops grown either in short rotation or in monocrop will eventually reach equilibrium of yield decline, and will remain consistent but lower than the same crop grown in a rotation (Bennett et al., 2012; Seifert et al., 2017; Stanger and Lauer, 2008). For example, Seifert et al. (2017) reported that the yield gap between rotated corn and continuous corn increased in each year of the treatments, and eventually plateaued after the third year, maintaining a 4.3% yield gap overall.

Cover Crops

Today, modern agricultural ecosystems are influenced not only by natural factors, but also by manmade factors that are necessary in order to achieve humanity's food and fiber production needs (Altieri, 1999). Generally, maintaining high production levels in these agroecosystems has been reliant upon a large amount of external inputs and continuous cropping, which has led to a lack in overall biodiversity and sustainability (Doran, 2002). Additionally, the misuse of these conventional agricultural systems has caused an increase in soil erosion, the eutrophication of water bodies, and decreased soil fertility and organic matter (Hargrove, 1991). Conversely, sustainable agricultural systems are those that meet humanity's needs for food and fiber via the safe and efficient utilization of natural resources and the necessary additional inputs (Ikerd, 1990). Sustainable agriculture today relies upon both technological advancements and improvements in management practices as a means to improve natural nutrient cycles, soil retention, and pest management. The implementation of cover crops is a significant tool that can be used to meet these goals (Parr et al., 1992).

Cover crops can broadly be defined as any living crops that are grown for seasonal ground cover and other soil management purposes (NRCS, 2016). They can be grown either between rows, in rotation with other crops, or during what would otherwise be fallow periods (Treadwell et al., 2010). Cover crops are used to improve soil health, nutrient cycling, reduce soil erosion, improve water retention and infiltration, and aid in the suppression of pests (Snapp et al., 2005). A wide variety of crop species are used as cover crops and selection should be based upon several factors, such as: climate, geography, soil type, cropping system, weed pressure, insect pressure, cost, and the overall goals of the producer.

Many different cover crop species are available to growers in the Midwest, and proper selection is crucial for improving soil health and cash crop performance. Common goals that producers have when evaluating which cover crop is most appropriate may include: reducing soil erosion, improve soil fertility, aid in nutrient cycling, conserve water, manage insect pressure, and suppress weeds (Reeves, 1994). Proper cover crop species selection also requires evaluating external factors, such as: climate, cropping system, soil type, weed pressure, insect pressure, accessibility of necessary equipment, and cost. The predominant cover crop species include grasses, legumes, and brassicas (Liu et al., 2005).

Legume cover crops are able to fix atmospheric nitrogen into a plant available form of ammonia (Islam and Reeder, 2014) through a symbiotic relationship with rhizobacteria (Snapp et al., 2005). Unfortunately, the amount of nitrogen that is fixed is limited by cool soil temperatures for legume cover crops planted in the fall and terminated in early spring (Wilke and Snapp, 2008). Legumes also produce significant amounts of aboveground biomass that can aid in protecting the soil surface and contributing to the soil's organic matter component (Meisinger et al., 1991). However, late fall and spring growth is reduced for most legume species because of cool temperatures. As a result, biomass production of most legume species planted after grain harvest in the fall and terminated early for grain planting in the spring could be small (Wilke and Snapp, 2008).

Cool season grass cover crop species possess rapid biomass accumulation in the fall that helps to cover and protect the soil surface through winter. Early planting and establishment improves the chances for adequate aboveground biomass accumulation (Bothwell et al., 2015). Some grass cover crops have allelopathic properties that can suppress weed growth (Weston, 1996). However, there is evidence that allelopathy is not limited to weeds, as rye (*Secale cereal* L.) cover crops have been shown to reduce corn yield (Vyn et al., 2000). Grass cover crops may aid in scavenging residual nitrogen and other mineral nutrients from the soil that would otherwise be lost to leaching (Wang et al., 2008). However, it is not clear at what time during the summer growing season the scavenged nutrients will become available (Ditch, 1991; Thapa et al., 2018). Rye is the most common grass cover crops in the United States (Myers et al., 2016), due to its winter-hardiness, quick establishment, and the ability to produce extensive amounts of biomass (SARE, 2012). Due to these attributes, rye is able to be planted much later in the fall as compared to other common cover crop species, making it an easy to establish even after the harvest of the previous cash crop.

Brassica cover crop plants produce large taproots, which may ease soil compaction and help scavenge nutrients from subsurface soil layers (Lawley et al., 2011;

Wright et al., 2017). Once the brassica plant dies and the large taproots decompose, the channels left in the soil help to improve water infiltration (Williams and Weil, 2004). Their aboveground biomass can also provide excellent soil cover, reducing soil erosion caused by wind or rain, and acting to suppress weeds from emerging (Alcántara et al., 2011). A common brassica cover crop is radish (*Raphanus sativus* L.)

Cover Crop Effects on Soil Health and Agronomic Performance

Erosion

Cover crops reduce soil erosion by protecting the soil surface from the force of raindrops and wind with their residues and by improving soil structure (Decker et al., 1994). Use of cover crops is a significant factor for reduced soil erosion in the Universal Soil Loss Equation (USLE) (Renard et al., 1991). A minimum cover crop seeding rate for preventing soil erosion has not yet been established, however, a cover crop that achieves at least 40% soil cover before the beginning of winter has been shown to provide significant protection from erosion (Clark, 2008).

Langdale et al. (1991) investigated how soil erosion was affected by a variety of different cover crop species used in varying cropping systems involving corn, soybean, and cotton. This research showed that the cover crop treatments significantly reduced soil erosion as compared to fallow treatments, regardless of tillage practice (conventional tillage vs. no-tillage). Kaspar et al. (2001) showed that oat (*Avena sativa* L.) and rye cover crops reduced soil erosion by 50% compared to fallow soil over a four-year study conducted in Iowa. Soil erosion that is caused by water runoff is decreased by cover crops via improving water infiltration rates and overall water holding capacity (Dabney et al., 2001; Fageria et al., 2005; Ketterings et al., 2015). A study by Rasnake et al. (1986) showed that soil erosion was reduced by up to 88% by double-cropping wheat after soybean as compared to a no cover crop control treatment in conventionally tilled soils in Kentucky.

Soil Water Content and Infiltration

Utilizing management practices such as cover cropping can lead to improved water infiltration rates by providing more residue cover on the soil surface. Different cover crops can have varying effects on soil moisture content and water infiltration rates. Infiltration of water through the soil surface is essential for proper plant growth and development, and cover crops can improve water infiltration by forming channels via their root systems and by improving soil structure. Cover crop residue on the soil surface has the potential to conserve soil moisture by shading the soil from direct sunlight exposure and decreasing water evaporation (Blanco-Canqui et al., 2011; Chen and Weil, 2011; Kaspar et al., 2001). Improving water infiltration rates can also translate to overall reductions in water runoff and soil erosion, ultimately leading to fewer nutrients leaching out of the rhizosphere and into groundwater supplies.

A rye cover crop has been shown to conserve soil moisture content better than several other cover crops (Chen and Weil, 2011) and to increase water infiltration rates compared to a no cover crop control (Kaspar et al., 2001). A rye cover crop has also shown to reduce sediment and nutrient losses through runoff water as compared to a no cover crop control (Korucu et al., 2018). When combined with a no-tillage management practice, cover crop treatments exhibited the fastest water infiltration rates compared to the no cover crop control in a study conducted by Mitchell et al. (2017) in California.

Bulk Density

The use of cover crops with large tap roots, such as radish, can alleviate soil compaction and help to improve bulk density and overall soil physical integrity (Chen and Weil, 2011). However, it is thought that bulk density changes slowly, and treatments greater than 3 years are typically needed in order to see significant reductions (Chen and Weil, 2011; Liebig et al., 2004). A study conducted by Villamil et al. (2006) showed that winter cover crops added to varying cropping rotations decreased bulk density by 7% in the top 5 cm. of soil, primarily due to the recurrent biomass being added and increased soil organic matter content. A rye cover crop was shown to decrease bulk density by 3.5% as compared to a no cover crop control in Missouri (Haruna and Nkongolo, 2015). A long-term no-tillage study conducted in Ohio showed that after four years of planting cover crop treatments, soil organic matter was increased and bulk density decreased (Islam and Reeder, 2014).

Aggregate Stability

The addition of cover crops can aid in improving aggregate stability of soils due to adding organic matter and increasing biological activity due to their residues. This organic matter and biological activity help to bind soil particles together via adhesive exudates that are produced (Grandy et al., 2006; Sapkota et al., 2012; Singh and Malhi, 2006). Generally, cover crops contribute positively to aggregate stability, but different species show more influence than others. An Ohio study conducted by Stavi et al. (2012) showed Austrian winter pea [*Pisum sativum* var. arvense (L.) Poir] alone and in combination with radish improved aggregate stability significantly more than radishes alone. In Indiana, Garvert (2013) investigated three cover crop treatments of hairy vetch [*Vicia hirsute* (L.) Gray], mustard (*Brassica rapa* L.), and rye. Their research showed significant improvements in aggregate stability compared to the no cover crop control, but the individual cover crop species did not show significant differences. An Illinois study conducted by Villamil et al. (2006) examined differences in soil physical properties in a corn/soybean rotation with cover crop treatments of hairy vetch, cereal rye, and a combination of both, along with a no cover crop control. This study showed significant improvements to aggregate stability by all of the cover crop treatments, but no significant differences were reported among the individual cover crop treatments.

Soil Organic Matter

Soil organic matter has been shown to be improved by cover cropping (Chen and Weil, 2010). However, increasing soil organic matter through use of cover crops can be slow. Often, several years are needed to realize improvements to soil organic matter from implementing cover crops (Moore et al., 2014). A study conducted in California by Steenwerth and Belina (2008) showed that five years were needed before any significant differences in soil organic matter content were measured. After that time, a rye cover crop improved soil organic matter compared to a no cover crop control. Villamil et al. (2006) found that after five years, a corn/soybean rotation that included a cover crop increased soil organic matter significantly compared to the treatment without a cover crop. The cover crop treatment of rye with hairy vetch showed the most improvement compared to the no cover crop control. In an Ohio experiment, Stavi et al. (2012) reported that a mixture of Austrian winter pea and radish increased soil organic matter significantly more than either cover crop in a treatment alone. Results from these studies show that the addition of cover crops to a cropping system can aid in improving soil organic matter.

Soil Microbial Community

The addition of cover crops to a cropping system may help to improve the soil microbial community diversity and overall population, largely due to the additional carbon source that the microorganisms receive from decomposing plant residues (Sapkota et al., 2012). Cover crops can also act as hosts for many species of mycorrhizal fungi, and can help to increase the amount of mycorrhizal inocula present in the soil for the succeeding cash crop (Dabney et al., 2001).

A study conducted in Italy by Sapkota et al. (2012) showed increased overall microorganism diversity under cover crop treatments of hairy vetch, clover (*Trifolium* L.), and brown mustard [*Brassica juncea* (L.) Czern] as compared to a no cover crop control. Utilizing oat and rye as a cover crop was shown to significantly increase the mycorrhizal inoculum along with improving soil aggregate stability compared to a no cover crop control (Kabir and Koide, 2002). Kabir and Koide (2002) also showed improvements in the density of soil mycorrhizal hyphae as a result of the cover crop treatments. These studies highlight the potential for improvement that cover crops may give to the soil microbial community.

Yield

A common concern when implementing any new management strategies is how cash crop yields will be affected. Cover crops have been shown to increase, decrease, and have no effect on yield in corn/soybean rotations (Steele et al., 2012; Strock et al., 2004). If yields are ultimately decreased, it is difficult to realize the benefits of implementing cover crops.

Cover crops can have negative effects on yield by: reducing nitrogen availability to the succeeding cash crop, contributing to poor soil to seed contact due to large amounts of residue, and from competition of volunteer cover crops after termination. In a study by Clark et al. (1994), multiple cover crop species were evaluated for their effect on corn yield in Maryland. The rye treatment terminated ten days prior to corn planting had significantly lower yields than the no cover crop control, hairy vetch, and rye/hairy vetch mixture treatments. A study by Acharya et al. (2017) also showed reduced yields in cover crop treatments that were terminated fewer than 10 days prior to cash crop planting. The authors also reported incidence of *Pythium* spp. increased as a result of shorter intervals between planting and cover crop termination, along with reduced corn emergence and shoot growth (Acharya et al., 2017). A study conducted in Ontario, Canada by Vyn et al. (2000) reported that the oat and rye cover crop treatments led to significantly lowered corn yields as compared to the no cover crop, radish, and red clover (*Trifolium pratense* L.) treatments when no additional nitrogen fertilizer was added to the corn. However, there were significant increases in corn yield when nitrogen fertilizer (150 kg ha^{-1}) was supplemented after cover crop termination. These studies show grass cover crop species leading to decreased corn yields, which may be due to the cover crops reducing nitrogen availability to the succeeding corn crop.

Cover crops have also been shown to increase cash crop yields by providing the succeeding cash crop with additional nutrients, by conserving moisture during dry times of the growing season, and by increasing soil organic matter content (Chen and Weil, 2011; Teasdale et al., 2008; Williams and Weil, 2004). A study by Chen and Weil (2010) comparing multiple cover crop species including radish, rapeseed, and cereal rye, showed that all treatments improved cash crop yield as compared to the no cover crop control. A Maryland study investigating cover crop effects on soybean yield showed that a radish and rye cover crop mixture significantly improved soybean yield compared to the other treatments (Williams and Weil, 2004). A study conducted by Ashworth et al. (2016) investigated cover crop effects on cash crop yield in corn/soybean rotations in Tennessee. Their research showed that cover crops had no effect on corn yield in a continuous rotation, but significantly improved corn yield when in a rotation with soybean.

Cover Crop Economics

Although the many benefits that cover crops have on soil health, water quality, and crop production are known, the financial and logistical obstacles surrounding their implementation must be addressed before widespread use of cover crops can occur (Strock et al., 2004). The economic component of cover crops is of concern to producers, and it is inconclusive whether implementing cover crops into crop rotations and nutrient management strategies will be economically viable. Due to most farming operations having profit maximization as the primary objective, the implementation of cover crops will be largely dependent upon if the additional revenue from the cash crop after planting a cover crop is greater than or equal to the cost of implementing the cover crop (Morton et al., 2006).

Currently, there are spreadsheet-based economic models available for determining the profitability of implementing cover crops into a farming operation. Generally, these models use a cost-benefit analysis to determine profitability of cover crops based upon yield changes in the succeeding cash crop (NRCS, 2014). Additional factors can be added to these models, including: erosion control, nutrient credits from cover crops, and reduced pesticide applications. However, there are limitations to these models. One example of a limitation in these models is that generally, nutrient credits are only given to leguminous cover crop species only, and mineral nutrients (notably N) scavenged and sequestered into the biomass of grass or brassica cover crop species are not included. Most likely due to the inability to accurately predict at what time during the growing season the scavenged nutrients will become available via their decomposing residues (Ditch, 1991; Thapa et al., 2018). Although these models may not give a precise prediction of the profitability of cover crops, they provide a general prediction that can be helpful for producers assessing the pros and cons of implementing cover crops on their operation.

A study by Morton et al. (2006) investigated the economic impact of cereal rye and crimson clover cover crops and their accumulated biomass in a corn-cotton rotation. Morton et al. (2006) developed a mathematical model to quantify the amount of biomass from rye and crimson clover cover crops needed to be economically viable. The authors included both direct and indirect effects of cover crops into their model, including weed suppression and N additions from the legume cover crop. They also used their experimental data to determine optimal planting dates and termination dates of the cover crop in order to best achieve the necessary amount of biomass to reach economic viability. The results from this study showed that for crimson clover, the minimum amount of biomass needed to make it economically viable to plant was 4,968 lbs. with the cost of planting and managing the cover crop being $33.54/acre. For cereal rye, the amount of biomass needed for economic viability was 5,072 lbs. with the cost of planting and managing the cover crop being $55.14/acre (Morton et al., 2006). Pratt et al. (2014) conducted a cost-benefit analysis of several cover crop species (annual ryegrass, cereal rye, crimson clover, hairy vetch, oat, and oilseed radish) and combinations (annual ryegrass with oilseed radish, and annual ryegrass with crimson clover). Four agronomic benefits were measured with each cover crop treatment, including: increased soil organic matter, added nutrient content, reduced erosion, and reduced compaction. The results from this study varied widely, as the on-site net benefit of cover crop treatments ranged from a net loss of $11.09/hectare to a net benefit of $87.32/hectare. Another study from Gabriel et al. (2013) assessed the economic value of cover crops and their residues. Their results showed the greatest economic net benefit was from selling the cover crop residue as forage rather than keeping them in the field and reducing fertilizer costs. These research findings highlight the variation in economic benefit from cover crops in different production scenarios. More research is needed in order to provide specific cover crop recommendations to producers.

Conclusions and Objectives

As outlined above, the agricultural management strategies of cover cropping and crop rotation have been linked to improvements in soil health and cash crop yields. However, there is a lack of long-term research on the effects of these management strategies, both individually and in combination, on soil health and yield in a corn-soybean system in Missouri. This research will investigate the impacts of cover crops and crop rotation on cash crop yield and several soil health indicators, including bulk density, potentially mineralizable nitrogen, organic carbon, active carbon, water stable aggregates, and soil microbial community population and diversity.

The objectives of this research for the second chapter are to quantify the changes in soil health quality indicators and cash crop yield as a result of implementing cover crops, crop rotation, and their combination. Specific objectives include determining: (1) if increased corn intensity in a rotation affects soil health and yield, (2) if the addition of cover crops will affect soil health and yield, and (3) if cover crops can correct the negative yield drag from soybean planted after soybean. The objectives of this research for the third chapter are to (1) evaluate the long-term effects of increased corn frequency within a corn-soybean rotation on several soil health quality indicators (bulk density, water stable aggregates, total nitrogen, potentially mineralizable nitrogen, total organic carbon, active carbon, soil organic matter, β-glucosidase, and soil microbial community biomass and diversity), and (2) evaluate the long-term corn and soybean yield responses to ten different corn-soybean rotations.

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Chapter II

Cover Crop and Rotation Effects on Soil Health and Yield in Corn-Soybean Cropping Systems

Abstract

The implementation of cover crops and crop rotation are widely promoted management strategies that have been shown to enhance soil health in agricultural systems, and may lead to increases in cash crop yields. This study examines the influence of cover crops, crop rotation, year, and their combination on several key soil health indicators and cash crop yield. Treatment combinations were assigned as a split-plot with whole plots arranged as a randomized complete block. The crop rotations examined were continuous soybean [*Glycine max* (L.) Merr.], continuous corn (*Zea mays* L.), and traditionally rotated corn and soybean. Cover crop treatments were a no-cover crop control and a cover crop treatment consisting of an 80% - 20% mixture of rye (*Secale cereal* L.) and radish (*Raphanus sativus* L.). Bulk density, water stable aggregates, soil moisture, total organic carbon, active carbon, potentially mineralizable nitrogen (PMN), and soil microbial community composition via a phospholipid fatty acid (PLFA) analysis were measured in 2017 and 2018 in Columbia, MO under no-till conditions. Seed yields of corn and soybean were recorded from 2016-2019. Crop rotation treatments improved water stable aggregates and corn yield. Implementing cover crops led to improvements in several soil health indicators (water stable aggregates, soil moisture, PMN, AMF, gram negative bacteria, and the gram positive / gram negative ratio) while maintaining yield in soybean and decreasing yield in corn.

Introduction

Soybean [*Glycine max* (L). Merrill] and corn (*Zea mays* L.) are the most economically important cash crops that are grown in the United States. In 2019, corn was grown on 37.1 million hectares of arable land, and soybean was grown on 32.4 million hectares of arable land (USDA-NASS, 2019). In the Midwest, corn-soybean is the dominant crop rotation, which covers approximately 75% of the arable land surface (Hatfield et al., 2007; Plourde et al., 2013). The simplest cropping system used in the Midwest is continuous cropping. Continuous cropping involves planting the same crop on the same land for two or more years. The amount of land area in the Midwest devoted to continuous cropping is not known because the USDA-NASS no longer provides that information. However, the use of continuous cropping can be inferred from the hectarages of corn and soybean. This is possible because corn and soybean are by far the most common crops in most Midwestern states (USDA-NASS, 2019). For example, in 2019 the nationwide hectarages of corn was 37.1 million and soybean 32.4 million. The ratio of corn to soybean hectarages is 1.15. With this ratio being larger than 1.0 there is more land area planted to corn than to soybean, so continuous corn is a likely result. In Missouri in 2019, the hectarages of corn was 1.3 million and soybean 2.1 million. Missouri's ratio of corn to soybean hectarages is 0.62. This ratio is much less than 1.0, and indicates that continuous soybean is common.

The intensification of continuous cropping in the Midwest has caused concern for maintaining soil health for the long-term (Bennett et al., 2012) and concerns about sustainability and environmental degradation. (Foley et al., 2011). Continuous cropping systems have been shown to be unsustainable for long-term yield and soil health

(Ashworth et al., 2017). Continuous cropping of corn and/or soybean has led to increases in weed pressure (Higgs et al., 1990) and pests (Chen et al., 2001), along with decreases in yield (Chen et al., 2001), soil quality (Karlen et al., 2006), and microbial biomass (McDaniel et al., 2014).

Crop rotation refers to a series of dissimilar crops being planted in the same field, typically following a defined order (e.g. corn – soybean). Crop rotation generally has multiple advantages over continuous cropping, including increased yield, improved soil fertility and soil structure, improved water retention, and a reduced risk of crop failure (Bullock, 1992; Sauerborn et al., 2000). It is also hypothesized that improving aboveground biodiversity will lead to improvements in belowground biodiversity, which can lead to significant effects on several soil health and crop productivity factors.

Cover crops are grown for many benefits, and can be broadly defined as any living crop, including grasses, legumes, and forbs, that are grown for seasonal ground cover and other conservation purposes (NRCS, 2016). Cover crops are used to improve soil health, nutrient cycling, reduce soil erosion, improve water retention and infiltration, and aid in the suppression of pests (Snapp et al., 2005). Cover crops have also been shown to increase cash crop yields by providing the succeeding cash crop with additional nutrients, by conserving moisture during dry times of the growing season, and by increasing soil organic matter content (Chen and Weil, 2011; Teasdale et al., 2008; Williams and Weil, 2004). It is further hypothesized that implementing cover crops with continuously grown soybean can ameliorate the negative yield drag associated with continuously planted soybean.

Research investigating the external management practices that influence yield and soil health can provide significant insights into ways to maximize production and profitability while also reducing the potential negative environmental impacts. Investigating the potential benefits of cover crops and crop rotation to soybean cropping systems and soil health is a research topic that warrants more attention. There is a lack of research investigating the long-term effects of these management strategies, both individually and in combination, on soil health and yield in a corn-soybean system in Missouri. The objectives of this research are to quantify the changes in (1) soil health quality indicators and (2) cash crop yield as a result of implementing cover crops, crop rotation, and their combinations over four growing seasons in Missouri.

Materials and Methods

Field Experiment and Site Specifications

Field trials were conducted in 2016, 2017, 2018, and 2019 at the University of Missouri Bradford Research Center (BRC) located near Columbia, Missouri (N38̊53’36.80”, W92̊12’53.21). The primary soil at the research site was a Mexico silt loam (Fine, smectitic, mesic Vertic Epiaqualfs) and ranged in slope from 0 to 2%.

Treatment combinations were assigned as a split-plot with whole plots arranged as a randomized complete block. Each treatment was replicated four times. Main plots were the three crop rotation treatments: continuous soybean, continuous corn, and rotated corn and soybean. Both corn and soybean components of the rotated system were planted each year. Two cover crop treatments were the subplots and consisted off a no-cover crop control and a cover crop treatment consisting of an 80% - 20% mixture of rye (*Secale cereal* L.) and radish (*Raphanus sativus* L.). Seeding rates were 89.7 kg ha^{-1} and 22.4 kg ha^{-1} for rye and radish. Cover crop seed was broadcast applied in mid-September of each year prior to the harvest of corn and soybean.

Plot dimensions were 12.2 m long and 6.1 m wide (8 rows). Row spacing for corn and soybean was 0.76m. Plantings of corn and soybean each year were conducted without tillage in early May. Cultivars were ‘P1197AM’ and ‘P37T09L’ for corn and soybean. To meet corn nitrogen requirements, ammonium nitrate was surface applied at 200 kg N ha^{-1}. Plots were irrigated on an as-needed basis.

Soil Sample Collection

Soil samples for physical and biological characterization were collected in early June 2018 and mid-July 2019 with a 7.62cm by 7.62cm bulk density sample ring inserted into the soil to a depth 7.62cm. Two samples per plot were taken with one between rows 2 and 3 and the other between rows 6 and 7 of the 8-row plots and combined to create a composite sample of each plot. The sampling area was cleared of any surface residue down to the bare soil surface prior to sampling in order to prevent residue contamination. The sample ring was driven into soil by hammering on a woodblock that was placed on the top of the ring. Next, the core was dug out with a shovel and any protruding soil from the bottom and plant roots were trimmed. Samples were placed in a plastic bag, labeled by plot and placed in a cooler to maintain temperature and moisture during transport to the laboratory. All samples were analyzed at the Soil Health Assessment Center, a soil health testing laboratory located at the South Farm Research Center in Columbia, MO.

Laboratory Analyses

At the Soil Health Assessment Center, soil samples were promptly processed. Samples from each plot were divided into two portions with one immediately freeze-dried for phospholipid fatty acid analysis. The remaining soil portions were weighed to determine gravimetric water content. Next, the soil samples were air-dried at room temperature, ground with a mortar and pestle, and passed through a 6-mm sieve for the rest of the analyses (Soil Survey Staff, 2014). Laboratory analyses summaries are included in Table 2.1.

Bulk Density

Bulk density was quantified using the ring excavation method due to it being simple, robust, and rapid. The excavated soil was air dried then oven dried at 110°C and weighed. Bulk density was computed as the ratio of oven-dry weight of soil sample to soil volume (Blake and Hartge, 1986).

Water Stable Aggregates

The method described by Kemper and Rosenau (1986) was used. Soil samples were air dried and gently pushed through a 2 mm sieve onto a 1 mm sieve. Soil retained on the 1 mm sieve was used to obtain one 3-g sample from each plot. Plastic pans were filled with 1.89 L of distilled water. Samples soaked in these pans overnight. The following day, samples were agitated by raising and lowering the sieves in the water pans 20 times within 40 seconds. The aggregates are then treated with sodium hexametaphosphate to disperse sand. Sand weight was subtracted after the samples were dried and reweighed. The sieves were then dried for approximately 2 hours at 110°C for the final calculation of percent of water stable aggregates (Kemper and Rosenau, 1986).

Total Organic Carbon

Total organic carbon (TOC) content was determined using the method described from the Kellogg Soil Survey Laboratory Methods Manual (Soil Survey Staff, 2014). Soil was air dried and passed through an 80-mesh sieve. A dry combustion method with a LECO C-144 carbon analyzer was used. A 0.5-g of sample of sieved soil was placed into a combustion boat. The sample was placed in a pure oxygen environment and heated to

927°C. Following combustion, CO_2 gas concentration was measured with a carbon infrared cell. TOC was converted to percent (%) of oven dried soil.

Active Carbon

Labile fractions of TOC are often designated as the active pool of carbon within soils (active carbon). This active pool consists of easily decomposed materials and is the most readily accessible energy source for soil microorganisms (Weil et al., 2003). The laboratory method described in Soil Survey Staff (2014) was used to determine active carbon. From oven dried soil, 2.5 g of dried soil were placed into 50 ml centrifuge tubes, and 18 ml of deionized water were added. Then, 2 ml of 0.2 M $KMnO_4$ potassium permanganate were added to each tube and tubes were shaken for 2 minutes. Tubes were left undisturbed for 10 minutes to allow soil to settle. A Cary 60 UV-Vis spectrophotometer was used to read the solution at 550 nm. Reactive carbon was reported as milligrams of carbon per kilogram of soil.

Potentially Mineralizable Nitrogen

Potentially Mineralizable Nitrogen (PMN) was determined using the method described by Waring and Bremner (1964). The procedure included loading 8 g of sieved, air dried soil into 50 ml tubes with 20 ml of deionized water. Tubes were incubated in a 40° C oven for 7 days. Next, 20 ml of 2 M KCl solution were added, and the tubes were shaken for 1 hour. Clear liquid was poured off into 15 ml plastic tubes and centrifuged at 3000 rpm for 10 minutes. The PMN content was the amount of ammonium nitrogen produced after a week of anaerobic incubation. Ammonium nitrogen was measured with a Spectronic 20D+ spectrophotometer.

Phospholipid Fatty Acid Analysis

Phospholipid fatty acid (PLFA) analysis is a commonly used method to provide information about microbial biomass and community composition in soils. The method used was developed by Buyer and Sasser (2012), in which PLFAs are extracted and esterified into fatty acid methyl esters (FAME). The FAME extracts were evaporated to dryness, dissolved in hexane, and then transferred to gas chromatography vials for analysis. An Agilent 6890 gas chromatograph was used to separate the FAMEs under a constant flow rate of 1.2 mL min^{-1} of H_2 carrier gas and a split ratio of 30:1. Oven temperature started at 190°C, increased to 285°C at 10°C min^{-1}, increased to 310°C at 60°C min^{-1}, and held constant at 310°C for 2 min. Injection and detector temperatures were 250°C and 300°C, respectively. The microbial identification system (MID) of Sherlock and Agilent Chemstation software was used to control the system, and the MIDI PLFAD1 software package was used to identify and categorize the FAMEs. MIDI software assigned markers to fungi, arbuscular mycorrhizal fungi (AMF), eukaryotes, and Gram-negative, Gram-positive, anaerobic, and actinobacteria microbial groups (Agilent, USA). Total fungi represented the sum of fungi, arbuscular mycorrhizal fungi (AMF), and eukaryotic microbial groups. Total bacteria was the sum of markers assigned to Gram negative, Gram positive, anaerobic, and Actinobacteria. Results were recorded in picomoles per gram of oven-dried soil (pmol/g).

Grain Yield

Plots were harvested using a Massey Ferguson 8XP3 small plot combine (Massey Ferguson Limited, Georgia, USA). Combine yield monitors equipped with HarvestMaster

Field Research Software were used to measure the weight and moisture to determine the yields of each plot (Juniper Systems, Inc., Utah, USA). Rows five, six, seven, and eight of the twelve rows of each plot were harvested for yield data. Corn and soybean grain yields were corrected to 15.5% and 13% moisture, respectively.

Statistical Analysis

Data were combined over yeas and analyses of variance (ANOVA) were conducted using the PROC GLIMMIX procedure in SAS 9.4 (SAS Institute, 2014). All factors were considered fixed in the analysis, with the exception of replication x rotation, and replication. (SAS Institute, 2015). Least squares means were estimated using the 'lsmeans' option, and significant differences between the least squares means were computed and compared using Fisher's least significant difference (LSD) by using the 'lines' option. The significance level of $\alpha = 0.05$ was used as the threshold to determine significant differences.

Results and Discussion

Soil Health Indicators – Physical Properties

Bulk density was affected by year, but not rotation or cover crop (Table 2.2, 2.3). This lack of cover crop effect contradicts previous research that found changes in crop rotation strategies and cover crop implementation can decrease bulk density (Stavi et al., 2012; Villamil et al., 2006). The cover crop treatments in this study have only been in place for five years, and several short duration studies have shown no significant effects on bulk density from cover crops because bulk density changes slowly (Chen and Weil, 2011; Liebig et al., 2004). Additionally, my experiment site has been under a no-till management system for over 20 years, which also has been shown to greatly improve soil bulk density regardless of other management practices (Sapkota et al., 2012). This may explain the lack of significant response of bulk density to crop rotation in this study.

Contradictory results from research studying the effects of crop rotation on bulk density have also been found. Sumner (1999) reported that bulk density has been shown to significantly change in response to crop rotation in some studies, but remain unchanged in others. For example, research from Hammel (1989), Kelley et al. (2003), and Huggins et al. (2007) found crop rotation and tillage to have little effect on bulk density. However, research by Pikul et al. (2008) and Riedell et al. (2013) has shown significant improvements in bulk density under more diverse cropping systems in no-till management systems.

Water stable aggregates were affected by the rotation system, cover crop, and year main effects, and there was a rotation x year interaction (Table 2.2, 2.4). There were no significant differences among rotation treatments in 2017, but continuous corn was significantly greater than the other two treatments in 2018 (Table 2.4). The primary difference between the two years was the timing of soil sampling. Perhaps the delayed sampling in 2018 allowed more of the greater amount of residues from continuous corn to be broken down by the soil microbes. This activity may form additional aggregates. Previous research has shown that increased corn residues significantly improve water stable aggregates (Nouwakpo et al., 2018), but other studies have reported that water stable aggregates are more sensitive to tillage regardless of crop rotation (Jung et al., 2008; Vyn and Raimbult, 1993). In the current study, the cover crop treatment significantly improved water stable aggregates by 19% compared to the no cover crop treatment. This confirms previous research conducted by Garvert (2013) and Villamil et al. (2006) showing significant improvements to aggregate stability due to the addition of cover crops. Further, rye was one of the cover crops included in our treatments, and has been shown to influence water stable aggregates. Studies by Steele et al. (2012), Garvert (2013), and Villamil et al. (2006) found significant improvements to water stable aggregates when a rye cover crop was included in treatments compared to no cover crop. Means in 2018 (29.9%) were significantly higher than in 2017 (25.6%). This may also be due to the delayed sampling time in 2018 compared to 2017, allowing soil microbes more time to break down the surface resides and produce adhesive exudates that bind soil particles together into aggregates (Grandy et al., 2006; Liebig et al., 2004).

Soil moisture content was significantly affected by cover crop, year, and rotation x year effects (Table 2.2, 2.5). Within rotation x year means, 2017 continuous soybean showed the lowest moisture means, with continuous corn in both years along with all other treatments in 2018 showing significantly higher soil moisture content. This is likely due to the increase in surface residues shading the soil leading to a reduction in evaporation. The cover crop treatment led to a significant increase of 5% in soil moisture compared to the no cover crop treatment. Cover crops have shown variable effects on soil moisture, as actively growing cover crops may reduce soil moisture through transpiration and cover crop residues may conserve soil moisture by reducing evaporation (Blanco-Canqui et al., 2011). Our results are consistent with studies by Chen and Weil (2011), showing that a rye cover crop had significantly higher soil moisture content during the summer compared to no cover crop. 2018 soil moisture content (24.70%) was significantly higher than 2017 (22.25%).

Soil Health Indicators – Chemical Properties

Total organic carbon was not significantly affected by rotation, cover crop, or any other measured effects (Table 2.2, 2.6). The lack of significant differences within our treatments may be due to the relatively short duration of our cover crop treatments. Change in organic carbon is slow and may take several years to realize benefits from management practices (Moore et al., 2014). As residue amounts on the soil surface increase, soil microorganisms incorporate them into the rhizosphere, leading to increases in total organic carbon and soil organic matter over time (Havlin et al., 1990; Whalen and Sampedro, 2010). However, previous research has shown cover crops leading to significant increases in total organic carbon over time (Islam and Reeder, 2014; Mukherjee and Lal, 2015; Stavi et al., 2012; Veum et al., 2014). Long-term studies conducted by Robinson et al. (1996) and Zuber et al. (2015) found that multi-year rotations greater than two years led to significant increases in total organic carbon compared to simple two year rotations or continuous corn and soybean. Conversely, short-term cover crop usage has been shown to not significantly increase total organic carbon (Bronick and Lal, 2005; Sainju et al., 2002). Reduced or no-tillage has also been shown to significantly increase total organic carbon over time, and Sapkota et al. (2012) reported total organic carbon was significantly improved under a long-term no-tillage system. This is relevant due to our experiment site being under a no-till management system for over 20 years.

Active carbon was only significantly affected by year (Table 2.2, 2.7). This labile pool of active carbon represents the easily decomposable organic substances serving as readily available substrates for the soil microbial community. Active carbon has been shown to be sensitive to management practices, usually showing increases with diversified crop rotations, cover crops, and under no-till systems (Culman et al., 2012; Huggins et al., 2007). It is not clear why none of the treatments affected active carbon.

Potentially mineralizable nitrogen (PMN) was significantly affected by the cover crop and year effects (Table 2.2, 2.8). Nitrogen is the most limiting nutrient for plant growth, and most of the soil nitrogen is stored in plant residues, soil organic matter, and the microbial community (Whalen and Sampedro, 2010). Soil microbes act together to convert these organic forms of nitrogen to plant available forms of ammonium and nitrate. Potentially mineralizable nitrogen is an indicator of the capacity of the soil microbial community to mineralize organic nitrogen and during the growing season (Drinkwater et al., 1997; Moebius-Clune, 2016). However, the timing of cover crop termination and C:N ratio of the residues can impact nitrogen cycling. Higher C:N ratios in residues can lead to net immobilization of nitrogen and reduce the overall amount available to the subsequent crop (Pantoja et al., 2016; Vigil and Kissel, 1991). In this study, the cover crop treatment led to a 19% increase in PMN as compared to the no cover crop treatment (Table 2.8). Significant increases in PMN have been shown to be influenced by residue additions from cover crops as they are broken down by the soil microbial community (Karlen et al., 2014; Spiegel et al., 2007; Veum et al., 2015). The addition of cover crops and no-till management practices was also shown to significantly increase PMN in other studies (Islam and Reeder, 2014; Karlen et al., 2013). Additionally, 2017 means (71.30 mg kg^{-1}) were significantly higher than 2018 means (53.78 mg kg^{-1}). This may be due to the delayed sampling during 2018 compared to 2017, allowing for more nitrogen uptake from the corn and soybean later in the growing season compared to the earlier 2017 sampling.

Soil Health Indicators – Biological Properties

Soil biological properties are the main drivers of agricultural ecosystems, performing key roles in regulating nutrient cycling, promoting soil aggregation, increasing water infiltration, and leading to reductions in soil erosion (Whalen and Sampedro, 2010). Crop rotation and cover cropping are management strategies that can influence soil biological properties, including the soil microbial community (Dick et al., 1997). Maintaining a stable and functionally diverse soil microbial population is crucial for building and maintaining soil health.

The soil microbial community is involved in many soil functional processes, including nutrient cycling and organic matter turnover (Yao et al., 2000). They have also been shown to be sensitive to soil management changes (Sylvia et al., 2005; Zhang et al., 2014). Phospholipid fatty acid (PLFA) analysis utilizes membrane lipids within microorganisms as biomarkers for specific constituencies of organisms, which creates an overall profile of the microbial community (Frostegård et al., 2011; Steenwerth et al., 2002).

Total PLFA was not significantly affected by any rotation or cover crops (Table 2.2). Overall, total PLFA values were large regardless of treatment, with the two year means for each rotation system and cover crop treatment greater than 100,000 pmol g^{-1} (Table 2.9). The lack of treatment effects were not expected. Cover crops have been shown to significantly improve the soil microbial community diversity and overall population, largely due to the additional carbon sources that the microorganisms receive from decomposing plant residues (Sapkota et al., 2012). Several studies have found no significant differences in soil microbial biomass from crop rotations compared to less diverse cropping systems (Liu et al., 2017; Zhang et al., 2014). Additionally, no-till has been shown to increase overall soil microbial activity, credited to less temperature and moisture fluctuations (Ferrari et al., 2015; Sapkota et al., 2012; Sun et al., 2016; Wardle, 1995). The use of a long-term no-till management system in this experiment may have contributed to these high PLFA values and may have muted any significant effects from the cover crop or rotation treatments.

Arbuscular mycorrhizal fungi (AMF) were significantly affected by the cover crop and year effects (Table 2.2, 2.10). Cover crops have been shown to be hosts for many species of mycorrhizal fungi and can increase the amount of mycorrhizal inocula present in the soil for the succeeding cash crop (Dabney et al., 2001). The cover crop treatment showed a 14% increase compared to the no cover crop treatment over two years. Lehman et al. (2012) also found significant increases in AMF values with cover crops compared to no cover crops. In this study, 2017 AMF values were significantly higher than those in 2018. Sampling time differences between years may be a factor. There was likely more living roots from the cover crops present at the time of sampling in 2017 compared to 2018. AMF were not significantly affected by crop rotation in this study. However, previous research has shown fungal groups to be the most sensitive PLFA biomarkers to crop rotation (Sun et al., 2016; Yin et al., 2004; Zhang et al., 2014), with significant increases in AMF and other fungi in corn-soybean rotations compared to continuous corn reported by Sun et al. (2016). Fungal groups have also been shown to be greatly affected by tillage, with previous research showing significant increases in AMF in no-till or conservation tillage treatments compared to traditional and deep tillage treatments (Vivekanandan and Fixen, 1991; Wortmann et al., 2008).

Gram negative bacteria was significantly affected by the cover crop treatment only (Table 2.2, 2.11). This bacterial group is hypothesized to utilize more plant derived, labile carbon sources (Fanin et al., 2019) and normally have monounsaturated fatty acid biomarkers (Frostegård et al., 2011). An 11% increase was observed in the cover crop treatment as compared to the no cover crop treatment. No other significant differences were detected.

The remaining microbial groups of gram positive bacteria, anaerobic bacteria, and actinobacteria showed no significant changes from any of the measured effects (Table 2.2). Gram positive bacteria are thought to utilize more recalcitrant, soil organic matter derived carbon sources (Fanin et al., 2019) and typically have terminal-branched saturated fatty acid biomarkers (Schmitt and Glaser, 2011). Anaerobic bacteria are important under low oxygen conditions, such as wet and deep soils, and can aid in reducing nitrogen compounds to release into the atmosphere (Frostegård et al., 1993; Frostegård et al., 2011). Actinobacteria are active in decomposing organic matter, are more resistant to water stress, and have mid-chain branched saturated biomarkers (Schmitt and Glaser, 2011). Previous research results on the effects of crop rotations and cover crops are conflicting. However, most studies report significant increases in soil bacterial groups and overall soil microbial populations from diversified crop rotations and increased residue additions (Acosta-Martínez et al., 2003; Miller and Dick, 1995; Yin et al., 2004). A meta-analysis by Venter et al. (2016) concluded that diverse cropping systems have been shown to increase, decrease, and have no effect on bacterial communities. A study from Zhang et al. (2019) showed significant increases in gram positive bacteria in more diverse cropping systems compared to continuous systems. Liu et al. (2017) found greater overall bacterial abundance and lower bacterial diversity in rotated corn compared to continuous corn.

The gram positive / gram negative ratio was significantly affected by the cover crop and year effects (Table 2.2, 2.15). This ratio is thought to increase with decreasing carbon availability or upon the depletion of labile substrates (Breulmann et al., 2014; Fanin et al., 2014). Previous research has shown that gram negative bacteria use more labile C sources, while gram positive bacteria use C sources that are more recalcitrant (Fanin et al., 2019). These results are consistent with this study's findings that the cover crop treatment led to a significant improvement as compared to the no cover crop treatment. Conversely, 2017 values (1.15) were significantly greater than 2018 values (1.21). This may be due to the delayed sampling in 2018 compared to 2017 and allowed for more decomposition of the surface residues.

Overall, total PLFA values were not significantly affected by any of the measured effects (Table 2.2). However, some of the measured microbial groups were improved by either the cover crop treatment (AMF, gram negative bacteria, and the gram positive / gram negative ratio) or year effect (AMF and the gram positive / gram negative ratio), with significant improvements observed with the cover crop treatment and in the 2018 results compared to 2017.

Grain Yield – Corn and Soybean

A common concern when implementing any new management strategies is how cash crop yields will be affected. The effects of cover crops on corn and soybean yield are conflicting, and they have been shown to increase, decrease, and have no effect on yield in corn-soybean rotations (Steele et al., 2012; Strock et al., 2004). Cover crops can have negative effects on yield by reducing nitrogen availability to the succeeding cash crop, contributing to poor stands due to large amounts of residue, and from competition of volunteer cover crops after termination. Cover crops have also been shown to increase cash crop yields by providing additional nutrients, by conserving moisture, and by increasing soil organic matter content (Chen and Weil, 2011; Teasdale et al., 2008; Williams and Weil, 2004). Crop rotation has been shown to lead to significant increases in seed yields of corn and soybean (Bennett et al., 2012; Foley et al., 2011), and continuous cropping generally leads to significant yield decreases in corn (Crookston et al., 1991; Porter et al., 1997) and soybean (Karlen et al., 2013; Wilhelm and Wortmann, 2004). This yield benefit from rotation has been referred to as the rotation effect, and the mechanisms involved are hypothesized to include mitigating pests, increases in nutrient cycling, and improved overall soil health (Bennett et al., 2012; Grover et al., 2009).

Soybean Yield

Soybean grain yield was significantly affected by year and there was a rotation x year interaction (Table 2.2, 2.16). Yield was variable across the four years of this experiment. The largest yields were in 2016 and 2017 (Table 2.16). The smallest yield was in 2018, and this year also received the least amount of rainfall (Table 2.18). Rotation affected soybean yield only in 2018. In 2018, the rotated soybean treatment yielded 2,952 kg ha^{-1}, 19% higher than the continuous treatment (2,475 kg ha^{-1}). These results are consistent with a meta-analysis conducted by Seifert et al. (2017), where continuous soybean yields were significantly worse in low yielding years, with an overall continuous soybean yield penalty of 10.3%. Additional research evaluating corn-soybean rotation effects on soybean yield shows positive impacts from rotating corn with soybean. Two long-term studies by Kelley et al. (2003) and West et al. (1996) showed significant yield improvements in soybean grown in a rotation compared to continuous soybean. Sindelar et al. (2015) reported that rotated soybean yielded significantly higher than continuous soybean in 67% of the 28 years in their study, and similar in the remaining 33%.

Soybean yield of the cover crop treatment was similar to the soybean yield of the no cover crop control (Table 2.16). Previous studies investigating cover crop effects on soybean yield are conflicting. The same cover crop combination of radish and rye significantly increased soybean yields in a study by Williams and Weil (2004). Conversely, a rye cover crop decreased soybean yield at one location in the study. A study by Strock et al. (2004) reported statistically similar soybean yields from a rye cover crop in corn-soybean rotations in Minnesota. Overall, it was encouraging that soybean yields were not significantly lowered due to the addition of the cover crop treatments.

Corn Yield

Corn seed yield was significantly affected by the rotation system, cover crop, and year (Table 2.17). There was no interaction between year and either rotation system or cover crops. Averaged over the four years, corn rotated with soybean yielded 12% more than continuous corn. These results are similar to many found in the literature evaluating long-term crop rotation effects on corn yield (Erickson, 2008). A 28-year study by Sindelar et al. (2015) found rotated corn to significantly out-yield continuous corn in 19 of the 28 years. The authors also concluded that crop rotation has a greater impact on corn and soybean yields than tillage in the Midwest (Sindelar et al., 2015). A study by Seifert et al. (2017) evaluated yield data from 2007 – 2012 across six states in the Midwest. Their results showed a 4.3% yield penalty for continuous corn compared to corn rotated with soybean, lower than our findings of 12%.

The cover crop treatment led to a significant 6% decrease in corn yield from 12,509 kg ha^{-1} to 11,836 kg ha^{-1} (Table 2.17). Results on similar studies from the literature are conflicting. Our results are similar to studies by Clark et al. (1994) and Vyn et al. (2000), who reported significant yield decreases from the use of rye cover crop treatments. These studies show grass cover crop species leading to significantly decreased corn yields, which may be due to the cover crops reducing nitrogen availability to the succeeding corn crop. However, rye cover crops have also been shown to have no significant effects on corn yield (Steele et al., 2012; Strock et al., 2004). Cover crops have also significantly improved corn yields compared to a no cover crop control in a studies by Chen and Weil (2011) and Ashworth et al. (2016).

Yield varied greatly among the four years of this experiment. Yield in 2016 was significantly less than all others. Yields in 2017 was significantly higher than 2018, but was similar to 2019 (Table 2.19). There were no clear rainfall related causes in these significant differences observed between years (Table 2.18).

Conclusions

These results confirm prior research that show corn and soybean grown in rotation with each other will often produce grain yields that are greater than, if not equal to, those grown continuously (Sindelar et al., 2015). Cover crop treatments did not significantly improve the yields of either corn or soybean in this experiment, and led to significant decreases in corn yields (Table 2.17). However, using cover crops in this experiment was shown to improve several soil health indicators, even under the no-till management system in place. The results from this research add to the existing literature of the effects of crop rotation and cover crops on soil health indicators and cash crop yield. Crop rotation treatments significantly improved water stable aggregates and corn yield. Implementing cover crops led to significant improvements in several soil health indicators (water stable aggregates, soil moisture, PMN, AMF, gram negative bacteria, and the gram positive / gram negative ratio) while maintaining yield in soybean and decreasing yield in corn.

Tables

Table 2.1. Summary of soil health laboratory analyses.

Property	Indicator	Abbreviation	Method	Reference
Physical Properties	Bulk Density	BD	Core method	Blake and Hartge, 1986
	Water Stable Aggregates	WSA	Wet-sieving	Kemper and Rosenau, 1986
	Gravimetric Moisture Content	Moisture	Air-dry	Soil Survey Staff, 2014
Chemical Properties	Toil Organic Carbon	TOC	Dry combustion by LECO C-144 carbon analyzer	Soil Survey Staff, 2014
	Active Carbon	AC	Potassium permanganate test	Soil Survey Staff, 2014
	Potentially Mineralizable Nitrogen	PMN	Anaerobic incubation test at 40°C	Waring and Bremner, 1964
Biological Properties	Microbial Community	PLFA	Bligh-Dyer liquid extraction	Buyer and Sasser, 2014

Table 2.2. Analysis of variance (ANOVA) including probability values for soil health quality parameters and yield as influenced by three rotation systems and two cover crop treatments over two years in Columbia, MO.

Effects	BD§	WSA	Mois.	TOC	Active C	PMN	PLFA	AMF
Rotation System	0.74	*	0.56	0.85	0.71	0.09	0.41	0.28
Cover Crop (CC)	0.61	**	*	0.57	0.13	*	0.07	*
Rotation x CC	0.87	0.19	0.78	0.63	0.50	0.78	0.44	0.50
Year	***	*	***	0.65	*	***	0.30	*
Rotation x Year	0.46	**	**	0.61	0.93	0.25	0.10	0.13
CC x Year	0.70	0.41	0.23	0.44	0.58	0.41	0.80	0.44
Rotation x CC x Year	0.83	0.96	0.40	0.31	0.56	0.71	0.30	0.29

Effects	Gram - Bacteria	Gram + Bacteria	Anaerobic Bacteria	Actino-bacteria	G+/G- Ratio	Yield Soybean	Yield Corn
Rotation System	0.43	0.49	0.61	0.54	0.58	0.24	*
Cover Crop (CC)	*	0.07	0.49	0.06	*	0.36	*
Rotation x CC	0.53	0.27	0.36	0.25	0.51	0.78	0.76
Year	0.99	0.38	0.06	0.053	*	***	**
Rotation x Year	0.12	0.07	0.58	0.13	0.47	*	0.10
CC x Year	0.63	0.84	0.83	0.72	0.42	0.30	0.96
Rotation x CC x Year	0.36	0.48	0.49	0.56	0.48	0.76	0.59

§ BD = Bulk Density (g cm^{-3}); WSA = Water Stable Aggregates (%); Mois. = Gravimetric moisture content (%); TOC = Total Organic Carbon (%); Active C = Active Carbon (mg kg^{-1}); PMN = Potentially Mineralizable Nitrogen (mg kg^{-1}); PLFA = Total phospholipid fatty acid content (pmol g^{-1}); AMF = Arbuscular Mycorrhizal Fungi (pmol g^{-1}); Gram – Bacteria = Gram Negative Bacteria (pmol g^{-1}); Gram + Bacteria = Gram Positive Bacteria (pmol g^{-1}); Anaerobic Bacteria (pmol g^{-1});

Actinobacteria (pmol g^{-1}); G+/G- Ratio = Gram Positive (G+) Bacteria / Gram Negative (G-) Bacteria Ratio; Yield Soybean = soybean seed yield (kg ha^{-1}); Yield Corn = corn seed yield (kg ha^{-1});

* significant at the 0.05 probability level.

** significant at the 0.01 probability level.

*** significant at the 0.001 probability level.

Table 2.3. Bulk density (g cm^{-3}) means as influenced by three rotation systems and two cover crop treatments over two years in Columbia, MO.

Rotation System	Cover Crop	2017	2018	2-Year Means
Continuous Soy	Yes	1.39a§	1.26a§	1.32a§
Continuous Soy	No	1.33a	1.25a	1.29a
Continuous Corn	Yes	1.43a	1.19a	1.31a
Continuous Corn	No	1.40a	1.22a	1.31a
Rotated	Yes	1.41a	1.28a	1.34a
Rotated	No	1.40a	1.24a	1.32a
Means		1.39a•	1.24b•	
Continuous Soy		1.36a¶	1.25a¶	1.31a¶
Continuous Corn		1.42a	1.20a	1.31a
Rotated		1.40a	1.26a	1.33a
	Yes	1.41a#	1.24a#	1.32a#
	No	1.38a	1.24a	1.31a

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Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.4. Water stable aggregates (%) means as influenced by three rotation systems and two cover crop treatments over two years in Columbia, MO.

Rotation System	Cover Crop	2017	2018	2-Year Means
Continuous Soy	Yes	30.66a§	25.00a§	27.83a§
Continuous Soy	No	23.30a	21.00a	22.15a
Continuous Corn	Yes	33.30a	46.25a	39.78a
Continuous Corn	No	19.48a	37.00a	28.24a
Rotated	Yes	25.36a	26.25a	25.81a
Rotated	No	21.20a	24.13a	22.66a
Means		25.55b•	29.94a•	
Continuous Soy		26.98b¶	23.00b¶	24.99b¶
Continuous Corn		26.34b	41.63a	34.01a
Rotated		23.28b	25.19b	24.23b
	Yes	29.77a#	32.50a#	31.14a#
	No	21.33b	27.34a	24.35b

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Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.5. Gravimetric moisture content (%) means as influenced by three rotation systems and two cover crop treatments over two years in Columbia, MO.

Rotation System	Cover Crop	2017	2018	2-Year Means
Continuous Soy	Yes	21.30a§	25.50a§	23.40a§
Continuous Soy	No	20.50a	24.80a	22.63a
Continuous Corn	Yes	25.30a	24.50a	24.90a
Continuous Corn	No	23.30a	23.30a	23.30a
Rotated	Yes	23.13a	25.00a	24.10a
Rotated	No	20.13a	25.00a	22.60a
Means		22.25b•	24.70a•	
Continuous Soy		20.90c¶	25.13a¶	23.00a¶
Continuous Corn		24.30a	23.90ab	24.10a
Rotated		21.63bc	25.00a	23.31a
	Yes	23.21b#	25.00a#	24.10a#
	No	21.30c	24.33ab	22.81b

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Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.6. Total organic carbon (%) means as influenced by three rotation systems and two cover crop treatments over two years in Columbia, MO.

Rotation System	Cover Crop	2017	2018	2-Year Means
Continuous Soy	Yes	1.96a§	2.05a§	2.00a§
Continuous Soy	No	2.13a	1.80a	1.96a
Continuous Corn	Yes	2.03a	2.13a	2.08a
Continuous Corn	No	1.93a	1.98a	1.95a
Rotated	Yes	1.98a	1.85a	1.91a
Rotated	No	1.95a	1.95a	1.95a
Means		1.99a•	1.96a•	
Continuous Soy		2.04a¶	1.93a¶	1.98a¶
Continuous Corn		1.98a	2.05a	2.01a
Rotated		1.96a	1.90a	1.93a
	Yes	1.99a#	2.01a#	2.00a#
	No	2.00a	1.91a	1.95a

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Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.7. Active carbon (mg kg^{-1}) means as influenced by three rotation systems and two cover crop treatments over two years in Columbia, MO.

Rotation System	Cover Crop	2017	2018	2-Year Means
Continuous Soy	Yes	637.20a§	713.00a§	675.10a§
Continuous Soy	No	616.70a	639.20a	627.94a
Continuous Corn	Yes	693.40a	735.10a	714.23a
Continuous Corn	No	587.00a	694.62a	640.80a
Rotated	Yes	620.64a	639.10a	629.90a
Rotated	No	579.00a	673.60a	626.30a
Means		622.30b•	682.42a•	
Continuous Soy		626.94a¶	676.10a¶	651.51a¶
Continuous Corn		640.20a	714.90a	677.51a
Rotated		599.80a	656.32a	628.10a
	Yes	650.40a#	695.72a#	673.10a#
	No	594.20a	669.13a	631.70a

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Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.8. Potentially mineralizable nitrogen (mg kg^{-1}) means as influenced by three rotation systems and two cover crop treatments over two years in Columbia, MO.

Rotation System	Cover Crop	2017	2018	2-Year Means
Continuous Soy	Yes	77.13a§	51.00a§	64.01a§
Continuous Soy	No	64.12a	34.50a	49.33a
Continuous Corn	Yes	82.50a	67.75a	75.13a
Continuous Corn	No	64.25a	64.50a	64.40a
Rotated	Yes	76.12a	53.43a	64.81a
Rotated	No	63.31a	51.50a	57.41a
Means		71.30a•	53.78b•	
Continuous Soy		70.65a¶	42.75a¶	56.70a¶
Continuous Corn		73.38a	66.13a	69.75a
Rotated		69.75a	52.46a	61.11a
	Yes	78.60a#	57.39bc#	68.00a#
	No	63.91b	50.17c	57.04b

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Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.9. Total PLFA (pmol g^{-1}) means as influenced by three rotation systems and two cover crop treatments over two years in Columbia, MO.

Rotation System	Cover Crop	2017	2018	2-Year Means
Continuous Soy	Yes	108,549a§	107,060a§	107,805a§
Continuous Soy	No	110,896a	95,779a	103,338a
Continuous Corn	Yes	115,898a	140,853a	128,375a
Continuous Corn	No	96,097a	118,673a	107,385a
Rotated	Yes	112,620a	102,622a	107,621a
Rotated	No	94,786a	109,323a	102,055a
Means		106,475a•	112,385a•	
Continuous Soy		109,723a¶	101,420a¶	105,571a¶
Continuous Corn		105,997a	129,763a	117,880a
Rotated		103,703a	105,972a	104,838a
	Yes	112,356a#	116,845a#	114,600a#
	No	100,593a	107,925a	104,259a

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Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.10. Arbuscular mycorrhizal fungi (pmol g^{-1}) means as influenced by three rotation systems and two cover crop treatments over two years in Columbia, MO.

Rotation System	Cover Crop	2017	2018	2-Year Means
Continuous Soy	Yes	5,860a§	4,830a§	5,345a§
Continuous Soy	No	5,980a	4,152a	5,066a
Continuous Corn	Yes	6,546a	6,302a	6,424a
Continuous Corn	No	4,940a	5,457a	5,198a
Rotated	Yes	6,369a	4,596a	5,482a
Rotated	No	4,972a	4,645a	4,808a
Means		5,778a•	4,997b•	
Continuous Soy		5,920a¶	4,491a¶	5,206a¶
Continuous Corn		5,743a	5,879a	5,811a
Rotated		5,670a	4,621a	5,145a
	Yes	6,258a#	5,243b#	5,750a#
	No	5,297b	4,751b	5,024b

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Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.11. Gram Negative Bacteria (pmol g^{-1}) means as influenced by three rotation systems and two cover crop treatments over two years in Columbia, MO.

Rotation System	Cover Crop	2017	2018	2-Year Means
Continuous Soy	Yes	34,207a§	32,491a§	33,349a§
Continuous Soy	No	34,653a	28,098a	31,375a
Continuous Corn	Yes	37,440a	41,338a	39,389a
Continuous Corn	No	29,464a	36,372a	32,918a
Rotated	Yes	35,487a	30,705a	33,096a
Rotated	No	29,851a	31,924a	30,888a
Means		33,517a•	33,488a•	
Continuous Soy		34,430a¶	30,295a¶	32,362a¶
Continuous Corn		33,452a	38,855a	36,154a
Rotated		32,669a	31,315a	31,992a
	Yes	35,711a#	34,845a#	35,278a#
	No	31,322b	32,131a	31,727b

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Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.12. Gram Positive Bacteria (pmol g^{-1}) means as influenced by three rotation systems and two cover crop treatments over two years in Columbia, MO.

Rotation System	Cover Crop	2017	2018	2-Year Means
Continuous Soy	Yes	24,113a§	23,621a§	23,867a§
Continuous Soy	No	24,156a	21,029a	22,593a
Continuous Corn	Yes	25,458a	30,179a	27,818a
Continuous Corn	No	20,702a	25,832a	23,267a
Rotated	Yes	23,532a	21,588a	22,560a
Rotated	No	21,399a	23,010a	22,204a
Means		23,227a•	24,210a•	
Continuous Soy		24,135a¶	22,325a¶	23,230a¶
Continuous Corn		23,080a	28,005a	25,543a
Rotated		22,466a	22,299a	22,382a
	Yes	24,368a#	25,129a#	24,749a#
	No	22,086a	23,290a	22,688a

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Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.13. Anaerobic Bacteria (pmol g^{-1}) means as influenced by three rotation systems and two cover crop treatments over two years in Columbia, MO.

Rotation System	Cover Crop	2017	2018	2-Year Means
Continuous Soy	Yes	912a§	994a§	953a§
Continuous Soy	No	942a	977a	959a
Continuous Corn	Yes	926a	1,133a	1,029a
Continuous Corn	No	820a	963a	891a
Rotated	Yes	869a	840a	855a
Rotated	No	808a	956a	882a
Means		879a•	977a•	
Continuous Soy		927a¶	985a¶	956a¶
Continuous Corn		873a	1048a	960a
Rotated		839a	898a	868a
	Yes	902a#	989a#	946a#
	No	857a	965a	911a

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Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.14. Actinobacteria (pmol g^{-1}) means as influenced by three rotation systems and two cover crop treatments over two years in Columbia, MO.

Rotation System	Cover Crop	2017	2018	2-Year Means
Continuous Soy	Yes	15,122a§	16,172a§	15,642a§
Continuous Soy	No	15,122a	14,709a	14,916a
Continuous Corn	Yes	15,832a	18,379a	17,106a
Continuous Corn	No	14,082a	16,219a	15,151a
Rotated	Yes	14,500a	14,217a	14,359a
Rotated	No	13,976a	14,551a	14,263a
Means		14,771a•	15,708a•	
Continuous Soy		15,117a¶	15,440a¶	15,279a¶
Continuous Corn		14,957a	17,299a	16,128a
Rotated		14,238a	14,384a	14,311a
	Yes	15,148a#	16,256a#	15,702a#
	No	14,394a	15,160a	14,777a

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¶ Rotation system treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.15. Gram Positive (G+) Bacteria / Gram Negative (G-) Bacteria Ratio (G+/G-) means as influenced by three rotation systems and two cover crop treatments over two years in Columbia, MO.

Rotation System	**Cover Crop**	**2017**	**2018**	**2-Year Means**
Continuous Soy	Yes	1.14a§	1.22a§	1.18a§
Continuous Soy	No	1.15a	1.28a	1.22a
Continuous Corn	Yes	1.12a	1.19a	1.16a
Continuous Corn	No	1.19a	1.18a	1.19a
Rotated	Yes	1.09a	1.18a	1.13a
Rotated	No	1.20a	1.22a	1.21a
Means		1.15b•	1.21a•	
Continuous Soy		1.15a¶	1.25a¶	1.20a¶
Continuous Corn		1.16a	1.18a	1.17a
Rotated		1.15a	1.20a	1.17a
	Yes	1.12b#	1.20a#	1.16b#
	No	1.18ab	1.23a	1.21a

§ Means followed by the same letter are not significantly different at the 0.05 probability level.

• Year means followed by the same letter are not significantly different at the 0.05 probability level.

¶ Rotation system treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.16. Soybean seed yield (kg ha^{-1}) means as influenced by two rotation systems and two cover crop treatments over four years in Columbia, MO.

Rotation System	Cover Crop	2016	2017	2018	2019	4-Year Means
Continuous	Yes	3,880a§	3,450a§	2,556b§	3,423a§	3,329a§
Continuous	No	3,759a	3,544a	2,394b	3,349a	3,262a
Rotated	Yes	3,436a	3,389a	3,026a	3,349a	3,302a
Rotated	No	3,329a	3,450a	2,872a	3,215a	3,215a
Means		3,601a•	3,458ab•	2,712c•	3,334b•	
Continuous		3,820a¶	3,497a¶	2,475b¶	3,383a¶	3,295a¶
Rotated		3,383a	3,416a	2,952a	3,282a	3,255a
	Yes	3,658a#	3,416a#	2,791a#	3,383a#	3,315a#
	No	3,544a	3,497a	2,636a	3,282a	3,235a

§ Means followed by the same letter are not significantly different at the 0.05 probability level.

• Year means followed by the same letter are not significantly different at the 0.05 probability level.

¶ Rotation system treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.17. Corn seed yield (kg ha-1) means as influenced by two rotation systems and two cover crop treatments over four years in Columbia, MO.

Rotation System	Cover Crop	2016	2017	2018	2019	4-Year Means
Continuous	Yes	8,205a§	13,316a§	10,088a§	12,710a§	11,029a§
Continuous	No	10,020a	13,517a	11,029a	12,845a	11,903a
Rotated	Yes	10,155a	13,652a	13,248a	13,181a	12,576a
Rotated	No	11,029a	14,459a	13,248a	13,921a	13,181a
Means		9,852c•	13,736a•	11,903b•	13,164ab•	
Continuous		9,079b¶	13,450a¶	10,558b¶	12,778b¶	11,500b¶
Rotated		10,558a	14,055a	13,248a	13,585a	12,845a
	Yes	9,146b#	13,517a#	11,702a#	12,979a#	11,836b#
	No	10,558a	13,988a	12,172a	13,383a	12,509a

§ Means followed by the same letter are not significantly different at the 0.05 probability level.

• Year means followed by the same letter are not significantly different at the 0.05 probability level.

¶ Rotation system treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Cover crop treatment means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2.18. Growing season rainfall amount (cm.) totals in Columbia, MO from May 1 to October 1 for each year.

Year	**Rainfall***
2016	68
2017	41
2018	36
2019	53

* cm

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Chapter III

Long-Term Rotation Intensity Effects on Soil Health and Yield in Corn-Soybean Cropping Systems

Abstract

In the Midwest, corn-soybean [*Glycine max* (L). Merrill] is the dominant cropping system, covering approximately 75% of the arable land surface (Hatfield et al., 2007; Plourde et al., 2013). The growing demand for corn (*Zea mays* L.) and its financial competitiveness as a cash crop over the past two decades has led to an increased use of more corn-intense cropping systems. This increase in corn intensity within corn-soybean rotations in the Midwest has caused concern for maintaining soil health and cash crop yields for the long-term. The objectives of this study were to evaluate the long-term effects of increased corn frequency within a corn-soybean rotation on several soil health quality indicators and evaluate the long-term corn and soybean yield responses to ten different corn-soybean rotations. Corn rotation frequency (CRF) ratings were assigned to each rotation treatment based on the percentage of corn within each rotation. Utilizing these ratings when evaluating the soil data allows for effects of increased corn within rotations to be more easily identified. Soil measurements were taken in 2014 and included several indicators of soil physical, chemical, and biological health. Yield data was collected from 2007 – 2019 to evaluate the long-term effects of various corn intensities within corn-soybean rotations. Overall, corn yields were significantly improved in the first year after soybean, and with fewer years of continuous corn in the rotation cycles. Soybean yields were most significantly improved after following two years of corn, and when avoiding consecutive years of soybean. For soil measurements, significant improvements from increased corn rotation intensity were seen in bulk density, total nitrogen, PMN, TOC, active carbon, SOM, β-glucosidase, overall microbial biomass and diversity, AMF, gram negative bacteria, gram positive bacteria, and

actinobacteria. These results provide valuable information to producers aiming to significantly improve soil physical, chemical, and biological function while also maintaining the highest yield potential in corn-soybean rotations.

Introduction

The growing demand for corn (*Zea mays* L.) and its financial competitiveness as a cash crop over the past two decades has led to an increased use of more corn-intense cropping systems. In order to meet the biofuel targets set by the Energy Independence and Security Act of 2007, a substantial increase in corn production will be necessary (Mehaffey et al., 2012). This demand will need to be met in areas already producing substantial amounts of corn, such as the Midwest United States. In the Midwest, corn-soybean [*Glycine max* (L). Merrill] is the dominant cropping system, covering approximately 75% of the arable land surface (Hatfield et al., 2007; Plourde et al., 2013). However, the intensification of continuous cropping in the Midwest has caused concern for maintaining soil health for the long-term (Bennett et al., 2012) and concerns about sustainability and environmental degradation (Foley et al., 2011). Although the exact amount of land area in the Midwest devoted to continuous corn is not known, we can infer the usage of continuous corn from the total hectarages of corn and soybean, as corn and soybean are by far the most common crops in most Midwestern states (USDA-NASS, 2019). For example, in 2019 the nationwide hectarages of corn was 37.1 million and soybean 32.4 million. The ratio of corn to soybean hectarages is 1.15. A ratio larger than 1.0 indicates more land area devoted to corn than soybean, so continuous corn is a likely result.

Continuous cropping systems have been shown to be unsustainable for long-term yield and soil health (Ashworth et al., 2017). Continuous cropping has led to yield decreases (Chen et al., 2001), likely due to increases in weed pressure (Higgs et al., 1990) and pests (Chen et al., 2001). Continuous cropping has also been shown to decrease soil quality (Karlen et al., 2006) and microbial biomass (McDaniel et al., 2014). Crop rotation has multiple advantages over continuous cropping systems, including: increased yield, improved soil fertility and soil structure, improved water retention, and a reduced risk of crop failure (Bullock, 1992; Sauerborn et al., 2000). It is also hypothesized that improving aboveground biodiversity will lead to improvements in belowground biodiversity, which can lead to significant effects on several soil health and crop productivity factors. Due to corn producing substantially more residues than soybean, soybean included in a rotation with corn can lead to increases in the relative amount of microbial biomass and diversity. This can ultimately lead to increases in nitrogen availability (Moore et al., 2000).

As outlined above, it is recognized that crop rotation aids in improving soil fertility and soil health, which may lead to increases in cash crop yields. Crops that are in a rotation often experience what is referred to as the “rotation effect”, which is the phenomenon of yield increases seen in crops grown for the first time on a piece of land, or when grown in a rotation (Bennett et al., 2012; Foley et al., 2011; Hilton et al., 2013). The rotation effect is not fully understood, however potential contributors are hypothesized to be: increased nutrient cycling, improved soil health, and the breakup of weed and disease cycles may be contributors (Hilton et al., 2013).

For corn and soybean, the phenomenon of the rotation effect has been well documented, and when these crops are grown without a rotation crop it generally leads to yield decreases (Karlen et al., 2013; Wilhelm and Wortmann, 2004). Long-term yield variability in corn and soybean cropping systems has been investigated by West et al. (1996), Kelley et al. (2003), and Grover et al. (2009), and these studies found that either corn or soybean grown in a rotation significantly out yielded those crops grown continuously. (Grover et al., 2009). Additionally, there is evidence that crops grown either in short rotation or continuously will eventually reach equilibrium of yield decline, and will remain consistent but lower than the same crop grown in a rotation (Bennett et al., 2012; Seifert et al., 2017; Stanger and Lauer, 2008). For example, Seifert et al. (2017) reported that the yield gap between rotated corn and continuous corn increased in each year of the treatments, and eventually plateaued after the third year, maintaining a 4.3% yield gap overall.

Although it is well documented in the literature that rotating corn and soybean is advantageous to producers, there is a lack of long-term research focusing on how increased corn intensity within corn-soybean rotations influences soil health and yield. The objectives of this study were to evaluate the long-term effects of increased corn frequency within a corn-soybean rotation on several soil health quality indicators and evaluate the long-term corn and soybean yield responses to ten different corn-soybean rotations.

Materials and Methods

Field Experiment and Site Specifications

This research evaluates data collected in 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2016, 2017, and 2019 for seed yield and in 2014 for soil measurements at the University of Missouri Bradford Research Center (BRC) located near Columbia, Missouri (N38°53'36.80", W92°12'53.21). Soil at the research site was a Mexico silt loam (Fine, smectitic, mesic Vertic Epiaqualfs) and ranged in slope from 0 to 2%.

The experiment was established in 2005 as a randomized complete block design with four replications and five rotation treatments with different ratios of corn and soybean: continuous corn (CCC), two years of corn followed by one year soybean (CCS), one year corn followed by one year soybean (CS), one year corn followed by two years of soybean CSS), and continuous soybean (SSS). In order to collect yield data for both crops in all phases within the rotations, five additional rotation treatments were included: one year soybean followed by one year corn (SC), the second-year corn in the CCS rotation (CSC), the soybean year in the CCS rotation (SCC), the first-year soybean in the CSS rotation (SSC), and the second year of soybean in the CSS rotation (SCS). These 10 rotation treatments were randomly assigned to plots in 2005, and the rotation sequence maintained in each plot from that year forward. For example, the plot that was assigned CCS in 2005 was CSC in 2006 and SCC in 2007. Names of the rotation treatments followed a specific and consistent order. The first letter was the crop harvested from the plot. The second letter was the crop harvested from the plot the previous year. In three phase rotations, the third letter was the crop harvest two years previous.

In order to determine the long-term impacts of corn frequency within rotations, corn rotation frequency indices (CRF) were assigned to each rotation treatment based on the percentage of corn within each rotation. For example, rotations CCC, CCS, CS, and CSS have 1.00, 0.67, 0.50, and 0.33 CRFs, respectively.

Plot dimensions were 12.2m long by 9.1m wide, Row spacing was 0.76m so each plot contained 12 rows. Plantings of corn and soybean each year were conducted without tillage and usually occurred in early May. Corn and soybean cultivars changed among the years, but were always selected to have high yield potential. Biotechnology traits of LibertyLink (BASF, Germany) were used as soon as they became commercially available. To meet corn nitrogen requirements, ammonium nitrate was surface applied at 200 kg N ha^{-1}.

Residue Sampling

Surface residue samples were collected by Jeremy Matson in late May of 2014 after crop emergence to determine differences in the amount of residues produced by each rotation treatment. Five residue samples were taken randomly from each individual plot using a square steel quadrat with sharpened edges (30 by 30 cm). All loose residues falling within the quadrat were removed from the soil surface and placed in ovens to dry at 48° C. Partially buried residues were cut at the surface. Each sample was then sifted to remove any foreign materials (e.g. soil particles), and dry weights were recorded.

Soil Sample Collection

Soil data for this experiment were collected in March of 2014 by Jeremy Matson in order to provide a snapshot of soil health conditions as a result of nine years of the ten rotation treatments being in place. All soil samples were collected at a depth increment of 0 – 5 cm. Within each plot, nine soil cores 3.18 cm in diameter were collected within rows in a fixed pattern to gather a representative sample of each plot (Figure 3.1). The sampling area was cleared of any surface residue down to the bare soil surface prior to sampling in order to prevent residue contamination. The nine samples from each plot were combined to form a composite sample. Samples were placed in a plastic bag, labeled by plot and placed in a cooler to maintain temperature and moisture during transport to the laboratory. All samples were analyzed at the Soil Health Assessment Center located at the South Farm Research Center in Columbia, MO.

Laboratory Analyses

At the Soil Health Assessment Center, soil samples were promptly processed. Samples from each plot were divided into two portions with one immediately freeze-dried for phospholipid fatty acid analysis. The remaining soil portions were weighed to determine gravimetric water content. Next, the soil samples were air-dried at room temperature, ground with a mortar and pestle, and passed through a 6-mm sieve for the rest of the analyses (Soil Survey Staff, 2014). Laboratory analyses summaries are included in Table 3.1.

Bulk Density

The soil core method developed by Blake and Hartge (1986) was used to determine bulk density values. A detailed description of the method is further described in the materials and methods section of Chapter II.

Water Stable Aggregates

The wet-sieving method developed by Kemper and Rosenau (1986) was used to determine water stable aggregates percentages. A detailed description of the method is further described in the materials and methods section of Chapter II.

Total Nitrogen

Total N dry combustion 4H2a method from the Soil Survey Staff (2014) was used for analysis. The total organic nitrogen analyzer LECO NFP-528 was used. An air dried 0.2 g and 80 mesh sieved sample was loaded into the sample holder where it is sealed and purged of any atmospheric gases during the loading process. The sample was dropped into the furnace and flushed with oxygen for quick combustion. The by-products of combustion passed through the furnace filter and cooler for collection in a ballast apparatus. The gases were equilibrated and a small portion was then measured by the thermal conductivity cell for nitrogen. The sample was then converted to percent (%) total nitrogen. The total nitrogen includes organic and inorganic forms of nitrogen including nitrate NO_{3^-} and ammonium NH_{4^+}.

Potentially Mineralizable Nitrogen

Potentially Mineralizable Nitrogen (PMN) values were determined using the method developed by Waring and Bremner (1964). A detailed description of the method is further described in the materials and methods section of Chapter II.

Total Organic Carbon

Total organic carbon (TOC) content was determined using the method developed from the Kellogg Soil Survey Laboratory Methods Manual (Soil Survey Staff, 2014). A detailed description of the method is further described in the materials and methods section of Chapter II.

Active Carbon

Active carbon values were determined using the method developed by the Soil Survey Staff (2014). A detailed description of the method is further described in the materials and methods section of Chapter II.

βeta-glucosidase

The methodology used to determine soil β-glucosidase enzyme activity was conducted according to measurements developed by Eivazi and Tabatabai (1988). Based on a colorimetric determination, the analysis reflects the amount of p-nitrophenol (PNP) released by β-glucosidase from a 1-gram sieved field-moist soil sample incubated in buffered (pH 6.0) p-nitrophenol-β-D-glucoside. The samples were incubated for 1 hour at 37° C with the substrate (p-nitrophenol-β-D-glucoside). Using filtration to extract the p-nitrophenol product, the amount released in the filtrate was determined colorimetrically at 410 nm. A calibration curve was established for the determination of p-nitrophenol concentration, expressing the β-glucosidase enzyme activity in μg g^{-1} soil hr^{-1}.

Phospholipid Fatty Acid Analysis

The method used to determine soil microbial biomass and composition via phospholipid fatty acid (PLFA) analysis was developed by Buyer and Sasser (2012). A detailed description of the method is further described in the materials and methods section of Chapter II.

Grain Yield

Plots were harvested using a Massey Ferguson 8XP3 small plot combine. Combine yield monitors equipped with HarvestMaster Field Research Software were used to measure the weight and moisture to determine the yields of each plot. Rows five, six, seven, and eight of the twelve rows of each plot were harvested. Corn and soybean grain yields were corrected to 15.5% and 13% moisture, respectively.

Statistical Analysis

Data of all characters were combined over years and analyses of variance (ANOVA) were conducted using the PROC GLIMMIX procedure in SAS 9.4. (SAS Institute, 2015). All factors were considered fixed in the analysis, with the exception of replication and replication x rotation (SAS Institute, 2015). Least squares means were estimated using the ‘lsmeans’ option, and significant differences between the least squares means were computed and compared using Fisher’s least significant difference (LSD) by using the ‘lines’ option. The 0.05 probability level was used as the threshold to determine significant differences.

Results and Discussion

Grain Yield – Corn and Soybean

Crop rotation has been shown to lead to significant increases in seed yields of corn and soybean (Bennett et al., 2012; Foley et al., 2011), and continuous cropping has shown to lead to significant yield decreases in corn (Crookston et al., 1991; Porter et al., 1997) and soybean (Karlen et al., 2013; Wilhelm and Wortmann, 2004). This yield benefit from rotation has been referred to as the rotation effect, and the mechanisms involved are hypothesized to include mitigating pests, increases in nutrient cycling, and improved soil physical and microbial properties (Bennett et al., 2012; Grover et al., 2009).

Corn Yield

Corn yields were significantly affected by rotation system, year, and their interaction (Table 3.2). Because of the significant rotation system x year interaction, it is necessary to evaluate how each rotation system affected corn yields within all years evaluated (Table 3.4).

Corn yields were affected by rotation system in six of the ten years evaluated in this study (2008, 2009, 2010, 2012, 2014, and 2019) (Table 3.4). Years 2011 and 2018 were not included in the analysis due to poor stands. Frequent rains in 2015 prevented planting until June and it was also eliminated from the analysis. In the six years in which rotation system affected corn yield, continuous corn (CCC) was among the least yielding rotations in all six years. It produced less yield than the commonly used two-year rotation (CS) in all of these years except 2010. The rotation yield advantage was 24% (2008), 22% (2009), 11% (2010), 16% (2012), 25% (2014), and 14% (2019), with an average effect of 19.7%. Yields of the CCS rotation were not different from the CCC yields in all six years in which rotation system affected yield. This means that the inclusion of a year of soybean did not improve yield in this study. And, the continuous cropping penalty occurred in the first of corn after corn. The decrease in yield was immediate and did not build over years. Similar information can be gained by comparing the CSS and CS rotations. Yields for these two rotations did not differ in any of the six years with significant rotation system effects. In this instance, the corn yield rotation advantage occurs following only one year of soybean and does not require more than that one year. Finally, CSC produced the same yield as CS. This means that the inclusion of an additional year of corn in the rotation did not affect the yield of corn following soybean.

As previously mentioned, corn yields were significantly affected by rotation system in six of the ten years evaluated (Table 3.4). Yield decreases from continuous corn in these years ranged from 11% to 25% compared to the highest yielding rotations of CSC, CS, and CSS. These results are similar to many found in the literature evaluating long-term crop rotation effects on corn yield. A 28-year study by Sindelar et al. (2015) found rotated corn to significantly out-yield continuous corn in 19 of the 28 years regardless of tillage system. The authors also concluded that crop rotation had a greater impact on corn and soybean yields than tillage in the Midwest (Sindelar et al., 2015). A study by Seifert et al. (2017) evaluated yield data from 2007 – 2012 across six states in the Midwest. Their results showed a 4.3% yield penalty for continuous corn compared to corn rotated with soybean, with low moisture years exacerbating this yield penalty.

Overall, our results show that corn yields are significantly improved in the first year after soybean, and with fewer consecutive years of corn in the rotation cycles. This is shown with the rotations of CSC, CS, and CSS yielding significantly higher than CCS and CCC overall. The CSC, CS, and CSS rotations are all following a year of soybean in the rotation, while the CCS and CCC rotations are all following corn. These results are consistent with previous research, showing that including at least one year of soybean in the rotation before corn will significantly improve or not change yields compared to continuous corn.

Soybean Yield

Overall, soybean yields were significantly affected by rotation system, year, and their interaction (Table 3.2). Because of the significant rotation system x year interaction, it is necessary to evaluate how each rotation affected soybean yields within all years evaluated (Table 3.6).

Soybean yields were significantly affected by rotation system in each of the eleven years evaluated (Table 3.6). The continuous soybean treatment (SSS) was among the rotation systems with the least yield in all 11 years. In six of the 11 years, the SSS yield was less than the yield of the commonly used two-year rotation, SC. The rotation yield advantages in those six years were 19, 12, 11, 25, 13, and 28% or an average yield advantage of 18%. The rotation advantage calculated across all years was 9.3%. In the five of the six years that exhibited a significant rotation yield advantage over continuous soybean, the yields of SSC and SSS were the same. Similar to corn, the yield penalty for continuous soybean is immediate in the second year of soybean. Soybean yield of SC did not differ from yield of SCC in 9 of 11 years. So, for most years, the addition of an extra year of corn did not increase soybean yield. But, in the other two years, SCC yielded more than SC. Soil health data has also been collected that may help explain this response.

Overall, soybean yields were significantly improved most after following two years of corn (SCC), and when avoiding consecutive years of soybean (Table 3.5). This is further shown as the SCC rotation yields were significantly highest or similar to the highest rotation in all eleven years of this study (Table 3.6). This is likely due to increased residue amounts from the consecutive years of corn before soybean, which has been shown to influence many soil physical, chemical, and biological functions (Miller and Dick, 1995; Moore et al., 2000; Robinson et al., 1996). The results from this long-term research is similar to other studies found in the literature. Two twenty-year studies by Kelley et al. (2003) and West et al. (1996) showed significant yield improvements in soybean grown in a rotation compared to continuous soybean. Sindelar et al. (2015) reported that rotated soybean yielded significantly higher than continuous soybean in 67% of the 28 years in their study, and similar in the remaining 33%. The previously mentioned multi-state and year study by Seifert et al. (2017) reported an overall continuous soybean yield penalty of 10.3%, which is similar to our findings of a 12.6% yield decrease from continuous soybean.

Soil Physical, Chemical, and Biological Health Indicators

In order to better represent the long-term impacts on soil health of increased corn rotation intensity within rotations, corn rotation frequency (CRF) ratings were assigned to each rotation treatment based on the percentage of corn within each rotation. CRF ratings included 0, 0.33, 0.5, 0.66, and 1.0. Utilizing these ratings when evaluating the data allows for the effects of increased corn within rotations to be more easily identified.

Soil Health Indicators – Physical Properties and Residues

Residue amounts were significantly affected by CRF (Table 3.7) and increased with the amount of corn in the rotation. Continuous corn (CRF = 1.00) residues were significantly higher than all other frequencies evaluated, with a 24% increase compared to the next highest CRF (0.66), and a 79% increase compared to continuous soybean (CRF = 0.00). These results were expected as corn produces substantially more residue than soybean (Lal, 2005). Increases in residue amounts has been shown to increase the relative amount of soil microbial biomass, soil organic carbon, and increase nitrogen availability (Moore et al., 2000; Wegner et al., 2015).

Bulk density values were significantly affected by CRF (Table 3.7) and was improved as CRF increased. Continuous corn (CRF = 1.0) showed the lowest bulk density. Continuous corn (CRF = 1.00) showed a 14% improvement compared to continuous soybean (CRF = 0.00), a 10% improvement compared to CRF = 0.33, and 6% improvement compared to CRF = 0.5 and CRF = 0.66. Bulk density has been shown to change in response to soil management practices such as differing crop rotations in some studies, and shown to be unaffected in others (Sumner, 1999). Research from Hammel (1989), Kelley et al. (2003), and Huggins et al. (2007) found crop rotation and tillage to have little effect on bulk density. Conversely, Pikul et al. (2008) and Riedell et al. (2013) reported significant improvements in bulk density under more diverse cropping systems in no-till management systems. Due to these conflicting results in the existing literature, data from our long-term rotation treatments under a no-till system provide valuable results on bulk density responses to increased corn intensity in corn-soybean rotations.

Water stable aggregates showed no significant differences across CRF (Table 3.7). This is likely due to these research plots being under a long-term no-till management system. Previous studies has shown water stable aggregates to be highest under no-till conditions, regardless of crop rotation (Jung et al., 2008; Vyn and Raimbult, 1993). Additionally, water stable aggregates has been shown to be very sensitive to tillage. Mannering et al. (1975) reported that a single year of tillage in a five year span produced similar water stable aggregate values in the succeeding year compared to the continually tilled treatments.

Overall, soil physical properties and crop residue amounts were shown to be greatly improved with the increased intensity of corn in the rotations, with the exception of water stable aggregates, where no significant differences were found (Table 3.7). These results may help to explain the yield differences observed in soybean, and it does not seem likely that the yield decline observed in continuous corn is a result of decreased soil physical properties.

Soil Health Indicators – Chemical Properties

Continuous corn (CRF = 1.00) differed for total nitrogen only with CRF = 0.33 (Table 3.8). Results from the literature on crop rotation effects on total nitrogen are variable. A long-term study by Carpenter-Boggs et al. (2000) found diverse crop rotations to significantly increase total nitrogen compared to continuous and simple rotation treatments, whereas no significant differences were reported in a similar study by

Mukherjee and Lal (2015). Additionally, soybean intensity in crop rotations have shown to deplete total nitrogen (Havlin et al., 1990), increase total nitrogen with corn residue additions (Havlin et al., 1990; Morachan et al., 1972), and have no effect on total nitrogen (Hickman, 2002). Results from this study do not show increased soybean in the rotation treatments depleting soil nitrogen, or show any significant differences associated within the corn-soybean rotations. Increased residue amounts over time have shown to slowly change the labile and recalcitrant pools of soil nitrogen (Abdollahi and Munkholm, 2014; Havlin et al., 1990), which correspond with the findings from this study.

Potentially mineralizable nitrogen (PMN) was significantly affected by CRF, but PMN was not associated with CRF value. The CRF with the largest PMN was 0.50 and 0.00, 0.33, and 0.75 did not differ. Nitrogen is the most limiting nutrient for plant growth, and most of the soil nitrogen is stored in plant residues, soil organic matter, and the microbial community (Whalen and Sampedro, 2010). Several microbial species act together to convert these organic forms of nitrogen to plant available forms of ammonium and nitrate. PMN values represent an indicator of the capacity of the soil microbial community to mineralize organic nitrogen and made available for plant use during the growing season (Drinkwater et al., 1997; Moebius-Clune, 2016). Together with the total nitrogen results discussed previously, these results on PMN allow us to conclude that increased corn intensity within rotations, especially continuous corn, lead to significant increases in both total nitrogen currently available and further nitrogen that could potentially be made available during the growing season.

Total organic carbon (TOC) was affected by CRF, with values increasing with higher percentages of corn in the rotations (Table 3.8). Continuous corn (CRF = 1.00) had significantly higher TOC than any other CRF. As residue amounts on the soil surface increase, soil microorganisms incorporate them into the rhizosphere, leading to increases in TOC and soil organic matter (Havlin et al., 1990; Whalen and Sampedro, 2010). There is ample evidence in the literature suggesting that greater amounts of residue contributed leads to increases in TOC. Long-term studies conducted by Robinson et al. (1996) and Zuber et al. (2015) found that multi-year rotations greater than two years led to significant increases in TOC compared to simple two year rotations or continuous corn and soybean. These findings were confirmed with our results, and were expected due to the greatest amount of residue being associated with increased corn intensity in the rotations.

Active carbon was affected by CRF and, like TOC, values increased with increasing frequency of corn in the rotation (Table 3.8). Continuous corn (CRF = 1.00) was significantly higher than all other CRF levels, and showed a 22% increase compared to the CRF = 0.33 level and a 17% increase compared to the continuous soybean rotation. These results were expected, as the same significant differences were seen in the results examining TOC. This labile pool of active carbon represents the easily decomposable organic substances serving as readily available substrates for the soil microbial community. Active carbon has been shown to be sensitive to management practices, generally showing increases with diversified crop rotations, cover crops, and no-till management practices (Culman et al., 2012; Huggins et al., 2007). Active carbon has also been shown to increase with water stable aggregates (Blanco-Canqui et al., 2011). The addition of no-till to this experiment has also been shown to increase active carbon, as described by Islam and Reeder (2014).

Similar to the significant differences observed for the soil physical properties, as corn rotation intensity increased, this led to significant increases in all soil chemical properties (Table 3.8). Continuous corn (CRF = 1.00) had the largest values for all measurements and was significantly different than every other CRF level with the exception of PMN, where it was similar to the highest value CRF level of 0.50. As CRF increased, the measured overall soil chemical properties also increased. These results help to explain the significant differences seen in cash crop yields.

Soil Health Indicators – Biological Properties

Soil biological properties are the main drivers of agricultural ecosystems, performing crucial roles in regulating nutrient cycling, promoting soil aggregation, increasing water infiltration, and leading to reductions in soil erosion (Whalen and Sampedro, 2010). Crop management strategies can influence soil biological properties, including the soil microbial community, making them a sensitive biological indicator of soil health (Dick et al., 1997).

Soil organic matter (SOM) showed significant responses across the CRF levels (Table 3.9). Continuous corn (CRF = 1.00) led to significantly higher SOM values than all other CRF levels with the exception of CRF = 0.5. Continuous corn exhibited a 15% significant increase in SOM compared to continuous soybean (CRF = 0.00). Crop rotations and residue management have been shown to influence SOM and nutrient availability through increasing nutrient inputs from decomposition of plant residues (Hatfield et al., 2001), which corresponds with our results as SOM significantly increased in the continuous corn CRF levels compared to continuous soybean. However results from the literature are conflicting, as crop rotation has been shown to increase (Murphy et al., 2011; Russell et al., 2006; Varvel, 2006), decrease (Benjamin et al., 2010; Coulter et al., 2009; Wang et al., 2006), and have no effect (Gijsman et al., 1997; Snapp et al., 2010; Soon et al., 2007) on SOM levels. Additionally, the effects of tillage on SOM are conflicting as well, likely due to the duration of studies. Tillage may lead to short-term increases in SOM, but in the long-term no-till systems generally lead to increases over time (Al-Kaisi et al., 2005; Grandy et al., 2006). These studies help to better understand our results, as our study was conducted under a long-term no-till management system.

Soil enzyme activities, particularly β-glucosidase, act as an indicator of the relative microbial activity in soils (Dick, 1994; Dick et al., 1997). These enzymes are also responsible for mediating and catalyzing numerous soil biochemical and nutrient cycling processes involved in soil function, and are indicators for changes due to soil management (Dick et al., 1997; Stott et al., 2010). In our study, β-glucosidase activity was significantly increased with corn rotation intensity increases (Table 3.9). Continuous corn (CRF = 1.00), CRF = 0.66, and CRF = 0.5 were all significantly larger than the lower CRF levels of 0.33 and continuous soybean (CRF = 0.00). Continuous corn showed a 38% increase compared to the continuous soybean treatments. Increases in β-glucosidase activity usually correspond with increases in overall soil microbial biomass, leading to an increased ability of soils to decompose plant residues and increase the availability of nutrients for the succeeding crop (Stott et al., 2010). Previous research has shown β-glucosidase activity to increase in more intensive cropping systems compared to continuous rotations and fallow periods (Acosta-Martinez et al., 2007; Acosta-Martínez et al., 2003). Large amounts of organic material has been shown to increase β-glucosidase activity (Martens et al., 1992), which correspond with our results in this study, as increased corn rotation intensity led to significant increases in both surface residues and β-glucosidase activity (Table 3.7; 3.9). Additionally, studies evaluating soil enzyme activity responses to tillage practices have shown β-glucosidase activity to increase with no-till compared to various tillage treatments (Balota et al., 2004; Veum et al., 2014).

The soil microbial community is involved in many soil functional processes, including nutrient cycling, energy flow, and organic matter turnover (Yao et al., 2000). They have also been shown to be sensitive to soil management changes (Sylvia et al., 2005; Zhang et al., 2014). Phospholipid fatty acid (PLFA) analysis utilizes membrane lipids within microorganisms as biomarkers for specific constituencies of organisms, which creates an overall profile of the microbial community (Frostegård et al., 2011; Steenwerth et al., 2002). PLFA analyses are more sensitive and culture independent compared to other methods, and are widely used as a means to estimate shifts in populations within the soil microbial community. Overall, PLFA biomass and diversity was significantly higher with more corn intensity in the rotations (Table 3.9). Continuous corn (CRF = 1.00) showed significantly greater total PLFA values compared to all other CRF levels, with a 26% increase compared to continuous soybean (CRF = 0.00). Overall microbial diversity, designated by number of unique PLFA peaks, was significantly highest in the CRF = 0.5 and continuous corn treatments, and all other groups were significantly greater than continuous soybean (Table 3.9). Results from the literature comparing to our results are conflicting. Many studies have credited an increase in surface residues to the increases seen in total soil microbial biomass (Miller and Dick, 1995; Moore et al., 2000; Robinson et al., 1996). Conversely, other studies have found no significant differences in soil microbial biomass from crop rotations compared to continuous systems (Zhang et al., 2014). These conflicting results may be due to varying sampling times, soil types, and climate. Additionally, no-till has been shown to increase overall soil microbial activity, credited to less temperature and moisture fluctuations (Sapkota et al., 2012; Wardle, 1995). The results from these studies crediting no-till and increased surface residues to improvements in soil microbial biomass and diversity correspond with our findings in this study.

There were varying results when evaluating arbuscular mycorrhizal fungi (AMF) and other fungi (all fungi minus AMF) (Table 3.9). Continuous corn (CRF = 1.00) had significantly higher AMF values compared to continuous soybean (CRF = 0.00) and CRF = 0.33, but was similar to both CRF = 0.5 and 0.66. Continuous corn led to a 20% increase in AMF compared to the continuous soybean treatment. Previous research has shown fungal groups to be the most sensitive PLFA biomarkers to crop rotation (Sun et al., 2016; Yin et al., 2004; Zhang et al., 2014), with significant increases in AMF and other fungi in corn-soybean rotations compared to continuous corn reported by Sun et al. (2016). Other fungi, which excludes AMF, were not significantly affected by CRF in our study (Table 3.9). This was surprising, as previous research has shown corn or soybean grown in a rotation to exhibit significantly larger fungi values compared to continuous systems (García-Orenes et al., 2013; Plenchette et al., 2005; Sun et al., 2016). Fungal groups have also been shown to be significantly affected by tillage, with previous research showing significant increases in overall fungi and AMF in no-till or conservation tillage treatments compared to traditional and deep tillage treatments (Vivekanandan and Fixen, 1991; Wortmann et al., 2008). Results from these studies can help to give additional context to our results, as our experiment is under a long-term no-till management system.

Of the four bacterial groups evaluated (gram negative, gram positive, anaerobic, and actinobacteria) continuous corn (CRF = 1.00) showed significant higher values compared to all other CRF levels, with the exception of anaerobic bacteria where there were no significant differences detected (Table 3.9). For the remaining three bacterial groups with significant differences, increased corn intensity in the rotation treatments led to significant increases compared to rotations with more soybean than corn. Compared to continuous soybean (CRF = 0.00), continuous corn led to a 19% increase in gram negative bacteria, a 24% increase in gram positive bacteria, and a 20% increase in actinobacteria (Table 3.9). Previous research on crop rotation intensity effects on soil bacterial groups is conflicting, and a meta-analysis by Venter et al. (2016) concluded that crop rotations have been shown to increase, decrease, and have no effect on bacterial communities. A study from Zhang et al. (2019) showed significant increases in gram positive bacteria in more diverse cropping systems compared to continuous systems. Liu et al. (2017) found greater overall bacterial abundance and lower bacterial diversity in rotated corn compared to continuous corn. Our results on CRF effects on soil bacterial groups is consistent with our previously mentioned results on soil biological properties (Table 3.9).

Overall, increased corn rotation intensity led to significant increases in every soil biological property measured with the exception of other fungi and actinobacteria (Table 3.9). This is consistent with much of the previous research reporting that the main driver behind changes in soil microbial abundance under crop rotations and tillage practices is due to higher inputs of organic matter and carbon in the soil profile (Miller and Dick, 1995; Moore et al., 2000; Robinson et al., 1996). This is likely due to soil microbial growth being a carbon limited process dependent upon the addition of residues to the soil surface (Lynch and Whipps, 1990; Wardle, 1995). Our results showed similar significant differences between soil biological properties, SOM, TOC, total N, and PMN (Tables 3.8; 3.9). These results show that increased corn rotation intensity contribute many benefits for soil physical, chemical, and biological health, indicating that overall soil function is improved with increased CRF. Further, these results show that the continuous corn yield penalty is not likely due to decreased soil function due to increased CRF.

Conclusions

In this study, we evaluated the long-term corn and soybean yield responses to ten different corn-soybean rotations. We also evaluated the long-term corn rotation intensity effects in corn-soybean rotation systems on several soil physical, chemical, and biological health indicators in one year to provide a snapshot of soil conditions after several years of the treatments being in place. Overall corn yields were significantly improved in the first year after soybean, and with fewer consecutive years of corn in the rotation cycles. These results are consistent with previous research, showing that including at least one year of soybean in the rotation before corn will significantly improve or not change yields compared to continuous corn. Soybean yields were most significantly improved after following two years of corn, and when avoiding consecutive years of soybean. Although the two-year corn-soybean rotation yields were statistically similar to soybean following two years of corn in 9 of 11 years in this study. For soil measurements, significant improvements from increased corn rotation intensity were seen in bulk density, total nitrogen, PMN, TOC, active carbon, SOM, β-glucosidase, overall microbial biomass and diversity, AMF, gram negative bacteria, gram positive bacteria, and actinobacteria. These results provide valuable information to producers aiming to significantly improve soil physical, chemical, and biological function while also maintaining the highest yield potential in corn-soybean rotations.

Tables

Table 3.1. Summary of soil health laboratory analyses.

Property	Indicator	Abbreviation	Method	Reference
Physical Properties	Bulk Density	BD	Core method	Blake and Hartge, 1986
	Water Stable Aggregates	WSA	Wet-sieving	Kemper and Rosenau, 1986
Chemical Properties	Total Organic Carbon	TOC	Dry combustion by LECO C-144 carbon analyzer	Soil Survey Staff, 2014
	Active C	AC	Potassium permanganate test	Soil Survey Staff, 2014
	Total Nitrogen	TN	Dry combustion by LECO FP-528 nitrogen analyzer	Soil Survey Staff, 2014
	Potentially Mineralizable Nitrogen	PMN	Anaerobic incubation test at 40°C	Waring and Bremner, 1964
Biological Properties	β-glucosidase	BG	Spectrophotometric measure of *p*-nitrophenyl	Eivazi and Tabatabai, 1988
	Microbial Community	PLFA	Bligh-Dyer liquid extraction	Buyer and Sasser, 2014

Table 3.2. Analysis of variance (ANOVA) for yield as influenced by five soybean rotation treatments and five corn rotation treatment over eleven years for soybean and ten years for corn in Columbia, MO.

Effects	**Yield Corn**	**Yield Soybean§**
Rotation	***	***
Year	***	***
Rotation x Year	*	***

§ Yield Soybean = soybean seed yield (kg ha^{-1}); Yield Corn = corn seed yield (kg ha^{-1})

NS not significant at the 0.05 probability level.

* significant at the 0.05 probability level.

** significant at the 0.01 probability level.

*** significant at the 0.001 probability level.

Table 3.3. Corn seed yield means as influenced by rotation system over ten growing seasons in Columbia, MO.

Rotation System†	Yield
CCC	10,199b§
CCS	10,260b
CSS	11,296a
CS	11,350a
CSC	11,452a

† Rotation system = frequency of corn (C) and soybean (S) in each rotation system. The first letter represents the current crop grown, and the following letters represent the preceding crops grown; Yield = corn seed yield (kg ha^{-1})

§ Corn seed yield means followed by the same letter are not significantly different at the 0.05 probability level.

Table 3.4. Corn seed yield (kg ha-1) means as influenced by rotation system x year across ten growing seasons in Columbia, MO.

Rotation†	**2007$**	**2008**	**2009**	**2010**	**2012**
CCC	10,254a•	8,898c•	10,702b•	9,185bc•	7,208b•
CCS	10,266a	9,150c	10,423b	8,879c	7,914ab
CSS	10,432a	10,715b	13,668a	10,219a	7,823ab
CS	10,522a	11,128ab	13,663a	9,767ab	8,360a
CSC	10,798a	11,747a	13,518a	9,817ab	7,871ab

Rotation	**2013**	**2014**	**2016**	**2017**	**2019**
CCC	12,734a•	10,671b•	10,359a•	11,136a•	10,843b•
CCS	12,430a	11,660ab	9,913a	10,490a	11,478b
CSS	12,691a	13,014a	10,514a	11,471a	12,418a
CS	12,363a	13,361a	10,238a	11,542a	12,562a
CSC	12,892a	13,116a	10,288a	11,801a	12,675a

† Rotation = frequency of corn (C) and soybean (S) in each rotation system. The first letter represents the current crop grown, and the following letters represent the preceding crop grown; Yield = soybean seed yield (kg ha-1).

$ Ten growing seasons from 2007 – 2019 are represented in this study. The data from years 2011, 2015, and 2018 were not included due to poor stands.

• Corn seed yield means within years followed by the same letter are not significantly different at the 0.05 probability level.

Table 3.5. Soybean seed yield means as influenced by rotation system over eleven growing seasons in Columbia, MO.

Rotation System†	Yield
SSS	3,310d§
SSC	3,379cd
SCS	3,470c
SC	3,619b
SCC	3,788a

† Rotation system = frequency of corn (C) and soybean (S) in each rotation system. The first letter represents the current crop grown, and the following letters represent the preceding crops grown; Yield = soybean seed yield (kg ha^{-1})

§ Soybean seed yield means followed by the same letter are not significantly different at the 0.05 probability level.

Table 3.6. Soybean seed yield (kg ha^{-1}) means as influenced by rotation system x year across eleven growing seasons in Columbia, MO.

Rotation†	**2007$**	**2008**	**2009**	**2010**	**2011**	**2012**
SSS	2,409c•	3,336c•	3,660c•	3,494bc•	2,821b•	3,508b•
SSC	2,542bc	3,431bc	3,892abc	3,385c	3,295a	3,463b
SCS	2,734ab	3,526abc	3,685bc	3,798ab	3,339a	3,499b
SC	2,847a	3,739a	3,988ab	3,648abc	3,531a	3,600ab
SCC	2,873a	3,672ab	4,062a	3,889a	3,541a	3,695a

Rotation	**2013**	**2014**	**2016**	**2017**	**2019**
SSS	4,032b•	3,173c•	3,981ab•	2,778c•	3,228b•
SSC	3,828b	3,353ab	3,746b	3,107bc	3,129b
SCS	4,060b	3,438a	3,677b	3,302ab	3,122b
SC	4,472a	3,267bc	3,843ab	3,349ab	3,526b
SCC	4,551a	3,433a	4,178a	3,563a	4,215a

† Rotation = frequency of corn (C) and soybean (S) in each rotation system. The first letter represents the current crop grown, and the following letters represent the preceding crop grown; Yield = soybean seed yield (kg ha^{-1}).

$ Eleven growing seasons from 2007 – 2019 are represented in this study. The data from years 2015 and 2018 were not included due to poor stands.

• Soybean seed yield means within years followed by the same letter are not significantly different at the 0.05 probability level.

Table 3.7. Soil physical property and residue means as influenced by corn rotation frequency (CRF) in 2014 in Columbia, MO.

CRF†	Residue	BD	WSA
0.00	2.2d§	1.19a	15a
0.33	5.1c	1.15a	13a
0.50	6.2c	1.10b	14a
0.66	8.2b	1.10b	14a
1.00	10.8a	1.04c	14a

† CRF = corn rotation frequency (percentage of years of corn in rotation system); Residue = surface residues (mg ha^{-1}); BD = bulk density (g cm^{-3}); WSA = water stable aggregates (%)

§ Soil physical property means followed by the same letter are not significantly different at the 0.05 probability level.

Table 3.8. Soil chemical property means as influenced by corn rotation frequency (CRF) in 2014 in Columbia, MO.

CRF†	TN	PMN	TOC	Active C
0.00	2.4ab	54c§	1.98bc	822bc
0.33	2.0b	63bc	1.95c	778c
0.50	2.3ab	83a	2.11b	891b
0.66	2.3ab	70abc	2.10b	849b
1.00	2.8a	81ab	2.38a	998a

† CRF = corn rotation frequency (percentage of years of corn in rotation system); PMN = potentially mineralizable nitrogen (mg kg^{-1}); TN = total nitrogen (%); TOC = total organic carbon (%); Active C = active carbon (mg kg^{-1})

§ Soil chemical property means followed by the same letter are not significantly different at the 0.05 probability level.

Table 3.9. Soil biological property means as influenced by corn rotation frequency (CRF) in 2014 in Columbia, MO.

CRF†	**SOM**	**β-gl.**	**PLFA**	**PLFA Peaks**	**AMF**	**Other Fungi**	**G -**	**G +**	**Anaero.**	**Actino.**
0.00	2.8c	201b	122,000c§	42b	6,200b	2,000a	41,000c	25,000d	1,300a	16,000c
0.33	2.9bc	224b	125,000c	45ab	6,100b	1,600a	41,000c	27,000cd	1,300a	17,000c
0.50	3.2abc	278a	135,000bc	46a	6,700ab	1,700a	45,000b	29,000bc	1,400a	18,000b
0.66	3.1bc	307a	138,000b	45ab	6,700ab	1,800a	45,000b	30,000b	1,500a	18,000b
1.00	3.3a	327a	154,000a	48a	7,800a	1,700a	51,000a	33,000a	1,600a	20,000a

† CRF = corn rotation frequency (percentage of years of corn in rotation system); PLFA = total PLFA (pmol g^{-1}); PLFA Peaks = number of unique PLFA peaks; β-gl. = β-glucosidase (µg PNP g^{-1} soil hr^{-1}); SOM = soil organic matter (%); AMF = arbuscular mycorrhizal fungi (pmol g^{-1}); Other Fungi = total fungi minus AMF (pmol g^{-1}); G - = gram negative bacteria (pmol g^{-1}); G + = gram positive bacteria (pmol g^{-1}); Anaero. = anaerobic bacteria (pmol g^{-1}); Actino. = actinobacteria (pmol g^{-1});

§ Soil biological property means followed by the same letter are not significantly different at the 0.05 probability level.

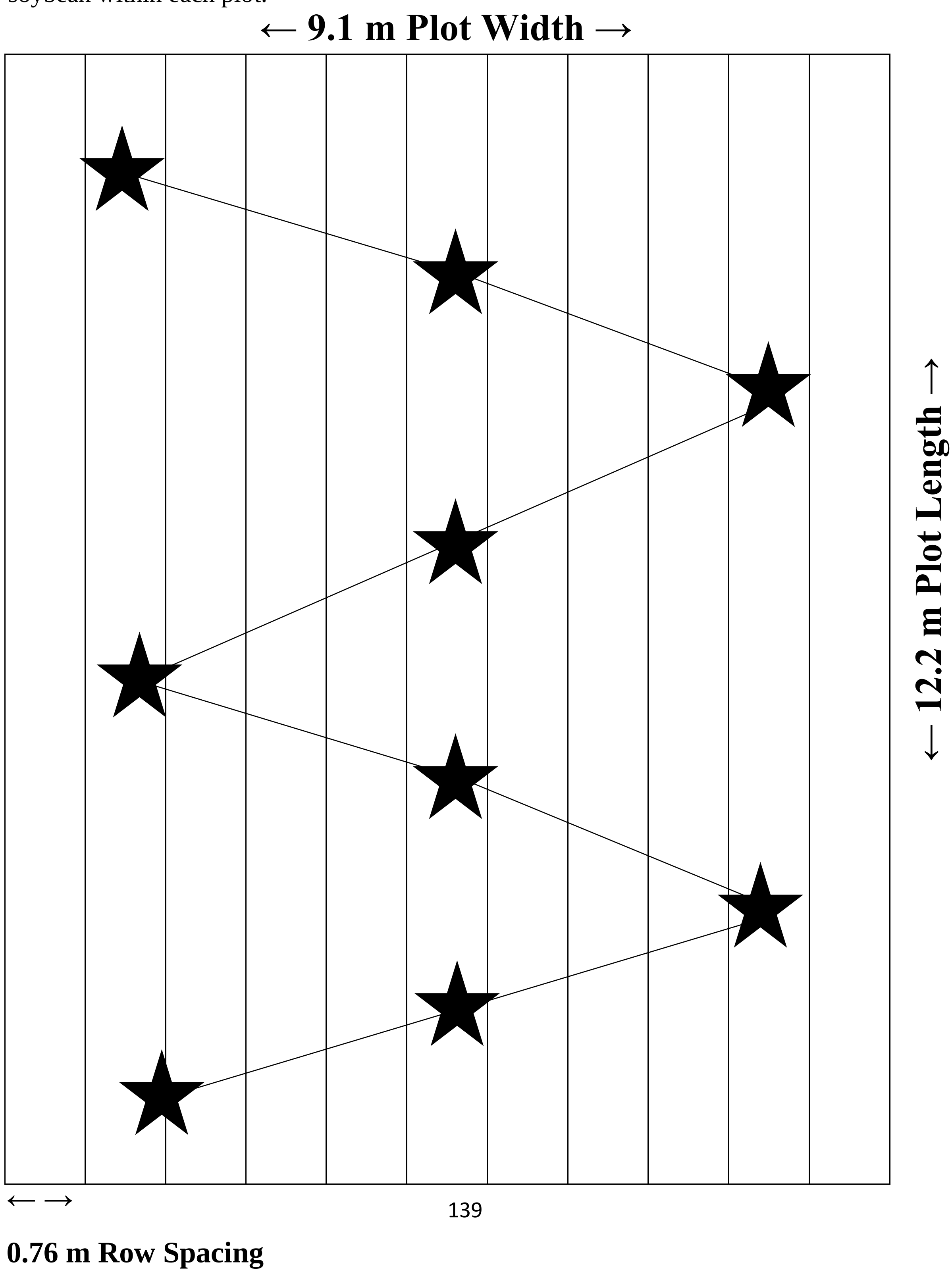
Table 3.10. Growing season rainfall amount (cm.) totals in Columbia, MO from May 1 to October 1 for each year.

Year	**Rainfall***
2007	31
2008	93
2009	59
2010	68
2011	38
2012	18
2013	44
2014	54
2015	60
2016	68
2017	41
2018	36
2019	53

*** cm**

Figures

Figure 3.1. Plot dimensions and soil sample patterns and locations for extracted soil cores (*****) within each individual plot. Each line represents the separate rows of corn or soybean within each plot.



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VITA

Jacob T. Young was born on May 25, 1993 in southeast Missouri. He graduated from Portageville High School in 2011. Jacob then went on to enroll at Southeast Missouri State University in Cape Girardeau, where he graduated magna cum laude with a Bachelor of Science degree in Agribusiness: Plant & Soil Sciences in 2014. He then earned a Master of Science degree in Plant, Insect, and Microbial Sciences in the program area of plant breeding, genetics, and genomics from the University of Missouri in 2017 under the advisement of Dr. Andrew Scaboo. Jacob then began working towards his Doctor of Philosophy degree in Plant, Insect, and Microbial Sciences in the program area of crop, soil, and pest management at the University of Missouri under the advisement of Dr. William Wiebold.