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# Evaluation of Winter Cover Crops on Nutrient Cycling, Soil Quality and Yield for Production Systems in the Mid-South

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EVALUATION OF WINTER COVER CROPS ON NUTRIENT CYCLING, SOIL QUALITY
AND YIELD FOR PRODUCTION SYSTEMS IN THE MID-SOUTH

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**Abstract**

The practice of planting cover crops during fallow periods has increased due to the benefits provided to the soil system including improved nutrient cycling, addition of organic matter and a more diverse soil fauna resulting in better crop yield and an overall improvement of soil health. Research has shown that microbial activity is sensitive to changes in management practices and is a good indicator of whether the changes are benefiting the production system. To study the effects of cover type on corn (*Zea mays* L.) harvest parameters and soil chemical and biological properties a field trial consisting of a split plot design was established at LSU Agcenter's Macon Ridge Research Station in northeast Louisiana. Treatments consisted of 8 covers: fallow, cereal rye (*Secale cereal* L.), forage radish (*Raphanus sativus* var. *longipinnatus*), berseem clover (*Trifolium alexandrinum*), crimson clover (*Trifolium incarnatum* L.), winter pea (*Pisium sativum* L.) and hairy vetch (*Vicia villosa* Roth) and 4 N rates (0, 235, 268 and 302 kg ha$^{-1}$).

Corn grain yield decreased by 20% after cover crop but responded to the addition of N both seasons. Cover crops had a positive effect on soil C:N over time, indicating active mineralization, and NO3$^-$ - N decreased almost three-fold between fall 2014 and spring 2015 ($p<0.05$). Cycling of C, N and S was also affected by cover crops; β-glucosidase and arylsulfatase activity were highest in spring 2015 (after cover crop termination) and averaged 73 and 32.9 mg p-nitrophenol kg$^{-1}$ soil h$^{-1}$, respectively. Microbial community structure shifted after cover crop with soil microbial communities under leguminous covers (hairy vetch, crimson clover, winter pea and berseem clover) separating from the brassica (forage radish) and grass (cereal rye) covers. Arbuscular Mycorrhiza Fungi (AMF) was higher (9.07 mol %) under the 0 N rate compared to the 263-302 kg ha$^{-1}$ N rates (average 7.28 mol %) indicating the

establishment of symbiotic relationship between plants and AMF as a response to nutrient deficient conditions. Cover crops established under Mid-South corn production systems show potential for improving the chemical and biological properties of soil.

**Chapter 1. Introduction**

**1.1 Changes in the Agriculture Landscape**

Agriculture has been a main contributor to Louisiana's economy since the 1800's with the production of sugarcane, cotton, rice, and soybean. The agriculture sector in Louisiana is a diverse one, while cotton and soybean dominated the northern part of the state, sugarcane and rice were mostly grown in the south (Reeves, 2003). Louisiana is the 2nd largest producer of sugarcane and sweet potatoes, the 3rd largest producer of rice and the 5th largest producer of cotton and pecans.

While cotton has traditionally been an important crop in Louisiana, not only in terms of acreage but also in terms of contribution to the local economy in the past decade there has been a noticeable shift from cotton to corn. This shift that can be traced to the introduction of the 1995 Federal Agriculture Improvement and Reform Act, also known as the Farm Bill. With this bill, farmers were able to plant a commodity different than their five-year history without losing any payment benefits and make crop selection decisions based on market demand and price. The steep decline in cotton acreage and number of producers began in 2002 when acreage decreased from 848,000 acres in 2001 to 490,000 acres in 2002, and from 2747 producers in 2001 to 2049 in 2002 (LSU AgCenter, 2002). This decline was attributed primarily to less competitive lint prices and the increased cost of inputs (i.e. irrigation and N-fertilizer) of cotton production in 2000 and 2001(Pettigrew and Zeng, 2014).

In 2007, corn acreage increased from 300,000 to more than 700,000 acres while cotton acreage planted decreased by approximately 50% (Figure 1.1). Several factors were influential in this shift, including the significance of the economic benefits of producing corn. In 2007, there was a difference of almost 37% between the costs of producing one acre of cotton versus corn;

additionally, the corn prices were near record levels while the price of cotton had decreased to near modern record lows (Fannin et al., 2008). High corn prices at the end of 2006 were driven mainly by an increase in national interest in ethanol production. With the demand for ethanol, traditional corn growing regions are all showing an increase in corn acreage (Scott et al., 2009).



Figure 1.1. Fluctuation in cotton and corn production in the state of Louisiana.

Corn production and management could be considered simpler than cotton, making this another possible reason why producers would favor corn production over cotton. Nutrient management in carbohydrate seed plants, such as corn, is less complicated than that of oil-seed plants such as cotton (Sabbe and Hodges, 2009). The estimated recovery of N by a cotton crop averages about 40% of the applied N while corn N recovery ranges between 30-70% (Fageria et al., 1997). In the absence of limiting factors such as reduced moisture availability, low temperatures, and reduction in photosynthetic activity, the cotton plant's indeterminate growth habit and vegetative branching provide a constant fruiting potential (Fageria et al., 1997). As a result, the use of plant growth regulators such as PIX (N,N-dimethyl piperidinium chloride) is

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necessary to deter the perennial characteristic of cotton therefore presenting an additional expense that producers need to incur to ensure good yields.

While the extensive tap root system of cotton makes it more efficient in scavenging for N further down the soil horizon (Deterling and El-Zik, 1982), after continuous cropping, the soil N reservoir is depleted and the addition of N fertilizers is needed. To ensure proper conditions for root expansion, conventional tillage has been the norm of cotton production systems; this type of tillage involves multiple disking, chisel plowing, harrowing, and bed formation. These implements destroy the soil surface, leaving no plant residues to protect the surface.

Another distinction that favored the increase in corn production was the opportunity to establish a crop early in the growing season in comparison to cotton. Corn can tolerate lower temperatures to 10 °C (Westgate et al., 2004) but cotton seeds require warm soil temperatures above 18 °C for at least three consecutive days for adequate germination (Smith and Cothren, 1999) but at 16 °C, cotton ceases to grow (Bradow and Bauer, 2009).

With the shift from cotton to corn, Bruns and Abbas (2005) set out to determine an adequate corn planting density since many farmers had adapted the same cotton planting and cultivating equipment that would plant at 101.6 cm between rows instead of the 76.2-81.3 cm being used in the Corn Belt. They found that neither corn yield or grain quality was negatively affected when planted at the wider row spacing at 76500 plants/ha. Today, row width planting distances for cotton and corn is traditionally set at 76 cm, and can be extended to 102 cm (Smith and Cothren, 1999; Westgate et al., 2004).

**1.2 Corn Production**

Corn is one of the most important grains in the world and its use is not limited to human food consumption; it is also an important component of livestock feed and a biofuel source in the

form of ethanol. Corn grown in developing countries is mostly for direct human consumption; therefore, it is not only a source of economic growth but also a vital component of food security and nutrition (Ransom et al., 2004). Corn is produced worldwide between latitudes ranging 58° N to 48° S and constant genetic improvement enables it to successfully adapt to different environmental conditions. It requires a minimum of 500 mm of rainfall with the optimum being 1200-1500 mm (FAO, no year). Production areas have been further classified into four broad environments: tropical, subtropical, temperate and highland (Ransom et al., 2004). The production systems are highly dependent on the environmental conditions and the corresponding development of technology in the producing countries.

The top five producers of corn in the world are the US, China (mainland), Brazil, Mexico and Argentina, with the US topping the list with more than 350M tonnes in 2013 (FAO Stat, 2015). Improvements in corn breeding such as the introduction of genetically modified corn and more efficient hybrids coupled with development and adoption of field technology has increased corn yield and reduced production costs.

As a $C_4$ plant, corn has lower photorespiration and is more efficient in using solar radiation than $C_3$ field crops such as rice, wheat, oats, barley and soybean which translates into higher photosynthesis rate and therefore yield (Fageria, 2009). The leaf area index (LAI) in corn is related to its capacity to intercept light and transform that energy into biomass production. Corn requires a high input of nutrients and uses most of these resources for producing leaf area which increases the efficiency of light interception in early growth stages. Nutrient requirement increases at the V6 growth stage to ensure optimum leaf growth and light interception at the flowering stage (Westgate et al., 2004). During the reproductive stage, the accumulated N in the leaves is translocated to the reproductive parts and used for grain development (Hagin and

Tucker, 1982). Overall corn is a more efficient plant in terms of photosynthetic rate in comparison to soybean, cotton, alfalfa and sorghum (Fageria, 1997).

A balance of both macro and micro nutrients is required for adequate growth and yield, however, N is one of the most limiting nutrients in corn production even though a hectare of land may contain approximately $8.4 \times 10^4$ Mg ha$^{-1}$ N in the atmospheric column (Prasad and Power, 1997). Corn N requirements depends on many factors such as genotype, environment, soil type management practices, expected yield, among others, therefore, N application rates vary from state to state. On average, N-fertilizer rate for the states in the Corn Belt ranged between 123 – 176 lb N ac$^{-1}$ yr$^{-1}$ (NASS-USDA, 2014) which may be applied all at once or in split applications. The LSU AgCenter (2015) recommends a split application of N with 50-75% at or before planting and the remainder when the corn seedling is at a height between 3-12 inches. Recommended N rate for Louisiana ranges between 134-302.4 kg ha$^{-1}$ depending on the soil type and whether the field will be irrigated. Adequate N rates in corn are necessary for adequate grain production and high economic returns, and an insufficiency in this element can lead to increased susceptibility of corn to *Aspergillus flavus*, a fungus which produces aflatoxin (Bruns and Abbas, 2004).

Producers use different types of fertilizers to supply the required N to their crops because corn takes up N as both nitrate ($NO_3^-$) and ammonium ($NH_4^+$) (Fageria, 2009; Havlin et al., 2005; Hagin and Tucker, 1982) with $NH_4^+$ being the preferred source since it requires less energy for protein synthesis (Havlin et al., 2005), and $NO_3^-$ uptake consumes five times more energy than $NH_4^+$ (Fageria, 2009). Additionally, N absorption as $NO_3^-$ affects both pH and micronutrient absorption by increasing the former and decreasing the latter; on the contrary, $NH_4^+$ uptake decreases rhizosphere pH and increases most micronutrient uptake (Fageria, 2009).

Blair et al. (1970) observed a similar effect in a study of corn grown in cultured solution; the treatment receiving N as $NO_3^-$ increased the solution pH from 6.5 to 7.1 while $NH_4^+$ decreased the pH from 6.5 to 4.2 over an eight day period. The study also reported that in the tops of plants fertilized with $NH_4^+$-N higher concentration of total sulfur as sulfate was measured while higher concentrations of P (as phosphate), $Ca^{2+}$ and $Mg^{2+}$ was observed in plants fertilized with $NO_3^-$-N.

## 1.3 Nitrogen in Agriculture Systems

Leguminous plants are able to utilize atmospheric N through biological fixation to supply their N requirement for optimum growth, but non-leguminous crops, such as corn, obtain most of their required N from organic and inorganic N sources. Organic sources of N are animal manure, sewer sludge and N fixed by leguminous plants while inorganic sources are those manufactured fertilizers that are obtained through the Haber-Bosch process which consists of producing anhydrous ammonia by reacting $N_2$ and $H_2$ gases (Havlin et.al, 2005).

In the soil system, N can be found in both organic (proteins, amino sugars, and humic substances) and inorganic ($NO_3^-$, $NO_2^-$, $NH_4^+$, $N_2O$, NO and $N_2$) forms and may be present as either labile (easily degradable) or non-labile (more resistant to decomposition) forms. In order for the corn plant to be able to absorb the required N from soil, organic N needs to undergo several transformations through a process called mineralization. Mineralization is the soil microbial process of transforming organic N into $NH_4^+$, and occurs in two phases: aminization and ammonification. During aminization, organic N in the form of protein from residue is converted into amino acids, amines and urea, and these are further converted into ammonia through ammonification. Both aminization and ammonification processes are carried out specifically by heterotrophic microorganisms (Prasad and Power, 1997). Environmental conditions, soil properties and management practices control soil N mineralization and also the

loss of soil mineralizable N pools (Nyiraneza et al., 2012). Besides being directly utilized by plants, $NH_4^+$ in the soil can be either fixed in the layers of clay minerals, lost through volatilization, immobilized by microorganisms during decomposition or converted to nitrates through nitrification (Havlin et al., 2005).

Another important transformation process of soil N is nitrification which involves the conversion of $NH_4^+$ to nitrite ($NO_2^-$) by *Nitrosomomas* bacteria, and $NO_2^-$ to $NO_3^-$ by *Nitrobacter*. Soil temperature, pH, moisture and oxygen concentration, as well as $NH_4^+$ supply and presence of nitrifiers all influence the rate of nitrification (Havlin et al., 2005). Nitrate is very mobile and can easily be lost through leaching so minimizing the concentration of $NO_3^-$ in soils by understanding the factors that govern nitrification is also important for N management. Besides leaching, soil $NO_3^-$-N can also be lost through denitrification, which is the direct reduction of $NO_3^-$ to gaseous N which occurs in waterlogged soils by anaerobic organisms.

Corn yield is highly related to N availability and uptake since the principal function of N in the plant is as a component of proteins and nucleic acid, which are needed for cell production and therefore growth processes (Sinclair and Rufty, 2012). However, receiving optimum corn yield as a response to N fertilization depends not only on the rate and source of N but also on multiple factors pertaining to the crop, soil and environment and the interaction among these three factors. Caviglia et al. (2014) studied 2 corn hybrids, 2 sowing dates and 2 N rates (0 and 200 kgN ha$^{-1}$) in Parana, Argentina and reported an increase of 204% with 200 kgN ha$^{-1}$ in the second corn crop versus no N. Additionally, planting corn at the right time increased N utilization efficiency since planting late resulted in a reduction of 34-48% total plant biomass which was reflected in yield reductions of 38-53%. When increasing rates of N as Ammonium Nitrate was applied to corn, Asghari and Hanson (1984) found positive yield response during the

first 3 years of the study. However, where precipitation was below normal and temperatures were above 32°C, yield was severely impacted and the corn did not respond to the varying N rates. This suggests that N may not be the only yield limiting factor in a corn production system, and adequate environmental conditions are needed for the benefits of N to be observed. In developing countries where more efficient genotypes are planted and improved technology has increased productivity, water availability is often the major limitation of crop yields (Sinclair and Rufty, 2012).

Notwithstanding the importance of N fertilization on corn yield, without proper management N can be easily lost from the system causing negative impacts not only on yield but also on the environment. According to Prasad and Power (2007) between 2.5-43% of applied N is lost through different loss mechanisms. These mechanisms include ammonia volatilization, surface run off, nitrate leaching or denitrification. N management is crucial in any production system that is dependent on N based inputs for optimum growth and yield. Tillman et al. (2002) reported an increase in N use efficiency (NUE) by at least 36% in corn production systems through the application of different management practices.

**1.4 Benefits of Integrating Cover Crops in Production Systems**

In recent years, the use of cover crops has increased as a tool to reduce N losses from soil through N recycling and scavenging by selected plant types. The term 'cover crops' was used to refer to those plant species that were established during the off season or between crops to provide a cover to soil (Weil and Kremen, 2006) which would reduce soil erosion either by wind or water. In most cases, cover crops are not grown for commercial purposes and are usually referred to as 'green manures' when incorporated in the soil (Fageria, 2009). The benefits of cover crops are many, in addition to nutrient management and the prevention of soil erosion the

benefits extend to weed and soil pest suppression, enhancement of soil physical and chemical properties, increase in soil organic matter content and a diversified soil biology. Cover crops can be divided into a variety of categories, namely, legumes, grasses and brassicas. Nair et al. (2014) suggest that cover crops can also be classified based on seeding time: summer annuals (established in spring or summer), winter annuals (seeded in fall), and perennials that persist for several years without replanting. The selection and integration of cover crop should be done based on the target benefit for the producer. The fibrous roots of grasses make them adequate for capturing nutrients and preventing soil erosion, and their high biomass production provides for N immobilization, which is released upon decomposition. Brassicas have a tap root system that enables them to scavenge for nitrate deep in the soil profile and many species contain glucosinolate, a class of chemical that breaks down into isothiocyanates (ITC's), which have been shown to control soil borne pathogens and weeds (Smith et al., 2011). Their ability to fix atmospheric $N_2$ makes leguminous species ideal if the reduction of synthetic N fertilizer use is the objective of integrating cover crops in a system (Duck and Tyler, 1991).

For the duration of the row crop growing season planned agronomic management controls and/or limits the adverse effects of pests and diseases. After harvest, most fields are usually left to fallow and different weeds become the primary plant species. In addition to weeds, certain insect pests and soil borne pests overwinter in the fallow field, posing potential deterrents to a successive row crop. The effects of cover crop specie on weeds, insects and soil pathogens has been studied in different cropping systems and the performance of the cover on pest suppression is mostly based on their characteristics. Cover crops from the Brassicaceae family have been reported to have positive effects on weed and soil pest suppression due to their ability to produce ITCs which are the products of the degradation of gluccosinolates (GSL). However,

the concentration GSL may vary between roots and shoots as well as among cover crops (Bangarwa et al., 2011). Furthermore, Bangarwa et al. (2011) found that spring-grown Brassicaceae cover crops had a higher concentration of GSL than when planted in fall. In conventional vegetable production in California, cereals and cereal/mustard mixes are commonly used for weed control while organic farmers prefer legume/cereal mixes because of the added benefits of N fixation (Brennan and Smith, 2005). To adequately suppress weeds cover crops must be able to produce biomass and provide ground cover early in the growing season, which could be accomplished by planting cover crops at high seeding rates however, the cost of seeds would be a determining factor. Brennan and Smith (2005) report that even though there was no difference in above ground biomass at the end of the growing season a mustard cover crop provided better control of burning nettle (*Urtica urens* L.) than an oat/legume mixture because it had greater above ground biomass early in the season.

Another study that evaluated the effect of oats, rye and wheat on broad leaf weeds in glyphosate resistant corn also found that while the oats cover had less biomass it produced the highest inhibition of weed emergence possibly due to an allelopathic effect, however, it was also harmful to the corn crop (Norsworthy, 2004). Cover crops can be an additional tool for the suppression of weed population however they have not been able to outperform weed control via chemical methods (Reddy et al., 2003; Norsworthy, 2004; Reddy and Koger, 2004; Norsworthy et al., 2011). Nonetheless, research by Gaston et al. (2003) and Locke et al. (2005) has shown that the presence of cover crop residue along with conservation management were conducive for the degradation and sorption of Fluometuron which would reduce the downward movement of this herbicide.

**1.5 Role of Cover Crops in N Management**

Row crops, such as corn, are highly dependent on N based fertilizers for adequate growth and development and recommendations vary between states. The LSU AgCenter recommends N rates for corn between 134-302.4 kg ha$^{-1}$ based on field evaluations carried out in different soils and production systems (LSU AgCenter, 2015); split applications are recommended to reduce losses due to volatilization and/or leaching. Excess fertilization and drought conditions can cause high residual N in soils and during a fallow season significant amounts of this residual N can be lost through leaching or denitrification. An application rate of 168 kg N ha$^{-1}$ to a corn crop grown in a well-drained Mattapex silt loam resulted in 87 kg N ha-1 as residual mineral N (Shipley et al., 1992).

During the growing season, plant water uptake reduces percolation therefore reducing the amount of nitrate leached but with the absence of a crop after harvest soil moisture increases and water moves below the root zone depositing nitrate into the groundwater. This is exacerbated when precipitation is high. Soil type may also affect the rate of leaching with courser soils being more conducive to leaching (Askegaard et al., 2011). In the Chesapeake Bay region in Maryland, nitrate concentration in groundwater was higher than that permitted by the U.S Environmental Protection Agency even when best management practices were carried out (Brinsfield and Staver, 1991). Integration of cover crops in row crop production is becoming increasingly popular primarily due to their impact on N cycling. Even though natural regeneration of plant species during a fallow period can capture some of the residual N, specific cover crop species have shown to be more efficient in capturing and using residual N. Legumes are able to supply their N requirements through fixation but grasses depend on fertilizer N. Shipley et al. (1992) reports that while legume cover crops have a higher N content the average fertilizer N uptake for

grass cover crop was 48 kg ha$^{-1}$ for cereal rye and 29 kg ha$^{-1}$ for annual rye grass compared to 9

and 8 kg ha$^{-1}$ for hairy vetch and crimson clover, respectively. Similarly, Brinsfield and Staver

(1991) observed that cereal rye had a greater assimilation of N than wheat, oats and barley but

concluded that planting date influenced the rate of assimilation with an early planting being more

beneficial.

Some grass species are better adapted and more widely used in different states with the

added benefit of reducing nitrate loss (Citations). Downy brome (*Bromus tectorum* L.) and

Canada blue grass (*Poa compressa* L.) decreased $NO_3^-$ losses by 74-75% when compared to a no

cover crop check in a no-till soybean system in Missouri (Zhu et al., 1989). Furthermore, studies

have shown that applying fertilizer N to a grass cover crop proved beneficial by increasing cover

crop biomass and N accumulation in the biomass (Torbert et al., 1996 and Rosolem et al., 2004).

The shift in the C:N ratio of the residue would determine the rate of decomposition and therefore

the availability of N to the successive row crop.

The C:N ratio of a cover crop is a determinant factor when choosing the type of cover to

use; C:N ratios higher than 30:1 result in immobilization of soil N while residues with C:N ratio

less than 20:1 decompose quickly, releasing N. With C:N ratios ranging from 11:1 to 16:1 hairy

vetch had a greater decomposition rate when compared to black oat and oil seed radish (Acosta

et al., 2014). The authors observed that the high N content and low C:N ratio of hairy vetch

resulted in the release of 50% of N in the first 30 days while black oat, with a C:N ranging from

39-44, resulted in net immobilization at the onset followed by mineralization at 60 days (Acosta

et al., 2014).

**1.6 Cover Crops and Soil C**

Because of its relationship to soil health, soil organic matter (SOM) is one of the important indicators included in many assessments. The organic materials in soil are composed of both humic and non-humic substances. The non-humic substances can still be distinguished and are mostly composed of simple proteins and carbohydrates. Soil microorganisms use these non-humic fractions of organic matter for their biological processes. The humic substances of SOM are more stable and their primary components are not distinguishable; these can be further broken down into fulvic acid, humic acid and humin.

SOM content affects nutrient and water retention, soil fertility, stability and structure. According to Reicosky et al. (2011), the content of SOM responds to changes in management such as tillage and C management and as such greatly influences soil quality. Cover crops add organic matter to the soil system through the addition of biomass. However, the rate of change in SOM would depend not only on cover crop species and biomass input but also on soil type and climatic conditions. Fibrous cover crops have a slower decomposition rate slowing the release of N, but they also promote more stable organic matter, which leads to better soil quality. Nascente et al. (2013) studied the effect of grass cover crops on total organic C and physical fractions of SOM under conventional tillage and no-tillage systems in Brazil. They reported higher amounts of organic C, at the 0-0.05 m depth, in the free light fraction (12.2 g kg$^{-1}$) and intra-aggregate light fraction (2.19 g kg$^{-1}$) of organic matter under the no-tillage system with cover crops than under a conventional tillage system with fallow (1.37 – 7.30 g kg$^{-1}$). Alvarez et al. (1998) also found higher C content in the light and medium fraction in the top 5 cm of soil under a no tillage system in an Argentine Rolling Pampa soil. Similarly, in a 10 year study conducted in New England (USA) on a fine sandy loam, Ding et al. (2006) found that a cover crop system with a

vetch + cereal rye mixture or cereal rye alone had significant effects on light fraction (LF) content versus a no-cover system. The LF (free LF + intra-aggregate LF) consists mostly of plant residues and other partially decomposed materials and makes up most of the labile soil organic matter and are more sensitive to short term changes in management practices. In this study, both OC and LF were higher in a cover crop system at a 0 N rate than with an N rate of 202 kg ha$^{-1}$. Reicosky et al. (2011) considers soil C contents as the main contribution to having a soil with good properties since it is linked to and influences the biological, physical and chemical properties of the soil. Carbon represents at least half of the total mass of SOM, and the soil is the largest terrestrial pool of organic C. Batjes (1996) estimated soil organic C at 684-724 Pg of C in the upper 30 cm and 1462-1548 Pg of C in the upper 100 cm. Increasing soil C increases the stability of soil aggregates which leads to lower bulk density, better root development and growth which in turn leads to increased productivity (Havlin et al., 2005).

Cover crops contribute to maintaining adequate levels of soil C by adding plant residue to the soil. Studying the effects of a legume crop (*Mucuna puriens* var. *utilis*) on a sandy loam Ultisol in Benin, Barthes et al. (2004) found that from March 1988 to November 1999, total C content increased from 5.2 to 11.6 g kg$^{-1}$ in the 0-10 cm layer when corn was planted into a mucuna mulch that was sown each year 1 month after corn. On the other hand, for corn grown under a traditional no-input cropping system total C slightly decreased while no change was observed in the mineral fertilized cropping system during the study period. The 19.9 t ha$^{-1}$ yr$^{-1}$ of dry matter in the corn-mucuna treatment represented 10.0 t C ha$^{-1}$ yr$^{-1}$ and most of this C returned to the soil was from both mucuna and corn above ground biomass or roots. Hubbard et al. (2013) found that sun hemp (*Crotalaria juncea* L.), a tropical legume, was a dominant factor controlling the response of soil C in different cover cropping systems and as a late summer cover

crop it added more C to the soil compared with a system using only crimson clover as a winter

cover crop. While reports have shown that cover crops alone have a positive effect on soil C

content, using additional soil management practices such as no-tillage can likewise increase the

quantity of organic C in the soil. In a three year study carried out in the Ajuno basin in Mexico,

Roldan et al. (2003) reported that under a no-tillage system, total organic C increased by 3.5 g

$kg^{-1}$ more than under conventional tillage practices. Furthermore, the authors did not find

significant differences in total organic C by planting a legume cover crop in addition to

maintaining a 33% residue cover, supporting the notion that better soil quality is best achieved

through the adoption of several conservation management practices.

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## Chapter 2. Effect of Cover Crops and N rate on Soil Physical and Chemical Properties and Corn Yield

### 2.1 Introduction

Agricultural land use in Louisiana has undergone a noticeable shift from cotton to corn. Acreage dedicated to corn production has increased in the past years with the greatest change occurring in 2007 when the total area planted increased from 121,405 ha in 2006 to 299,467 ha. While a shift in land use can be traced to the introduction of the 1995 Federal Agriculture Improvement and Reform Act, the recent spike in corn acreage may stem from several factors, including a lower cost of production and higher returns in comparison to cotton (Fannin et al., 2008) and increased demand for ethanol (Scott et al., 2009).

Corn is typically produced under an intensive production system with high input requirement. As with all crops, a balance of both macro- and micro- nutrients is required for adequate growth and yield. However, N is the most limiting nutrient in irrigated corn production (Prasad and Power, 1997). In Louisiana, N recommendations range from 134 and 302 kg ha$^{-1}$ depending on soil type and typically applied as a split application (LSU AgCenter, 2015). Corn responds favorably to applied N but the response depends on factors related to the crop, the agro-environment (climate, soil type, spacing) and fertilizer applied (Asghari and Hanson, 1984; Liu and Wiatrak, 2011; Sinclair and Rufty, 2012; Caviglia et al., 2014).

Intensive farming has generated significant economic and environmental costs but the high productive capacity of these systems is still appealing to farmers (de Santa Olalla, 2001). These costs necessitate alternative management practices that will maintain or increase productivity while conserving the soil's productive capacity. The continuous monoculture row-crop production in the Mid-South has depleted the soil of its nutrients, organic matter and overall

productivity, thereby necessitating external inputs to meet crop demand. To address this problem, conservation practices must be adopted and and included as a vital part of land management. Using cover crops in agronomic systems is one of the many alternatives available to producers. Cover crops are planted during the fallow period between cash crops and, though initially used to reduce soil loss by erosion (Weil and Kremen, 2006), they also have the potential to improve nutrient cycling.

The benefits of planting a cover crop are many, including pest suppression and buildup of natural predators (Tillman et al., 2004; Lundgren and Fergen, 2010; Hooks et al., 2013), as well as influencing soil nematode communities (Ito et al., 2015). Cover crop species also increase and affect both physical (Nascente, Li and Crusciol, 2013) and chemical (Ding et al., 2006) properties of soil organic matter, as well as total C and N in the surface, thereby influencing nutrient cycling in the soil system.

Several researchers have also highlighted the effect that cover crops have on N cycling. Corn production systems have a high fertilizer demand, therefore, the careful and efficient management of N is a priority, which can translate into less N loss and/or a reduced dependency on inorganic fertilizer applications. Depending on the specie, cover crops can contribute to N cycling by either uptaking residual N or by adding N to the system. Certain grass cover crop species have shown to be more efficient in capturing and using residual N (Brinsfield and Staver, 1991; Shipley et al., 1992; Kramberger et al., 2009).  Baggs, Watson and Rees (2000) reported an overall reduction of available soil $NO_3^-$-N, between 10-20 kg ha$^{-1}$, by cover crops and green manure treatments, which would have reduced leaching potential. They highlighted the importance of selecting cover crops based on geographic location and climate that would enhance the N uptake capacity of the covers. In an oxisol in Parana Brazil, yield response to

cover crops were different, depending on the crop. Soybean yields were highest under a black oat (*Avena strigosa* Schieb.) cover (2.7 vs 2.0 t ha^{-1}), maize responded better to a lupin (*Lupinus albus* L.)cover (6.4 vs 6.1 t ha^{-1}) and kidney bean (*Phaseolus vulgaris* L.) yield increased under black oat and oil seed radish (*Raphanus sativus* L. var *oleiferus*) (0.7 vs 0.4 t ha^{-1}) (Derpsch, Sidiras and Roth, 1986).

Although research on the effects of cover crops on different production systems and geographic regions is ongoing, the integration of cover crops in the Mid-South is still recent and more information is needed to enable effective decision making. It is expected that alternative production practices would lead to a more efficient use of resources while maintaining and increasing the long-term fertility and biological activity of the soil. The objective of this trial was to evaluate the effects of cover crops and N rates on corn grain yield, yield characteristics, and soil chemical properties under a Mid-South production system.

**2.2 Materials and Methods**

2.2.1 Site Description

The field study was conducted in 2014 and 2015 and was located at the Louisiana State University AgCenter's Macon Ridge Research Station near Winnsboro, Louisiana (32°09′48″N 91°43′24″W). The soil at the research station is classified as a Gigger-Gilbert silt loam (fine-silty, mixed, thermic Typic Fragiudalfs). The area receives an average rainfall of 124 cm (Figure 2.1) and average high and low temperature of 24 °C and 11 °C, respectively

2.2.2 Experimental Design

Treatments included eight types of cover (seven cover crops and a fallow treatment) and four N rates (0, 235, 268 and 302 kg ha^{-1}, applied as Urea). Cover crop species consisted of cereal rye (*Secale cereale*) planted at 78.5 kg ha^{-1}; forage radish (*Raphanus sativus* var.

*longipinnatus*) planted at 10.1 kg ha$^{-1}$, berseem clover (*Trifolium alexandrinum*) planted at 22.4 kg ha$^{-1}$; crimson clover (*Trifolium incarnatum* L) planted at 16.8 kg ha$^{-1}$; winter pea (*Pisium sativum* L) planted at 78.5 kg ha$^{-1}$; hairy vetch (*Vicia villosa* Roth) planted at 22.4 kg ha$^{-1}$and a forage radish + cereal rye mix planted at 4.5 and 72.9 kg ha$^{-1}$, respectively. An untreated fallow plot served as a control treatment with native winter vegetation composed mostly of henbit (*Lamium amplexicaule*) and ryegrass (*Lolium* sp.).

Treatments were arranged in a split-plot with a randomized complete block design in the subplot for a total of 32 treatments (Table 2.1). To conduct the trial, a field measuring 0.72 ha was divided into eight plots, in each plot one type of cover was planted using its corresponding planting rate.
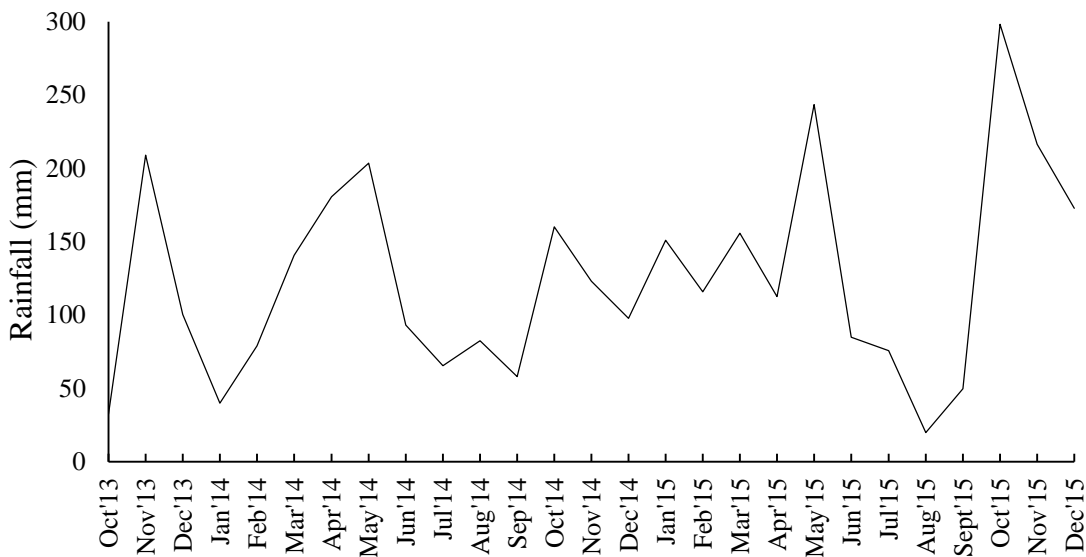


Figure 2.1. Monthly rainfall measured at Winnsboro, LA from October 2013 – December 2015.

Table 2.1 Description of treatments in the cover crop by N-rate field experiment.

| Treatment Number | Cover Crop | N rate (kg ha$^{-1}$) |
|---|---|---|
| 1 | Cereal Rye + Forage Radish | 0 |
| 2 | Cereal Rye + Forage Radish | 235 |
| 3 | Cereal Rye + Forage Radish | 268 |
| 4 | Cereal Rye + Forage Radish | 302 |
| 5 | Forage Radish | 0 |
| 6 | Forage Radish | 235 |
| 7 | Forage Radish | 268 |
| 8 | Forage Radish | 302 |
| 9 | Fallow | 0 |
| 10 | Fallow | 235 |
| 11 | Fallow | 268 |
| 12 | Fallow | 302 |
| 13 | Hairy Vetch | 0 |
| 14 | Hairy Vetch | 235 |
| 15 | Hairy Vetch | 268 |
| 16 | Hairy Vetch | 302 |
| 17 | Crimson Clover | 0 |
| 18 | Crimson Clover | 235 |
| 19 | Crimson Clover | 268 |
| 20 | Crimson Clover | 302 |
| 21 | Cereal Rye | 0 |
| 22 | Cereal Rye | 235 |
| 23 | Cereal Rye | 268 |
| 24 | Cereal Rye | 302 |
| 25 | Winter Pea | 0 |
| 26 | Winter Pea | 235 |
| 27 | Winter Pea | 268 |
| 28 | Winter Pea | 302 |
| 29 | Berseem Clover | 0 |
| 30 | Berseem Clover | 235 |
| 31 | Berseem Clover | 268 |
| 32 | Berseem Clover | 302 |

Covers were planted in mid-October in 2013, 2014 and 2015 and were seeded by broadcast using a Gandy broadcast spreader (Owatonna, MN) and shallowly incorporated using a custom-built row shaper. No planting nor chemical control was conducted in the fallow treatment in an attempt to simulate the natural regeneration of winter weeds during the fallow period between summer corn crops.

After planting, covers were allowed to grow without any further application of fertilizer or agricultural chemicals. Winter temperatures in the Mid-South are not sufficient to cause a natural termination of the covers therefore broad spectrum herbicides were used. Four weeks prior to intended planting, February in 2014 and 2015, covers were terminated with an application of 2,4-D and Glyphosate (Roundup) at a rate of 1.17 and 2.34 L ha$^{-1}$, respectively.

Following termination, each cover crop plot was further divided into 16 sub-plots (for a total of 128 sub-plots; Figure 2.2). Each sub-plot consisted of a four-row plot, measuring 13.7 m in length and 4 m in width with 1 m row spacing. Plots were sown with Pioneer 1319HR at the rate of 79,040 plants ha$^{-1}$ using a John Deere MaxEmerge 2 (Moline, IL) planter. The N rates were hand-applied to the corn crop at emergence (VE; Hicks and Thomison, 2004) as urea (46-0-0) and incorporated within 48 hours of application with at least 1.25 cm of rainfall or irrigation water. Additional fertilizer inputs applied to summer corn were based on soil test reports from the LSU AgCenter Soil Test and Plant Analysis Laboratory (STPAL). For both years, P and K were applied at a rate of 67.3 kg ha$^{-1}$ as triple super phosphate (0-46-0) and potassium chloride (0-0-60), respectively. Pest management was carried out based on current LSU AgCenter recommendations.

The effect of cover and N rate treatments on corn production was determined by measuring corn grain yield, 100 grain weight, SPAD (Minolta Chlorophyll Meter SPAD-502, Konica Minolta, Ramsey, NJ) leaf chlorophyll content and extractable nutrients in grain. Data on these variables were collected from the corn crop grown during summer 2014 and 2015; further details are provided in section 2.2.4. Treatment effects on soil chemical properties was determined by collecting soil samples at three different times during the experiment.

| | | | |
|---|---|---|---|
| Crimson clover | Cereal rye | Winter pea | Berseem clover |
| Cereal rye + Forage radish | Forage radish | Fallow | Hairy vetch |

**(A)**

**(B)**

| $N_2$ | $N_4$ | $N_1$ | $N_3$ |
|---|---|---|---|
| $N_3$ | $N_1$ | $N_4$ | $N_2$ |
| $N_4$ | $N_3$ | $N_1$ | $N_2$ |
| $N_1$ | $N_2$ | $N_3$ | $N_4$ |
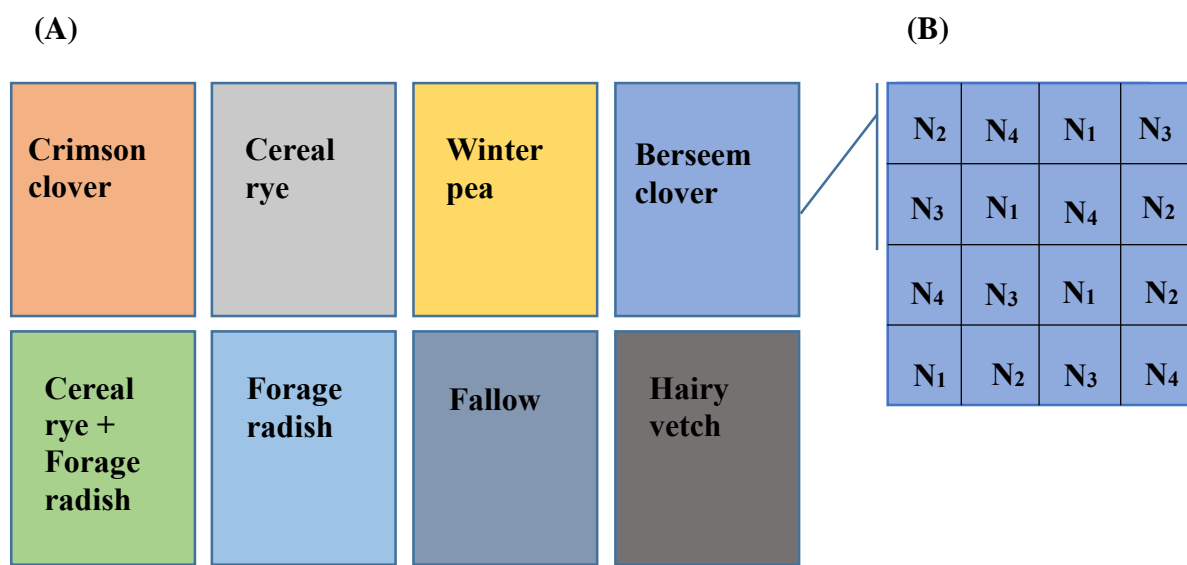
Figure 2.2. Field layout of eight cover treatments (A) for the cover crop x N rate field trial situated at the LSU AgCenter's Macon Ridge Research Station, Winsboro, Louisiana. (B) Expanded view of a cover section and the distribution of the four N-rate used in the study. Not drawn to scale.

### 2.2.3 Measurement of Corn Variables

#### 2.2.3.1 Grain Yield and 100 Grain Weight

Grain yield data was collected in August 2014 and 2015. A plot combine was used to harvest the two middle rows of each sub-plot. Grain moisture was adjusted to 155 g kg$^{-1}$ and dry weight was used to calculate yield. Grain harvested from the two middle rows represented yield per 300 ft$^2$ which was then converted to bushels ac$^{-1}$ and further converted into kg ha$^{-1}$. The 100 seed weight was determined by taking a random sample of grain collected from each sub-plot and recording the weight of 100 grains.

#### 2.2.3.2 Leaf Chlorophyll Content Determination using a SPAD Meter

A SPAD meter was used to take in-season readings of leaf chlorophyll content of the summer corn crop. Readings were taken in June 2014 and 2015 when the corn plants were at the

VT or tassel emergence stage. Readings were taken from the ear leaf of twenty randomly

selected corn plants located in the middle two rows in each sub-plot. Three readings were taken

along the leaf blade as an attempt to minimize variability within a single plant.  The average of

three readings per leaf was used as the SPAD reading for a specific plant while the average of the

20 randomly selected plants was recorded as the SPAD reading for the plants in the sub-plot.

### 2.2.3.3 Grain Nutrient Analysis

Grain samples from each sub-plot were submitted to the LSU Agricultural Center's Soils,

Tissue and Plant Analysis Lab to determine N, P, K and S content and trace nutrients (Al, B, Ca,

Cu, Fe, Mg, Mn, Na and Zn).

### 2.2.4 Soil Variables Measured

### 2.2.4.1 Soil Sampling

Samples were collected from the 0-8 cm depth using a 5 cm in diameter soil probe in

October 2014 (after corn harvest, before cover crop planting), February 2015 (after cover crop

termination, before corn planting) and again in October 2015 (after corn harvest, before cover

crop planting). Six samples were collected from each sub-plot – three from between planting

rows and three from the harvested rows within the 2 middle rows of the plots – and composited.

Samples were air dried at room temperature for five days, sieved to < 2 mm and submitted for

further analysis.

### 2.2.4.2 Soil Nutrient Status

Soil samples were analyzed for soil pH (1:1 in deionized water), Total C and N (via dry

combustion method by Leco CN Analyzer; St. Joseph, MI) and Mehlich-III extractable

micronutrients (measured via Inductively Coupled Plasma; Lexington, KY) were determined.

Separately, subsamples were also analyzed for inorganic N ($NO_3^-$-N and $NH_4^+$-N) through 1$M$

KCl extraction and analyzed using flow injection analyzer (Lachat Quickchem 8500; Loveland, CO).

### 2.2.4.3 Soil Organic Matter

Soil organic matter (SOM) was determined as percent Loss-on-Ignition as described in Lowther et al (1990). Soil was weighed (3.5 g) and placed in ceramic crucibles to dry for 24 hours at 105°C. The weight of the oven dried soil (soil $wt_{105}$) was taken and the samples were transferred to a muffle furnace for 16 hours at 450°C for combustion of organic matter. Heating at 450°C or less and at long periods of time would result in removal of organic matter while not decomposing other inorganic constituents that may be present in the soil.  At the end of the combustion period the final weight (soil $wt_{400}$) of the sample was recorded. LOI (%) was calculated using the following equation:

$$LOI(\%) = \frac{\text{soil wt}_{105} - \text{soil wt}_{400}}{\text{soil wt}_{105}} \times 100$$

The data recorded required correction to obtain SOM content from LOI % using the following linear equation SOM (%) = (b + LOI) + a, where b = 0.914 and a = 0. Using a regression coefficient "b" less than one accounts for the loss of constituents other than organic matter during ignition (Lowther et al., 1990).

### 2.2.4.4 Particle Size Analysis

The modified hydrometer method as described in Grossman and Reinsch (2002) was used to determine particle size distribution of the soil samples. A 50 g sample of ground, air dried soil was placed in 500 ml Nalgene bottles with 15 ml of 10% sodium hexametaphosphate to facilitate

particle dispersion. Bottles were filled with 250 ml DI water and placed on a reciprocal shaker

for 2 h on high speed. The soil solution was transferred to 1 L graduated cylinders, filled to the 1

L mark with DI water and mixed with a plunger.  Hydrometer reading at 40 seconds and 24

hours was used to determine sand and clay content, respectively, while silt content was

calculated thereafter. Temperature (°C) was recorded at the time of hydrometer reading to correct

for variation in water viscosity by adding 0.36 for every degree above 20° C.  The soil's texture

was determined by plotting the sand, silt and clay content using the USDA textural triangle.

### 2.2.4.5 Gravimetric Moisture Content

Soil moisture was determined gravimetrically using convective oven drying. Moist soil

(10 g) was placed into tared drying tins and oven dried for 24 h at 105° C and reweighed. Water

content was determined using the following formula:

$$\theta_m = \frac{(\text{mass of moist soil} - \text{tin}) - (\text{mass of dry soil} - \text{tin})}{\text{mass of dry soil} - \text{tin}}$$

### 2.2.5 Litter bag study

The litter bag study consisted in placing a known quantity of cover biomass into a mesh

bag which is then buried. The bags are extracted at periodic intervals, dried and reweighed to

determine the amount of mass lost. Since the bags are buried in situ, they are exposed to the

normal temperature and moisture conditions.  We were interested in determining nutrient loss

over time but due to the limited amount of biomass remaining lab analysis was limited to total C

and N.

In this study, nylon bags of mesh size 50μm were filled with a known weight of biomass

collected from each cover section. The weight of the litter bags containing the plant material was

recorded. The top of the bags were then folded, heat sealed and labeled with an aluminum tag.
The final weight of the bag + biomass + tag was recorded. Forty bags were filled for each cover
type and were buried at a depth of 5 cm in the subplots receiving 0 and 268 kg N ha-1 in their
respective cover sections. Based on the field design, each cover section had four subplots for
each of the two N rates selected. Therefore, in each cover section 5 bags were buried in each of
the four 0 and 268 kg N ha-1 subplots. One bag from each subplot of was extracted every month
for 5 months (March-July, 2015). Extracted bags were washed to remove soil, dried at 105 $^0$C
and the contents were weighed and submitted, for C and N analysis, to the Soil Test and Plant
Analysis Lab at LSU main campus in Baton Rouge. C and N results for March is not included in
the analysis because bags were not separated by N rate

2.2.6 Data Analysis

Statistical analyses were performed on soil and corn variables using the PROC MIXED
in SAS 9.4 (SAS Institute, 2012). Mean separation was done using Tukey's Honestly Significant
Difference (HSD) method at a 5% confidence level. A formal test could not be done to evaluate
the main effect of cover crop and interpretation was limited to its interaction with N rate or
sampling time (for soil data) or year (for crop data), when significant. The means model used for
the soil and corn output response variables can be described as follows

$$Y_{year*N\ rate*row(cover)} = \mu + \pi_{sampling\ time} + \alpha_{cover} + \beta_{N\ rate} + \pi\alpha_{sampling\ time*cover} + \pi\beta_{sampling\ timer*N\ rate}$$

$$+ \pi\alpha\beta_{sampling\ time*cover*N\ rate} + \rho_{row/cover} + \delta_{N\ rate*row/cover} + \varepsilon_{sampling\ time*N\ rate*row(cover)},$$

where:

Y = output response variable (soil and corn variables),
$\mu$ = the overall mean,
$\pi$, $\alpha$, and $\beta$ = fixed effects,
$\rho$ and $\delta$ = random effects
$\varepsilon$ = residual error

For the litter bag study, C and N data was analyzed using PROC MIXED in SAS 9.4 (SAS Institute, 2012) using the following means model:

$$Y_{bag/sampling\ time*N\ rate*row(cover)} = \mu + \pi_{sampling\ time} + \alpha_{cover} + \beta_{N\ rate} + \pi\alpha_{sampling\ time*cover} +$$

$$\pi\beta_{sampling\ timer*N\ rate} + \pi\alpha\beta_{sampling\ time*cover*N\ rate} + \rho_{row/cover} + \delta_{N\ rate*row/cover} + \gamma_{sampling\ time*N}$$

$$rate*row(cover) + \varepsilon_{bag/sampling\ time*N\ rate*row(cover)}$$

where:

Y = output response variable ,
μ = the overall mean,
π, α, and β = fixed effects,
ρ, δ, and γ = random effects
ε = residual error

## 2.3 Results and Discussion

2.3.1 Treatment Effects on Corn Grain Yield

A yield decrease was recorded in 2015, compared to 2014, in cover crop treatments, with the exception of cereal rye + forage radish and hairy vetch treatments. The greatest reduction in yield occurred in the cereal rye (3.34 Mg ha$^{-1}$) and crimson clover (3.23 Mg ha$^{-1}$) cover crop treatments. Corn yield in the fallow treatment was comparable with cover crop treatments except crimson clover and cereal rye + forage radish in 2014 (10.94 Mg ha$^{-1}$ vs 13.12 and 8.74 Mg ha$^{-1}$, respectively) and hairy vetch in 2015 (8.09 Mg ha$^{-1}$ vs 10.67 Mg ha$^{-1}$) (Table 2.2). Kramberger et al. (2009) suggests that a corn crop followed by a bare fallow can use available N resulting from mineral fertilizers, residual soil mineral N available at planting, and N released from soil OM mineralization occurring in season.

In 2014, grain yield did not differ between the N rates applied (235, 268 and 302 kg Urea ha$^{-1}$) (Table 2.2). While the recommended N rate for the Northeast region of Louisiana is 268 kg ha$^{-1}$, a significant increase of 4.8 Mg ha$^{-1}$ was obtained by applying the lower rate of 235 kg N

ha$^{-1}$ compared to not adding any N, suggesting that, in 2014, no benefits was observed from N rates greater than 235 kg N h$^{-1}$. Despite higher yields in 2014, there was a greater benefit of N application in 2015. In 2014 adding 235 kg N ha$^{-1}$ rather than 0 N resulted in a yield increase of 4.8 Mg ha$^{-1}$ while in 2015 the yield increase was 6.2 Mg ha$^{-1}$. In 2015 grain yield increased from 0 to 268 kg N ha$^{-1}$ applications, however no yield benefit was observed at the 302 kg ha$^{-1}$.

Table 2.2. Effect of cover crop and N rate treatments on corn grain yield.

| Treatment | 2014 | 2015 |
|---|---|---|
| | ----------------Mg ha$^{-1}$------------------ | |
| Cover Crop | | |
| Cereal Rye + Forage Radish | 8.74 A$^†$c$^‡$ | 8.58   Abc |
| Forage Radish | 11.47  Aab | 9.04  Babc |
| Fallow | 10.94   Ab | 8.09    Bc |
| Hairy Vetch | 12.085  Aab | 10.67    Aa |
| Crimson Clover | 13.12   Aa | 9.89  Babc |
| Cereal Rye | 11.93  Aab | 8.59   Bbc |
| Winter Pea | 12.68  Aab | 10.40   Bab |
| Berseem Clover | 11.81  Aab | 9.74  Babc |
| N rate (kg ha$^{-1}$) | | |
| 0 | 7.74   Aa | 3.83    Ba |
| 235 | 12.62   Ab | 10.08    Bb |
| 268 | 12.94   Ab | 11.45    Bc |
| 302 | 13.09   Ab | 12.14   Acd |
| Analysis of Variance (LSD protected p ≤ 0.05) | | |
| Cover Crop | <0.0001 | |
| N rate | <0.0001 | |
| Cover Crop x N rate | NS§ | |
| Year | <0.0001 | |
| Year x Cover Crop | 0.0039 | |
| Year x N rate | <0.0001 | |
| Year x Cover Crop x N rate | NS | |

† Same upper case letter are not significantly different (p ≤ 0.05) between sampling time within a cover crop or N rate.
‡ Same lower case letters are not significant (p ≤ 0.05) within sampling time across cover crops or N rates.
§ Not Significant at 0.05 level.

Higher yields in 2014 could be attributed to previous field management since a soybean crop was planted in summer 2013 prior to cover crop establishment, resulting in additional residual N entering the system.

Pederson and Lauer (2003) reported significant effects of rotation sequence on corn and soybean yield evaluated during 1997-2001 on a Plano silt loam soil in Arlington, Wisconsin. Corn yields after five years of continuous soybean and annually rotated corn-soybean had 17% more grain yield in first year than continuous corn. Furthermore, lower yields in 2015 for both cover crop and N rate treatments could be attributed to higher rainfall in early spring. Measured rainfall for early spring 2014 was 259.6 mm and 422.4 mm in 2015. This excess rainfall could have resulted in loss of available N from the soil system. Pederson and Lauer (2003) also contributed differences in yield across years in their study to wet springs that caused run-off and N deficiency symptoms.

Seed weight per 100 seeds, as seen in Figure 2.3 was significantly higher in 2014 (29.57 g 100 seeds $^{-1}$) than 2015 (56.10 g 100 seeds $^{-1}$) which coincides with the yield performance of the corn crop in each year. Seed dry weight is considered an important contributor to seed yield in addition to number of seeds per land area (Borras et al., 2004). A reduction in 100 seed weight can be attributed to environmental and physiological factors (Eck, 1986). Within specific genetic material, decreased kernel weight translates into lower yields, due to a reduction in net photosynthesis or translocation of dry material during grain filling

Seed weight was affected by the addition of N and year x N rate interaction (Table 2.3). There was no significant differences in seed weight for N rates in 2014. In 2015, the greatest seed weight was obtained at an N rate of 302 kg ha$^{-1}$ which was significantly greater than that obtained from the 0 and 235 kg ha$^{-1}$ rates in the same year but was similar to those obtained at all
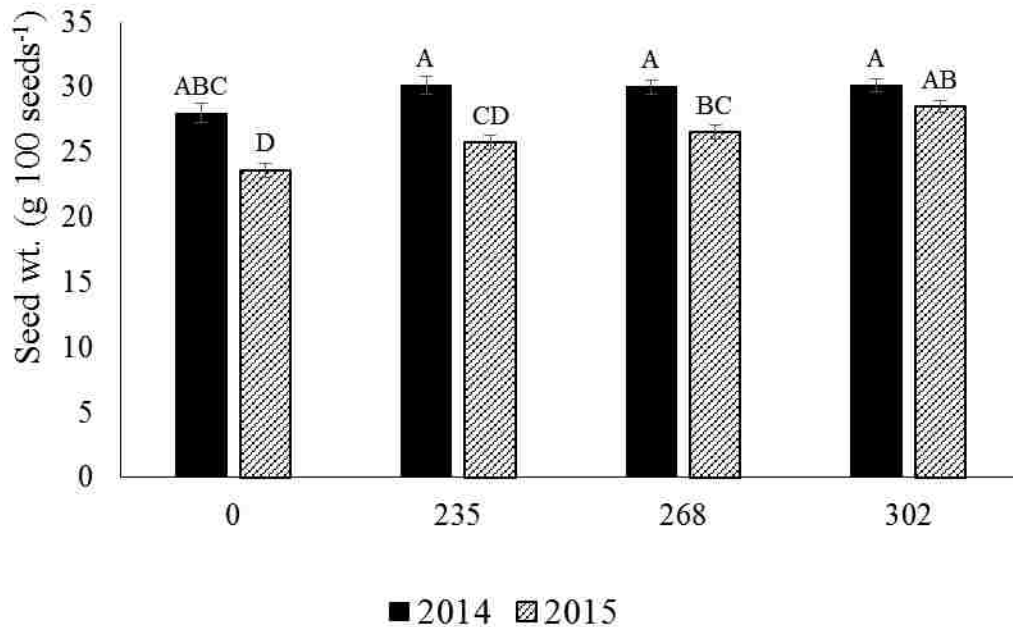
Figure 2.3. Effect of N rate on 100 seed weight measured in 2014 and 2015. Same upper case letter are not significantly different (p ≤ 0.05).

N rates in 2014. Bassegio et al. (2013), found similar effects of N on corn seed weight and observed a significant linear regression between N rate and 100 seed weight in corn.

Table 2.3.  ANOVA p-values for 100 seed weight as affected by sampling time, cover crop, N rate and their interactions.

| Source | 100 seed weight |
|---|---|
| Year | <0.0001 |
| Covercrop | <0.0001 |
| Year x Cover crop | NS[†] |
| N rate | <0.0001 |
| Year x N rate | 0.0438 |
| Cover crop x N rate | NS |
| Year x Cover crop x N rate | NS |

†Not Significant at 0.05 level.

2.3.2 Effects of Cover Crop and N rate on Nutrient Content of Corn Grain

Cover crop treatments significantly affected B, Mn, S, Zn and N content in corn grains, while N rate affected Fe, Mg, S, Zn, N, P and K content. Only a Year x N rate interaction was observed for Mg, Mn, P, and K. Higher nutrient concentrations were also observed when corn was fertilized with 302 kg N ha$^{-1}$. This was significantly different from 0 N except for Fe, Mg, S, N, P and K. On the other hand, grain Zn concentrations was highest for the treatments where no N was applied. This is contrary to the findings of Li et al. (2007) who did not observe any changes in Zn concentrations in grains and above ground parts that received different fertilization practices including no fertilization.

2.3.3 Treatment Effects on Leaf Chlorophyll Content

Relative amount of leaf chlorophyll content was measured using a SPAD meter from each plot to determine whether cover crop or N-rates, or their interaction, had any effect on leaf chlorophyll content, which could aid in N management in corn (Bullock and Anderson, 1998). The SPAD readings ranged from 47.6-51.8 and 43.7-54.7 in 2014 and 2015, respectively. Significant cover crop and N-rate main effects as well as sampling time x cover crop and sampling time x N-rate interaction was observed for SPAD readings (Table 2.4).

Table 2.4. ANOVA p-values for SPAD readings (unit less) as affected by sampling time, cover crop, N rate and their interactions.

| Source | SPAD |
|---|---|
| Sampling time | NS[†] |
| Covercrop | <0.0001 |
| Sampling time x Cover crop | 0.0013 |
| N rate | <0.0001 |
| Sampling time x N rate | <0.0001 |
| Cover crop x N rate | NS |
| Sampling time x Cover crop x N rate | NS |

† Not Significant at 0.05 level.

The SPAD readings taken from the fallow treatment (Figure 2.4) was among the lowest values for both years and was significantly lower than the other cover crop treatments except cereal rye +forage radish in 2014 and cereal rye in 2015. Contrast analysis comparing fallow to
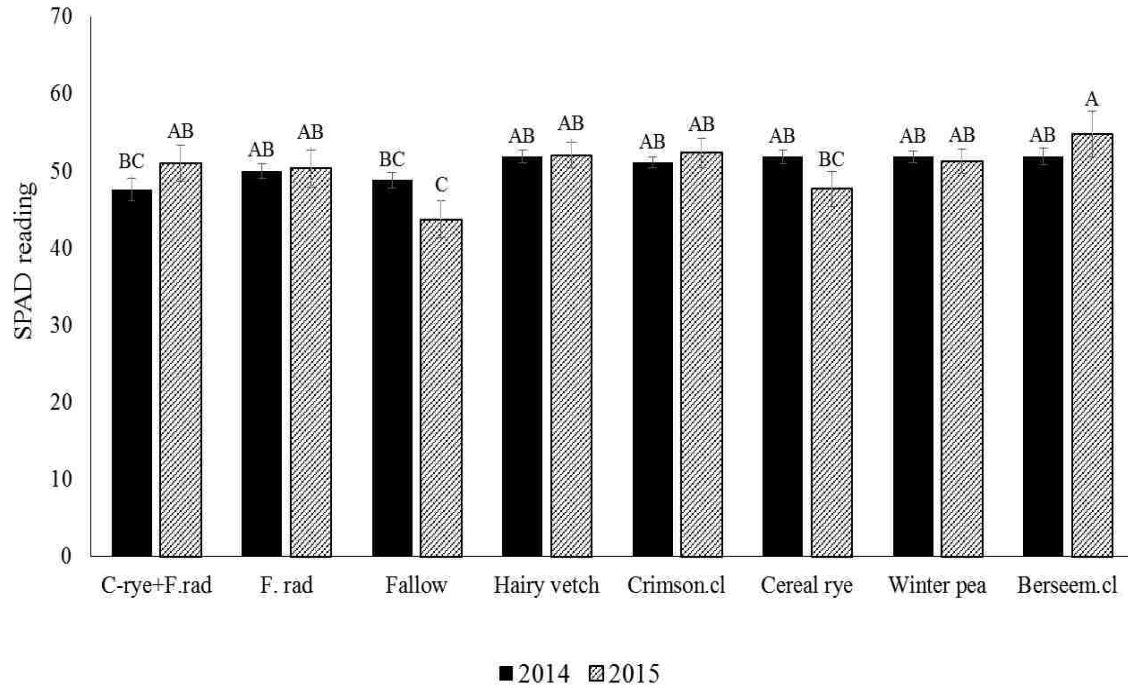


Figure 2. 4. SPAD reading measured from corn ear leaf at the VT stage within each cover crop treatment in 2014 and 2015. Same upper case letter are not significantly different ($p \leq 0.05$).

all covers showed a significant difference ($p < 0.0001$). Further contrast analysis to compare the four legume cover crops (hairy vetch, crimson clover, winter pea and berseem clover) to the non-legume covers (cereal rye + forage radish mix, forage radish, cereal rye and fallow) also showed a significant difference ($p < 0.0001$). While we could not determine whether one of the eight cover treatments had a greater impact on leaf chlorophyll content, as interpreted by the SPAD meter, the contrasts show that a legume cover crop increased SPAD readings.

The main effect of N treatment showed a significant difference between applying and not applying N, though the leaf chlorophyll content did not differ between higher N rates. The

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significant sampling time x N rate interaction showed that the 0 kg ha^{-1} N rate had a significantly lower leaf chlorophyll content in the second year of the experiment (2015) which can be the result of stripping the soil from any N reserves by both the cover crop and corn crop. The contrary can be observed at the highest N rate of 302 kg ha^{-1} where SPAD values for 2015 was significantly higher than those obtained in 2014 (Figure 2.5).
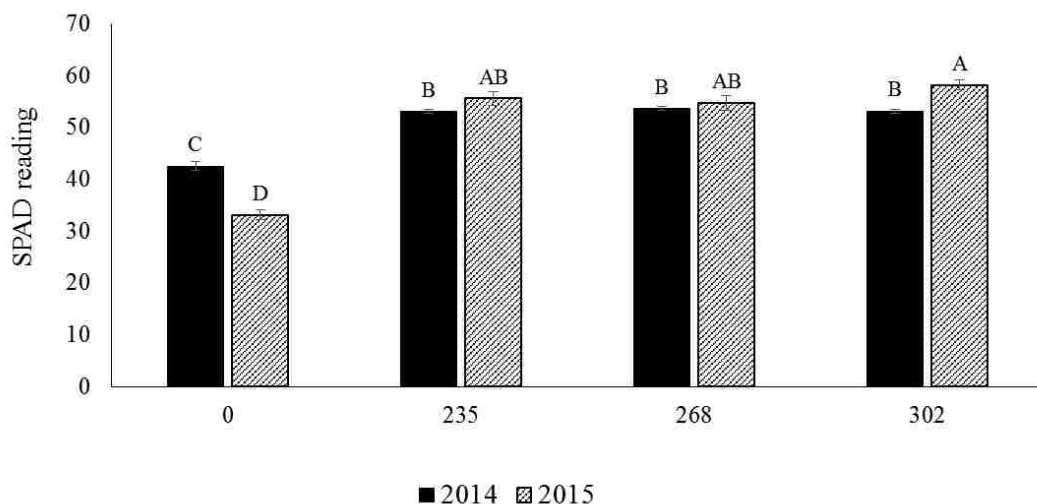


Figure 2.5. Effect of varying N rates on corn ear leaf SPAD reading measured in 2014 and 2015. Same upper case letter are not significantly different (p ≤ 0.05).

The main effect of N treatment showed a significant difference between applying and not applying N, though the leaf chlorophyll content did not differ between higher N rates. The significant sampling time x N rate interaction showed that the 0 kg ha^{-1} N rate had a significantly lower leaf chlorophyll content in the second year of the experiment (2015) which can be the result of stripping the soil from any N reserves by both the cover crop and corn crop. The contrary can be observed at the highest N rate of 302 kg ha^{-1} where SPAD values for 2015 was significantly higher than those obtained in 2014.

Though higher SPAD values were obtained in 2015 at the 235, 268 and 302 kg N ha$^{-1}$ these did not result in higher yields compared to that observed in 2014. According to Piekielek and Fox (1992), different hybrids may have different luxury consumption of N, which would reflect in high SPAD meter readings but not necessarily in higher yield. SPAD meter readings can be influenced by environmental and/or physiological conditions, including growth stage. Piekielek and Fox (1992), found that SPAD readings taken from leaves four and five were better predictors of relative grain yield using Cate-Nelson plots than leaves at leaf 6 stage. On the other hand, Blackmer and Schepers (1995) found that readings early in the season had poor predictability of crop yield response to N and correlations were higher with readings at R4-R5 stage.

2.3.4 Changes in Soil pH as a Result of Treatment Effects

Soil pH levels for sampling conducted at fall 2014 (after corn harvest and prior to cover crop planting) averaged at 6.3 and did not differ significantly from that obtained after cover crop termination i.e. spring 2015. However, the average soil pH of the samples collected in fall 2015 decreased to 5.8 and differed significantly from the previous sampling time which would mean that soil pH was more affected by management during the corn crop than a cover crop treatment. N rate also had a significant effect on soil pH and can be stated as follows 302<268>235<0.

Management practices, such as the use of inorganic N fertilizers, can decrease soil pH, particularly when the fertilizer is ammonium based (Brady and Weil, 2004). Nitrogen was applied in the form of urea and, while its incorporation in the soil initially results in an increase in pH due to the reactions involved in urea hydrolysis (Chien, Gearhart and Collamer, 2008), its long term use may result in an overall decrease in soil pH.

Liu et al. (2014) conducted a study to determine the effect of urea application and simulated acid rain on soil acidification and microbial community structure. Their results showed that the concentration of $H^+$ and $Al^{3+}$ were higher when urea was applied combined with a simulated acid rain than when no fertilizer was applied indicating the effect of the fertilizer on decreasing soil pH. Soil pH tends to decrease after the continuous use of inorganic fertilizer, specifically those that are ammonium based (Juo et al., 1995 and Chien et al., 2008).

2.3.5 Changes in Soil Extractable Nutrients

Cover crop significantly affected changes in nutrient levels for Ca, Cu, Mg, and K. N rates significantly affected extractable Mg which was highest at the 0 N rate and only significantly different from the 268 and 302 kg N ha$^{-1}$; no significant difference between the 235, 268 and 302 kg N ha$^{-1}$ was observed. While a significant effect of N was not observed for all extractable nutrients Thomson, Marschner and Römheld (1993) reported increases in micronutrients such as Fe, Zn, Mn, Cu when the fertilizer was $NH_4^+$-N based, suggesting that it was mostly due to the effect of the fertilizer acidifying the rhizosphere.

Of the eight extractable nutrients, all, except Cu, were affected significantly by the time of sampling by either increasing or decreasing during the duration of the experiment. Similar to soil pH, the results show that changes in extractable nutrient levels was more affected by a corn crop rather than a cover crop since, at the end of the corn season, their concentration increased from that measured in fall 2014. Changes in pH, redox potential, biological activity, SOM, cation-exchange capacity during the corn season could have resulted in the changes in extractable nutrient concentrations (Fageria, Baligar and Clark, 2002).

Treatment interaction of sampling time x cover crop was only significant for K and Na (Table 2.5). The greatest change in nutrient concentration across sampling time was observed

with Na that had an initial concentration of 65.58 mg kg$^{-1}$ in fall 2014 and increased to 295.09 mg kg$^{-1}$ in fall 2015 (Figure 2.6). There was also a significant interaction between sampling time and cover crop with the fallow treatment having the highest concentration of Na in fall 2015; however the fallow treatment was only significantly different from berseem clover, forage radish, hairy vetch and crimson clover cover treatments.

A significant sampling time x cover crop interaction for K$^+$ show that the effect of cover crops was dependent on the time of sampling. Soil extractable K+ was highest in fall 2015 (Figure 2.7) and significant differences between sampling time for each covercrop was observed in all cover crops, except crimson clover and berseem clover. Based on the soil test data, extractable K+ in the soil was similar for sampling conducted in Fall 2014 and Spring 2015 (before and after cover crop, respectively) across all cover crop treatments.

2.3.6 Effect of Cover Crops on Inorganic N Determined at Three Sampling Times

Prior to the establishment of the cover crops (fall 2014) the cereal rye plot had the highest concentration of residual soil $NO_3^-$ - N at 51.51 kg ha$^{-1}$ which was not different from that observed in the berseem clover and crimson clover plots (Table 2.6). Soil $NO_3^-$ - N decreased noticeably at spring 2015 sampling across all cover crops and returned to those concentrations previously recorded in fall 2014. The cereal rye plot had the greatest decrease in soil $NO_3^-$ - N, although it was not significantly different from the other cover crop treatments. Shipley et al. (1992) reported higher N uptake for grass cover crop compared to legumes at a rate of 48 kg ha$^{-1}$ for cereal rye and 29 kg ha$^{-1}$ for annual rye grass compared to 9 and 8 kg ha$^{-1}$ for hairy vetch and crimson clover, respectively. Similarly, Brinsfield and Staver (1991) observed higher N assimilation by cereal rye when compared to other grass species such as wheat, oats and barley.

Table 2.5. ANOVA p-values for soil pH and extractable nutrients as affected by sampling time, cover crop, N rate and their interactions.

| Source | Soil pH | Ca | Cu | Mg | P | K | Na | S | Zn |
|---|---|---|---|---|---|---|---|---|---|
| Sampling time | <0.0001 | <.0001 | 0.3019 | <.0001 | 0.0001 | <.0001 | <.0001 | 0.0204 | 0.0009 |
| Covercrop | <.0001 | <.0001 | 0.0001 | <.0001 | NS | <.0001 | NS | NS | NS |
| Sampling time x Cover crop | NS[†] | NS | NS | NS | NS | 0.0192 | 0.0382 | NS | NS |
| N rate | 0.0032 | NS | NS | 0.0242 | NS | NS | NS | NS | NS |
| Sampling time x N rate | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Cover crop x N rate | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Sampling time x Cover crop x N rate | NS | NS | NS | NS | NS | NS | NS | NS | NS |

†Not Significant at 0.05 level.
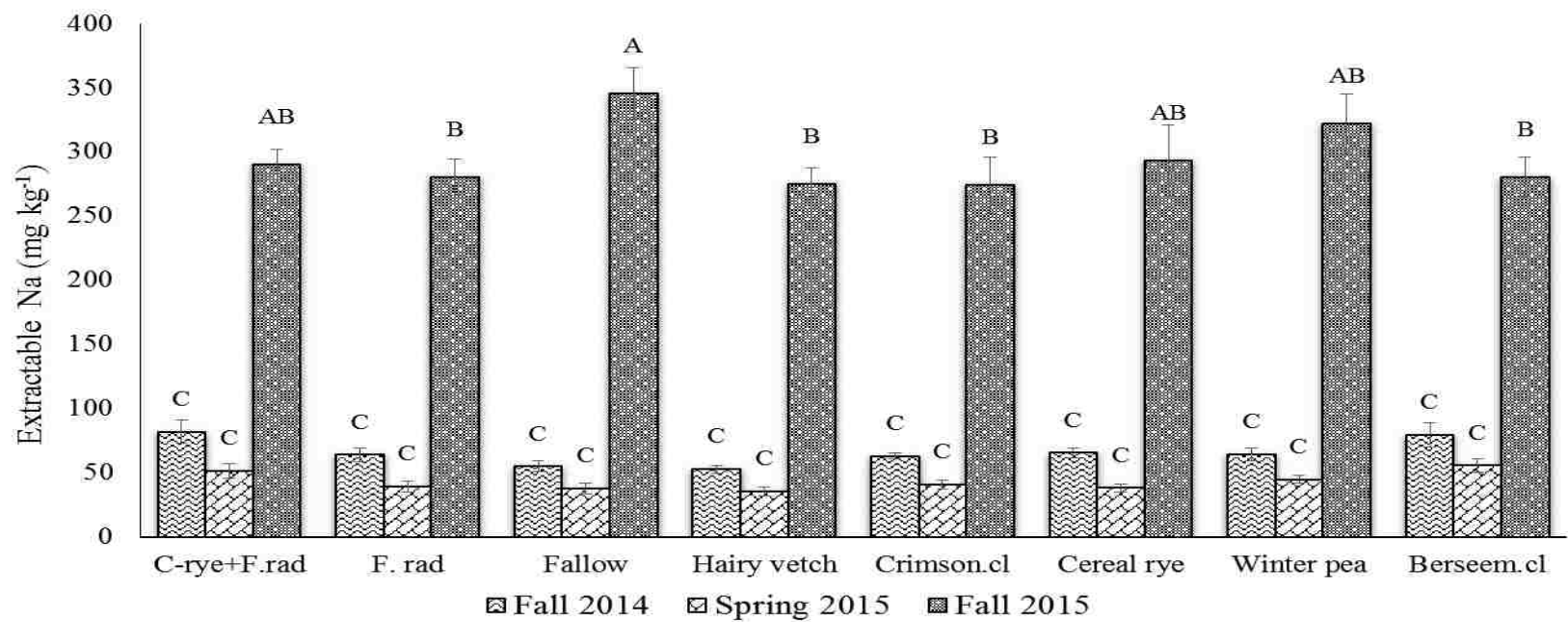
Figure 2.6. Effect of cover crop on soil extractable Na measured in fall 2014, spring 2015 and fall 2015. Same upper case letter are not significantly different at 0.05 level
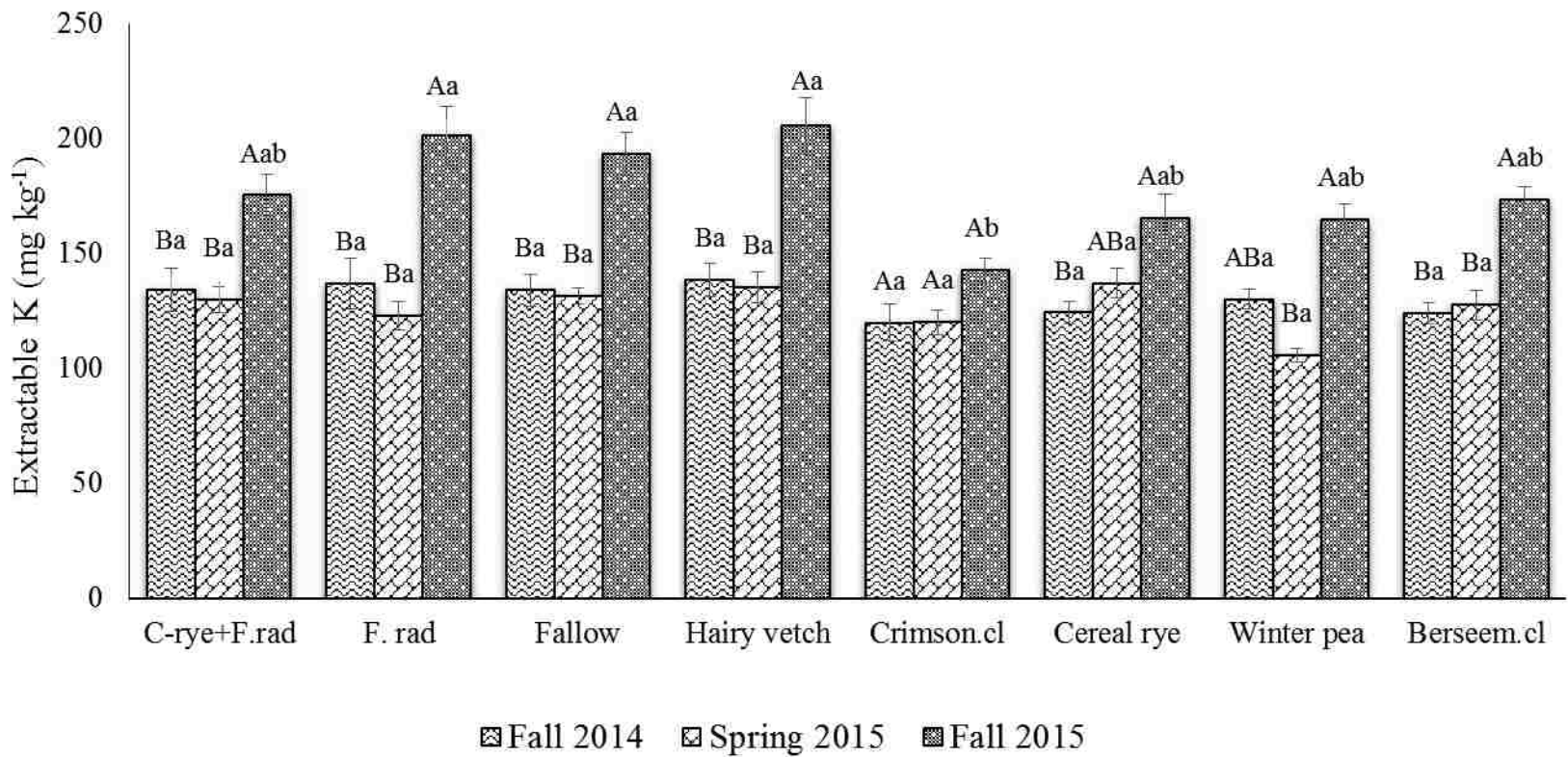
Figure 2.7. Effect of cover crop on soil extractable K measured in fall 2014, spring 2015 and fall 2015. Same upper case letter are not significantly different between sampling time within a cover crop (p ≤ 0.05). Same lower case letters are not significant within sampling time across cover crops at 0.05 level.

Table 2.6. Soil inorganic N concentrations measured at three sampling times.

| Cover crop | Inorganic N | | | | | |
| | Fall 2014 | | Spring 2015 | | Fall 2015 | |
| | $NO_3^-$ | $NH_4^+$ | $NO_3^-$ | $NH_4^+$ | $NO_3^-$ | $NH_4^+$ |
| | ------------------------------------------------kg ha$^{-1}$----------------------------------------------- | | | | | |
| Cereal rye+Forage radish | 24.07  A[†]b[‡] | 19.06  Ba | 8.62 Ba | 32.92  Ab | 31.54  Aa | 21.76 Ba |
| Forage radish | 27.48  Ab | 19.32  Ba | 8.59 Ba | 33.70  Ab | 33.97  Aa | 22.94 Ba |
| Fallow | 27.67  Ab | 24.56  Ba | 8.31 Ba | 35.03  Aab | 36.63  Aa | 22.64 Ba |
| Hairy vetch | 31.21  Ab | 23.59  Ba | 9.55 Ba | 41.49  Aab | 41.22  Aa | 25.74 Ba |
| Crimson clover | 38.83  Aab | 21.81  Ba | 9.61 Ba | 40.91  Aab | 39.70  Aa | 22.01 Ba |
| Cereal rye | 51.51  Aa | 20.20  Ba | 8.45 Ba | 43.89  Aa | 43.41  Aa | 24.13 Ba |
| Winter pea | 33.26  Ab | 23.25  Ba | 15.21 Ba | 42.94  Aa | 38.05  Aa | 22.67 Ba |
| Berseem clover | 37.03  Aab | 20.66  Ba | 10.51 Ba | 33.37  Ab | 44.23  Aa | 24.82 Ba |

† Same upper case letter are not significantly different (p ≤ 0.05) between sampling time within a cover crop or N rate.
‡ Same lower case letters are not significant (p ≤ 0.05) within sampling time across cover crops or N rates.

Planting of cover crops during a fallow period is recommended due to their ability to perform as catch crops thereby improving nutrient management, specifically N. Various research on the effect of cover crops on soil N have found similar effects of cover crops on decreasing $NO_3^-$-N loss (Zhu et al., 1989; Baggs, Watson and Rees, 2000; Gabriel, Munoz-Carpena and Quemada, 2012; Gabriel, Garrido and Quemada, 2013). While soil $NO_3^-$ - N was significantly reduced in spring 2015 $NH_4^+$ - N was highest at this time of sampling. $NO_3^-$, $NH_4^+$ is not readily leached into subsoil due of its adsorptive capacity to organic matter and negatively charged surfaces of clay (Brady and Weil, 2002).

Ammonium based fertilizers as well as decomposing organic matter are potential sources of $NH_4^+$ fraction of soil inorganic N (Bronson, 2008). The increase in soil $NH_4^+$-N observed at spring 2015 sampling (i.e. after cover crop) can be the result of cover crop and corn residue decomposition. The increase of $NH_4^+$ -N coupled with adequate soil conditions would have resulted in nitrification and the supply of available $NO_3^-$ - N to the successive corn crop. However, the benefit of available soil inorganic N was not reflected in better crop yield in 2015, possibly as a result of high rainfall, which could have resulted in leaching of the more mobile $NO_3^-$ - N and/or runoff of surface $NH_4^+$ -N. Additionally, noticeable yield was not observed even with minimal differences between each N rate applied. Optimal N cycling and management is one of the principal benefits of integrating cover crops to a production system. It is therefore important that time of burndown of cover crop and N release/mineralization coincides with crop N demands.

2.3.7 Soil Total C, N and Organic Matter

Cereal rye cover crop had the highest soil C and soil N at 10.18 and 1.23 g kg$^{-1}$, respectively, which was higher than the fallow treatment. Cereal rye is capable of reducing N

leaching and scavenge for residual N in the soil, tying N in its biomass for subsequent release into the soil. Other research has found that higher soil N was found in legume cover crops due to their ability to fix N while Roldan et al. (2003) reported no influence of leguminous cover crops on either soil N or C but, rather, that the rate of residue incorporation was a more dependent factor than cover type.

The rate of decomposition of the cover crop influences changes in total C and N (Piotrowska and Wilczewski, 2012). C and N in the soil was highest in spring 2015 when samples were collected a week after cover crop termination (Table 2.7). Our findings agree with those of Blevins, Thomas and Cornelius (1977) in which total N measured in soil generally follows changes in soil C.

Table 2.7. Changes in soil C, N, C:N ratio and organic matter across three sampling times.

| Parameter | Fall 2014 | Spring 2015 | Fall 2015 |
|---|---|---|---|
| C (g kg$^{-1}$) | 9.00 B[†] | 9.43 A | 9.39 AB |
| N (g kg$^{-1}$) | 1.10 B | 1.20 A | 1.11 B |
| C:N ratio | 8.24 B | 7.86 C | 8.49 A |
| Organic Matter (OM) (%) | 2.19 B | 2.11 C | 2.51 A |

†Same upper case letter in a row are not significantly different at the 0.05 level.

Our results show soil C increasing in spring to 9.43 g kg$^{-1}$ from 9.00 g kg$^{-1}$ measured in fall 2014 and decreasing again in fall 2015 to 9.39 g kg$^{-1}$. Soil C:N was the lowest at spring sampling and, in the presence of microorganisms, can be used as a good indicator of mineralization of nutrients since mineralization is expected at C:N ratio < 25:1 (Brady and Weil, 2004). Furthermore, Hubbard, Strickland and Phatak (2013) report improvements in soil structure and fertility as a result of increased soil C and N provided by the input of cover crop biomass. Cover crops and N rate did not have an effect on organic matter during the course of the study (data not shown). However, organic matter increased by 15% from 2014 to 2015 which

could have been as a result of accumulation of both cover crop and corn residue. OM concentration was the lowest after the cover crop treatment (spring 2015) even though total C concentrations were higher. Organic matter comprises the more labile fraction of soil C suggesting that the more complex forms of C is contributing to the accumulation of soil C in this production system (Post and Kwon, 2000).

2.3.8 Litter bag study

Cover residue C:N ratio in April was the lowest for forage radish (9:1) and highest for fallow (15:1) and winter pea (13:1). C:N ratio continued to increase until June when it seemed to be levelling off except for the cereal rye+forage radish mix that increased drastically from 11 to 14 (Figure 2.8).
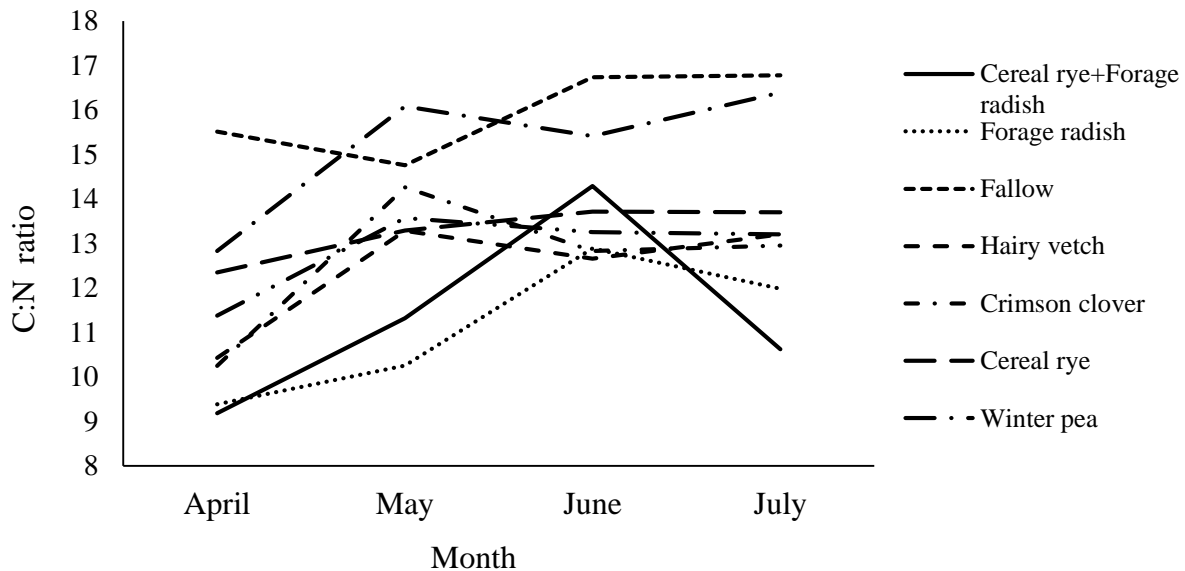


Figure 2.8. Changes in C:N ratio of cover crop biomass buried in nylon mesh bags during a summer corn crop in 2015.

The C:N ratio of legume covers increased in May and decreased in June. N content for cover residue decreased over time indicating that active mineralization was ongoing. Also, the C:N ratio for all the residue remained well below the cutoff point of 25:1; a C:N ratio >25:1

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would have result in immobilization.  The low C:N ratio and indication of ongoing mineralization would mean that the N was being made available for the corn crop. The release of N can also explain why NAGase activity was higher in fall 2015.

## 2.4 Conclusions

The effect of cover crop and N rate on corn grain yield and soil chemical properties was determined with this study. An overall yield decrease of up to 20% was recorded in 2015 compared to 2014 for the cover treatments, except for the cereal rye + forage radish mix and hairy vetch, which maintained their yields across both years.

Furthermore, the legume covers hairy vetch and winter pea increased yield in 2015 compared to the fallow treatment. Management and climatic factors could have influenced the decrease in yield. Although the study was not able to determine whether a cover crop can compensate for lower N rates, grain yield did respond favorable to the addition of N fertilizers and applying more than the recommended rate did not result in significant yield increase.

The availability of soil extractable nutrients was higher after a corn crop rather than after planting the cover treatments. This could be as a result of the applied N rates acidifying the soil thereby increasing the availability of some micronutrients. The loss of residual $NO_3^-$-N was abated with the planting of cover crops during the fallow season. These covers were able to tie up the residual N in their biomass which would eventually be released into the soil system. Additionally, with the adequate C:N ratio of <10:1 observed in this study and high $NH_4^+$-N measured in spring 2015 (average, 38.05 kg ha$^{-1}$) mineralization should have resulted in N availability for the summer corn crop. Cover crop termination was scheduled 6 weeks prior to corn planting and the high rainfall recorded during this wait period could have resulted in N loss from the system. This highlights the fact that the performance of a cover crop is not only

determined by the specie but also the interaction of many environmental and management

factors.

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**Chapter 3. An Evaluation of Soil Biological Properties of a Mid-South Corn Production System Produced under Eight Different Cover Crops and Nitrogen Rates**

**3.1 Introduction**

The suitability of soil for sustaining life is a function of its physical, chemical and biological properties (Lal, 1999). Conservation agriculture- a management strategy that (1) reduces soil disturbance by reducing tillage, (2) maintains soil cover, and (3) incorporates the rotation of crops (Hobbs, Sayre and Gupta, 2008) promotes a more sustainable form of crop production which contributes to maintaining soil quality. The usage of cover crops, for example, is one of the many tools used in conservation agriculture. While many studies have focused on the effect of management systems on soil physical and chemical properties, researchers have begun integrating biological properties, including plant roots and soil organisms, which is more sensitive to management changes in the short term (Dick, 1992; Loynachan, 2012). Soil organisms can be grouped by size: micro, meso or macro (Loynachan, 2012), based on functional guild: microfood web, litter transformers or ecosystem engineers (Kladivko and Clapperton, 2011) or based on their physiological states: active, potentially active, dormant and dead (Blagodatskayaa and Kuzyakova, 2013).

The importance of studying soil microorganism lies in their role in the decomposition of organic matter that results in the production of metabolites needed for their growth. Soil organisms produce enzymes to be able to breakdown complex substances in organic matter such as cellulose which cannot be transported across the cell membrane due to its size (Fuhrmann, 2005). Soil organic matter, and microbial biomass (Dick, 1992), are sinks for C, N, S and P (Stirling et al., 2016) which be made available to plants through the action of specific enzymes. Soil microbes also promote soil aggregation either due to hyphae formation by fungi, through the

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secretion of bacterial and fungi mucilage or due to burrowing or cast formation by earthworms (Six et al., 2004). Furthermore, the soils' ability to suppress soil-borne disease is said to be influenced by microbial diversity (Garbeva, van Veen, and van Elsas, 2004). Weller et al. (2002) describes two types of suppression: 'general suppression', which is not transferable, and is due to the total microbial biomass and 'specific suppression', a transferable type of suppression due to the effect of individual or select groups or microbes.

Early methods for studying soil microbial communities, such as classical plating, have underestimated the diversity of these communities mostly because a large portion of soil microorganisms cannot be cultured (Cavigelli, Robertson and Klug, 1995) leading to the adoption of other methods that can better study soil microorganism and their role in soil biogeochemical processes, such as nutrient cycling. Enzyme assays are used to estimate microbial activity since microbial biomass is a main source of extracellular enzymes (Fuhrmann, 2005). Enzymes involved in C cycling include β-glucosidase, N-acetylglucosaminidase, lipase, amylase, chitinase among other while amidase, amidohydrolase, urease and arylamidase are involved in N cycling (Caldwell, 2005). Other enzymes that are widely studied include phosphatase and arylsulfatase that are involved in P and S cycling. Soil enzymes have been correlated with soil organic C and N content (Dick et al., 1988) assays and can indicate the response of microbial community to changes in the environment (Caldwell, 2005).

The composition of microbial communities can be determined through the analysis of microbial lipids using either the Phospholipid Fatty Acid (PLFA) or Fatty Acid Methyl Esters (EL-FAME's) method (Cavigelli, Robertson and Klug, 1995; Schutter and Dick, 2000). Both methods have been widely used to characterize microbial communities under tropical plant diversity gradients (Carney and Matson, 2006), native forest and agricultural soils (Ibekwe and

Kennedy, 1999; Chaer et al., 2009). These methods provide information on total microbial composition as well as the identification of microbial groups (bacteria, fungi, and actinomycetes) based on specific biomarkers.

Hamido and Kpomblekou (2009) studied the effect of tillage systems and cover crops (crimson clover, black oat and a crimson clover + black oat treatment) on amidohydrolase (L-asparaginase, L-glutaminase, and urease) and arylamidase activity in tomato fields. Arylamidase activity in soil was increased when cover crop was planted, specifically in black oat (10.0 mg $\beta$-naphthylamine kg$^{-1}$ h$^{-1}$ vs 3.13 mg $\beta$-naphthylamine kg$^{-1}$ h$^{-1}$ and 7.43 mg $\beta$-naphthylamine kg$^{-1}$ h$^{-1}$ for the weed plot and a black oat + crimson clover mix, respectively) but they found possible allelopathic effects of black oat on L-asparaginase activity. Mbuthia et al. (2015) found that cover crop had the greatest effect on soil microbial biomass N and total FAME while total FAME increased with increasing N rate and the activity of C, N & P cycling enzymes ($\beta$-glucosidase, $\beta$-glucosaminidase, and phosphodiesterase) was higher in no-till plots. DNA extraction and real-time PCR of cucumber rhizosphere soil showed that N fertilizer, applied as urea, changed the composition of ammonia oxidizing bacteria, fungal and bacteria composition in a sandy loam soil (Harbin, China) though the authors mention that bacteria, fungi and ammonifiers were estimated using the most probable number method  (Zhou, Guan and Wu, 2015). Perucci et al. (1997) and Simmons and Coleman (2008) concluded that organic C is a determining factor in microbial community response to management changes.

Understanding the changes in microbial composition, size and activity would assist in choosing management practices that can better improve ecosystem services (Acosta-Martinez et al., 2010).  With this in mind, the objective of the experiment was to determine the effects of cover crops and varying N rates, applied as urea, across three sampling times on microbial

activity and community composition in a Gigger silt loam. We hypothesized that cover crops planted during winter would positively affect soil biological properties including nutrient cycling and microbial composition.

**3.2 Materials and Methods**

3.2.1 Site Description

A field experiment to evaluate the effects of cover crops and N rates on soil biological properties was established in spring 2014. The experiment was located at the LSU AgCenter's Macon Ridge Research Station in Winnsboro, Louisiana (32°09′48″N 91°43′24″W). The station has an average rainfall of 124 cm and average ambient temperature of 24° C (high) and 11° C (low). The soil is classified as a Gigger silt loam (fine-silty, mixed, thermic Typic Fragiudalfs) with <2% slope.

3.2.2 Experimental Design

Treatments consisted of eight types of cover (seven cover crops and a fallow treatment) and four N rates (0, 235, 268 and 302 kg ha$^{-1}$, applied as Urea). A field measuring 0.717 ha was divided into eight plots and a type of cover was randomly assigned to each section.The eight cover treatments consisted of cereal rye (*Secale cereale*) planted at 78.5 kg ha$^{-1}$; forage radish (*Raphanus sativus* var. *longipinnatus*) planted at 10.1 kg ha$^{-1}$, berseem clover (*Trifolium alexandrinum*) planted at 22.4 kg ha$^{-1}$; crimson clover (*Trifolium incarnatum* L) planted at 16.8 kg ha$^{-1}$; winter pea (*Pisium sativum* L) planted at 78.5 kg ha$^{-1}$; hairy vetch (*Vicia villosa* Roth) planted at 22.4 kg ha$^{-1}$and a forage radish + cereal rye mix planted at 4.5 and 72.9 kg ha$^{-1}$, respectively. A fallow treatment was included to serve as a control and consisted of native winter vegetation composed of henbit (*Lamium amplexicaule*) and ryegrass (*Lolium* sp.).

During both study years (2014 and 2015), cover crops were seeded by broadcasting in mid-October. No planting was done in the fallow treatment. The cover crops did not receive any fertilization or pest and disease control and were terminated with a mix of 2,4-D + Glyphosate at a rate of 1.17 and 2.34 liters ha-1, respectively, during the first week of February in 2014 and 2015.

In spring 2014 and 2015, each section or cover plot was further divided into 16 subplots measuring 55.6 $m^2$ each and corn (Pioneer 1319 HR) was planted at a rate 32,000 seeds $ac^{-1}$ using a John Deere MaxEmerge 2 Planter. The four N rates used in the study (0, 235, 268 and 302 kg $ha^{-1}$) was randomly assigned to each subplot and applied at planting. Four rows of corn were planted to each subplot. Agronomic management of the corn crops was based on the recommendations of the LSU AgCenter. The field design was arranged as a split plot, with a cover type as the main plot and N rates as the split plot.

3.2.3 Soil Sampling

Soil samples were collected on three occasions: the 'before cover crop' period were collected in the first week of October, 2014 and 2015 while 'after cover crop' samples were collected in February 2015 (one week after cover crop termination). At each sampling time, six samples were collected from between and within the middle two rows in each subplot from the 0-8 cm depth using a 5 cm soil probe. The samples were composited to get a representative sample and transported to the lab in a cooler. Soils were sieved to pass through a <4.75 mm mesh after which 100 g were air dried and the remainder stored in a freezer at -20 ⁰C for further analysis.

3.2.4 Soil Enzyme Assays

3.2.4.1 β-glucosidase

Sieved (<4.75 mm), air dried soil samples were used to determine β-glucosidase activity in soil, measured as µmol p-nitrophenol $kg^{-1}$ $h^{-1}$, using the method described by Tabatabai (1994). Three (two replicates and a control) 0.5 g samples were placed in separate 50 ml Erlenmeyer flask, to which 2 ml of Modified Universal Buffer pH 6 and 0.5 ml of 0.05$M$ p-Nitrophenyl- β-D-glucoside (PNG) solution was added. Controls did not receive the PNG solution. The flasks were capped incubated for one hour at 37 $^{o}$C after which 0.5 ml of 0.5$M$ $CaCl_2$ and 2 ml 0.1$M$ Tris (hydroxylmethyl) aminomethane (THAM) adjusted to pH 12 was added. Following incubation and the addition of $CaCl_2$ and THAM controls received 0.5 ml of PNG solution. The soil suspension was filtered through a Whatman no. 2 filter paper and a 200 µL aliquot was pipetted into a 96-well plate. The intensity of the yellow color filtrate was quantified at 420 nm and concentration of p-nitrophenol was determined based on a standard curve.

3.2.4.2 N-acetyl-β-D-glucosaminide (NAGase)

NAGase activity in soil, measured as µmol p-nitrophenol $kg^{-1}$ $h^{-1}$, was quantified from sieved (<0.475 mm), air dried samples using the method described by Parham and Deng (2000). Three 0.5 g samples were placed in separate 50 ml Erlenmeyer flasks (2 replicates and a control) to which 2 ml of 0.1$M$ Acetate buffer pH 5.5 and 0.5 ml of 10m$M$ p-Nitrophenyl-N-acetyl-B-D-glucosaminide (pNNAG) solution was added. Controls did not receive the pNNAG solution. The flasks were capped and placed in an incubator at 37° C for 1h after which 0.5 ml of 0.5$M$ $CaCl_2$ and 2 ml 0.1$M$ THAM pH 12 was added. Controls received 0.5 ml of pNNAG solution after the addition of 0.5$M$ $CaCl_2$ and THAM. The soil suspension was filtered through a Whatman no. 2

filter paper and a 200 μL aliquot was pipetted into a 96-well plate. The intensity of the yellow

color filtrate was quantified at 420 nm.

3.2.4.3 Arylsulfatase

Sieved air dried samples were used to estimate arylsulfatase activity in soils following a

modification of the method described by Tabatabai and Bremner (1970). Half gram of soil was

weighed into three (2 replicates and a control) separate Erlemeyer flasks and 2 ml of 0.5*M*

Acetate buffer pH 5.8 and 0.5 ml of 0.05 *M* p-Nitrophenyl sulfate solution was added to the

flasks, except the control. The flasks were mixed gently, stoppered and placed in an incubator at

37° C for 1h after which 0.5 ml of 0.5*M* $CaCl_2$ and 2 ml 0.5*M* Sodium Hydroxide was added; 0.5

ml of 0.05 *M* p-Nitrophenyl sulfate solution was then added to the control. The soil suspension

was filtered through a Whatman no.2 filter paper and 200 μL aliquot was pipetted into a 96-well

plate. The yellow coloration of the filtrate was measured with a spectrophotometer adjusted to

420 nm.

To obtain a standard curve for calculation of the activity of the three enzymes 0, 1, 2, 3,

4, and 5 ml aliquots of a diluted standard p-nitrophenol solution was pipetted into glass test tubes

and the volume adjusted to 5 ml therefore corresponding to 0, 10, 20, 30, 40 and 50 μg p-

nitrophenol. One ml 0.5*M* $CaCl_2$ and 4 ml of 0.1*M* THAM were added. The suspension was

filtered through a Whatman no. 2 filter paper and a 200 μL aliquot was pipetted into a 96-well

plate. The absorbance was quantified at 420 nm.

3.2.5 Ester-linked Fatty Acid Methyl Ester (EL-FAME) analysis

Soil microbial community composition was determined using EL-FAME profiles based

on the procedure described by Shutter and Dick (2000). Three grams of field moist sample was

extracted with 15 ml of a methylation agent (0.2*M* Potassium Hydroxide in methanol) over 1

hour incubated in a 37 °C water bath undergoing periodic mixing. After the hour, the samples were cooled to ambient temperature for 5 minutes and pH was neutralized with 3 ml of 1.0$M$ acetic acid. Following pH neutralization 3 ml of hexane was added to separate the EL-FAME's into an organic phase followed by centrifuging at 2200 rpm for 5 minutes. After centrifugation, the top organic phase was carefully transferred to clean test tube and concentrated with a flow of $N_2$ gas to evaporate the hexane. Each sample was read on a gas chromatograph with a flame ionization detector and nitrogen as the carrier gas.

The concentration of FAME (nmol g$^{-1}$ soil) were calculated using the 19:0 internal standard and relative abundance (mol%) was calculated by dividing each FAME by the total sum of all identified EL-FAMEs in a sample. Identified EL-FAMEs are named by the number of C atoms, a colon, and the number of double bonds followed by the position of the first double bond from the methyl (ω) end. Notations include Methyl (ME), cyclic (cy), *cis* (*c*), and *trans* (*t*) isomers, and iso (*i*) and anteiso (*a*) branched EL-FAMEs. Biomarkers included: *i*15:0, *a*15:0, *i*17:0, and *a*17:0 for Gram-positive bacteria; cy7:0 and cy19:0 for Gram-negative bacteria; 10Me 16:0 for Actinomycetes; 18:1 ω9c and 18:2 ω6c for saprophytic fungi; and 16:1 ω5c for arbuscular mycorrhizal fungi (AMF) (Zelles, 1999).

3.2.6 Data Analysis

Enzyme activity, total EL-FAME's and selected FAME's groups were analyzed using the PROC MIXED procedure for fixed effects in SAS 9.4 (SAS Institute, 2012). Mean separation was done using Tukey's Honest Significant Difference method at a 5% confidence level. A formal test could not be done to evaluate the main effect of cover crop and interpretation was limited to its interaction with N rate or sampling time, when significant. The means model used for the soil and corn output response variables can be described as follows

$$Y_{\text{year*N rate*row(cover)}} = \mu + \pi_{\text{sampling time}} + \alpha_{\text{cover}} + \beta_{\text{N rate}} + \pi\alpha_{\text{sampling time*cover}} + \pi\beta_{\text{sampling timer*N rate}}$$

$$+ \pi\alpha\beta_{\text{sampling time*cover*N rate}} + \rho_{\text{row/cover}} + \delta_{\text{N rate*row/cover}} + \varepsilon_{\text{sampling time*N rate*row(cover)}},$$

where:

Y = output response variable (soil and corn variables),
$\mu$ = the overall mean,
$\pi$, $\alpha$, and $\beta$ = fixed effects,
$\rho$ and $\delta$ = random effects
$\varepsilon$ = residual error

To examine soil microbial community composition principal coordinate analysis (PCoA) was performed on the relative abundances of fatty acids using the *vegan* package in R (RCore Team, 2016). Ordination plots were used to show differences in soil microbial community structure. Vectors were used to indicate maximum correlation between microbial community composition and environmental variables using the *envfit* package.

## 3.3 Results and Discussion

3.3.1 Soil Enzyme Activity

β-glucosidase hydrolyzes various β-glucosides such as cellobiose and cellulose resulting in energy sources for soil microorganisms (Kanazawa and Filip, 1986) and is considered a good indicator of changes within the soil system resulting from management practices (Bandick and Dick, 1999; Acosta-Martinez, Mikha and Vigil, 2007). β-glucosidase activity under cereal rye + forage radish, forage radish, fallow, hairy vetch and cereal rye treatments did not change during the experiment. On the other hand, crimson clover, winter pea and berseem clover (77.4, 69.8, and 101.4 mg p-nitrophenol kg$^{-1}$ soil h$^{-1}$, respectively) all had significantly higher β-glucosidase activity at spring 2015 sampling compared to fall 2014 (46 mg p-nitrophenol kg$^{-1}$ soil h$^{-1}$) and 2015 (53.4 mg p-nitrophenol kg$^{-1}$ soil h$^{-1}$) (Figure 3.1). There was no significant difference among cover crop treatments at either of the fall samplings.
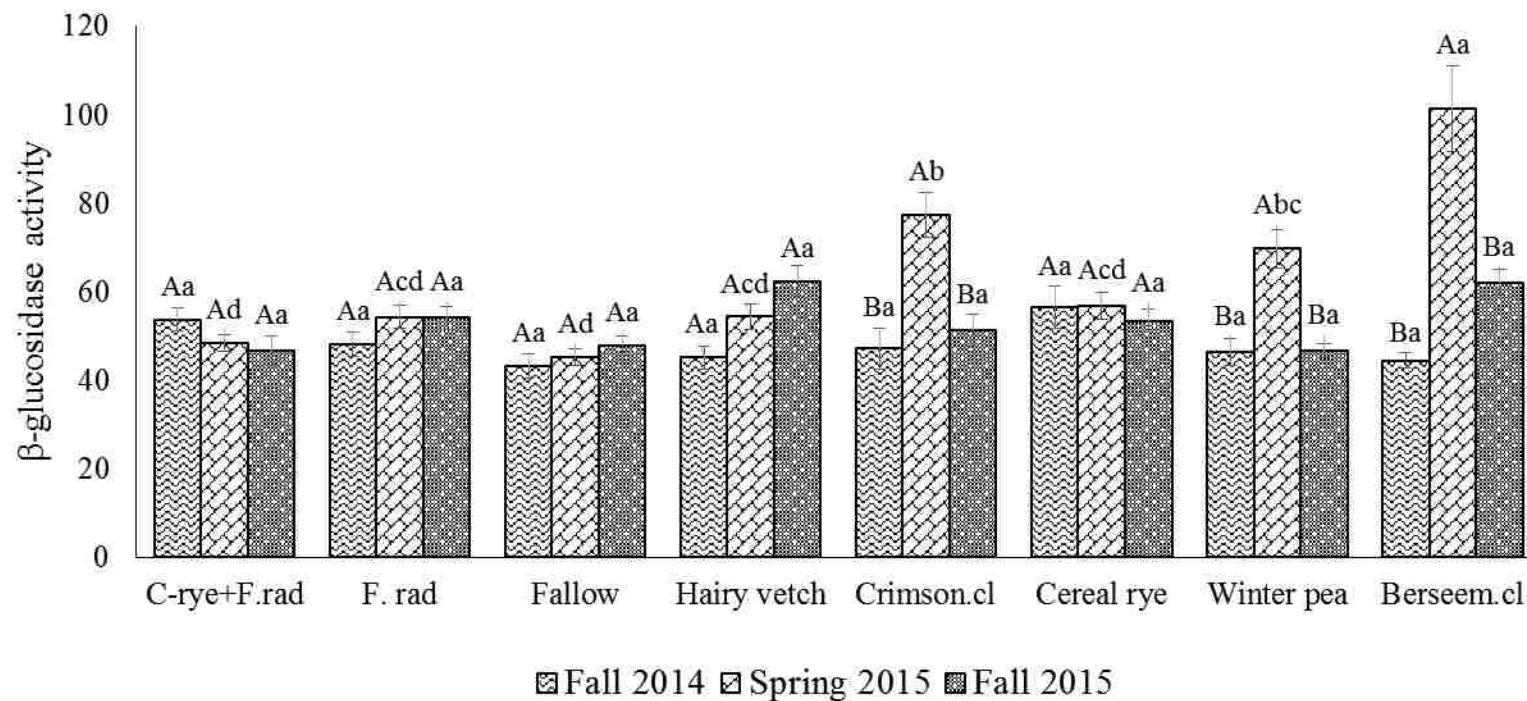
Figure 3.1. Effect of cover crop on β-glucosidase activity measured in fall 2014, spring 2015 and fall 2015. (β-glucosidase activity: mg p-nitrophenol kg-1 soil h-1; C-rye+F.rad: Cereal rye+Forage radish mix; F.rad: Forage radish; Crimson.cl: Crimson clover; Berseem.cl: Berseem clover). Same upper case letter are not significantly different (p ≤ 0.05) between sampling time within a cover crop. Same lower case letters are not significant within sampling time across cover crops (p ≤ 0.05).

Initial β-glucosidase activity measured in soil collected in fall 2014 (before cover crop) ranged from 43.2 to 56.7 mg p-nitrophenol kg$^{-1}$ soil h$^{-1}$ and was highest in the cereal rye, cereal rye+forage radish mix and lowest in the fallow plot, although not significant. β-glucosidase activity was highest in spring 2015 (after cover crop termination) and ranged from 45.2 to 101.4 mg p-nitrophenol kg$^{-1}$ soil h$^{-1}$ with three legume treatments, namely, berseem clover, crimson clover and winter pea treatments having the highest activity at 101.4, 77.4 and 69.8 mg p-nitrophenol kg$^{-1}$ soil h$^{-1}$, respectively. These values were significantly different from the fallow treatment ($p < 0.0001$).

Liang, Grossman and Shi (2014) studied microbial response to winter legume cover crops in a sandy loam (siliceous, termic Aquic Hapludults) at North Carolina and found that soil enzymes, including β-glucosidase, showed more response to cover type than termination methods and was higher in cover crop treatments compared to plots with no additional residues. Furthermore, β-glucosidase activity was more positively affected by Austrian winter pea and crimson clover than hairy vetch. Bandick and Dick (1999), also found positive response of enzyme activity to cover crops in vegetable cover crop and crop rotation plots established in Oregon on a Willamette silt loam (Pachic Ultic Argixeroll). They observed higher α- and β-glucosidase activity in soils under cover crops compared to soils under fescue or winter fallow treatments possibly due to the higher C input and increased microbial activity.

β-glucosidase activity is not only sensitive to changes in management but is also affected by temporal fluctuations (Bandick and Dick, 1999; Mendes et. al, 1999). These temporal variations can be a result of added residue or crop and climatic factors (Debosz, Rasmussen and Pedersen, 1999). In this study the temporal fluctuation of β-glucosidase activity is likely due to a combination of management and climatic factors. Even though temperatures were lower prior to

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spring 2015, higher activity was likely a result of added residue provided by the cover crop, furthermore soil moisture was lower in fall 2014 and 2015 therefore contributing to a decrease in β-glucosidase activity during this period.

NAGase activity in soil hydrolyzes N-acetyl-β-D-glucosamine (NAG) residues, found in chitin, and has been linked to C and N cycling in soils (Parham and Deng, 2000; Ekenler and Tabatabai, 2003). Results of this study indicate significantly higher NAGase activity in fall 2015 (Figure 3.2), ranging from 17.7 - 26.2 mg p-nitrophenol $kg^{-1}$ soil $h^{-1}$ with the crimson clover treatment having the greatest activity, though only different from winter pea, hairy vetch and fallow treatments.  Among the cover crop treatments, fallow and hairy vetch had similar NAGase activity in spring and fall 2015 while enzyme activity in the berseem clover plots increased steadily at different sampling time (10.4, 16.9, 21.7 mg p-nitrophenol $kg^{-1}$ soil $h^{-1}$ for fall 2014, spring 2015 and fall 2015, respectively). Ekenler and Tabatabai (2003) reported higher NAGase activity in soils under no-till/double mulch and reported significant correlation between NAGase activity and organic C content in the surface soil. β-glucosidase and NAGase activity can follow similar patterns in soil (Acosta-Martinez et al., 2007), but in this study, β-glucosidase activity was higher in spring while NAGase activity was higher in fall likely a result of changes in C and N coupled with the application of N fertilizer.

Enzymes also have a role in making organic sources of sulfur available to plant; arylsulfatase is responsible for the release of plant available $SO_2$ from sulfate esters (Hai-Ming et al., 2014). Statistical analysis showed a significant 3-way interaction ($p \leq 0.05$) between sampling time, cover crop, and N rate (Table 3.1) on arylsulfatase activity; the significant interaction was evident in berseem clover and forage radish cover crops.
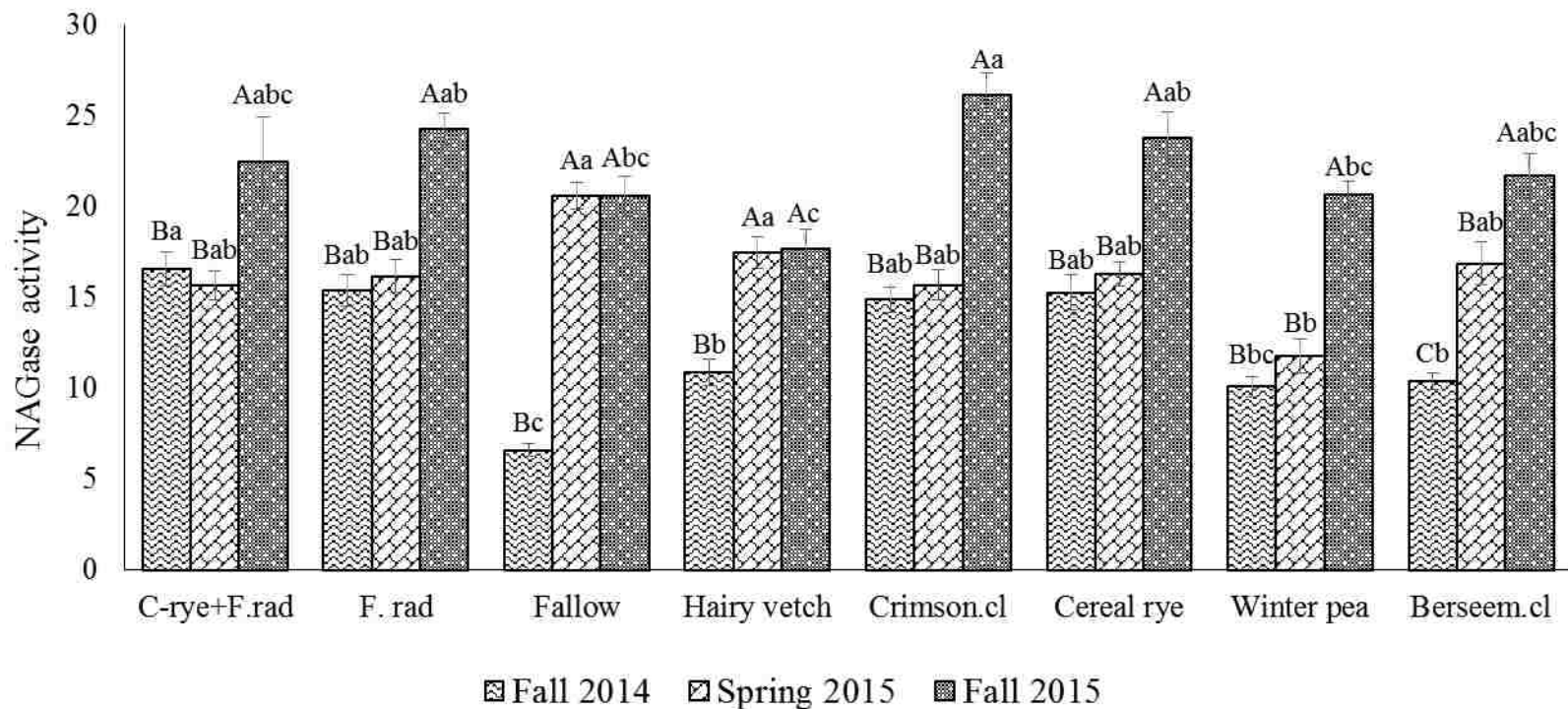
Figure 3.1. Effect of cover crop on NAGase activity measured in fall 2014, spring 2015 and fall 2015. (NAGase: N-acetyl-β-D-glucosaminide activity; NAGase activity: mg p-nitrophenol $kg^{-1}$ soil $h^{-1}$; C-rye+F.rad: Cereal rye+Forage radish mix; F.rad: Forage radish; Crimson.cl: Crimson clover; Berseem.cl: Berseem clover). Same upper case letter are not significantly different (p ≤ 0.05) between sampling time within a cover crop. Same lower case letters are not significant within sampling time across cover crops (p ≤ 0.05).

Table 3.1. ANOVA p-values for β-glucosidase, NAGase and Arylsulfatase as affected by sampling time, cover crop, N rate and their interactions.

| Source | β-glucosidase | NAGase | Arylsulfatase |
|---|---|---|---|
| Sampling time | $<.0001$ | $<.0001$ | $<.0001$ |
| Covercrop | $<.0001$ | $<.0001$ | $<.0001$ |
| Sampling time r x Cover crop | $<.0001$ | $<.0001$ | $<.0001$ |
| N rate | NS† | NS | 0.0005 |
| Sampling time x N rate | NS | NS | 0.0281 |
| Cover crop x N rate | NS | NS | NS |
| Sampling time x Cover crop x N rate | NS | NS | 0.0379 |

† Not significantly different at 0.05 level.

No changes were observed in arylsulfatase activity for berseem clover within fall 2014 and spring 2015 samples at the different N rates but comparing each N rates by sampling time showed a higher arylsulfatase activity (47 mg p-nitrophenol $kg^{-1}$ soil $h^{-1}$) at the 302 kg $ha^{-1}$ N rate in 2015 (Figure 3.3).

Arylsulfatase activity was similar across all N rates in fall 2014, but decreased at the 302 kg $ha^{-1}$ N rate after a forage radish cover crop (spring 2015). Also, when comparing between the two sampling times arylsulfatase increased from 14.4 to 45.1 mg p-nitrophenol $kg^{-1}$ soil $h^{-1}$ at the 0 N rate and from 20.6 to 59.3 mg p-nitrophenol $kg^{-1}$ soil $h^{-1}$ at 268 kg $ha^{-1}$. Since enzyme activity was comparable in fall 2014 across N rates the changes in enzyme activity could be attributed to the cover crop though the residual effect of the N rates on cover crop growth should also be considered. Knauff, Schulz and Scherer (2003) studied arylsulfatase activity in the rhizosphere of different crop species and found higher enzyme activity in soil samples that had direct contact with the roots. They suggest that higher microbial biomass in the rhizosphere was a result of increased root exudates. While arylsulfatase is both an intracellular and extracellular enzyme, Klose et al. (1999) reported extracellular enzymes accounting for 45% of the total arylsulfatase activity and 55% was associated with the microbial biomass. Residual N in the N

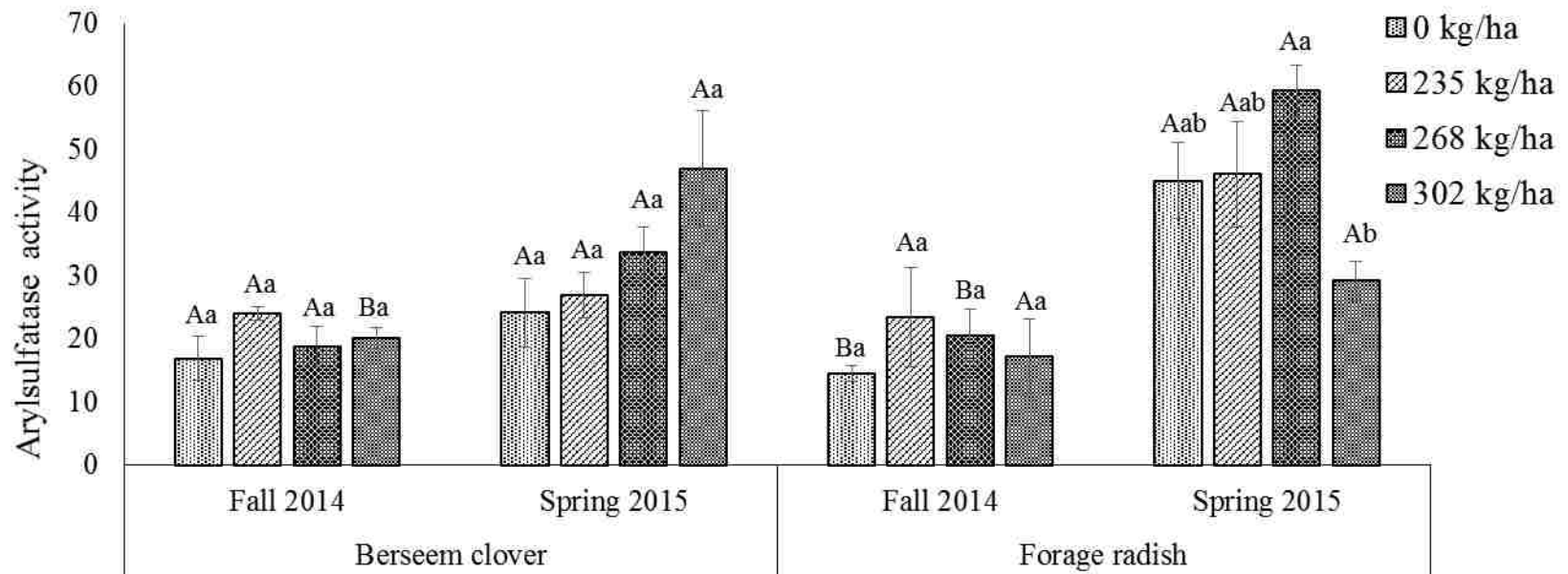Figure 3.3. Effect of Berseem clover and Forage radish at four N rates on Arylsulfatase enzyme before (fall 2014) and after cover crop (spring 2015). Arylsulfatase activity: mg p-nitrophenol kg$^{-1}$ soil h$^{-1}$. Same upper case letter are not significantly different (p ≤ 0.05) between sampling time within a cover crop or N rate. Same lower case letters are not significant (p ≤ 0.05) within year across cover crops or N rates.

treated plots could have promoted plant growth resulting in greater root expansion in both

berseem clover and forage radish resulting in more sites for arylsulfatase activity in the

rhizosphere.

Various studies have shown the effect of residue management, different tillage practices,

soil types and nutrient management on soil biochemical processes (Dick, 1992) and significant

correlation with soil C and soil N (Waldrop et al., 2000; Ekenler and Tabatabai, 2003; Mankolo

et al., 2012) under different production systems and soils. In this study enzyme activity was

positively correlated with soil C and N and $NH_4^+$-N (Table 3.2), and glucosidase showed a

higher correlation of the three enzymes evaluated.  Furthermore, glucosidase and NAGase

correlated positively with soil organic matter.

3.3.2 Microbial Community Composition

A significant interaction between cover crop and sampling time showed total FAMEs

being higher in fall 2014 and 2015 (129.60 nmol $g^{-1}$ and 149.69 nmol $g^{-1}$, respectively) and

decreasing to 105.79 nmol $g^{-1}$ in spring 2014 across cover crop treatments.  Total EL-FAME

remained at comparable values for forage radish (127.85 nmol $g^{-1}$), hairy vetch (116.32 nmol $g^{-1}$), cereal rye (140.00 nmol $g^{-1}$) and winter pea (120.98 nmol $g^{-1}$) among the three sampling times

(Figure 3.4).

Analysis of individual FAME biomarkers showed that arbuscular mycorrhiza fungi

(AMF), fungi, Gram+ bacteria, Gram- bacteria, actinomycetes and total bacteria (summation of

Gram+ bacteria, Gram- bacteria and actinomycetes) were highest during the fall (8.59, 28.16,

24.44, 14.91, 10.08, 50.43 mol %, respectively) than in spring (6.03, 20.19, 17.69, 10.09, 6.80,

34.59 mol %, respectively) except for fungi:bacteria ratio which did not change across sampling

Table 3.2. Correlations between soil chemical properties and soil enzyme activity.

| | Glucosidase | NAGase | Arylsulfatase | pH | Total C | Total N | C:N | Organic matter | $NO_3^-$-N | $NH_4^+$-N |
|---|---|---|---|---|---|---|---|---|---|---|
| Glucosidase | 1 | 0.255† | NS‡ | NS | 0.414 | 0.410 | NS | 0.173 | NS | 0.431 |
| NAGase | | 1 | NS | -0.366 | 0.297 | 0.199 | 0.207 | 0.412 | 0.229 | 0.104 |
| Arylsulfatase | | | 1 | -0.146 | 0.169 | 0.199 | NS | NS | -0.149 | 0.284 |
| pH | | | | 1 | -0.139 | -0.110 | NS | -0.392 | -0.373 | 0.107 |
| Total C | | | | | 1 | 0.878 | 0.220 | 0.391 | 0.230 | 0.349 |
| Total N | | | | | | 1 | -0.269 | 0.284 | 0.115 | 0.432 |
| C:N | | | | | | | 1 | 0.214 | 0.239 | -0.168 |
| Organic matter | | | | | | | | 1 | 0.404 | -0.110 |
| $NO_3^-$-N | | | | | | | | | 1 | -0.362 |
| $NH_4^+$-N | | | | | | | | | | 1 |

† Significant at the 0.05 level

‡ Not significant
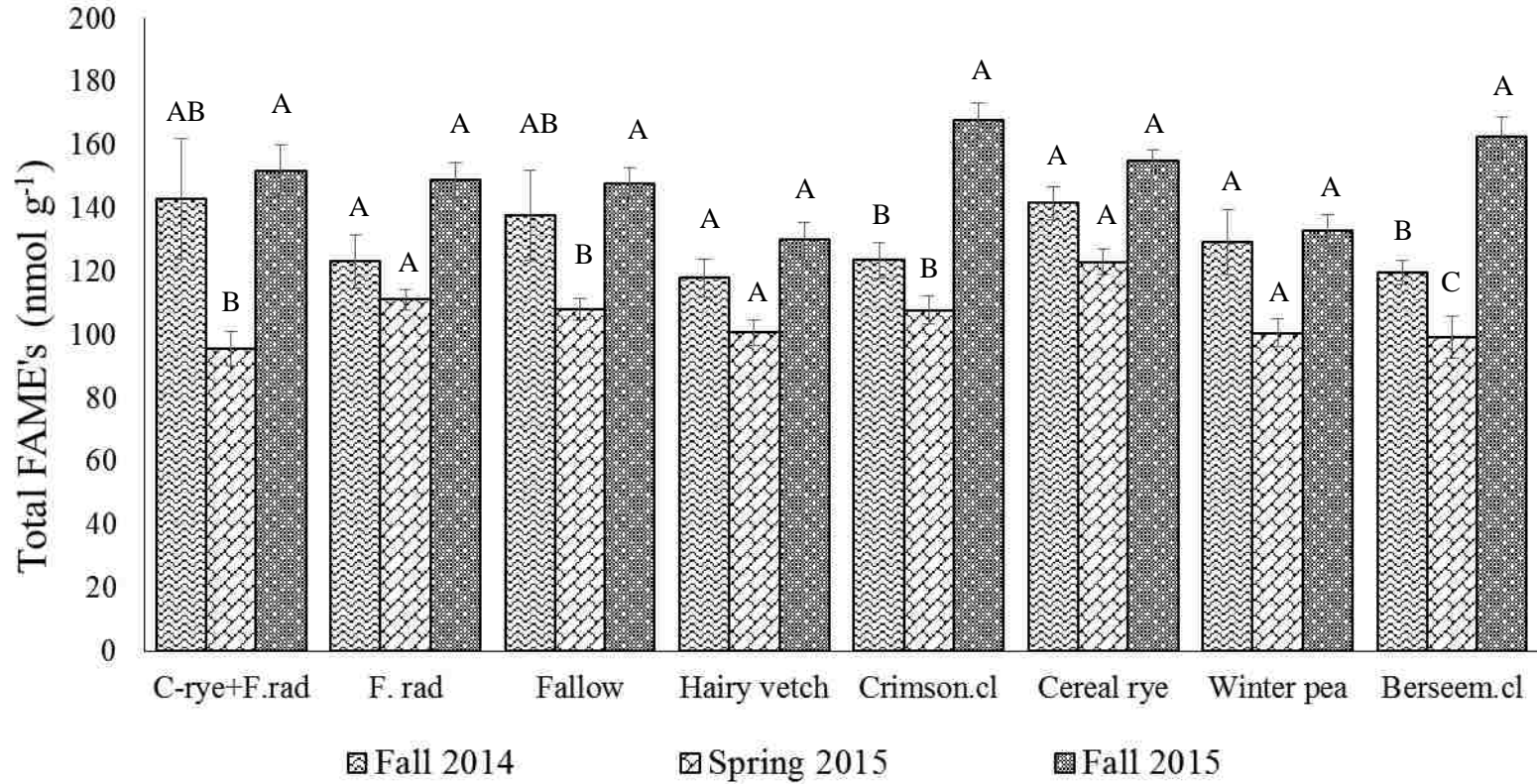
Figure 3.4. Total EL-FAME's measured by cover crop between three sampling times. Same upper case letter are not significantly different (p ≤ 0.05) between sampling time within a cover crop or N rate.

time (Table 3.3). Additionally, only AMF showed a response to N rates being significantly higher at the 0 rate (9.07 mol %) compared to the treatments receiving N (7.28 mol %).

Table 3.3. Changes in concentration of microbial groups at three sampling times.

| Variable | Fall 2014 | Spring 2015 | Fall 2015 |
|---|---|---|---|
| | ---------------------------mol %--------------------------- | | |
| Microbial Groups | | | |
| Arbuscular mycorrhiza fungi (AMF) | 8.52 A† | 6.03 B | 8.65 A |
| Fungi | 24.54 B | 20.19 C | 28.16 A |
| Actinomycetes | 8.49 B | 6.80 C | 10.08 A |
| Gram + | 21.38 B | 17.69 C | 25.44 A |
| Gram - | 11.54 B | 10.09 C | 14.91 A |
| Total bacteria | 41.42 B | 34.59 C | 50.43 A |
| Fungi:bacteria | 0.59:1 A | 0.58:1 A | 0.55:1 A |

† Same letters in a row not significantly different at $p \leq 0.05$.

The effect of cover crop, N rate and sampling time was evident on relative abundance of soil microbial community and further analysis within each sampling time showed varying response of the soil microbial community to the treatments. In fall 2014 and 2015, no differences were observed in microbial community structure using PCoA suggesting that the residual effect of cover crop management did not influence soil microbial communities after eight months.

However, microbial community structure was affected by N rate with increased AMF populations at the 0 rate and all others increasing following applications of urea (Figure 3.5). This suggests that no synthetic N input may have resulted in nutrient deficiency thereby promoting the establishment of AMF relationship with the plants and thus explaining the higher concentration of AMF at the 0 N rate. The treatments receiving varying rates of urea did not present a nutrient stressed environment and plant photosynthates were allocated to above ground biomass rather than to the formation of AMF-plant root symbiotic relationships (Mbuthia et al., 2015).

In spring 2015, PCoA showed a shift in the microbial community as a response to cover crop with soil microbial communities under leguminous covers (hairy vetch, crimson clover, winter pea and berseem clover) separating from the brassica (forage radish) and grass (cereal rye) covers (Figure 3.6). The relative abundance of Gram– bacteria, AMF and total bacteria appeared to be greater in treatments under forage radish.
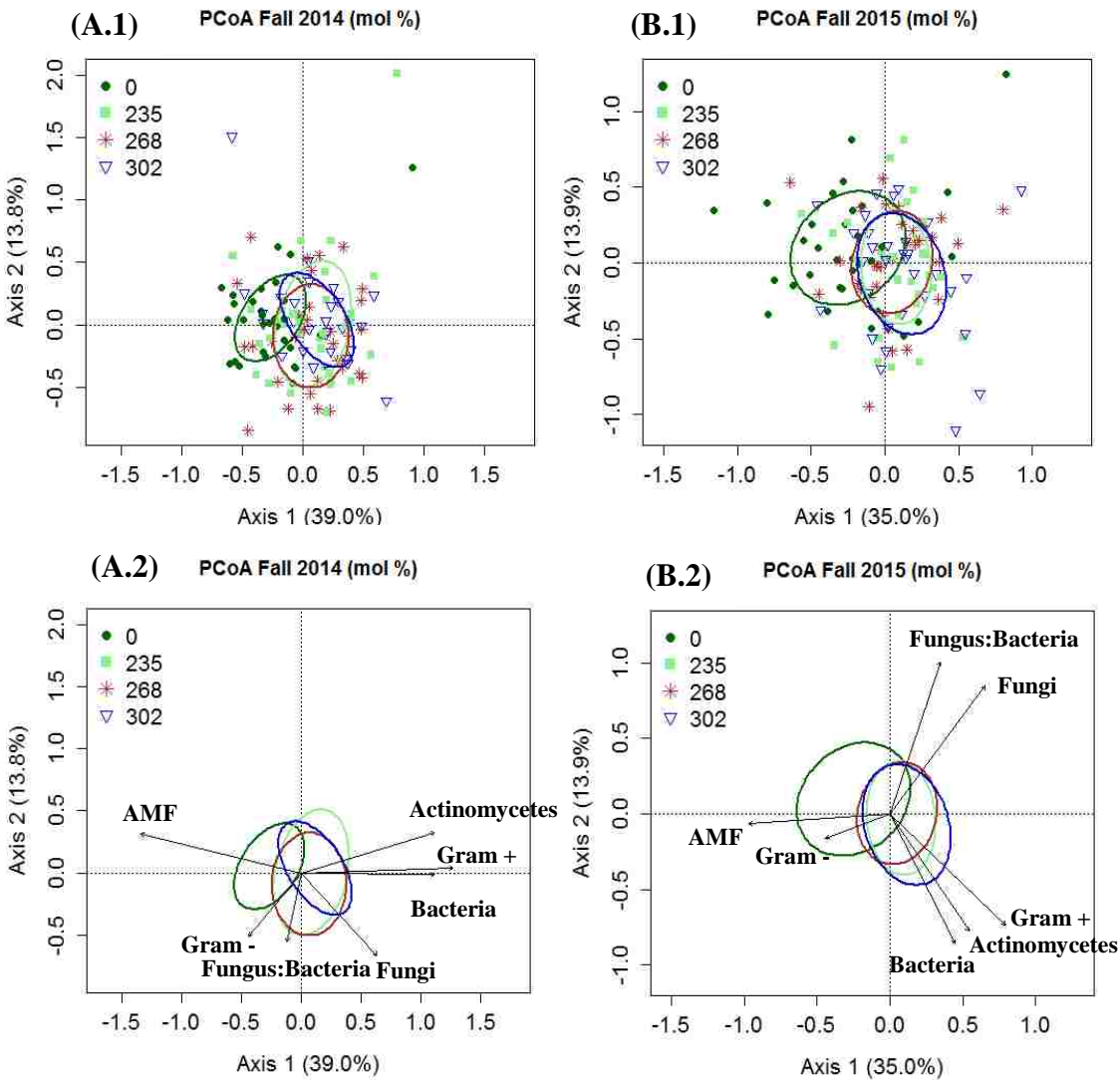


Figure 3.5. Microbial community structure according to EL-FAMEs influenced by N rates at 2014 (A.1 and A.2) and 2015 (B.1 and B.2) fall sampling.
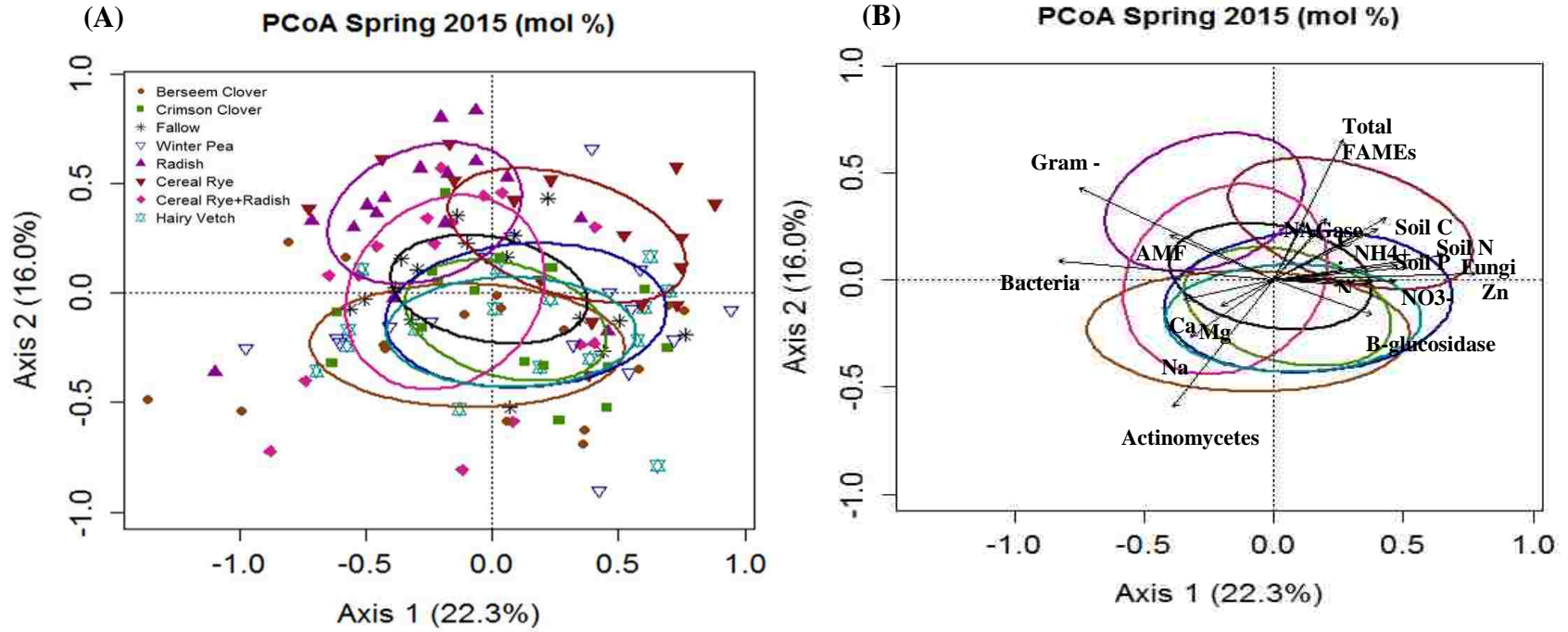
Figure 3.6. Microbial community structure according to EL-FAMEs at spring sampling (A and B).

It was interesting to find a greater relative abundance of AMF in the forage radish cover because brassicas are known to have negative effects on AMF colonization (Gavito and Miller, 1998) due to the production of isothiocyanates. White and Weil (2011) suggest that the negative effect of a forage radish cover on AMF colonization is reduced since the forage radish shoots decompose on the soil surface and isothiocyanates released during decomposition would be diffused into the atmosphere. The greater relative abundance of Gram– bacteria in the forage radish treatment could be explained by their affinity to easily degradable plant material (Hu et al., 1999). The fleshy tap root of forage radish can range from 3-6 cm in diameter and 15-30 cm in length (White and Weil, 2011) and is a prime source of easily degradable material thereby stimulating greater Gram- bacteria.

Since the lower C:N ratio of legumes, compared to the higher C:N ratio of grasses, provides for faster decomposition of organic matter (Balota et al., 2014) and microbial proliferation, we expected to find a relationship between soil C, N, and NAGase with microbial communities in legume covers rather than in the grass (cereal rye) treatment; however, the reverse was observed in this study. A possible explanation could be that sampling was done at a time when legume biomass had not started to decompose and did not provide the residues needed to influence microbial proliferation (Simmons and Coleman, 2008). Furthermore, analysis of C:N content (data not presented) of cereal rye and legumes taken one month after termination were similar, ranging from 12-19:1 and 11-16:1 for cereal rye and legumes, respectively. This would indicate that cereal rye would have similar potential like that of legumes of providing easily degradable residue to support microbial processes explaining the close relationship with soil C, N and NAGase.

## 3.4 Conclusion

The effect of cover crop and N rate on microbial activity and composition was determined by estimating enzyme activity and microbial community profiling with EL-FAME's. Carbon cycling enzyme (β-glucosidase) activity was highest in spring and legume covers (crimson clover, winter pea and berseem clover) had higher β-glucosidase activity at spring 2015 sampling compared to fall 2014 and 2015. Even though the lower temperatures contributed to a decrease in total microbial biomass during spring, the additional cover crop biomass would have provided substrate for β-glucosidase to act on. Activity of the N cycling enzyme, NAGase, was higher at fall 2015 sampling and activity in the fallow plot was comparable to winter pea and vetch. Arylsulfatase activity was the highest in spring 2015 for forage radish and berseem clover and was affected by N rates.

The activity of the enzymes assayed as well as microbial community structure were sensitive to management practices and time of sampling suggesting that they can provide information on the impact of that these practices may have on C, N and S cycling and ecosystem function and health. These results show that cover crops and N rates affected soil biological components, but other factors such as C:N ratio, time of termination and the carryover effect of previous management influenced microbial activity and composition.

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## Chapter 4. Conclusions

The increasing adoption of cover crops as a winter fallow rotation in row crop agriculture systems and their proven benefits on soil properties led to the establishment of this study. A vast amount of research has been conducted in corn cropping systems in the north of the U.S but little is known about the performance and/or benefits of cover crops in the Mid-South where mild winters and wet springs prevail. With this in mind, we set out to evaluate whether different types of winter cover crops would affect the yield of corn planted under different N rates. Contrary to what was hypothesized, corn yield decreased by 20% after a winter cover crop. Cover crops had C:N ratios on average of <15:1 and soil C:N did not exceed 10:1; furthermore, soil C and N, and $NH_4^+$-N was highest in spring after the cover crop and 6 weeks before corn planting. These soil chemical parameters were significantly and positively correlated with the activity of C and/or N cycling soil enzymes. These conditions would have resulted in mineralization and a subsequent increase in nutrients for the summer corn crop, but instead, a yield decrease was obtained. This leads to the conclusion that while cover crops were able to cycle nutrients both climate and time of cover crop termination are key factors that must be considered for Mid-South production systems. A shorter wait period between cover crop termination and corn planting would likely ensure that released nutrients are utilized by corn plants rather than being lost from the soil due to rainfall.

Soil microbial community, determined through the analysis of extracted EL-FAMEs, responded to both cover crops and N rates, but treatment effects were influenced by time of sampling. A distinct separation in community structure could not be established in the fall samples, indicating that cover crops did not drive microbial communities; any residual effect that cover crops may have had on fall microbial communities was lost during the 8 months between

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sampling in 2015. Interestingly though, there was a greater abundance of AMF in the 0 N plots at both fall samplings likely the result of established symbiotic relationships between corn plants and AMF as a response to the nutrient deficient conditions in the 0 N plots. The same was not noticeable in spring indicating that nutrient deficiency was abated in the 0 N plots by either the nutrient scavenging/cycling and/or fixation ability of cover crop species or that cover crop nutrient demand was lower than corn. Microbial communities grouped separately in spring depending on whether it was a legume, grass or brassica covers and the majority of soil chemical variables, including enzyme activity, had a higher relationship with legumes and grasses.

The results of this study show that even though crop productivity decreased, the question as to whether cover crops were advantageous to the system should not be answered only based on productivity variables but should include the impact on ecosystem services which have a long term effect. Furthermore, it supports the notion that sustainable management strategies such as the use of cover crops should not be taken as a 'one size fit all' approach because they are easily influenced by soil properties and climatic and environmental factors. Overall, the cover crops did not outperform the fallow treatment suggesting that by just keeping the ground covered provided benefits to the system. However, the vegetative composition of the fallow should not be ignored since certain species considered weeds in the Mid-South, such as the rye grass that dominated our fallow treatment, have responded well as cover crops in other areas.

Further research is required to better evaluate the benefits of cover crops in the Mid-South under different soils and with different termination dates and over a longer time frame so that variability can be reduced and well-informed recommendations can be made.

**Vita**

Ina Iris Sanchez was born in San Ignacio, Belize. She attended EARTH University in Costa Rica, where she obtained her Bachelor of Science Degree in Agricultural Sciences in December 2010. Upon completion, she returned to Belize and began working with the Ministry of Agriculture promoting urban agriculture and later in areas related to research and development. In August 2014 she was admitted into the Master of Science program in the School of Plant, Environmental and Soil Sciences at Louisiana State University Agricultural and Mechanical College, and she also obtained a 2 year study leave from the Ministry of Agriculture to be able to work towards her degree. Upon completion of her degree she will return to Belize to resume her duties with the Ministry of Agriculture.