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# COVER CROPS AND NITROGEN RATES IMPACT ON SOIL CHEMICAL AND BIOLOGICAL PROPERTIES IN LOUISIANA NO-TILL CORN PRODUCTION

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**Abstract**

Use of cover cropping systems to improve soil health is still limited in Louisiana. This study aimed to examine the interaction between cover crops and nitrogen (N) fertilizers rates on crop yield, soil chemical and biological properties. Winter cover crops, including legumes, a grass & a brassica, and a fallow control, were combined with N fertilizer application at four rates (0, 90, 179, 269 kg N ha-1) in continuous corn production as part of a no-till system. Soil samples were collected at 0-8 cm before and after cover crop termination in 2017 and 2018. Soil nutrients, organic matter, inorganic N, microbial community composition, and soil enzymes were analyzed. Legumes increased corn grain yield overall and maximized yield at 90 kg N ha-1 compared to grass & brassica treatments which maximized corn grain yield at 179 kg N ha-1. Regardless of cover crop type, nitrogen fertilizer applications increased soil organic matter by 8% compared to no nitrogen applications. The concentrations of soil phosphorous from legume was 19% higher than the grass & brassica treatment, while grass & brassica had a greater soil potassium concentration than legume. Cover crops and N applications improved soil enzymes for carbon and N cycling. Nitrogen rates applied for the main crop promoted microbial biomass in spring soil sampling. Arbuscular mycorrhizal fungi were greatest in the grass & brassica treatment and when no N was applied. Overall, the incorporation of winter legumes could reduce N fertilizer input, sustain corn production, and benefit soil health.

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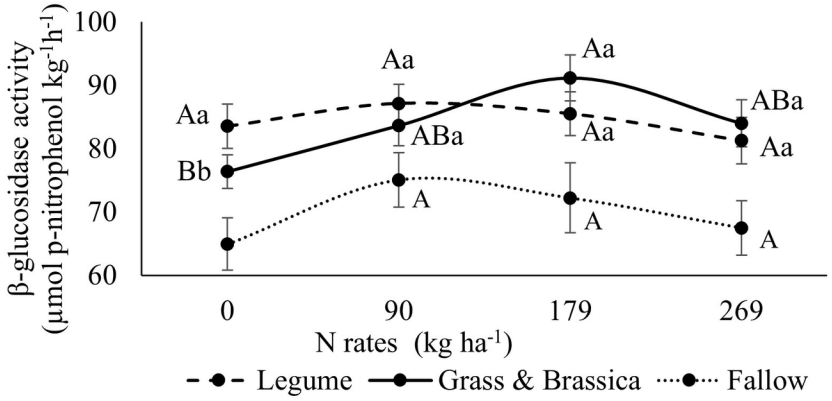


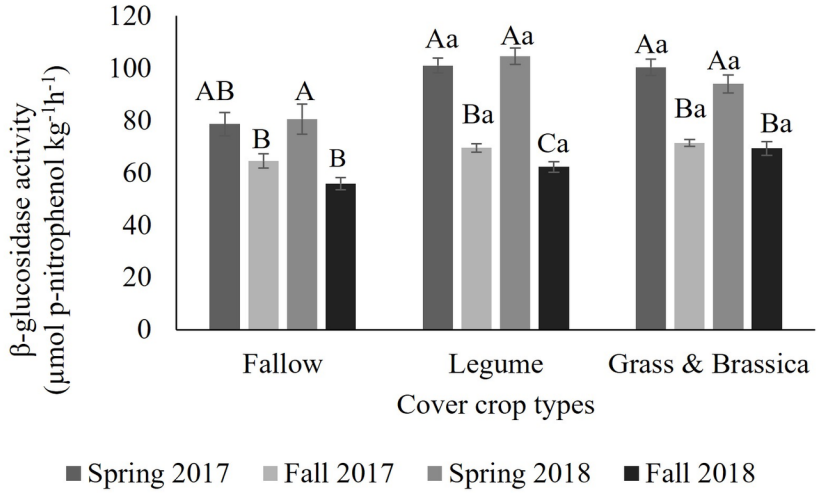


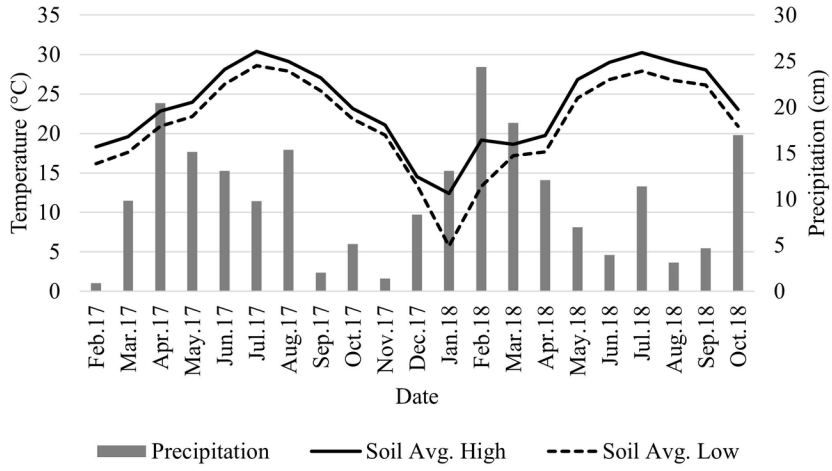

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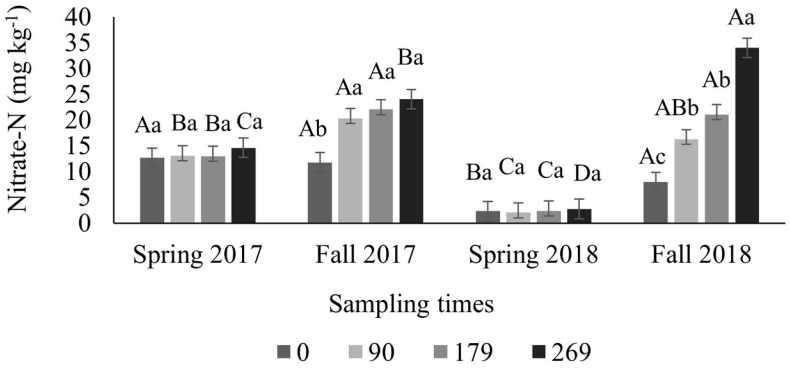


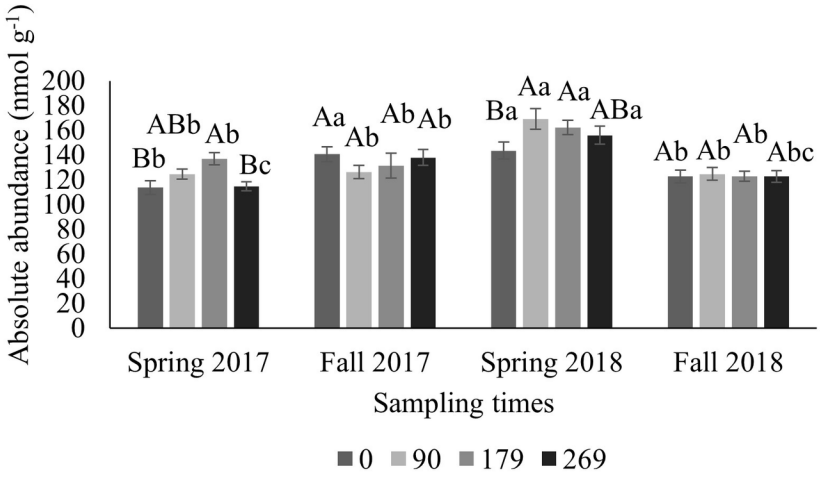


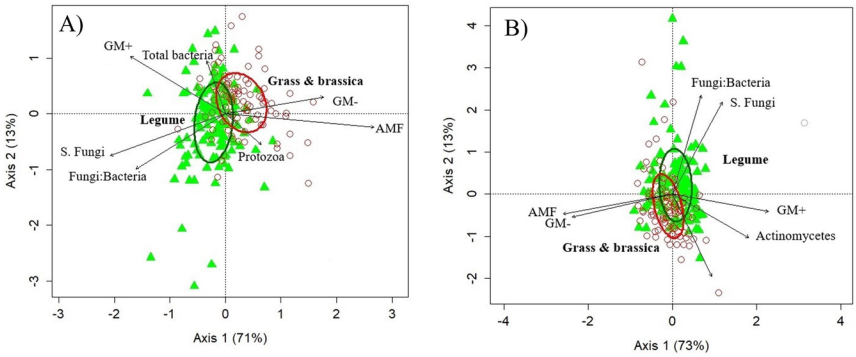

# COVER CROPS AND NITROGEN RATES IMPACT ON SOIL CHEMICAL AND BIOLOGICAL PROPERTIES IN LOUISIANA NO-TILL CORN PRODUCTION

## Core ideas

- Legumes increased corn grain yield and reduced EONR
- Legumes promoted soil nitrate-N and P while grass & brassica had a greater K
- Cover crops increased β-glucosidase and NAGase enzyme activities


## ABSTRACT

Use of cover cropping systems to improve soil health is still limited in Louisiana. This study aimed to examine the interaction between cover crops and nitrogen (N) fertilizers rates on crop yield, soil chemical and biological properties. Winter cover crops, including legumes, a grass & a brassica, and a fallow control, were combined with N fertilizer application at four rates (0, 90, 179, 269 kg N $ha^{-1}$) in continuous corn production as part of a no-till system. Soil samples were collected at 0-8 cm before and after cover crop termination in 2017 and 2018. Soil nutrients, organic matter, inorganic N, microbial community composition, and soil enzymes were analyzed. Legumes increased corn grain yield overall and maximized yield at 90 kg N $ha^{-1}$ compared to grass & brassica treatments which maximized corn grain yield at 179 kg N $ha^{-1}$. Regardless of cover crop type, nitrogen fertilizer applications increased soil organic matter by 8% compared to no nitrogen applications. The concentrations of soil phosphorous from legume was 19% higher than the grass & brassica treatment, while grass & brassica had a greater soil potassium concentration than legume. Cover crops and N applications improved soil enzymes for carbon and N cycling. Nitrogen rates applied for the main crop promoted microbial biomass in spring soil sampling. Arbuscular mycorrhizal fungi were greatest in the grass & brassica

23 treatment and when no N was applied. Overall, the incorporation of winter legumes could reduce
24 N fertilizer input, sustain corn production, and benefit soil health.
25 **Abbreviations:** AMF, arbuscular mycorrhizal fungi; Gram-, Gram negative bacteria; Gram+,
26 Gram positive bacteria; NAGase, N-acetyl-β-D-glucosaminidase
27

## 1. INTRODUCTION

In 2020, corn (*Zea may*s L.) was one of the major cash crops across the U.S. (37,231,079 hectares) and in Louisiana (234,718 hectares) (USDA-NASS, 2020). Corn is widely used for grain and ethanol production, and macro-and micronutrients are needed to achieve optimal production, particularly nitrogen (N), often the most limiting nutrient. In general, N fertilizer recommendations in the Mid-South ranged from 135 to 235 kg N $ha^{-1}$ depending on soil texture (LSUAgCenter, 2019). In conventional corn farming practices, high N fertilizer usage caused land degradation, in turn, leading farmers to need high N fertilizer inputs to maintain high crop production. Consequently, this increases farming costs, and causes environmental issues, such as eutrophication which contributes to the dead zone in the Gulf of Mexico (Rabalais et al., 2002).

Adaptation of conservation agriculture practices, such as no-till and cover crops has been recommended in many states across the US (Dabney et al., 2010; Mbuthia et al., 2015; Mitchell et al., 2017). A no-till system increases plant residues remaining on the soil surface after crop harvest and enhances water infiltration (Govaerts et al., 2007; Mitchell et al., 2017; Nouri et al., 2019). Cover crops have been used for many decades to prevent soil erosion. They provide a wide range of benefits to the soil ecosystem, including enriching the biological, chemical, and physical properties, in particular contributing N, diversified soil biota, and improved aggregate stability (Adetunji et al., 2020; Alvarez et al., 2017; Dabney et al., 2001; Langdale et al., 1991; Ryu et al., 2010). Hence, combining cover crops and no-till could bring a myriad of advantages, including enhanced yield and soil nutritional, biological, and biochemical soil properties (Mitchell et al., 2017; Mullen et al., 1998; Sanchez et al., 2019a). For example, Chen and Weil (2011) found that under a no-till system in cool to temperate, humid climates, a mixture of forage radish (*Raphanus sativus* var. *longipinnatus* ) and cereal rye (*Secale cereale*) was the most

practical and advantageous cover crop before summer crops because it alleviated soil compaction and increased maize yield. Several studies examined the interaction of cover cropping, tillage system, and fertilizer input to maintain or improve soil productivity and increase crop yield. Mullen et al. (1998) found that a hairy vetch (*Vicia villosa* Roth) grown under zero-tillage in corn production promoted significant organic carbon (C) accumulation without and with N fertilizer addition (0 and 168 kg N $ha^{-1}$), and the cover crop increased bacterial population and β-glucosidase activity under no N input. However, the N rate at 168 kg N $ha^{-1}$ increased enzyme activities in the wheat (*Triticum aestivum* L.) treatment.

Another study indicated that agricultural conservation practices in reduced tillage, cover crops, and fertilizer application rates were associated with microbial biomass and activities related to soil health and cotton production in west Tennessee (Mbuthia et al., 2015). In this study, hairy vetch significantly increased β-glucosaminidase (NAGase) activity while mycorrhizal fungi were decreased relative to wheat and no cover crop and with increased N fertilizer applications. The results suggest that cover crops in long-term no-till significantly shift microbial communities and activities in favor of C, N, and phosphorus (P) cycling and improved yield (Mbuthia et al., 2015). Liang et al. (2014) reported that Austrian winter pea (*Pisum sativum*) could enhance soil productivity during organic transition management. Moreover, planting potatoes after forage radish and winter pea increased potato yield while reducing N fertilizer need (Emad et al., 2017). In a no-till system of corn/soybean productions, winter cover crops increased soil organic matter to 30 cm in depth in Illinois (Villamil et al., 2006). Additionally, the use of winter cereal rye as a cover crop reduced soil erosion by 11 to 29 % without affecting crop yield during a 45-year period (Basche et al., 2016).

The living organisms in soils help break down organic residues and mineralize nutrients into

the soil (Kuehn et al., 2000). Fungi are essential in degrading some complex compounds in dead plant materials, including cellulose, hemicellulose, and lignin, by secreting extracellular enzymes to catalyze those recalcitrant compounds (Ahmed et al., 2009; Baldrian & Valášková, 2008; Purahong et al., 2016). Labile soil C inputs could alter the soil microbial community structure and regulate decomposition more than recalcitrant C compounds (De Graaff et al., 2010). Cover crop residues increased fatty acid methyl esters (FAMEs) diversity and shifted microbial community composition (Schutter et al., 2001). The significant factors that influenced microbial community structure were season, soil type, and soil physical and chemical properties (Schutter et al., 2001).

Soil enzymes are the mediators of organic matter decomposition and soil nutrient transformations. Soil β-glucosidase and NAGase enzymes are good indicators for soil health because they are engaged in soil nutrient cycling, in particular C and N (Bandick & Dick, 1999; Makoi & Ndakidemi, 2008) and can be used to measure the C and N demand of microbes in the soil (Sinsabaugh & Moorhead, 1994). β-glucosidase activity, a soil C cycling indicator of cellulose degradation, was sensitive and quick to respond to changes in soil management practices and could be used as an early indicator of biological changes (Bandick & Dick, 1999). The β-glucosidase activity was affected by cover cropping and N fertilizer application which linked to C substrate sources and N demand during litter decomposition activities to allow microorganisms access to energy and nutrients (Allison & Vitousek, 2005; Averill & Finzi, 2011; Sinsabaugh, 1994).

In Louisiana, the effects of cover crops have been reported since 1990 and primarily focused on planting crimson clover, hairy vetch, and wheat species to reduce soil erosion, minimize N fertilizer, add soil organic matter, and maintain the main crop yield (Boquet & Coco, 1993;

Boquet & Coco, 1991; Hutchinson et al., 1993). The recent research from Sanchez et al. (2019a) and Sanchez et al. (2019b) demonstrated that leguminous cover crops could reduce the N fertilizer application for corn production under a no-till system compared to non-legumes in Louisiana. Soil C concentration increased under no-till with cover crop inclusion, and non-legume cover crop increased soil potassium (K), sulfur (S), calcium (Ca), and magnesium (Mg) availability and promoted higher microbial biomass and all FAME markers, with the exception of saprophytic fungi, which was enriched under the legume. Also, all soil C, N, and S cycling enzyme activities were increased after the use of cover crops in a no-till system. However, N fertilizer application reduced AMF populations and P concentrations. One limitation of this study was the narrow difference between high N fertilizer rates (0, 235, 268 and 302 kg N $ha^{-1}$) which prompted the continuation of the study with a more representative range of fertilizer application rates.

Although there have been studies on the cover crops in the Mid-South, the information regarding growing cover crops and N fertilizer in the crop production systems in a humid subtropical climate is limited. Updating and investigating new information will help producers make decisions to maintain and enhance long-term soil fertility and biological activity, thus it is imperative to study the effects of cover crops in the Mid-South into current agricultural systems. Our study's goal was to examine the interaction of cover crops and N rates on corn yield, soil biological properties, nutrient cycling, and microbial composition in a conservation tillage corn production system in Louisiana.

## 2. MATERIALS AND METHODS

### 2.1 Site description

The two-year study was conducted at the Louisiana State University AgCenter Macon Ridge Research Station, Winnsboro, Louisiana (32°0′94″N 91°43′24″W) in 2017 and 2018. The soil type was a Gigger-Gilbert silt loam (fine-silty, mixed, thermic Typic Fragiudalfs) which received an average rainfall of 216.5 cm. High and low soil temperatures were 30°C and 6°C, respectively in 2017 and 2018 (Figure 1).

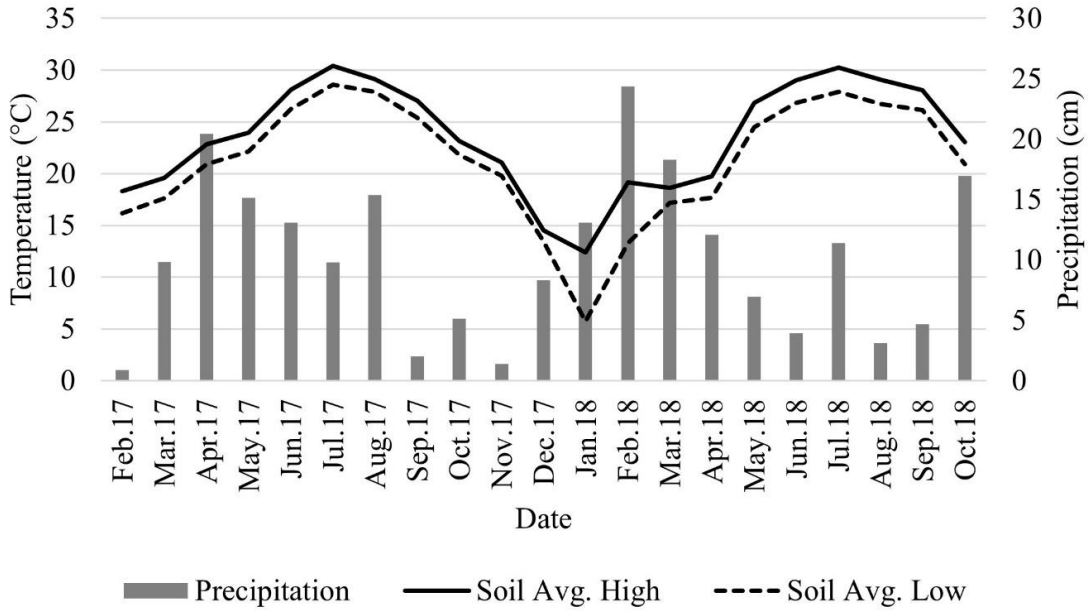


Figure 1. Average monthly high and low temperatures and total monthly precipitation from February 2017 through October 2018. * Due to equipment failure data from Jan.18, Feb.18 and Mar.18 was obtained from the Dean Lee Research Station, Alexandria, LA.

## 2.2 Experimental design

The research design was described in detail in Sanchez et al. (2019a); however, changes in the N fertilizer rate were initiated in 2017. Briefly, the experimental design was a split-plot with a randomized complete block design with subplots for a total of 12 treatments and four replications. This trial was conducted in a no-tillage field of 0.72 ha. There were two types of covers as the main plot consisting of legumes and non-legumes (grass & brassica). Legume treatment included four monoculture legume cover crops [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 44.8 kg $ha^{-1}$, and hairy vetch (*Vicia villosa* Roth) planted at 22.4 kg $ha^{-1}$]. For the grass & brassica treatment, there were three grasses and a brassica [cereal rye (*Secale cereale*) planted at 78.5 kg $ha^{-1}$, forage radish (*Raphanus sativus* var. *longipinnatus*) planted at 10.1 kg $ha^{-1}$, and forage radish and cereal rye mix planted at 4.5 and 72.9 kg $ha^{-1}$]. A fallow treatment was used as a control. In the fallow plot native winter weeds, primarily henbit (*Lamium amplexicaule* L.) and ryegrass (*Lolium* spp.), were allowed to grow with no mechanical nor chemical control. Cover crop treatments were divided into 16 subplots (4 m x 13.7 m) to which four N fertilizer rates of 0, 90, 179 and 269 kg N $ha^{-1}$ were randomly applied as urea (46-0-0) by hand at planting. Triple superphosphate (0-46-0) and potassium chloride (0-0-60) were applied at 67.3 kg $ha^{-1}$ rate for P and K fertilizer, respectively. All fertilizers were applied only for corn crop. Cover crops were seeded into each plot in mid-October after harvesting corn in 2017 and 2018 by broadcast seeding using a Gandy 10T-series drop spreader (Gandy Company, Owatonna, MN). Cover crops were grown over the winter without any further fertilizer, pesticide, or herbicide application until termination in February. They were terminated by application of 2,4-D at a rate of 0.5 kg ai $ha^{-1}$ and glyphosate (Roundup) at a rate of 1.5 kg ai $ha^{-1}$ prior to corn seeding 6 weeks after termination. Corn was seeded by Pioneer 1329HR at the rate of 79,040 plants $ha^{-1}$ and using a John Deere MaxEmerge 2 planter (John Deere Manufacturing Co., Moline, IL).

Corn grain yield was recorded following harvest in September of 2017 and 2018 using a Kincaid 8-XP (Kincaid Equipment, Haven, KS) plot combine. The corn grains were harvested in each sub-plot from the two middle rows. A small sample from each sub-plot was used to determine grain moisture immediately following harvest using a Dickey-John Grain Moisture

Meter (Dickey-John Corp., Auburn, IL). Corn grain moisture was used to adjust grain yields to 15.5 g $kg^{-1}$.

**2.3 Soil sampling**

Treatment effects on soil nutrient parameters, soil biological properties, and microbial composition were determined by collecting soil samples after corn grain harvest in October and after cover crop termination in February of each year. Soil samples were collected at 0-8 cm soil depth using a 5 cm diameter soil probe. Six samples were collected from each sub-plot. After collection, samples were sieved to <4.75 mm, air-dried at room temperature for five days, and used for analysis including soil nutrient concentrations, inorganic N, and enzyme activities. Field moist soils were kept in the freezer at -20 °C and used for the analysis of soil moisture, soil organic matter, and microbial community composition.

**2.4 Soil chemical properties analysis**

Soil samples were analyzed for soil pH, soil organic matter, total C and total N, soil extractable P and K, and inorganic N including nitrate-N ($NO_3^-$-N) and ammonium-N ($NH_4^+$-N). Soil pH was measured in deionized water at a 1:1 ratio. Soil organic matter was determined by the weight loss-on-ignition method as described in Nelson and Sommers (1996). Briefly, five grams of field-moist soil was oven-dried at 105 °C for 18 hours and weighted after cooling in a desiccator. After cooling, the soil samples were transferred to a muffle furnace at 400 °C for 24-hour (Barthès et al., 2004) for ignition. Following combustion, sample weight was recorded again for the determination of mass loss-on-ignition. Total C and total N were measured using the dry combustion method by LECO CN Analyzer (St. Joseph, MI). Mehlich-III extractable nutrients (P and K) were measured via Inductively Coupled Plasma (Lexington, KY). Inorganic N ($NO_3^-$-N and $NH_4^+$-N) was determined following the method of Mulvaney (1996). Briefly, one

gram of air-dried soil was extracted using 10 mL of 2 *M* potassium chloride (KCl) and shaken for one hour. Samples were filtered through Whatman 42 filter paper and the filtrate was analyzed by colorimetric analysis using the microplate method (Hood-Nowotny et al., 2010).

**2.5 Soil enzyme assays**

Potential β-glucosidase activity for C cycling and β-glucosaminidase (N-acetyl-b-D-glucosaminidase or NAGase) activity for C and N cycling in soil samples, were measured in mg p-nitrophenol $kg^{-1}$ $h^{-1}$. Soil β-glucosidase analysis was conducted using the method described by Tabatabai (1994), while NAGase was assessed using the method described by Parham and Deng (2000). Each sample had a duplicated sample and a control. Briefly, the air-dried samples were mixed with a buffer solution and substrate specific to each enzyme and incubated for 1-hr at 37 °C. Following incubation, a buffer and flocculant were added, along with the substrate to the control, before filtering through a Whatman No.2 filter paper. The filtrate was analyzed according to color change using an EON spectrophotometer (Bio Tek, Vermont).

**2.6 Ester-linked Fatty Acid Methyl Ester (EL-FAME) analysis**

Soil microbial community composition was determined using EL-FAME profiles (FAMEs) following Schutter and Dick (2000). Extraction was done by adding 15 mL of 0.2 M potassium hydroxide in methanol to three grams of field-moist soil. Samples were then placed in a 37 °C water bath for 1-hr with mixing every 15 minutes. The pH was adjusted to neutral with 3 mL of 1.0 *M* acetic acid followed by the addition of 3 mL of hexane, and centrifugation at 2200 rpm for 5 minutes. The organic phase was transferred into a clean test tube and concentrated using $N_2$ gas to evaporate the hexane. Fatty acids in the soil samples were analyzed by gas chromatography (Agilent 7890B) using a fused silica capillary column and flame ionization detector using hydrogen for carrier gas, with temperatures ramped from 190 to 250°C at 5°C per minute

201 followed by a ramp to 300°C for 2 min to clear the column. The concentration of FAMEs (nmol
202 $g^{-1}$ soil) was determined using a 19:0 internal standard for calculation. Relative abundance
203 (mol%) was calculated based on the total FAMEs extracted. The MIDI (Microbial ID, Inc)
204 library was used to identify the FAMEs. FAMEs are identified based on the number of C atoms,
205 and number of double bonds when present, and the position of the first double bond from the
206 methyl (w) end of the molecule. The branched EL-FAMEs included Methyl (Me), cyclic (cy), *cis*
207 (c), and *trans* (*t*) isomers, and iso (*i*) and anteiso (*a*). Biomarker indicators included: Gram
208 negative bacteria (Gram-) using cy17:0, cy19:0, 16:1ω7, 16:1ω9c, 18:1ω5c, 18:1ω7c, and
209 19:1ω6c; Gram positive bacteria (Gram +) using i14:0, *i*15:0, *a*15:0, i16:0, a16:0, *i*17:0, *a*17:0
210 and 18:0; saprophytic fungi using 18.1 w9c, 18:2 w6c, 18:3ω6,9,12c and 20:1 ω 9c;
211 actinomycetes using 10Me 16:0, 10Me17:0 and 10Me18:0; arbuscular mycorrhizal fungi (AMF)
212 using 16:1 w5c; and protozoa using 20:3 w6,9,12c and 20:4 ω6,9,12,15c (Frostegård & Bååth,
213 1996; Madan et al., 2002; Pennanen et al., 1996; Zak et al., 1996; Zelles, 1997; Zogg et al.,
214 1997).

215 **2.7 Data analysis**

216 The experimental design was a split-plot with four replications. The main plot was two types
217 of cover crops (legumes and grass & brassicas) with four N fertilizer rates as the sub-plot. Corn
218 grain yield, soil chemical parameters, and soil biological properties were analyzed by SAS 9.4
219 software (SAS institute, 2015) using the PROC MIXED procedure for fixed effect. Comparison
220 of mean was done by Tukey's Honest Significant Difference method at a 5% confidence level.
221 Because the fallow treatment (control) was not replicated throughout the field, it was not used for
222 statistical comparison among cover crop treatments. However, statistical analysis within the

fallow treatment was done over time and between N fertilizer application rates, and it was used for qualitative comparison.

The quadratic-plateau statistical model was fitted to data using R-Studio (version 3.5.1, R-Core Team, 2018). The models were done on corn grain yield across years and fertilizer N rates to calculate the economically optimum N rate (EONR) for each cover crop treatment. The EONR was described as the N application rate where $1 of additional N fertilizer returned $1 in grain yield. The assumptions for this analysis were that all costs were fixed and only N fertilizer was the variable cost (Colwell, 1994). The ratio of the cost of N fertilizer ($0.212 $kg^{-1}$) to the price of corn grain ($0.144 $kg^{-1}$) was 1.47 (CP). An equation was used to calculate the EONR as

$$\mathrm{EONR} = \frac{\mathrm{CP}-\mathrm{b}}{2\mathrm{c}}$$

where b and c are the linear and quadratic coefficients from the quadratic equation, respectively.

Principal coordinate analysis (PCoA) was used to analyze soil microbial community structure across three factors, including cover crop types (legume and grass & brassica), N rates (0, 90, 179 and 269 kg N $ha^{-1}$) and soil sampling seasons (fall and spring). The PCoA was determined by relative abundances of FAMEs using the *vegan* package (Oksanen, 2018) in RStudio (version 3.5.1, R-Core Team, 2018). Of two points, the greater distance indicated a greater dissimilarity between microbial communities. The *envfit* function was used to create vectors that indicated the correlation between microbial community composition and environmental parameters, in this case identified microbial groups. The angles between vectors are indicative of the correlations between the microbial community groups. The smaller shape angles show a positive correlation while the angles greater than 90° are negative (Calderon et al., 2016).

## 3. RESULTS

### 3.1 Corn grain yield response

The analysis of variance for corn grain yield indicated that there were significant interactions between N fertilizer rate and cover crop (*P=0.0121*), N fertilizer rate and sampling time (*P<0.0001*), and cover crop and sampling time (*P=0.0436*). Corn grain yield increased with the additions of N fertilizer following both types of cover crops (Table 1). The greatest yield was

Table 1. Interaction of N rate, cover crop type, and sampling year on corn grain yield. Standard error in parentheses.

| | N fertilizer rate (kg N ha$^{-1}$) | | | |
|---|---|---|---|---|
| | 0 | 90 | 179 | 269 |
| | Corn grain yield (Mg ha$^{-1}$) | | | |
| **Cover crop type** | | | | |
| Legume | 3.9 (0.26) A†b‡ | 8.3 (0.35) Aa | 9.3 (0.37) Aa | 9.1 (0.42) Aa |
| Grass & Brassica | 2.4 (0.33) Bc | 6.9 (0.24) Bb | 8.8 (0.38) Aa | 9.0 (0.43) Aa |
| Fallow | 2.0 (0.28) c | 6.5 (0.46) b | 8.4 (0.75) a | 8.4 (0.69) a |
| **Sampling year** | | | | |
| 2017 | 3.3 (0.38) A†c‡ | 8.8 (0.31) Ab | 10.6 (0.25) Aa | 10.6 (0.34) Aa |
| 2018 | 3.0 (0.26) Ac | 6.4 (0.18) Bb | 7.5 (0.21) Bab | 7.6 (0.28) Ba |

| | Cover crop type | | |
|---|---|---|---|
| **Sampling year** | Legume | Grass & Brassica | Fallow |
| | Corn grain yield (Mg ha$^{-1}$) | | |
| 2017 | 9.0 (0.39) Aa | 7.7 (0.53) Ab | 7.4 (0.9) |
| 2018 | 6.4 (0.26) Ba | 5.9 (0.33) Bb | 5.2 (0.6) |

†Same uppercase letters are not significant (α = 0.05) between cover crop treatments or sampling year. ‡Same lower letters are not significant (α = 0.05) across N rates with cover crop treatments. Fallow values are provided for quantitative comparison only and were used in statistical analysis within N rates.

found in corn grown following leguminous cover crops at a fertilizer rate of 90 kg N ha$^{-1}$, which increased corn production by 128% relative to 0 kg N ha$^{-1}$ treatment. Corn planted following the grass & brassica treatment maximized yield at a fertilizer rate of 179 kg N ha$^{-1}$, with a 271% increase compared to 0 kg N ha$^{-1}$. Increasing fertilization to 269 kg N ha$^{-1}$ did not promote greater yield in either cover crop type. When no N fertilizer was applied, corn planted after no cover crops produced the lowest yield (2 Mg ha$^{-1}$) which was similar to the grass & brassica treatment (2.4 Mg ha$^{-1}$). As N fertilizer rates increased in the fallow treatment, corn production increased with the highest yield observed at 179 kg N ha$^{-1}$ (8.4 Mg ha$^{-1}$). Average corn grain yield at all N fertilizer rates across this two-year experiment significantly decreased in 2018

except for 0 kg N $ha^{-1}$ (Table 1). Corn grain yield under legume and grass & brassica treatments also experienced significant losses, 29% and 23%, respectively, in the second year (Table 1).

Using quadratic-plateau regression to estimate corn grain yield response to N fertilizer rates, legumes had the lowest (149 kg N $ha^{-1}$) economic optimum N rate (EONR) followed by the grass & brassica (184 kg N $ha^{-1}$), and control (193 kg N $ha^{-1}$) (Table 2). Following legumes, 151 kg N $ha^{-1}$ (328 kg urea $ha^{-1}$) was needed to achieve the maximum yield (9.2 Mg $ha^{-1}$) while under grass & brassica, 207 kg N $ha^{-1}$ (450 kg urea $ha^{-1}$) was required to reach the highest yield, 8.9 Mg $ha^{-1}$ (Table 2).

Table 2 Corn yield response parameters† at economic optimum nitrogen (N) fertilizer rate (EONR) for each cover crop treatment as predicted by the quadratic-plateau regression model

| Cover crops | a | b | c | N rate at the plateau | EONR | Yield at plateau |
|---|---|---|---|---|---|---|
| | | Mg $ha^{-1}$ | | kg N $ha^{-1}$ | kg N $ha^{-1}$ | Mg $ha^{-1}$ |
| Legume | 3.92 | 0.06987 | -0.00023 | 151 | 149 | 9.23 |
| Grass &brassica | 2.39 | 0.06356 | -0.000169 | 207 | 184 | 8.31 |
| Fallow | 1.95 | 0.06569 | -0.000167 | 197 | 193 | 8.43 |

† a, b, c are intercept, linear coefficient and quadratic coefficient, respectively

**3.2 Treatment effects on soil chemical properties**

There was an interaction between sampling time and cover crop type (*P=0.0144*) and sampling time and N fertilizer rate (*P=0.0013*) on soil pH. Following increased N rates, soil pH decreased from 6.1 where no fertilizer was applied to 5.7 where fertilizer was applied at 90 kg N $ha^{-1}$, and it continued to decrease to 5.5 at 269 kg N $ha^{-1}$ (Table 3). Moreover, compared to grass & brassica, and fallow treatments, the legume treatment had lower soil pH over time. Regardless of cover crop or N rate treatment, soil pH was consistently higher in the spring samplings compared to the fall (Table 3).

Soil organic matter was affected by N fertilizer rate (*P<0.0001*) and sampling time (*P<0.0001*) but not cover crop treatments (*P=0.5230*). Nitrogen fertilizer input of 90 kg N $ha^{-1}$

Table 3. Interaction of N rate, cover crop type, and sampling year on soil pH. Standard error in parentheses.

| | Sampling time | | | | |
|---|---|---|---|---|---|
| | Spring 2017 | Fall 2017 | Spring 2018 | Fall 2018 | Average |
| **Cover crop type** | | | | | |
| Legume | 5.8 (0.04) A†b‡ | 5.3 (0.04) Bc | 6.2 (0.08) Aa | 5.3 (0.06) Bc | 5.6 (0.04) B |
| Grass & Brassica | 6.0 (0.05) Aa | 5.5 (0.07) Ac | 6.2 (0.06) Aa | 5.7 (0.08) Ac | 5.9 (0.04) A |
| Fallow | 6.1 (0.05) a | 5.6 (0.06) b | 6.3 (0.13) a | 5.6 (0.14) b | 5.9 (0.06) |
| **N Rate** (kg N $ha^{-1}$) | | | | | |
| 0 | 6.1 (0.06) Ab | 5.7 (0.07) Ac | 6.4 (0.08) Aa | 6.0 (0.07) Ba | 6.1 (0.04) A |
| 90 | 5.8 (0.05) Ab | 5.4 (0.07) ABc | 6.2 (0.11) Aa | 5.5 (0.08) Cb | 5.7 (0.04) B |
| 179 | 5.9 (0.06) Ab | 5.3 (0.07) Bc | 6.2 (0.10) Aa | 5.4 (0.08) Cbc | 5.7 (0.05) B |
| 269 | 5.8 (0.06) Ab | 5.1 (0.07) Bc | 6.0 (0.13) Ba | 5.1 (0.08) Cc | 5.5 (0.06) C |

†Same uppercase letters are not significant (α = 0.05) between cover crop treatments or sampling year. ‡Same lower letters are not significant (α = 0.05) across sampling time with cover crop treatments or N rates. Fallow values are provided for quantitative comparison and were used in statistical analysis across sampling times.

increased soil organic matter by 8% (26.9 to 29.1 g $kg^{-1}$) compared to no N fertilizer application. Furthermore, soil organic matter concentration was boosted over time from 26.9 g $kg^{-1}$ in spring 2017 to 30.2 in fall 2018, an increase of 12%. At the same time, compared to the 0 kg N $ha^{-1}$, the 90 kg N $ha^{-1}$ fertilizer application also increased total carbon (*P<0.0001*) by 12% (11.5 to 12.9 g $kg^{-1}$) and total N increased (*P<0.0001*) by 7% (1.4 to 1.5 g $kg^{-1}$) at 179 kg N $ha^{-1}$.

Extractable $NH_4^+$-N was not affected by the application of N fertilizer (*P =0.2841*) or cover crop type (*P=0.3532*), but it was influenced by sampling time (*P<0.0001*). In spring 2017, extractable $NH_4^+$-N concentration was highest (9.0 mg $kg^{-1}$) before decreasing to 5.6 mg $kg^{-1}$ in fall 2017 and remained unchanged in spring 2018 (5.4 mg $kg^{-1}$). A significant increase was measured again in fall 2018 (6.5 mg $kg^{-1}$). In contrast, extractable $NO_3^-$-N responded to cover crop types (*P*=0.0099). Moreover, there was an interaction between N fertilizer rate and sampling time (*P<0.0001*) on extractable $NO_3^-$-N. Leguminous cover crops averaged 17% greater $NO_3^-$-N level than the grass & brassica treatment (15.0 and 12.6 mg $kg^{-1}$ respectively). Soil $NO_3^-$-N levels were greater in the fall sampling, following the corn harvest, compared to spring after cover crop termination in both years (Figure 2). In fall samples, $NO_3^-$-N was greater

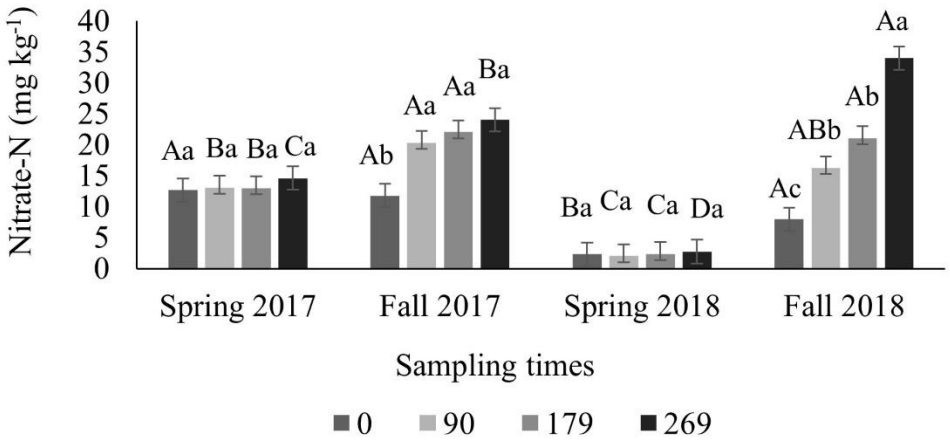


Figure 2. Soil nitrate-N concentration under different nitrogen fertilizer rates at different sampling times. Error bars represent standard error. Same uppercase letters are not significant ($\alpha = 0.05$) within an N rate across sampling times. Same lowercase letters are not significant ($\alpha = 0.05$) between N rates within a sampling time.

following N fertilizer application rates higher than 90 kg N $ha^{-1}$ for both years while there was no difference in spring between N fertilizer application rates (Figure 2). In fall 2018, $NO_3^-$-N reached the highest concentration following 269 kg N $ha^{-1}$ compared to no N fertilizer application, with more than 4 times (8.0 to 34.0 mg $kg^{-1}$) the $NO_3$-N (Figure 2). In fall 2017, the concentration of $NO_3^-$-N was 88% greater in the 269 kg N $ha^{-1}$ treatment compared to no N application (Figure 2). However, compared to all other sampling times, $NO_3^-$-N was substantially less in spring 2018 following cover crops for all N rates (Figure 2).

Different cover crop types, N rates, and sampling times affected soil macronutrient concentrations. Increasing N rates resulted in significantly decreased concentrations of soil extractable P (*P<0.0001*), and K (*P=0.0014*). The legume treatment had 19% higher concentrations of soil extractable P than grass & brassica (Table 4). In contrast, the grass &

Table 4. The effect of cover crop treatments, N fertilizer rates, and soil sampling times on soil extractable phosphorus and potassium concentrations.

| Parameters | Phosphorus | Potassium |
|---|---|---|
| Cover crop type (CC) | | mg $kg^{-1}$ |
| Legume | 33.9 (1.2) A[†] | 183.9 (2.0) B |
| Grass & Brassica | 28.0 (1.1) B | 208.4 (3.5) A |
| Fallow | 29.4 (1.7) | 196.1 (5.0) |
| N fertilizer rates (N) | | |
| 0 | 39.0 (1.6) A | 207.4 (3.7) A |
| 90 | 27.0 (1.4) B | 191.1 (3.5) B |
| 179 | 28.8 (1.7) B | 196.2 (4.2) B |
| 269 | 29.0 (1.6) B | 189.9 (4.1) B |
| Sampling times (S) | | |
| Spring 2017 | 34.0 (1.7) A | 195.7 (2.8) B |
| Fall 2017 | 29.2 (1.5) A | 213.6 (4.6) A |
| Spring 2018 | 29.8 (1.6) A | 185.4 (3.7) C |
| Fall 2018 | 30.8 (1.7) A | 189.8 (4.0) BC |
| ANOVA | | *P value* |
| Cover crop type | *0.0002* | *<0.0001* |
| Nitrogen rate | *<0.0001* | *0.0014* |
| Sampling time | *0.1317* | *<0.0001* |
| Cover crop type x Nitrogen rate | *0.3930* | *0.7433* |
| Sampling time x Nitrogen rate | *0.9998* | *0.9442* |
| Cover crop x Sampling Time | *0.9615* | *0.0815* |
| Cover crop type x Nitrogen rate x Sampling time | *0.9960* | *0.9765* |

[†]Same uppercase letters are not significant ($\alpha = 0.05$) between cover crop treatments, N fertilizer rates, or sampling times.

brassica treatment had greater K concentrations than legume species by 13% (Table 4). In addition, extractable K was also influenced by sampling times (*P<0.0001*). From the data, the fall samplings following corn harvest tended to have higher concentrations of soil extractable K than spring samplings following cover crop termination. In particular, fall 2017 had the greatest concentration of soil K at 213.6 mg $kg^{-1}$ which was 8% greater than spring 2017 and 11% greater than fall 2018.

### 3.3 Soil biological properties

#### 3.3.1 Soil enzyme activity response as affected by cover crops, N input, and sampling time

Soil β-glucosidase for soil C enzyme activity was influenced by the interaction between cover crop type and N fertilization application (*P=0.0554*) and cover crop type and sampling times (*P=0.0064*). At 0 kg N $ha^{-1}$, β-glucosidase activity was higher in the legume treatment than in the grass & brassica by 9% but did not differ at the other N rates (Figure 3). Following grass

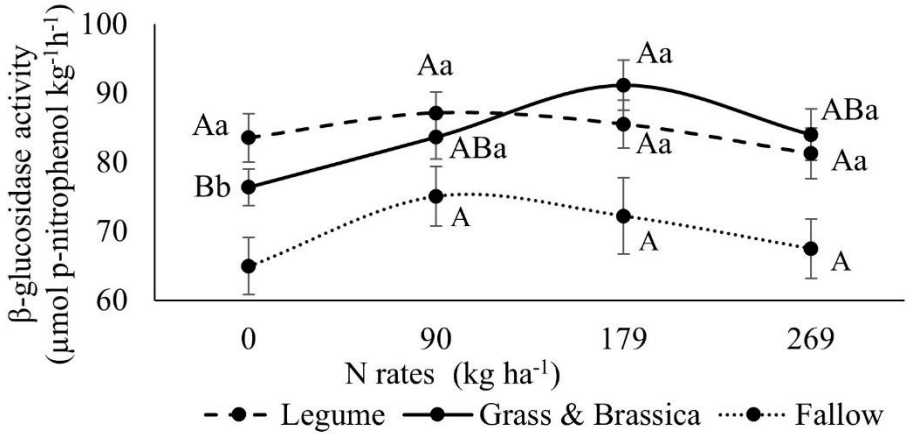


Figure 3. Potential enzyme activity of soil β-glucosidase under different cover crop types and nitrogen fertilizer rates. Error bars represent standard error. Same uppercase letters are not significant (α = 0.05) between N rates. Same lowercase letters are not significant (α = 0.05) between cover crop treatments. Fallow values are provided for quantitative comparison between cover crop treatments and was used in statistical analysis only within N rates.

& brassicas, the input of N fertilizer at 179 kg N $ha^{-1}$ resulted in 18% greater C enzyme activity relative to no N fertilizer input. However, enzyme activity under legume cover crops or fallow treatments did not respond to N inputs (Figure 3). In both cover crop types, β-glucosidase was high in spring and low in fall, with a difference of about 38% (Figure 4). A similar trend was measured in the control treatment.

NAGase activity did respond to different types of cover crops (*P=0.0011*) and sampling times (*P<0.0001*) whereas it did not respond to N fertilizer application (*P=0.6582*). The grass & brassica cover crop treatment demonstrated greater NAGase activity than legume cover crops, 27.7 and 25.8 μmol p-nitrophenol $kg^{-1}$ $h^{-1}$, respectively. NAGase activity was 24.5 μmol p-

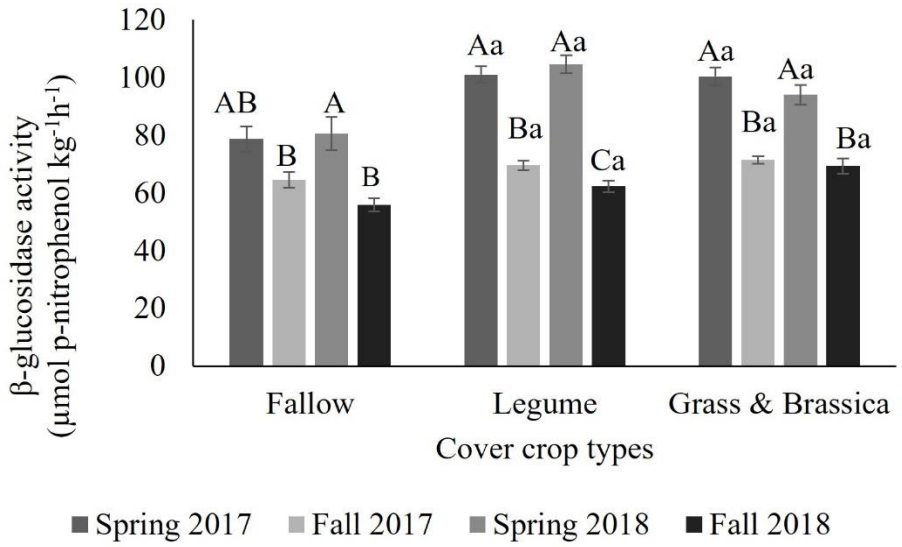


Figure 4. Potential enzyme activity of soil β-glucosidase under different cover crop types at different sampling times. Error bars represent standard error. Same uppercase letters are not significant (α = 0.05) within a cover crop type across sampling times. Same lowercase letters are not significant (α = 0.05) between sampling times across legume and grass & brassica types.

nitrophenol $kg^{-1}$ $h^{-1}$ in spring 2017 and decreased to 22.2 μmol p-nitrophenol $kg^{-1}$ $h^{-1}$ in fall 2017. In spring 2018, NAGase activity decreased by 58%, from 35.0 to 25.3 μmol p-nitrophenol $kg^{-1}$ $h^{-1}$ in fall 2018.

### 3.3.2 Changes in microbial community composition

In this experiment, we found that the interaction of cover crops and sampling time impacted AMF absolute abundance (*P<0.0001*, Table 5). The grass & brassica treatment had greater in absolute abundance of AMF than leguminous species at all sampling times except fall 2018 (data not shown). Microbial biomass (estimated from total FAMEs) was impacted by the interaction between N fertilizer rates and sampling time (*P=0.0432*). In the spring of both years, total microbial abundance tended to increase with addition of N fertilizer while there was no response in either fall samplings (Figure 5). In spring 2017, the amount of total microbial biomass was greatest at 179 kg N $ha^{-1}$ and in spring 2018, the greatest microbial abundance was measured at

Table 5. Absolute abundance of fatty acid methyl esters (FAMEs) from soil samples collected from different cover crop types, N fertilizer rates, and sampling times from 2017 to 2018.

| | Total FAMEs | Gram+ bacteria | Gram- bacteria | AMF | Actino-mycetes | Saprophytic fungi | Total bacteria | Protozoa | Fungi: Bacteria |
|---|---|---|---|---|---|---|---|---|---|
| Cover crop types (CC) | | | | | Absolute abundance (nmol $g^{-1}$) | | | | |
| Legume | 131.9 (2) † | 25.0 (0.4) | 14.0 (0.3)b | 5.8 (0.2) b | 4.9 (0.2) | 28.0 (0.8) | 43.9 (0.7)b | 1.8 (0. 06) | 0.64 (0.01)a |
| Grass & Brassica | 136.9 (3) | 25.2 (0.4) | 16.1 (0.4) a | 8.2 (0.3) a | 4.5 (0.2) | 26.2 (0.6) | 46.3 (0.8)a | 1.6 (0. 08) | 0.57 (0.01)b |
| Fallow | 124.4 (4) | 22.9 (0.7) | 13.8 (0.4) | 6.5 (0.5) | 4.7 (0.3) | 26.8 (1.5) | 41.4 (1.3) | 1.7 (0. 10) | 0.65 (0.04) |
| N rates (kg $ha^{-1}$) (N) | | | | | | | | | |
| 0 | 130.2 (3)a | 22.7 (0.5)b | 15.9 (0.5)a | 8.7 (0.4) a | 4.5 (0.2)a | 25.1 (0.9)b | 43.1 (1.0)a | 1.8 (0. 14) a | 0.58 (0.02)a |
| 90 | 136.2 (3)a | 25.3 (0.6)a | 15.1 (0.4)a | 7.1 (0.4)b | 4.9 (0.2)a | 28.2 (1.3)a | 45.3 (1.0)a | 1.8 (0. 07) a | 0.63 (0.02)a |
| 179 | 138.4 (4)a | 26.5 (0.7)a | 15.2 (0.5)a | 6.7 (0.3)b | 5.2 (0.3)a | 28.0 (1.1)a | 46.9 (1.3)a | 1.7 (0. 06)a | 0.60 (0.02)a |
| 269 | 132.9 (3)a | 25.9 (0.6)a | 13.8 (0.4)b | 5.6 (0.3)c | 5.3 (0.2)a | 27.2 (0.8)ab | 45.0 (1.1)a | 1.6 (0. 07)a | 0.60 (0.01)a |
| Sampling times (S) | | | | | | | | | |
| Spring 17 | 122.5 (2)c | 23.3 (0.5)c | 15.1 (0.4)b | 5.2 (0.2)c | 3.8 (0.2)b | 24.0 (0.7)c | 42.1 (0.8)bc | 1.3 (0. 03)c | 0.57 (0.01)a |
| Fall 17 | 134.2 (4)b | 25.3 (0.8)b | 14.2 (0.4)b | 7.0 (0.3)b | 5.5 (0.2)a | 27.9 (1.1)b | 45.0 (1.3)b | 2.0 (0. 10)a | 0.63 (0.02)a |
| Spring 18 | 157.7 (4)a | 27.8 (0.6)a | 18.9 (0.5)a | 9.6 (0.5)a | 5.3 (0.3)a | 31.6 (1.3)a | 51.9(1.2)a | 2.0 (0. 13)a | 0.61 (0.02)a |
| Fall 18 | 123.3 (2)c | 24.0 (0.5)bc | 12.0 (0.3)c | 6.2 (0.2)b | 5.4 (0.2)a | 24.9 (0.8)c | 41.3 (0.8)c | 1.6 (0. 06)b | 0.61 (0.02)a |
| ANOVA | | | | | *P value* | | | | |
| CC | *0.1134* | *0.7559* | *<0.0001* | *<0.0001* | *0.7900* | *0.0899* | *0.0262* | *0.0851* | *<0.0001* |
| N | *0.2606* | *<0.0001* | *0.0008* | *<0.0001* | *0.0583* | *0.1160* | *0.0906* | *0.1864* | *0.4057* |
| S | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *0.1731* |
| CC x N | *0.3960* | *0.3771* | *0.7033* | *0.5241* | *0.3976* | *0.1154* | *0.6604* | *0.2602* | *0.1267* |
| CC x S | *0.6657* | *0.8961* | *0.0713* | *<0.0001* | *0.7941* | *0.5284* | *0.7198* | *0.6734* | *0.5472* |
| N x S | *0.0432* | *0.1300* | *0.0188* | *0.0004* | *0.1201* | *0.1332* | *0.1685* | *0.5928* | *0.3235* |
| CC x N x S | *0.6657* | *0.6928* | *0.9504* | *0.3592* | *0.8582* | *0.5658* | *0.8557* | *0.6163* | *0.5057* |

†Same lowercase letters are not significant (at $\alpha = 0.05$) between cover crop treatments or N rates or sampling times. (Gram+ bacteria= Gram positive bacteria; Gram- bacteria = Gram negative bacteria; AMF= Arbuscular mycorrhizal fungi; Fungi:Bacteria= Saprophytic fungi to total bacteria.

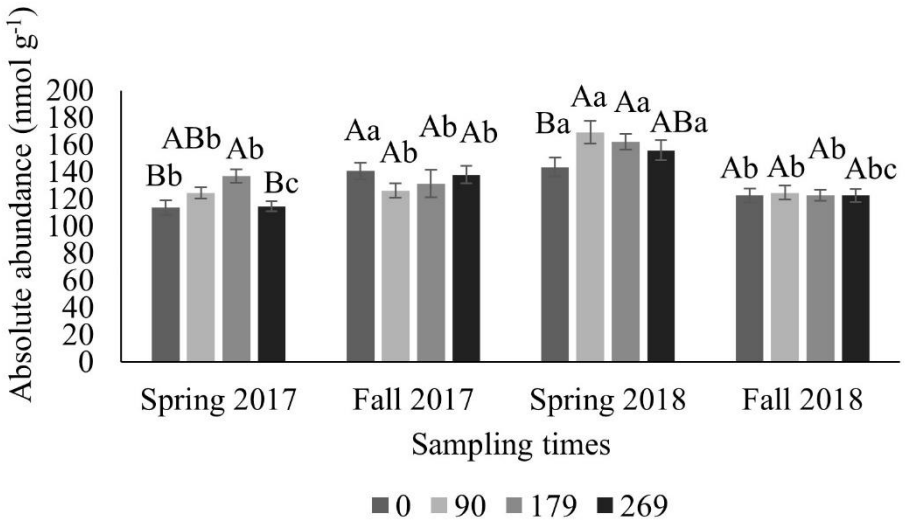


Figure 5. Total microbial abundance according to N rate and sampling time. Error bars represent standard error. Same uppercase letters are not significant ($\alpha = 0.05$) within a sampling time. Same lowercase letters are not significant ($\alpha = 0.05$) within N rates across sampling times.

341

342 90 kg N $ha^{-1}$ but was not different from the total microbial abundance at 179 and 269 kg N $ha^{-1}$

343 (Figure 5).

344 Principal coordinate analysis using the relative abundance of extracted FAMEs was

345 conducted to determine the microbial community structure differences between types of cover

346 crops, N fertilizer rates, and sampling seasons. Seventy-two fatty acids present in soil samples

347 were used to observe the difference in the structure of the microbial community within the three

348 main factors. This analysis revealed that seasons (fall and spring seasons) had the greatest impact

349 on variation; therefore, to determine the effect of cover crop types (Figure 6) and N fertilizer

350 rates (Figure 7), PCoAs were conducted between fall and spring seasons. Soil microbial groups

351 separated more distinctly by cover crop types in spring sampling time (*P=0.001*) (Figure 6A)

352 compared to fall (Figure 6B). The relative abundance of AMF and Gram- bacteria was higher in

353 grass & brassica treatments in spring while legumes had greater total fungi, Gram+ bacteria, and

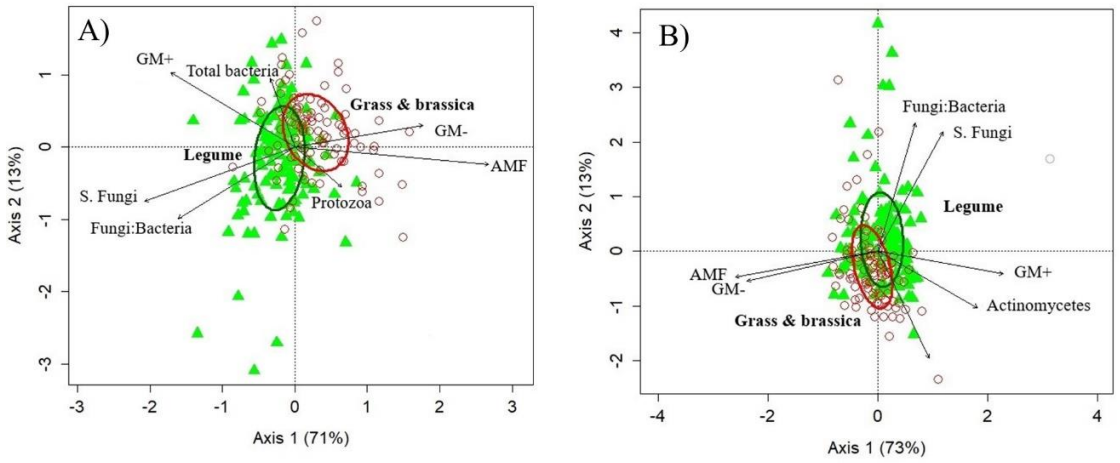


Figure 6. Ordination plots of distance-based redundancy analysis (db RDA) derived from fatty acid profiles (relative abundance) under legume and grass & brassica treatments collected in spring (A) and fall (B). Saprophytic fungi (S. Fungi), total bacteria, actinomycetes, Gram positive bacteria (GM+), Gram negative bacteria (GM-), Arbuscular mycorrhizal fungi (AMF), S. fungi:bacteria ratio (Fungi:Bacteria).

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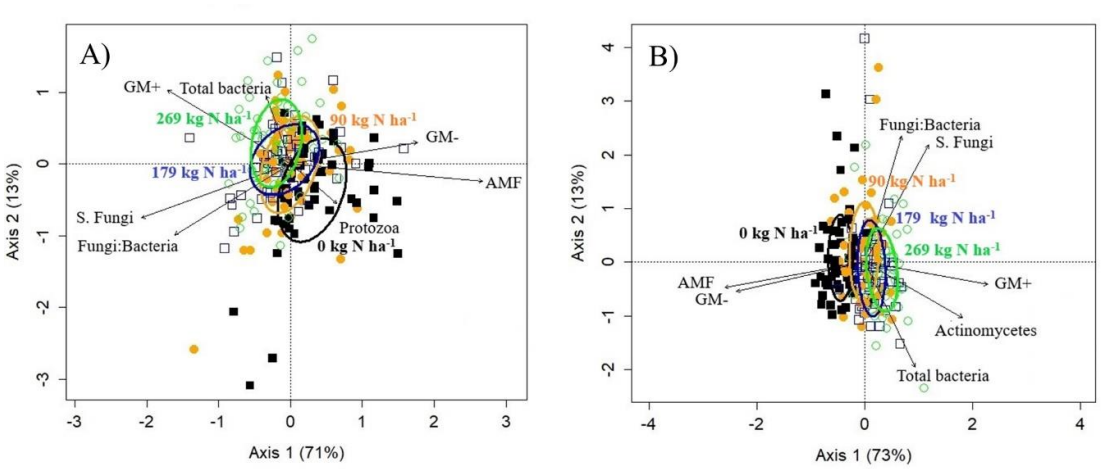


Figure 7. Ordination plots showing distance-based redundancy analysis (db RCA) of the relative abundance of fatty acid profiles under to different N rates (0, 90, 179, 269 kg N $ha^{-1}$) collected in the spring (A) and fall (B) over 2 years. Saprophytic fungi (S. Fungi), total bacteria, actinomycetes, Gram positive bacteria (GM+), Gram negative bacteria (GM-), Arbuscular mycorrhizal fungi (AMF), S. fungi:bacteria ratio (Fungi:Bacteria).

355

356 a higher ratio of fungi to bacteria (Figure 6A).In spring, total bacteria and Gram + bacteria

357 increased following N fertilizer rate of 269 kg N $ha^{-1}$ treatments while protozoa flourished in the

358 no N treatment (Figure 7A). However, distinct dissimilarities between N fertilizer rates were

observed in fall (*P=0.001*) (Figure 7B). In fall sampling, the 0 kg N $ha^{-1}$ separated from 179 kg N $ha^{-1}$ and 269 kg N $ha^{-1}$. The AMF and Gram - bacteria favored 0 kg N $ha^{-1}$, while treatments at 179 kg N $ha^{-1}$ were dominated by saprophytic fungi, and an increased F: B ratio. Finally, the actinomycetes were affected and strongly correlated to Gram + bacteria at the 269 kg N $ha^{-1}$ fertilizer rate.

# 4. DISCUSSIONS

## 4.1 Corn grain yield response

The significance of cover crop types and N fertilizer rates interaction indicates that cover crop types had different responses to N fertilizer applications and that the corn yield varied with changes of N fertilizer rates. While corn production decreased over time, corn grown after legume species cover crop had higher production than grass & brassica species at both 0 and 90 kg N $ha^{-1}$ application rates. In a similar soil type, Boquet et al. (2004a) showed that the optimum cotton lint yield under wheat cover crop was observed at 118 kg N $ha^{-1}$ under no-till, whereas no N fertilizer addition was needed following a hairy vetch cover crop. Another study conducted in the USA and Canada found that cool-season legume cover crops increased corn grain yield by 34% compared to no cover crop at a low N fertilizer rate whereas a cool-season grass cover crop did not affect corn yield (Miguez & Bollero, 2005). This is due to legumes fixing $N_2$ from the atmosphere in addition to scavenging residual N from the soil, which accumulates in their biomass, to be made available to the subsequent cash crop through microbial degradation. Consequently, planting corn after the termination of leguminous species, which may decompose easily and act as an available N source, could be beneficial to corn seedling growth and development. Even though the cover crops were terminated in the early stage (in February),

legumes accumulated N by fixing $N_2$ in a greater amount than grasses which concurs with Lawson et al. (2015).

On the other hand, the lower corn grain yield following non-legume cover crops may be explained by the lack of biomass, N immobilization (Tollenaar et al., 1993; Wyland et al., 1995), or an allelopathic impact (Raimbault et al., 1990). In the warm temperate zone of the Pampas, Alvarez et al. (2017) reported that compared to a fallow treatment, corn production was increased (>7%) following a legume cover crop but decreased (8%) following a non-legume cover because of $NO_3^-$ depletion. In our case, limited growth of non-legume cover crops due to the early termination (February) of cover crops to prepare for corn planting reduces N accumulation opportunity. Unfortunately, we did not record the cover crop biomass, but our observations and subsequent studies of cover crop biomass degradation and soil N availability support this hypothesis. Additionally, the non-legumes likely degraded slowly, especially cereal rye as reported by Jahanzad et al. (2016), and therefore N was slowly released.

Previous reports regarding corn grain yield response to N fertilization revealed that the quadratic-plateau model was the most appropriate model to obtain a valid prediction of EONR (Alotaibi et al., 2018; Cerrato & Blackmer, 1990; Nyiraneza et al., 2010). In our study, the range of EONR rates was somewhat similar to that obtained for corn in Illinois, USA (114-203 kg N $ha^{-1}$) (Coulter & Nafziger, 2008) and in Quebec, Canada (123-173 kg N $ha^{-1}$) (Alotaibi et al., 2018). The presented EONR for legume (149 kg N $ha^{-1}$) and grass & brassica (184 kg N $ha^{-1}$) were lower than the fertilizer rate recommendation for corn in Louisiana (235 kg N $ha^{-1}$) (LSUAgCenter, 2019). Our results suggest that growing corn following legumes could reduce EONR while still maintaining yield. However, the higher EONR following grass & brassica

403 treatments further supports our hypothesis regarding immobilization of N (Coulter & Nafziger,
404 2008) and its impact on corn grain yield.

405 **4.2 Treatment effects on soil chemical properties**

406 Soil pH is one of the most widely used physio-chemical parameters for agricultural soil
407 quality indices (Bastida et al., 2008; Singh, 2018). It is no surprise that soil pH decreased under
408 the corn fertilized by urea ($NH_4^+$-based fertilizer) since ammonium nitrifying bacteria convert
409 $NH_4^+$ to $NO_3^-$ in a process that releases $H^+$, and acidifies the soil (McCauley et al., 2009; Singh,
410 2018). The more N fertilizer applied, the more intense acidification is seen, resulting in the low
411 soil pH following corn harvest. This pattern was found in fall samplings of this study, and other
412 similar studies (Belay et al., 2002; Mbuthia et al., 2015). Additional acidification also occurred
413 when corn released $H^+$ to absorb $NH_4^+$ (Becking, 1956; Bolan et al., 1991; Tang & Rengel,
414 2003). However, in our study, planting both types of cover crops after corn harvest increased soil
415 pH. One explanation is that pH can be increased by the production of $HCO_3^-$ and/or $OH^-$
416 following residual $NO_3^-$ uptake by cover crop. Leguminous cover crops did not increase soil pH
417 as much as non-leguminous cover crops which may be caused by symbiotic N fixation
418 (Marschner, 2011), in which $NH_4^+$ was produced and excreted from the nodules into the soil, or
419 after the cover crop was terminated when $NH_4^+$ was released as the nodules decomposed (Bolan
420 et al., 1991).

421 Soil organic matter (SOM) is used as a soil health indicator because it is involved in soil
422 biological, chemical, and physical properties that affect nutrient mineralization (McCauley et al.,
423 2009). Although cover crop types did not affect SOM, the addition of N fertilizer did increase
424 SOM levels (Ladha et al., 2011; Mahal et al., 2019). Moreover, SOM was increased following
425 the inclusion of cover crops and minimum N fertilizer input under no-tillage for over 2 years in

426 our study (continued from 3 years under cover crop x N rates on the no-till system). Although,
427 the cover crop biomass was not recorded, our $NO_3^-$-N data in the fall samplings showed the
428 residual N fertilizer was left-over from the main crop. We hypothesized that the residual $NO_3^-$-N
429 promoted cover crop growth and increased biomass. Therefore, more residue would be left on
430 the soil surface after cover crop termination, building SOM. Nitrogen fertilizer input at the rate
431 of 90 kg N $ha^{-1}$ resulted in the greatest amount of soil total C and total N. Potentially, the residual
432 fertilizer N left over after harvest was absorbed by cover crop residues and accumulated into
433 biomass tissues (Boquet et al., 2004b; Jahanzad et al., 2016; Singh et al., 2020). A previous study
434 from Boquet et al. (2004b) on the impact of tillage, cover crops, and N fertilizer input on cotton
435 production at the same location indicates that increased N applied for the main crop significantly
436 supported the growth of grass cover crop, which is consistent with our findings. Additionally,
437 under no-tillage, the biomass residue is left on the soil surface, which reduces contact with soil
438 microorganisms and slows decomposition, building SOM (Lin, 2017; Mazzoncini et al., 2011;
439 Mbuthia et al., 2015; Sainju et al., 2002). Additionally, Mahal et al. (2019) demonstrated that
440 synthetic N fertilizer (ammonium nitrate) increased soil organic matter by suppressing the soil
441 organic matter mineralization process. Overall, SOM was increased by the presence of cover
442 crops and the use of N fertilizer.
443 In general, $NH_4^+$ and $NO_3^-$ forms can be obtained directly from N fertilizers and/or soil
444 organic matter degraded by microbes (Sollins et al., 1996). In our study, the $NH_4^+$-N did not
445 respond to cover crop types or N fertilizer rates but responded to different sampling times. It is
446 notable that in spring 2017, there was a transition of the experiment from high N fertilizer rates
447 (235, 268, 302 kg N $ha^{-1}$) (Sanchez et al., 2019a) to lower N fertilizer rates (90, 179, and 269 kg
448 N $ha^{-1}$). Hence, in spring 2017, $NH_4^+$-N levels might be impacted by the previous N fertilizer rate

input. An increase of $NH_4^+$-N level in spring and decrease in fall after corn harvesting was observed by Sanchez et al. (2019a). Since cover crops took up residual $NH_4^+$-N leftover from previous corn production, it is then returned to the soil after termination in the spring. However, in this study, the N fertilizer rates were lower, and compared to Sanchez et al. (2019a), our $NH_4^+$-N tended to increase in fall 2018 (the last sampling time) after the corn season when fertilizer was added for corn production. This caused a greater concentration of excess ammonium leftover in the soil that was prone to lose or uptake by cover crops established following corn harvest. Brackin et al. (2015) proved that applying urea-based fertilizer resulted in a high ammonium flux concentration, and it exceeded sugarcane uptake.

Unlike $NH_4^+$-N, the level of $NO_3^-$-N was greater in the legume treatment than grass & brassica. This was because legumes have the ability for biological N fixation, which added N back to the soil (Jahanzad et al., 2016; Singh et al., 2020). A study from Tonitto et al. (2006) revealed that legumes in a fertilizer application system following a cash crop reduced $NO_3^-$-N leaching by 40% compared to a bare fallow treatment. They also found that N fixation of legumes during legume development added N to the N pool, and ultimately, nutrients could be utilized by the subsequent cash crop. This aligns with our finding that under legumes with- and without N fertilizer input, the corn grain yield was greater than grass & brassica. Differences in $NO_3^-$-N were influenced by the interaction of N fertilizer application and time of sampling. Following the corn harvest in fall, soil samples contained significantly greater concentrations of $NO_3^-$-N than spring following cover crop termination. Also, in the fall samplings, $NO_3^-$-N concentrations were greater in higher N fertilizer rate application treatments. However, there is no difference across N rate treatments in the level of $NO_3^-$-N in samples collected in spring. Our study indicates that some amount of $NO_3^-$-N was not completely taken up by the main crop and

remained in the soil after corn harvest. Consequently, it can be lost via cover crop uptake, runoff, leaching, and the denitrification process (Baggs et al., 2000; Dabney et al., 2010; Francis et al., 1998; Schjønning et al., 2003). Moreover, following the corn harvest in fall 2018, the highest level of $NO_3^-$-N through the experiment was under the 269 kg N $ha^{-1}$ treatment. This suggests that the N fertilizer application at 269 kg N $ha^{-1}$ may surpass corn requirements as we did not measure a significant improvement in corn yield. A substantial decrease of $NO_3^-$-N concentration at all N rates was observed in spring 2018. Although, we did not measure the runoff or infiltration in this study, this loss was possibly in response to climate effects or cover crop management practices. Cover crops and microorganisms can uptake some $NO_3^-$-N (Kaspar et al., 2012; Pantoja et al., 2016). However, the sudden decline in $NO_3^-$-N level in spring 2018, was likely due to loss through leaching, and runoff during heavy rain events (Fang et al., 2007), especially in this geographic region of this trial which received a high amount of cumulative precipitation, exceeding 40 cm, during cover crop establishment throughout winter until termination in spring.

Extractable soil P and K were affected by cover crop types and N rates, and only K was affected by sampling time. Soil P concentration was greater in legumes than grass & brassica cover crops. This is related to legumes requiring more P for biological N fixation (McLaughlin et al., 1990; Weisany et al., 2013). Similarly, Villamil et al. (2006) found that incorporated hairy vetch in a corn-soybean rotation resulted in significantly higher available soil P than that of cereal rye. However, our results showed a greater concentration of K in grass & brassica than the legumes. This was likely due to the more extensive root system of grasses with a longer length and high amounts of root biomass (Caradus, 1980; Jackman & Mouat, 1972). It is reported that grass cover crops needed and absorbed more K than leguminous cover crops and could absorb K

near the soil surface (Eckert, 1991). The available soil P and K responded to the absence and presence of N fertilizer in this study. Both soil P and K concentrations were greatest in the 0 kg N $ha^{-1}$ treatment. It is possible that increased N fertilizer application rates, and increased growth of cover crop biomass resulted in higher demands of soil P and K (Mbuthia et al., 2015). Whereas, the plots receiving no N addition were still receiving P and K fertilizer bud had the lower yield of the main crop causing a build-up P and K reserves (Belay et al., 2002). The lower P concentration in the N fertilizer application treatments may also be due to soil acidification resulting from N fertilizer input and reducing soil P availability in this study (Havlin et al., 2016; Schroder et al., 2011).

### 4.3 Soil biological properties

#### 4.3.1 Soil enzyme activity

With no N fertilizer addition, the greater β-glucosidase activity was found following leguminous treatments than grass & brassica. Even though the soil total C and total N were the same between cover crop types at no fertilizer treatment, there was a positive effect from N-fixation in legumes resulting in greater N concentration assimilated in their biomass and the soil (Boquet et al., 2004b; Piotrowska & Wilczewski, 2012). We saw greater available $NO_3^-$-N concentration under legumes than grass & brassica cover crops that could promote microbial activities and the C-acquiring enzyme activities. Liang et al. (2014) reported that an Austrian winter pea, a legume, had the highest β-glucosidase activity. We found that N fertilizer at any rate under legume cover crops did not affect β-glucosidase activity while β-glucosidase activity did respond to N fertilizer input at 179 kg N $ha^{-1}$ under the grass & brassica treatment. Grasses tend to contain more C or cellulose and N, compared to legumes leading to N immobilization and slow decomposition (Jahanzad et al., 2016; Lupwayi et al., 2006). However, when soil

provided available N compounds from fertilizer, it quickly stimulated microbial activities to obtain C for an energy source (Allison & Vitousek, 2005). A study from Piotrowska and Wilczewski (2012) revealed that increasing N fertilizer rate up to 80 kg N $ha^{-1}$ stimulated the greatest β-glucosidase activity level in both legume (field pea) and brassica (oilseed radish) before it decreased when applying N fertilizer more than 120 kg N $ha^{-1}$. A meta-analysis by Xiao et al. (2018) showed that low and medium N fertilizer application encouraged β-glucosidase activity because N addition induced the demand for C resulting in production of β-glucosidase to hydrolyze soil organic matter or soil organic C. Nonetheless, increments of N fertilization could negatively reduce the enzyme yield as the acidification process can decrease soil pH to below 5.5 at 269 kg N $ha^{-1}$ rate in our study. Other studies have also reported reduced β-glucosidase caused by high N fertilizer input lowering the soil pH (Ullah et al., 2019; Xiao et al., 2018; Zhang et al., 2015). Nitrogen fertilization in corn affected soil pH in the fall as shown in our results, which may shift microbial community ultimately decreasing β-glucosidase activity than in the spring after cover cropping due to higher soil pH.

NAGase activity represents C and N cycling which results in N mineralization in the soil (Ekenler & Tabatabai, 2002; Sinsabaugh et al., 1993). This study showed that the potential NAGase activity was affected by cover crop types and soil sampling time while it did not respond to N fertilizer application. Similarly, a meta-analysis study found that NAGase did not react to N fertilizer addition because fertilization inhibited the N-acquisition enzyme activity (Xiao et al., 2018). From our results, the grass & brassica treatment promoted more NAGase activity than legumes. This may be due to the extensive root system of grass, in particular cereal rye, which increased the rhizosphere, an area high in microbial population and enzyme activity (Acosta-Martinez et al., 2007; Bandick & Dick, 1999). The study from Averill and Finzi (2011)

confirmed that N-degrading enzyme level is positively associated with the growth of roots which provided labile C. Like the β-glucosidase enzyme activity pattern, the enzyme concentration tended to be greater in spring than the fall season. Cover crops in spring improved NAGase activity regardless of cover crop types because organic C and N from the cover crop biomass residue on the soil surface in spring acted as substrates for microorganisms to consume. This was consistent with Sanchez et al. (2019b)'s study.

### 4.3.2 Changes in microbial community structure

Results from this study demonstrated that total microbial biomass (total FAME) did not respond to cover crop types, but it was affected by the interaction between N fertilizer rates and sampling time. On the other hand, looking at individual microbial groups, the interaction between cover crop types and sampling time impacted the absolute abundance of AMF only. The grass & brassica treatment had greater AMF than leguminous species across almost all of the sampling times due to their ability to acquire nutrients (Smith & Read, 2010). Although grass cannot fix N as the legumes did, it scavenged nutrients, especially N and P via a robust root system and was likely enhanced by mycorrhizal fungi (Murrell et al., 2020). Similarly, a study conducted in Tennessee (Mbuthia et al., 2015) reported that wheat cover (grass) had more AMF abundance than hairy vetch (legume). Another study showed that oats and rye increased mycorrhizal colonization effectively for sweet corn (Kabir & Koide, 2002). Several studies confirmed that AMF from cover crops had the potential to enhance mycorrhizal colonization of the next cash crop at an early stage (Lehman et al., 2012; Njeru et al., 2014; White & Weil, 2010). This validates our finding that AMF under grass & brassica treatment was greater than legumes over time (excepting the first spring).

As we expected, the microbial biomass (total FAMEs) was promoted in spring after cover crops were terminated and was lower in fall after the main crop harvest because cover crops provide a simple source of C in root exudation through the winter months. In a study by Calderon et al. (2016), they observed that the presence of roots at cover crop termination had the greatest impact on microbial community growth than at the main crop planting. Furthermore, more substrates were added by the residue after cover crops termination, thereby supplying both C and N for the microbial community. The application of urea as N fertilizer during corn planting acidified the soil pH to below 5.5 which may have also contributed to lower microbial abundance in fall samplings (Dequiedt et al., 2011; Geisseler & Scow, 2014). Verdenelli et al. (2019) reported that during the growing season, mineral fertilizer reduced bacterial and fungal richness. However, at the fall samplings of our study, there was no difference in response to microbial abundance among N fertilizer rates. The only effect of N fertilizer rate and sampling time was in the spring samplings. In spring 2017, a N fertilizer rate of 179 kg N $ha^{-1}$ increased microbial biomass. The same response was measured in spring 2018 and was likely due to the presence of N fertilizer leftover from corn. With increased N fertilizer, more residue N could support the growth of cover crop biomass productivity from photosynthesis and transfer the substrates to the rhizosphere and soil (Calderon et al., 2016; Verdenelli et al., 2019; Wang et al., 2003) to promote soil microorganism growth. The study from Sanchez et al. (2019b), however, did not find the effect of different N rates on microbial biomass. This could be due to the small difference in N rates of 235, 268, and 302 kg N $ha^{-1}$ that were higher than our study. Because this study was conducted on the same field as Sanchez et al. (2019b) the effect of the highest N rates input (302 kg N $ha^{-1}$) from Sanchez's study influenced our results in spring 2017 with a decline in microbial biomass.

586 Proportions of AMF and Gram- bacteria populations were relatively higher under the grass &
587 brassica treatment, while saprophytic fungi, Gram+ bacteria, and the fungi:bacteria ratio were
588 higher under the legume treatment. This distinct separation was more apparent in spring than fall.
589 Greater mycorrhizal populations under grass & brassica was in agreement with previous studies
590 (Acosta-Martinez et al., 2007; Sanchez et al., 2019b). Acosta-Martinez et al. (2007) observed
591 that a crop rotation including wheat increased mycorrhizal populations, which were vital in a
592 semiarid and arid environment. Frey et al. (1999) observed that grasses had greater belowground
593 biomass compared to legumes in a no-till system, and this allowed an increased hyphal network
594 of AMF. Notably, we did not expect the increase of the AMF populations because brassicas are
595 an AMF non-host cover crop. Furthermore, the production of glucosinolates (can form
596 isothiocyanates) released from brassica potentially inhibit mycorrhizae (Glenn et al., 1988; Hill,
597 2006). Therefore, the increase of the AMF population likely resulted from grass plots. Because
598 AMF symbionts can facilitate plant nutrient uptake and increase water and carbohydrates in root
599 exudates, they may create a preferable environment around the AMF hyphae and increase
600 populations of Gram- bacteria (Toljander et al., 2006). The pattern of greater relative abundance
601 of fungi, fungi:bacteria ratio, and Gram+ bacteria in legume treatments in all times of sampling
602 from our study was in agreement with previous reports (Mbuthia et al., 2015; Sanchez et al.,
603 2019b). For example, Sanchez et al. (2019b) measured a greater relative abundance of fungi and
604 fungi:bacteria ratio under legume treatment in the same climate.
605 The most obvious demonstration of N fertilizer impacts on microbial populations was the
606 greater relative abundance of AMF in the 0 N treatment that was also reported in many previous
607 studies (Mbuthia et al., 2015; Sanchez et al., 2019b; Tian et al., 2013; Wang et al., 2009). In
608 unfertilized soil, AMF reside in plant host roots and carbohydrates were supplied by the hosts,

609 and in return, the AMF contributed soil P and possibly N to plant hosts (Hodge et al., 2010; Liu
610 et al., 2015). Our finding revealed that Gram- bacteria were closely correlated to AMF under no
611 N fertilizer. This supports the possibility that these bacteria obtained benefits from the fungi and
612 plant hosts symbiosis. Moreover, Fanin et al. (2019) studied Gram+ and Gram- bacteria
613 indicators for C availability and illustrated that Gram- bacteria prefer more simple C compounds
614 while Gram+ bacteria utilized more complex C compounds. Actinomycetes, a type of Gram+
615 bacteria that form fungal-like filaments, play a major role in decomposing complex compounds
616 in plant residues along with other soil microbes. Additionally, they can survive in unfavorable
617 environmental conditions (Bhatti et al., 2017). Actinomycetes were present with Gram+ bacteria
618 and might take over the fungal role in the decomposition of corn residue after harvesting in the
619 fall (Helfrich et al., 2015). Saprophytic fungi and the fungi:bacteria ratio were promoted by N
620 fertilizer input in our study which affected substrates added to the soil, and supported fungal
621 growth (Belay et al., 2002).

622 **5. CONCLUSION**

623 From our two-years of data, we found that the use of winter cover crops in the fallow season
624 under no-till corn production could reduce the N fertilizer rate input for corn. Soil organic matter
625 did not respond to cover crops but did increase under N fertilizer input. Fertilizer applications
626 also significantly increased total C and total N. Moreover, leguminous cover crops had greater
627 soil $NO_3^-$-N and available P concentrations, and β-glucosidase activity than the grass & brassica
628 treatment while the grass & brassica treatment had greater K concentrations than legume species.
629 The input of N fertilizer increased the C enzyme activity under grass & brassica covers. Also, the
630 grass & brassica cover crop treatment demonstrated greater NAGase activity and generally, had
631 greater AMF abundance than legume cover crops. Total microbial abundance responded to N

fertilizer. The effect of cover crop types on soil microbial groups was more significant in spring sampling times compared to fall. Overall, the incorporation of legume cover crops for crop rotation reduced N fertilizer input, sustained corn grain production, and benefited soil health parameters.

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Figure 1. Average monthly high and low temperatures and total monthly precipitation from February 2017 through October 2018. * Due to equipment failure data from Jan.18, Feb.18 and Mar.18 was obtained from the Dean Lee Research Station, Alexandria, LA.

Figure 2. Soil nitrate-N concentration under different nitrogen fertilizer rates at different sampling times. Error bars represent standard error. Same uppercase letters are not significant ($\alpha = 0.05$) within an N rate across sampling times. Same lowercase letters are not significant ($\alpha = 0.05$) between N rates within a sampling time.

Figure 3. Potential enzyme activity of soil β-glucosidase under different cover crop types and nitrogen fertilizer rates. Error bars represent standard error. Same uppercase letters are not significant ($\alpha = 0.05$) between N rates. Same lowercase letters are not significant ($\alpha = 0.05$) between cover crop treatments. Fallow values are provided for quantitative comparison between cover crop treatments and was used in statistical analysis only within N rates.

Figure 4. Potential enzyme activity of soil β-glucosidase under different cover crop types at different sampling times. Error bars represent standard error. Same uppercase letters are not significant ($\alpha = 0.05$) within a cover crop type across sampling times. Same lowercase letters are not significant ($\alpha = 0.05$) between sampling times across legume and grass & brassica types.

Figure 5. Total microbial abundance according to N rate and sampling time. Error bars represent standard error. Same uppercase letters are not significant ($\alpha = 0.05$) within a sampling time. Same lowercase letters are not significant ($\alpha = 0.05$) within N rates across sampling times.

Figure 6. Ordination plots of distance-based redundancy analysis (db RDA) derived from fatty acid profiles (relative abundance) under legume and grass & brassica treatments collected in spring (A) and fall (B). Saprophytic fungi (S. Fungi), total bacteria, actinomycetes, Gram positive bacteria (GM+), Gram negative bacteria (GM-), Arbuscular mycorrhizal fungi (AMF), S. fungi:bacteria ratio (Fungi:Bacteria).

Figure 7. Ordination plots showing distance-based redundancy analysis (db RCA) of the relative abundance of fatty acid profiles under to different N rates (0, 90, 179, 269 kg N $ha^{-1}$) collected in the spring (A) and fall (B) over 2 years. Saprophytic fungi (S. Fungi), total bacteria, actinomycetes, Gram positive bacteria (GM+), Gram negative bacteria (GM-), Arbuscular mycorrhizal fungi (AMF), S. fungi:bacteria ratio (Fungi:Bacteria).