Effects of Cover Crop Species on Residue Decomposition and Soil Nitrogen Availability in a Corn-Soybean System

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DEDICATION

I would like to dedicate this thesis to my fiancée, Hannah Lorenzen, for sticking with me throughout this journey. Through thick and thin. You by my side through all of this, whether it is flying across the country and spending your vacations sampling in the field or spending countless hours in the lab to forgiving me for the lack of communication at times because of certain deadlines, you made this endeavor all the easier. I would like to also dedicate this thesis to her family for also helping me get to this point in my career and being another form of support.

Lastly, I dedicate this thesis to my mother, father, brother, and sister. Without your support before the initiation of and throughout my program, whether it be emotional, financial, or being a champion for what I am doing, this thesis would not be possible. To give all the thanks in the world, would still not come close to how important you all were in the process of obtaining this degree.

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ABSTRACT

There is limited knowledge concerning the effects of multi-species cover crops (CC) on soil nitrogen (N) dynamics in the row-crop systems of the southeastern US. This study was conducted with two objectives: (i) to determine the rates of *in-situ* CC residue decomposition and N release in response to CC species and degree of soil contact, and (ii) to understand the seasonal changes in soil N pools across different CC treatments compared to a CC-free control at different soil depths.

Based on a 21-week *in situ* litterbag experiment, CC residue decomposition rates and N release were determined. Litterbags were either placed on the soil surface (SP) or in the subsurface (SSP). The SSP residues decomposed and released N significantly faster than SP residues. By week 4, proportion of residues decomposed was 43% and 72%, and the amount of N remained was 30 and 8 kg N ha^{-1} for SP and SSP, across the treatments. For SP, the cereal rye (*Secale cereal* L.) and hairy vetch (*Vicia villosa* Roth) mixture consistently had the greatest N remaining, whereas the single-species cereal rye had the greatest N remaining for SSP.

Soils were sampled 5 times between October 2017 and November 2018 from the 0-5, 5-10, and 10-20 cm depth and analyzed for plant available N, water extractable total soluble N (WEN), and potentially mineralizable N. Cover crop type, soil depth, and soil sampling event had no significant effect on any of the analyzed parameters. However, the soil depth and sampling time interaction were significant for all parameters. Plant available N was greatest in the top soil layer

in April and May 2018 (27 and 24 mg N kg^{-1}), whereas WEN was greatest in May 2018 with a 167% increase from April 2018.

Overall, this study determined the rate of N release in soil in response to different CC species and characterized available soil N pools. This study indicated that no-till planting of row-crops within a few weeks of CC termination is a strategic management method for utilizing CC as a supplemental N source for row-crop production.

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CHAPTER I

GENERAL INTRODUCTION

The practice of growing cover crops (CC) during fallow periods has been gaining popularity amongst producers due to the potential beneficial services CC offer (CTIC, 2017). Cover crops have shown the potential to immobilize nitrogen (N) and decrease N leaching, decrease soil erosion, and combat weeds, amongst other services (Constantin et al., 2011; De Notaris et al., 2018; García-González et al., 2018; Weber et al., 2017; Zotarelli et al., 2009). In addition to all these benefits, producers are interested to know how the integration of CC affect fertilizer N management for succeeding row-crops (CTIC, 2017).

Several factors must be considered for the successful establishment and management of CC. One of the important factors is species selection, which is determined by factors that include climate, seed availability, rotation scheme, and soil type (Reeves, 1994). Climate is an important deciding factor for species selection because in areas where precipitation is low, CC can reduce the soil moisture available for the subsequent growing season and suppress row-crop yields (Blanco-Canqui et al., 2015; Lewis et al., 2018). According to the National Oceanic and Atmospheric Administration, Tennessee experiences humid subtropical climates, characterized by hot and humid summers followed by mild winters. Therefore, it is important to choose species of CC that are well-suited for this climate. Due to frequent frosts during the winter months in the mid-south, CC species that can overwinter should be utilized. Duiker (2014) reported that cereal rye (*Secale cereal* L.) proved to be more winter-hardy compared to other common grasses and legumes. Furthermore, important row-crop soils in Tennessee are loess-derived and erosion-prone, containing low levels of soil organic carbon (SOC), ranging from 17 to 24 Mg ha^{-1} to a depth of 15 cm (Jagadamma et al., 2019). An effective cover cropping strategy should not only reduce soil erosion and weed pressure but also enrich soils with organic carbon.

Ecosystem Services from Cover Cropping

Cover crops are a useful management tool to protect soil environments from wind and water erosion, because bare soil erodes at faster rates under intense precipitation (Labrière et al., 2015). Not only does cover cropping reduce erosion, but it can have a significant impact on overall soil quality. When soil quality was ascribed to microbial biomass and activity in soils of west Tennessee, cover cropping showed improvements to soil quality (Mbuthia et al., 2015). When practicing no-tillage along with cover cropping, there is a sustainable improvement in soil quality over time due to an increase in SOC content which leads to improved soil structure and water availability for row-crops (Nascente et al., 2013). Cover cropping can have an even more significant effect on the croplands of west Tennessee as these loess-derived soils are prone to deterioration due to erosional impacts, hindering productivity. Barnes et al. (2016) reported that CC help prevent the loss of productivity of soils developed from loess deposits. Not only can CC improve soil quality, but CC have shown to be a strategic tool to suppress weed pressure (Baraibar et al., 2018; Finney et al., 2016). In addition to these benefits, producers' motivation to adopt CC can be enhanced if cover cropping can also provide N to subsequent row-crops (CTIC, 2016).

Nitrogen Credits from Cover Cropping

Nitrogen is the most important macronutrient for achieving high row-crop yields. However, Pantoja et al. (2015) reported reduced N use efficiency with increased N rates. With synthetic N fertilization causing an increase in production cost of row-crops while potentially deteriorating water and environmental quality, there is a need to minimize the use of external N inputs into soil. In response, leguminous CC can reduce the use of synthetic N fertilizer as they can supply

N to soil by atmospheric N fixation. The University of Tennessee Extension reported that if a single-species legume CC produces high enough biomass, it can contribute 67 to 90 kg N ha^{-1} (McClure et al., 2018). In another study conducted in southwestern France, Plaza-Bonilla et al. (2017) determined that winter pea (*Pisum sativum subsp. Arvense* L.) CC contributed 40 to 49 kg N ha^{-1}. These N contribution levels are significant because it is expected to directly inform producers' decision-making processes around the selection of CC species likely to be the most profitable and sustainable, especially in the southeastern region which represents 30% of total croplands in the US (O'Connell et al., 2015).

Nitrogen Transformation in Soil

About 98% of soil N is present in organic forms, meaning that plant available N [PAN; ammonium (NH_4) and nitrate (NO_3)], comprises only 1-2% of the total soil N pool (Brady, 2010). The process of converting organic N to PAN is called *mineralization,* which includes two processes. The first process is *ammonification*, which is the conversion of organic N to NH_4-N. The second process is *nitrification,* which is the conversion of NH_4-N to NO_3-N through the nitrite-N (NO_2-N) intermediary. *Nitrobacter* and *Nitrosomonas* are primarily responsible for the conversion of NH_4-N to NO_2-N and NO_2-N to NO_3-N, respectively (Fageria, 2016). However, there is also a symbiotic relationship between leguminous plants and some bacteria, primarily *Rhizobium* and *Bradyrhizobium*; which convert atmospheric N gas (N_2) into inorganic forms by a process known as *N fixation*.

Nitrogen Fixation

Our atmosphere is composed of 78% N_2. Although plants need N to grow and produce, this gaseous form of N is inert and cannot be directly used by plants. Nitrogen gas is composed of three covalent bonds (N≡N) that are stable, requiring a significant amount of energy to break and create two individual N atoms. Legumes exude carbohydrates for the N fixing bacteria and in return the bacteria supply the legume, and subsequently the soil environment, with NH_4-N (Evans et al., 1987). This occurs at the root tip of the legume; the bacteria infects the host and forms a nodule on the root tip which is the site where N fixation occurs (Mylona et al., 1995). Nitrogen fixation would not occur without the nitrogenase enzyme, which catalyzes the reaction of splitting N_2 using 16 moles of adenosine tri-phosphate to produce 2 moles of ammonia (NH_3) (Giller, 2001). It has been reported that legumes can provide between 8 to 350 kg N ha^{-1} through N fixation (Tonitto et al., 2006). Also, 82% of the N produced by legume CC can be directly available for corn (*Zea mays* L.) after CC termination (Ketterings et al., 2015). Studies have also shown that biological N fixation improved the yield of various silage grains (Frasier et al., 2017; Iannetta et al., 2016; Parr et al., 2011).

Inorganic (Ammonium & Nitrate) Nitrogen in Soil

Ammonia gas produced by the N fixing bacteria is converted to NH_4-N in the presence of water: NH_3 (g) + H_2O (*l*) → NH_4^+ (aq) + OH^- (aq). Ammonification, the transformation of amino groups in organic compounds to NH_3 or NH_4, can also provide NH_4^+ to soil (Strock, 2008). Ammonium formed by both these means undergoes oxidation and transform to first NO_2: $2NH_4^+$ (aq) + $3O_2$ (g) → NO_2^- (aq) + $2H_2O$ (*l*) + $4H^+$ (aq), followed by NO_3: $2NO_2^-$ (aq) + O_2 (g) → 2 NO_3^- (aq) (Fageria, 2016), which is together known as *nitrification*. Nitrification is an energy generating

process, therefore it readily occurs if favorable soil conditions exist for the bacteria (Tanaka et al., 2014). Plants mostly take up N in the form of NO_3-N; therefore, it is quite important to supply NO_3-N at the right time for the row-crop. Nitrate is highly mobile with water and can be leached out of the root zone during rain events or with irrigation; potentially ending up in larger bodies of water. In addition to leaching loss, N can also be lost through *denitrification*, which is a reductive process of transforming NO_3 to gases such as nitric oxide (NO), nitrous oxide (N_2O), and N_2 (Stenger et al., 2018). This process has the potential to lower NO_3 concentrations in soil and reduce leaching of NO_3 to waterways, but it increases N_2O, a potent greenhouse gas, emission to the atmosphere potentially contributing to air pollution and global warming (Matocha et al., 2012). Although denitrification removes N loads, emphasizing the need to decrease N inputs into agricultural soils, it may only remove up to 33% in some instances (Slone et al., 2018). Also, in soils with C to N ratios of 18 and higher, microbes scavenge available NO_3 and NH_4 for protein synthesis (Cheng et al., 2017). The process of converting mineral N to organic N is termed *immobilization*.

RATIONALE AND SIGNIFICANCE

This research seeks to examine the impact of CC on soil N dynamics in hot and humid environments. Anthropogenically sourced N inputs have become a topic of concern for environmental and public health. These N inputs potentially contaminate groundwater, which millions of people globally rely on for drinking water (Eller and Katz, 2017; Ledoux et al., 2007). A great proportion of NO_3 contaminated groundwater can be traced back to fertilizer inputs (Eller and Katz, 2017). Several agriculturally productive areas such as Fresno, CA have shown that close to 90% of its inhabitants face the risk of consuming drinking water with

dangerous levels of NO_3 (Harter et al., 2012). Higher concentrations of NO_2 in contaminated drinking water by the reduction of NO_3 can elicit methemoglobinemia, or "blue baby syndrome," in infants. Higher levels of NO_3 have also been identified as carcinogenic, especially of the stomach and esophagus (Bogárdi et al., 2013). This problem needs to be addressed upstream, as groundwater remediation costs can range into the hundreds of millions of dollars (Mayzelle et al., 2014).

Nitrogen pollution in oceans creates large algal blooms causing hypoxia for aquatic organisms resulting in large dead zones (Dodds, 2006; Scavia et al., 2017). Dead zones have grown in numbers since the 1960s as a result of fertilizer runoff and the effect of eutrophication (Diaz and Rosenberg, 2008). The Gulf of Mexico contains a large dead zone due to eutrophication resulting from the discharge of N from the Mississippi River. It is estimated that the Mississippi River discharges 91% of the N detected in the Gulf of Mexico (Rabalais et al., 2007). The Mississippi River receives almost all of its N from conventional corn fields along the Corn Belt, indicating the need to better manage soil N, such as through the reduction in synthetic N inputs (McLellan et al., 2015). Tonitto et al. (2006) reported that N leaching can be reduced by up to 70% with cover cropping. Therefore, this research can have a profound broader impact on helping to reduce the impacts of N leaching losses.

Locally, this project can help producers achieve profitable farming through N credits. Nitrogen fixation through growing leguminous CC can supply inorganic N at various rates depending on species and environmental conditions. The addition of inorganic N through biological methods can reduce the need for synthetic inputs which can save producers revenue. By reducing the need

to apply fertilizers over large areas of land, farmers can cut down on the usage of farm machinery.

The effect of different cover cropping species on soil N availability has been studied across the US (Finney et al., 2016; Lawson et al., 2013; Liebman et al., 2018). However, limited studies have been done to show how different CC species influence soil N availability in the hot and humid regions of the southeastern US. This study can have a profound impact on croplands across the hot and humid southeastern US.

OBJECTIVES AND HYPOTHESES

Objective 1. To determine the rates of *in-situ* CC residue decomposition and N release in response to species types and degree of soil contact.

Hypotheses: (i) The multi-species CC mixture will show the fastest rate of decomposition and N release compared to the single- and double-species CC treatments due to increased species of legumes and functional diversity. (ii) Residue decomposition and N release will increase with degree of soil contact.

Objective 2. To determine how different cover cropping species change plant available N at different soil depths during the CC and row-crop growing season.

Hypotheses: (i) Legume-included treatments will lead to higher plant available N concentrations compared to single-species grass CC, due to the N fixing capabilities of legumes. However, the increased plant available N from legume-included CC will only last for a short period of time after CC termination due to the rapid decomposition of

legumes. (ii) Grass/legume mixtures will have a higher potential to provide plant available N throughout the growing season as a result of the different rates of decomposition of grasses and legumes in the mixtures.

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CHAPTER II

COVER CROP RESIDUE DECOMPOSITION AND NITROGEN RELEASE IN A CORN-SOYBEAN PRODUCTION SYSTEM IN WEST TENNESSEE

A version of this chapter will be submitted for publication to the Agronomy Journal by Manuel J. Sabbagh, Sindhu Jagadamma, Forbes R. Walker, Jaehoon Lee, Michael E. Essington, Amanda J. Ashworth, and Liesel G. Schneider.

ABSTRACT

There is limited knowledge about the potential for multi-species cover crops (CC) to be used as a supplemental nitrogen (N) source in comparison to single- and double-species CC in the hot and humid southeastern US. A litterbag study was conducted to determine the *in-situ* rates of CC residue decomposition and N release after CC termination and during row-crop production. Cover crop residues of single-species grasses [cereal rye (*Secale cereal* L.) and wheat (*Triticum aestivum* L.)], double-species grass/legume mixtures [cereal rye and crimson clover (*Trifolium incarnatum* L) and cereal rye and hairy vetch (*Vicia villosa* Roth)], and a multi-species mixture [cereal rye, whole oats (*Avena sativa* L.), crimson clover, hairy vetch, and daikon radish (*Raphanus sativus var. Longipinnatus*)] were either surface placed (SP) to mimic no-tillage or subsurface placed (SSP) to a depth of 5 cm to mimic conventional tillage. The percentage of CC biomass and N content remaining was measured at 5 timepoints throughout the soybean [*Glycine max* (L.) merr.] growing season. The SSP residues decomposed and released N significantly faster than SP residues. By week 4, proportion of residues decomposed was 43% and 72%, and the amount of N remained was 30 and 8 kg N ha^{-1} for SP and SSP, across the treatments. For SP, the cereal rye and hairy vetch mixture consistently had the greatest N remaining, whereas the single-species cereal rye had the greatest N remaining for SSP. This study revealed that when not

tilled, producers can potentially maximize the utilization of CC as a supplemental N source if row-crop planting is done within a few weeks of CC termination.

INTRODUCTION

The cover crop (CC) planted acreage has risen steadily during the past decade (CTIC, 2017). This increase is a consequence of the ability of CCs to control weeds (Baraibar et al., 2018), reduce nitrogen (N) leaching (Frasier et al., 2017; Komatsuzaki, 2017; White et al., 2017), control soil erosion (Roth et al., 2018), and improve soil health (Crowley et al., 2018). According to the Natural Resources Conservation Service (NRCS) of the United States Department of Agriculture, soil health is achieved by minimal or no tillage and by enhancing biodiversity by increasing plant diversity. Nunes et al. (2018) showed that continuous no-tillage with diversified cropping systems compared to conventional tillage had greater soil health benefits across three different soil types in the northeastern US. In a study conducted in the San Joaquin Valley in central CA, Mitchell et al. (2017) reported that cover cropping with no-tillage have a pronounced beneficial effect on soil health compared to conventional tillage. In regions such as the hot and humid southeastern US, in which 20 to 50% of corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and soybean [*Glycine max* (L.) merr.] croplands are under no-tillage management (Claassen et al., 2018), producers are enthusiastic to further promote sustainable production by adopting cover cropping. Mbuthia et al. (2015) reported an improvement in soil quality in the cropping systems of west Tennessee by adopting cover cropping along with no-tillage in comparison to a conventional tillage system with no CC.

Cover crops can enhance soil health and plant productivity by their influence on nutrient cycling; particularly N cycling (Mbuthia et al., 2015). There is an increased interest to provide N to row-crops through cover cropping, especially in corn production systems which require high inputs of mineral N for proper growth and development (Kablan et al., 2017). However, there are CC family-specific interactions that affect the N cycle and influence the availability of N for succeeding row-crops. The main families of CC that are utilized by producers include legumes and grasses, with *Brassicas* gaining increased attention in recent years. Legumes supply plant available N to succeeding row-crops through their ability to fix atmospheric N. Ketterings et al. (2015) reported that 82% of the N produced by legumes can be directly available for corn after CC termination. In a meta-analysis study that looked at N inputs by legumes across different soil types and climates, Tonitto et al. (2006) determined that leguminous CC can provide 8 to 350 kg N ha^{-1} depending on the N content of aboveground biomass. The University of Tennessee Extension for agricultural research also reported that if a single-species legume CC produces high enough biomass, it can contribute 67 to 90 kg N ha^{-1} (McClure et al., 2018). Grasses, in contrast, take up or scavenge for residual N from the soil system. The ability of grasses to scavenge N more efficiently than legumes make them a strategic tool to reduce N leaching which can contaminate nearby water sources (Meisinger and Ricigliano, 2017). *Brassicas* function similarly to grasses. They are N scavengers, and additionally they have an extensive deep tap-root system which allows them to remove N in deeper soil depths. Furthermore, *Brassicas* have been shown to improve soil moisture content because their deep tap-roots create channels through the soil profile which help alleviate compaction and increase water infiltration (Wang et al., 2018; Williams and Weil, 2004).

It is important to know the rate of N turnover by the CC in order to determine the potential N savings from incorporating CC into a row-crop management scheme. Sievers and Cook (2018) determined that grasses, such as cereal rye (*Secale cereal* L.), due to having greater carbon to N ratios (C/N) decompose more slowly and release N slower than legumes, such as hairy vetch (*Vicia villosa* Roth), which have lower C/N. Ramirez-Garcia et al. (2015) also showed that a narrower C/N results in a faster decomposition rate. It is common practice to employ CC mixtures of N fixers and scavengers to harness a more functional diversity of ecosystem services in order to get a more even spread of N input from CC for subsequent row-crops. Legume monocultures have been shown to have an asynchrony between N turnover and corn demand of N (Stute and Posner, 1995), moreover Wallace et al. (2017) reported that CC mixtures had a better synchrony between N release and corn demand.

If the N in the CC residue is released too quickly before planting the row-crops, there is a great risk that the N will be lost from the soil (Nagumo and Nakamura, 2013). Therefore, it is important to know the mineralization rate of CC residues. There are various factors that influence the rate of CC decomposition. Temperature, moisture, residue type and quality, and method of incorporation (surface mulch vs. tilled into the soil) are the main variables that dictate decomposition rates of CC residues. Because of the hot and humid conditions, it is possible that CC residues decompose rapidly in the southeastern US, regardless of method of incorporation and CC type (Jani et al., 2016).

Residue type controls the decomposition and nutrient release to a great extent. The content of different biochemical components (e.g., lignin, cellulose, hemicellulose, polyphenol) vary in

different CC types (Chauvin et al., 2015; Redin et al., 2014; Stubbs and Kennedy, 2017; Tian et al., 1992). Some species may have a greater percentage of lignin, while others may have greater amounts of cellulose. It is the difference in their compositional content that influences the decomposition rate of each individual CC species. Xu et al. (2017) determined that as lignin concentration in residues increases, the rate of decomposition decreases.

Tillage enhances CC residue decomposition by providing better soil-to-CC residue contact, which ensures better microbial decomposition of plant residues. Mulvaney et al. (2017) reported that residues placed in the subsoil, mimicking tillage management, resulted in faster rates of decomposition, compared to surface placed residues. Tillage allows for more oxygen diffusion into deeper soil depths, creating favorable conditions for microbial oxidation of organic residues. Buried residues decomposed consistently faster compared to surface placed residues in studies conducted in Alabama and Massachusetts which included legume and non-legume species (Jahanzad et al., 2016; Mulvaney et al., 2010). Tillage positively impacts the rate of residue decomposition and N release, but this may not be synchronized with row-crop demand, especially with single-species legumes or grasses. Legume and grass mixtures along with no-tillage management may provide a better synchrony of N release and row-crop demand (Poffenbarger et al., 2015a). Although row-crop production in west Tennessee is predominantly no-tillage, tillage management is still practiced, so it is important to know the effect of degree of soil-to-CC residue contact on residue decomposition.

Few studies in the southeastern US have focused on the residue decomposition and N release of multi-species CC mixtures compared to single- and double-species CC. Therefore, we conducted

this study to determine the *in-situ* rates of CC residue decomposition and N release in response to CC species type and level of soil-to-CC residue contact. We hypothesized that due to a narrower C/N as a result of an increase in legume species present, increase in functional diversity, and overall increase in biodiversity, the multi-species CC treatment will have the fastest rate of decomposition and N release. We also hypothesized that CC residue decomposition and N release will increase with subsurface placed residues compared to surface placed residues due to enhanced oxygen diffusion into the lower soil depths and increased soil-to-CC residue contact, resulting in greater microbial oxidation of CC residues.

MATERIALS AND METHODS

Study Site Description

The litterbag study was conducted at the University of Tennessee Research and Education Center in Milan, TN (34.9421° N, 88.7164° W; elevation of 129 m) on an existing CC field trial that began in 2013 on plots that have been under no-tillage management for more than 30 years. This is a no-tillage corn-soybean production system with soybean in 2018, planted on 14 May 2018. The CC treatments were wheat (*Triticum aestivum* L.; WHT); cereal rye (CR); cereal rye with crimson clover (*Trifolium incarnatum* L.; CR/CC); cereal rye with hairy vetch (CR/HV); and a 5-crop mixture (soil health mix, SHM) of cereal rye, whole oats (*Avena sativa* L.), crimson clover, hairy vetch, and daikon radish (*Raphanus sativus var. Longipinnatus*). The study was a randomized complete block experimental design (RCBD) with four blocks for each CC treatment. Each experimental unit measured 4.06 x 9.14 m (equivalent to 5 crop rows). Seeding rates for all treatments followed the NRCS recommendations for Tennessee (Table 2.1). Cover

crops were drill seeded using a 1.83 m Tye drill on 10 October 2017. Cover crops were subsequently terminated on 30 April 2018 with the application of glyphosate [N-(phosphonomethyl)-glycine]. Subsequent pre- and post-emergence plant protection chemicals applied by broadcast spraying on an as-needed basis is provided in Table 2.2. Soybeans received fertilizer applications; rates were soil-test based recommendations by the University of Tennessee. Fertilizers were broadcast applied on 11 April 2018. Diammonium phosphate (18-46-0), muriate of potash (0-0-60), and ammonium sulfate (21-0-0-24) were applied at 20.6 kg P_2O_5 ha^{-1}, 74 kg K_2O ha^{-1}, and 2.4 kg S ha^{-1}. Cover crop and soybean management followed production practices recommended by the University of Tennessee for no-tillage systems. The soil of this site is characterized as a Lexington silt loam (fine-silty, mixed, active, thermic Ultic Hapludalfs).

Litterbag Preparation and Placement

Cover crop residues were randomly obtained from each treatment plot. Residue samples were sheared as close to the soil surface as possible and placed into paper bags prior to CC termination. The CC samples were oven-dried at 60°C for 48 hours and then cut into 10 cm segments before being placed into litterbags. Each litterbag, measuring 10 x 20 cm with a mesh size of 55 μm, was filled with 10 g of oven-dried CC residues, which is equivalent to 5 Mg ha^{-1}. This litterbag design is similar to methods described by Hutchens and Wallace (2002), Graça (2005), and Partey et al. (2017). One set of CC residues were placed horizontally on the soil surface (SP) of each plot after CC termination and fastened into place with metal stakes to ensure the litterbags stayed in place. Another set of CC residues were placed into the soil at a depth of 5 cm to mimic soil disturbance from conventional tillage operations (SSP). Litter bag placement

was done on 17 May 2018. In total, there were 400 bags (5 CC treatments x 2 placement methods x 5 retrieval dates x 2 bags retrieved per method per plot at each retrieval date x 4 blocks).

Calculations and Lab Analysis

Prior to placement of the litterbags in the field, oven-dried CC samples, after being ground with a spice grinder (Krups, Solingen, Germany), were analyzed for total C and N content by dry combustion using a CN analyzer (vario MAX cube, Elementar, Langenselbold, Germany). Two bags were randomly collected 1, 2, 4, 9, and 21 weeks after litterbag placement from each plot at each soil depth (SP and SSP). After collecting the litterbags from the field, they were placed into plastic bags and placed over ice until taken to the lab where they were cleaned by gently removing any soil particles and invertebrates. The residues were then oven-dried at 60°C for 48 hours. After oven drying, the residues were weighed to determine the dry matter content (DM) of each litterbag. A subsample of the oven-dried and ground CC residues was corrected for mineral soil by placing a subsample in a muffle furnace for 8 hours at 450ºC. The ash-free dry weight (AFDW) is the difference between the oven-dried residue subsample weight and the weight of the 450ºC dried subsample (Hutchens and Wallace, 2002; Partey et al., 2017). The concentration of total C and N in the residues at each sampling time were measured using a CN analyzer. The amount of N that remained in the litterbag was computed from the combination of the AFDW and the N concentration data. A C/N was computed using the mass-based residue compositions.

Statistical Analysis

Means and statistical significances for the decomposition rates of DM and N content across the five CC treatments were determined using PROC GLIMMIX analysis of variance through SAS v9.4 software (SAS Institute Inc., Cary, NC, USA). The percentage of DM remaining (MR) and N content remaining (NR) at each time point was calculated as:

$$MR \text{ or } NR = (X_t/X_0)\times 100 \quad [1]$$

where X_t is the DM or N content at time t, in terms of weeks, and X_0 is the DM or N content at week 0. Week 0 was included in our statistical analysis. This statistical model, which is an RCBD with a factorial arrangement of treatments, had CC treatment, placement method, and time as the fixed effects and block and a block by time interaction as the random effects. The Slicediff option in PROC GLIMMIX was utilized to determine the difference of one fixed effect within another. Significances were determined at $P \leq 0.05$. The DM decomposition and N release rates were determined by fitting the data to a single-parameter exponential decay model using the NLIN procedure in SAS:

$$DM(t) \text{ or } N(t) = P_0 e^{-kt} \quad [2]$$

where $DM(t)$ and $N(t)$ is the percentage of DM or N content remaining at time t (weeks), P_0 is the initial content of DM or N in percentage (100%) or mass area^{-1} (kg N ha^{-1}), and k is the decomposition rate of DM or N release. Goodness-of-prediction parameters were calculated to assess the fit of each model to its set of datapoints. Goodness-of-prediction was calculated as:

$$G = \left(1 - \left[\frac{\sum_i^n \{A(i) - A'(i)\}^2}{\sum_i^n \{A(i) - \bar{x}\}^2}\right]\right) \times 100 \quad [3]$$

where G is the calculated goodness-of-prediction value; $A(i)$ is the measured percentage of DM or N content remaining, $A'(i)$ is the predicted value of DM or N content remaining, and $\bar{x}$ is the mean of the measured datapoints (Essington, 2015).

RESULTS

Litterbag placement method had a significant effect on DM remaining and N release, therefore data was statistically analyzed separately for each litterbag placement method.

Climate

Climate data was collected from the National Oceanic and Atmospheric Administration website (https://www.ncdc.noaa.gov/cdo-web/datatools). Throughout the study period (May to October 2018), precipitation at this location totaled 884 mm (Fig. 2.1). The mean monthly temperature at this location for the study period was 23.5°C. July had, numerically, the greatest mean monthly temperature (26.7°C) and October had the lowest (15.9°C).

Cover Crop Dry Matter Loss

There was a significant CC treatment by time interaction for SP (P=0.023; Table 2.3). Within all weeks except week 1, CC treatment had a significant effect on DM remaining (P<0.05; Table 2.4; Fig. 2.2). Soil health mix had the lowest percentage of DM remaining after two weeks (61%), which was significantly lower than CR/HV (68%). At week 4, SHM still had the lowest percentage of DM remaining (53%), which was significantly lower than all other CC treatments (57-60%). The WHT residues decomposed faster after week 4, resulting in the lowest DM remaining at week 21 (19%) compared to all other treatments except SHM.

There was a significant CC treatment by time interaction for SSP (P=0.0005; Table 2.3). Throughout the experiment except at the end (week 21), CC treatment had a significant effect on

DM remaining (P<0.05; Table 2.4; Fig. 2.2). Unlike SP, WHT had the lowest percentage of DM remaining (28%) compared to all other CC treatments at week 2. While SHM had the second lowest percentage of DM remaining (32%), which was significantly lower than CR/HV (36%) and CR (45%). The percentage of DM remaining for CR was significantly greater compared to all other treatments. At week 4, WHT continued to have the lowest DM remaining (21%), which was significantly lower than all other treatments except the SHM (25%). Soil health mix and CR/HV (27%) had the second lowest DM remaining which were significantly lower than CR (38%) and CR/CC (32%). At the end of the study, no statistical differences across the CC treatments was found, although WHT numerically had the lowest percentage of DM remaining (10%) compared to all other CC treatments (13-14%). Overall for both methods of residue placement, CR and rye/legume mixtures consistently had greater DM remaining indicating that CR had the greatest potential to hinder residue decomposition. Within the first week, 47% of SSP DM remained while 74% remained for SP. After 4 weeks, 57% DM was still remaining in the SP whereas a small proportion of the SSP DM was still remaining (29%). After 21 weeks of decomposition 23% DM still remained for SP, with only 13% remaining for SSP.

Nitrogen Release

The N content in CC residues, before *in-situ* decomposition, was significantly greater for CR/HV (98 kg N ha^{-1}) followed by SHM (90 kg N ha^{-1}; Table 2.5; Fig. 2.3). The SP had a significant CC treatment by time interaction (P<0.0001; Table 2.3) on N release from CC decomposition. In weeks 1, 2, and 4, CC treatment had a significant effect on N remaining (P<0.05; Table 2.5; Fig. 2.3). One week after initial bag placement, the SHM and CR/HV residues contained the greatest

amount of N (64 and 65 kg N ha^{-1}) compared to all other CC treatments (49-54 kg N ha^{-1}). Within week 2, CR/HV had the greatest N remaining in the residues (46 kg N ha^{-1}) compared to all other CC treatments (33-38 kg N ha^{-1}) except SHM (40 kg N ha^{-1}). At week 4, CR/HV continued to have the greatest N remaining (37 kg N ha^{-1}), which was significantly greater than all other CC treatments (27-30 kg N ha^{-1}). At the end of the experiment, only 4.4 to 7.5 kg N ha^{-1} N remained, and the treatment differences were not significantly different.

For the SSP, there was a significant CC treatment by time interaction ($P<0.0001$; Table 2.3). Until week 4, CC treatments had a significant effect on N remaining ($P<0.05$; Table 2.5; Fig. 2.3). In week 1, WHT contained the lowest N remaining (20 kg N ha^{-1}) compared to all other CC treatments (24-26 kg N ha^{-1}). At week 2, CR had significantly the greatest N remaining in its residues (16 kg N ha^{-1}) and WHT had the least (9.3 kg N ha^{-1}). The same trend was observed at week 4 where CR had the greatest N remaining (11 kg N ha^{-1}) and WHT the least (5.3 kg N ha^{-1}) except for CR/CC (10 kg N ha^{-1}). There was a consistent trend with WHT containing the lowest and CR containing the greatest N remaining throughout the study. At the end of the experiment, no significant treatment differences were found and the amount of N remaining in the residues was negligible (1.2 to 1.9 kg N ha^{-1}). Overall, within the first 2 weeks the SSP had released 84% of its available N, whereas SP only released 52%. By week 4, 89% and 62% of the available N had been released for SSP and SP. By the end of the study, only 8% and 2% of the available N had remained for SP and SSP.

Carbon to Nitrogen Ratio

Over the course of the study, there was a significant CC treatment by time interaction on C/N for SP ($P<0.0001$) and SSP ($P=0.0012$; Table 2.3). For both placement methods, CC treatments had a significant effect on C/N at all sampling times ($P<0.05$) except for SSP in week 21 ($P>0.05$; Fig. 2.4). Across the CC treatments, there was a consistent trend of C/N increasing from week 0 to week 1 for SP (21-31 to 24-34) and SSP (21-31 to 38-46). After week 1, C/N generally showed a decreasing trend with time for both SP and SSP. Data also showed that after week 1, CR and SHM had the greatest and lowest C/N.

Exponential Decay Modeling for Dry Matter Mass Loss

We modeled the cumulative DM decomposition over the 21 weeks of this study. The single-parameter exponential decay model provided a satisfactory fit for the cumulative DM loss data in response to CC treatment and placement method, determined by the goodness-of-fit parameter (G; 78-92%; Table 2.6). For the SP, decomposition rates (k; percent loss week^{-1}) across CC treatments was -0.09 to -0.11 with no significant differences across the CC species. For the SSP, the greatest k was observed from WHT (-0.63), SHM (-0.51), and CR/HV (-0.44) and the lowest from CR (-0.21). On average, there was a 4-fold increase in the decomposition rates for SSP (-0.43) compared to SP (-0.1).

Exponential Decay Modeling for Nitrogen Loss

The single-parameter exponential decay model provided a satisfactory fit for the N loss over time in response to CC treatment and soil disturbance (G= 90-98%). For the SP, the k was greatest for single-species CR (-0.33) and SHM (-0.3) compared to CR/CC (-0.21; Table 2.7). This trend was

not seen in the SSP, for which the greatest *k* was observed from SHM (-1.18) and WHT (-1.14) compared to CR/CC (-0.87). The presence of CR in either the mixtures or as in the single-species treatment did not hinder the release of N from the CC residues over time. Similar to DM loss, there was an average 4-fold increase in the N release rates when comparing the *k*-values of the SSP (-1.07) to the SP (-0.26).

DISCUSSION

The main objective of this study was to compare the *in-situ* rates of DM decomposition and N release from multi-species CC in comparison to single- and double-species CC under soybean management. Overall, CR/HV under SP method had the greatest N remaining after week 4 (Table 2.5; Fig 2.3). Our results are not consistent with a study conducted in Maryland which showed that more N remained for single-species CR in comparison to mixtures of legumes and grasses as well as single-species legumes (Poffenbarger et al., 2015a). Unfortunately, our field experiment does not include a single-species legume CC treatment to make a direct comparison of N release of single-species cereal and legume CC.

Effects of Carbon to Nitrogen Ratios on Residue Decomposition and Nitrogen Release

Cover crops in this study had a C/N ranging between 21 to 31, with WHT and CR/CC containing the greatest C/N at the start of the study followed by CR, SHM and CR/HV (Fig. 2.4). Although, another study reported single-species CR having a C/N of 83 (Poffenbarger et al., 2015b), our lower C/N for single-species CR (25) and WHT (29), at the start of this study could stem from the fact that these were terminated before full maturity (Roth et al., 2018). Aher et al. (2017)

reported narrower C/N of CC biomass in the vegetative growth stage compared to full maturity. The C/N of the rye/legume and 5-crop mixtures did not match with other studies that have reported C/N of various CC mixtures of legumes, grasses, and *Brassicas* below 20 (Finney et al., 2016; Tosti et al., 2012). The narrower C/N of CR/HV and SHM at the start of the experiment was a result of N remaining in these residues for both placement methods (Table 2.5; Fig. 2.3). Throughout the study, WHT, CR/HV, and SHM consistently had significantly lower C/N compared to CR and CR/CC; however, only WHT and SHM consistently had the lowest percentage of DM remaining for both placement methods (Table 2.4; Fig. 2.2). Following week 4, C/N aligned more with the DM remaining for CR and WHT for both SP and SSP; WHT had significantly lower percentage of DM remaining compared to CR. In weeks 9 and 21, CR had a significantly great C/N compared to WHT.

Overall, there was a decrease in C/N with time. Prior studies have reported enrichment of N in CC residues over time due to fungal translocation from soil to CC residues, which was attributed to increased fungal genes detected on the CC residues (Frey et al., 2000; Wells et al., 2017; Williams et al., 2018). Sievers and Cook (2018) also reported decreased C/N for single-species CR, although an increase in C/N for single-species hairy vetch. Studies have reported soil C/N below 12 and decreasing with soil depth (Cates and Ruark, 2017; Fan et al., 2015). The loessal soils of west Tennessee have an average C/N of 11 to a depth of 30 cm (Personal communication; Essington, ME). Although CC residues were corrected for soil contamination, the decrease in C/N for all CC treatments may by the result of the CC residue C/N equilibrating with soil C/N due to increased residency in the soil. When comparing the C/N of SP with SSP

between weeks 4, 9, and 21, SP had 22% greater C/N values than SSP. Indicating that over time, the CC residues were equilibrating with their soil surroundings.

Cereal Rye Allelochemicals and Their Influence on Decomposition and Nitrogen Release

Although there was efficient decomposition of CC residues, the presence of CR, whether as a single-species or rye/legume mixture, hindered CC residue decomposition in the SSP. Cereal rye's allelopathic effect contributing to its well-established role as a weed suppressor has been extensively studied (Barnes and Putnam, 1987; Reiss et al., 2018; Schulz et al., 2013). Because CR is an efficient weed suppressor, its allelochemicals may also inhibit microbial communities, especially when incorporated into the soil as in the case of our SSP method (Rice et al., 2007). Consequently, our results showed that WHT had a significantly lower percentage of DM remaining in comparison to CR. Although we did not study allelopathic interactions, future studies on the effect of allelochemicals from CR on soil microbial communities and its response on N release are needed to gain a better understanding of the mechanisms behind residue decomposition and N release from different CC species.

Influence of Soil Management Practices on Decomposition and Nitrogen Release

The faster rates of CC decomposition and N release for SSP than SP is supported by the results of other studies (Jahanzad et al., 2016; Lynch et al., 2016; Mulvaney et al., 2010; Radicetti et al., 2017). Improved gas diffusion into the soil and better soil-to-CC residue contact as a consequence of residue burial facilitated faster oxidation of organic CC residues (McCourty et al., 2018). Several field studies have reported greater respiration rates when residues were incorporated into soil by conventional tillage compared to reduced or no-tillage (Bojarszczuk et

al., 2017; Schwen et al., 2015; Silva et al., 2019). These greater respiration rates are potentially indicative of increased oxidation of organic substances by soil microbes. Henriksen and Breland (2002) determined reduced decomposition of surface applied straw residues due to delayed colonization of microbes. The SSP treatment enhanced decomposition and the release of N at greater rates, with more than 80% of the N in the CC residues lost within the first 2 weeks after litterbag placement. If the succeeding crop is corn, this can cause an asynchrony with corn N demand, which is greatest approximately 3 weeks after plant emergence (Ransom and Enders, 2013).

CONCLUSIONS

In this study, we found that increased contact of CC residues with soil, which mimicked a conventionally tilled system, significantly enhanced the decomposition of CC residues and the subsequent release of N after termination. This supported our hypothesis that DM decomposition and N release would be greater with increased soil disturbance. A majority of the N release from the SSP occurred rapidly within the first 2 weeks after termination. For example, WHT had lost 60 kg N ha^{-1} in 2 weeks leaving only 9 kg N ha^{-1}. For the SP method, there was a pronounced delay in CC residue decomposition compared to SSP. Double-species CR/HV and the 5-species SHM had the greatest N remaining 2 weeks after litterbag placement, while at week 4, CR/HV alone had the greatest N remaining. Overall, our hypothesis that the SHM would have the greatest rate of N release was partially correct. Four weeks after litterbag placement, N remaining across the CC species for SP and SSP was 33 to 44% and 8 to 14%. As a result, we suggest that producers decrease the lag time between CC termination and row-crop planting in

order to utilize the N from CC residues to subsequent row-crops. This study lessens the knowledge gap regarding diverse cover cropping strategies and their potential as a supplemental N source for row-crops. Additional research is needed to document the inhibitory effect of CR included CC on residue decomposition and N release. Further studies are also needed to determine the combined effect of cover cropping, row-crop planting dates, and N fertilizer rates on row-crop yield to gain a better understanding of the benefits of CC as a supplemental N source for increased profitability and sustainability in the hot and humid southeastern US.

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APPENDIX II

Table 2.1 Seeding rates for cover crop treatments. Seeding rates followed the recommendations by the Natural Resources Conservation Service of the United States Department of Agriculture for Tennessee.

Treatment	Crop	Seeding Rate
		---------kg ha^{-1} --------
WHT	Wheat	84.20
CR	Cereal Rye	84.20
CR/CC	Cereal Rye	25.82
	Crimson Clover	15.72
CR/HV	Cereal Rye	25.82
	Hairy Vetch	23.58
SHM	Cereal Rye	16.84
(Soil health mix)	Whole Oats	22.45
	Crimson Clover	4.49
	Hairy Vetch	6.74
	Daikon Radish	2.25

Table 2.2 List of herbicides (H) and fungicides (F) applied for the 2018 soybean growing season.

Pesticide Type	Application Date	Commercial Name	Chemical Type
H	May 8	Liberty	Pre-emergence
H	May 14	Gramoxone SL 2.0	Pre-emergence
H	May 14	Prowl H2O	Pre-emergence
H	May 14	Canopy	Pre-emergence
H	June 6	Roundup PowerMax	Post-emergence
H	June 6	Dual II Magnum	Post-emergence
F	July 19	Quadris Top SBX	Post-emergence
F	July 19	Endigo XC	Post-emergence

Table 2.3 Analysis of variance for the percentage of dry matter content (DM) remaining, nitrogen (N) content remaining, and carbon to nitrogen mass ratio as a function of cover crop treatment and weeks after litterbag placement.

Effect	% DM Remaining	N Content Remaining	C/N
		Pr > F†	
SP‡			
Treatment	<0.0001	<0.0001	<0.0001
Week	<0.0001	<0.0001	<0.0001
Treatment x Week	0.023	<0.0001	<0.0001
SSP			
Treatment	<0.0001	<0.0001	<0.0001
Week	<0.0001	<0.0001	<0.0001
Treatment x Week	0.0005	<0.0001	0.001

†Significance was determined at $P \leq 0.05$

‡SP, surface placed litterbags; SSP, subsurface placed litterbags

Table 2.4 Percentage of dry matter content (DM) remaining for surface and subsurface placed litterbags. Standard errors of the mean are shown in parenthesis.

Treatment†	%DM Remaining				
Week	**1**	**2**	**4**	**9**	**21**
SP‡					
WHT	74 (0.77)	64 (0.75) b§	57 (1.73) a	31 (0.73) c	19 (0.53) c
CR	75 (0.54)	65 (0.78) ab	59 (2.19) a	42 (1.47) a	27 (0.60) a
CR/CC	72 (0.51)	63 (2.11) b	57 (1.83) a	36 (1.24) b	24 (0.63) ab
CR/HV	75 (1.12)	68 (1.74) a	60 (1.81) a	38 (1.42) b	24 (0.60) ab
SHM	72 (0.72)	61 (0.65) b	53 (1.2) b	32 (0.48) c	23 (0.72) bc
MSDV¶	3.86	3.86	3.86	3.86	3.86
SSP					
WHT	41 (1.07) b	28 (1.44) d	21 (0.79) d	15 (0.53) c	10 (1.01)
CR	54 (0.75) a	45 (1.44) a	38 (1.8) a	27 (3.03) a	14 (1.41)
CR/CC	51 (0.8) a	36 (1.57) bc	32 (1.69) b	21 (0.84) b	14 (0.77)
CR/HV	43 (0.4) b	36 (0.74) b	27 (0.57) c	17 (2.5) bc	14 (0.84)
SHM	45 (0.94) b	32 (1.54) c	25 (0.92) cd	18 (1.26) bc	13 (0.95)
MSDV	3.89	3.89	3.89	3.89	3.89

†WHT, wheat; CR, cereal rye; CC, crimson clover; HV, hairy vetch; SHM, soil health mix (cereal rye, whole oats, crimson clover, hairy vetch, and daikon radish)

‡SP, surface placed litterbags; SSP, subsurface placed litterbags

§Within a column for each placement method (surface and subsurface), means followed by the same lowercase letter are not significantly different at $P \leq 0.05$ (n=4). Absence of lowercase letters indicate fixed effects were not significantly different

¶Minimum significant difference value

Table 2.5 Nitrogen (N) content remaining for surface and subsurface placed litterbags for each treatment. Standard errors of the mean are shown in parenthesis.

Treatment†	N Remaining					
Week	**0**	**1**	**2**	**4**	**9**	**21**
SP‡	**kg N ha^{-1}**					
WHT	68.7 d§	52.8 (11.8) b	36.5 (1.2) bc	30.4 (2.4) b	11.2 (0.7)	4.4 (0.3)
CR	83 c	49.1 (8.7) b	32.6 (1) c	27 (1.8) b	14.7 (0.3)	7.4 (0.1)
CR/CC	67.6 d	53.6 (10.2) b	38.1 (1.8) bc	28.5 (2) b	12.8 (1.1)	6.8 (0.3)
CR/HV	97.7 a	63.7 (6.5) a	46 (2.2) a	37 (2.7) a	15.6 (1.5)	7.5 (0.2)
SHM	89.7 b	64.5 (3.7) a	40.1 (0.4) ab	29.8 (1.1) b	11.6 (0.3)	6.2 (0.3)
MSDV¶	6.31	6.31	6.31	6.31	6.31	6.31
SSP						
WHT	68.7 d	19.8 (1.6) b	9.3 (0.8) d	5.3 (0.4) c	2.6 (0.3)	1.2 (0.3)
CR	83 c	25.3 (0.8) a	15.5 (0.8) a	11.4 (0.3) a	5.4 (0.7)	1.6 (0.3)
CR/CC	67.6 d	26 (1.1) a	11.4 (1.3) cd	9.7 (0.8) ab	5 (0.5)	1.5 (0.1)
CR/HV	97.7 a	24.8 (1.5) a	14.5 (0.2) ab	9 (0.5) b	4 (0.9)	1.9 (0.3)
SHM	89.7 b	24.1 (1.5) a	12.5 (0.9) bc	8.1 (0.3) b	3.7 (0.5)	1.4 (0.3)
MSDV	2.27	2.27	2.27	2.27	2.27	2.27

†WHT, wheat; CR, cereal rye; CC, crimson clover; HV, hairy vetch; SHM, soil health mix (cereal rye, whole oats, crimson clover, hairy vetch, and daikon radish)

‡SP, surface placed litterbags; SSP, subsurface placed litterbags

§Within a column for each placement method (surface and subsurface), means followed by the same lowercase letter are not significantly different at $P \leq 0.05$ (n=4). Absence of lowercase letters indicate fixed effects were not significantly different

¶Minimum significant difference value

Table 2.6 Exponential decay model (Equation [2]) parameters for the cover crop dry matter content (DM) loss in response to cover crop species and residue placement method. Standard errors of the mean are shown in parenthesis.

Treatment†	Intercept	95% CI‡	Decomposition Rate	95% CI	G§
SP¶	**%DM**		**%loss week^{-1}**		**%**
WHT	0.892 (0.029)	0.833 – 0.952	-0.113 (0.011)	-0.136 – -0.089	91.8
CR	0.859 (0.030)	0.797 – 0.921	-0.076 (0.009)	-0.095 – -0.058	86.6
CR/CC	0.859 (0.033)	0.791 – 0.927	-0.091 (0.011)	-0.115 – -0.068	86.4
CR/HV	0.88 (0.028)	0.822 – 0.937	-0.086 (0.009)	-0.104 – -0.067	90
SHM	0.871 (0.034)	0.801 – 0.942	-0.111 (0.014)	-0.139 – -0.083	87.5
SSP					
WHT	0.961 (0.051)	0.855 – 1.07	-0.63 (0.074) a#	-0.785 – -0.476	88.9
CR	0.848 (0.056)	0.733 – 0.964	-0.214 (0.037) b	-0.292 – -0.137	78.2
CR/CC	0.912 (0.06)	0.788 – 1.034	-0.374 (0.058) ab	-0.493 – -0.254	80.9
CR/HV	0.923 (0.061)	0.804 – 1.06	-0.436 (0.065) a	-0.571 – -0.302	82.9
SHM	0.943 (0.057)	0.824 – 1.06	-0.511 (0.069) a	-0.655 – -0.367	84.6

†WHT, wheat; CR, cereal rye; CR/CC, cereal rye and crimson clover; CR/HV, cereal rye and hairy vetch; SHM, soil health mix (cereal rye, whole oats, crimson clover, hairy vetch, and daikon radish)

‡Confidence interval

§Goodness-of-prediction parameter (Equation [3])

¶SP, surface placed litterbags; SSP, subsurface placed litterbags

#Within a column for each placement method (surface and subsurface), means followed by the same lowercase letter are not significantly different at $P \leq 0.05$ (n=4). Absence of lowercase letters indicate fixed effects were not significantly different

Table 2.7 Exponential decay model (Equation [2]) parameters for the cover crop nitrogen content loss in response to cover crop species and residue placement method. Standard errors of the mean are shown in parenthesis.

Treatment†		Intercept	95% CI‡	Decomposition Rate	95% CI	G§
SP¶		**%DM**		**%loss week^{-1}**		**%**
	WHT	66.1 (2.45) b#	61 – 71.1	-0.219(0.022) ab	-0.264 – -0.175	93.9
	CR	77 (3.75) ab	69.2 – 84.8	-0.33 (0.039) a	-0.41 – -0.25	89.9
	CR/CC	65.5 (2.12) b	61.1 – 69.9	-0.21 (0.018) b	-0.248 – -0.172	94.9
	CR/HV	90.9 (4.12) a	82.4 – 99.5	-0.263 (0.03) ab	-0.326 – -0.201	91.3
	SHM	87.2 (2.74) a	81.5 – 92.9	-0.302 (0.023) a	-0.35 – -0.254	96
SSP						
	WHT	68.3 (1.55) c	65.1 – 71.5	-1.14 (0.063) a	-1.27 – -1.01	98.4
	CR	81.8 (2.85) b	75.9 – 87.7	-0.988 (0.08) ab	-1.15 – -0.823	97.7
	CR/CC	66.9 (2.17) c	62.5 – 71.4	-0.871 (0.064) b	-1 – -0.734	96.6
	CR/HV	97 (2.59) a	91.6 – 102	-1.16 (0.083) ab	-1.33 – -0.99	97.9
	SHM	89.1 (2.24) ab	84.5 – 93.8	-1.18 (0.073) a	-1.33 – -1.03	98

†WHT, wheat; CR, cereal rye; CR/CC, cereal rye and crimson clover; CR/HV, cereal rye and hairy vetch; SHM, soil health mix (cereal rye, whole oats, crimson clover, hairy vetch, and daikon radish)

‡Confidence interval

§Goodness-of-prediction parameter (Equation [3])

¶SP, surface placed litterbags; SSP, subsurface placed litterbags

#Within a column for each placement method (surface and subsurface), means followed by the same lowercase letter are not significantly different at $P \leq 0.05$ (n=4). Absence of lowercase letters indicate fixed effects were not significantly different

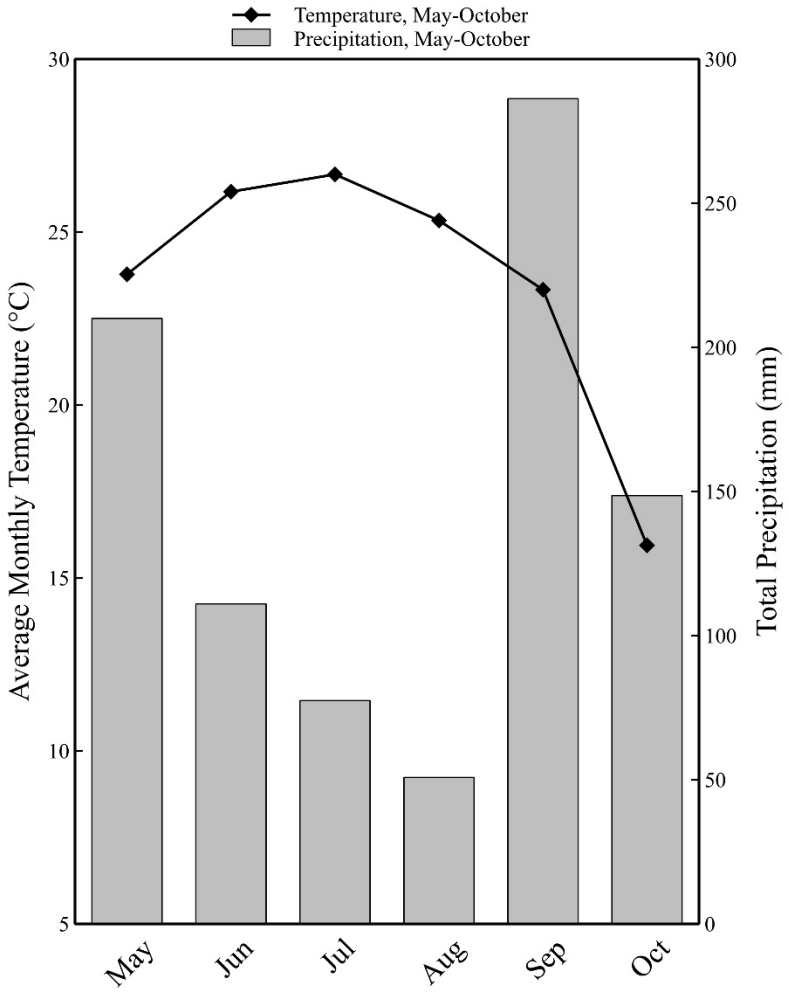


Figure 2.1 Total monthly precipitation and mean monthly temperatures for the months of May to October of 2018 at the University of Tennessee Research and Education Center in Milan, TN.

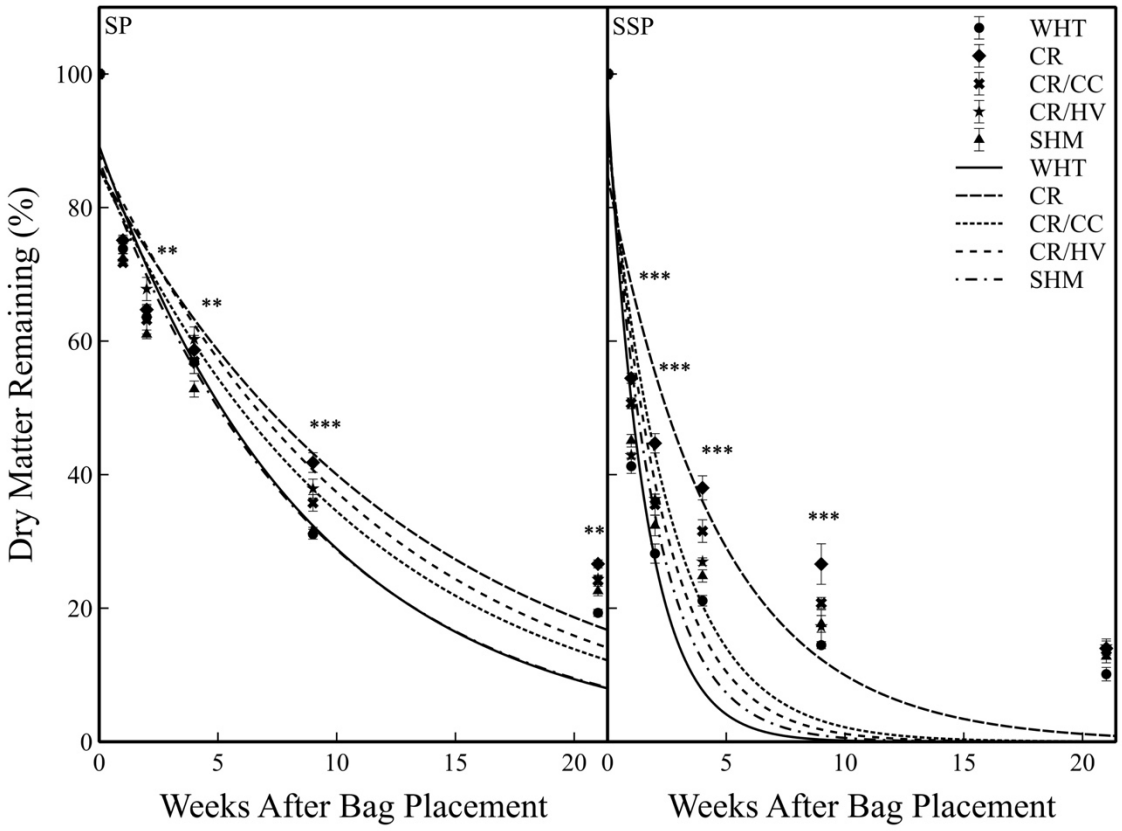


Figure 2.2 Percent cover crop (CC) dry matter content remaining at different times in response to residue placement methods: surface placed (SP) and subsurface placed (SSP).
WHT, wheat; CR, cereal rye; CR/CC, cereal rye and crimson clover; CR/HV, cereal rye and hairy vetch; SHM, soil health mix (cereal rye, whole oats, crimson clover, hairy vetch, and daikon radish). Error bars represent standard error of the mean. Asterisks (*) above error bars indicate that CC type had a significant effect on percentage of CC residue remaining in that week. Absence of * indicates that CC treatment had no significant effect on CC dry matter remaining. Significance was determined at $P \leq 0.05$ (n=4). The lines derived from the single-parameter exponential decay model used to predict the rate of CC residue decomposition over time (Equation [2]); the model parameters for each CC treatment is given in Table 2.6.

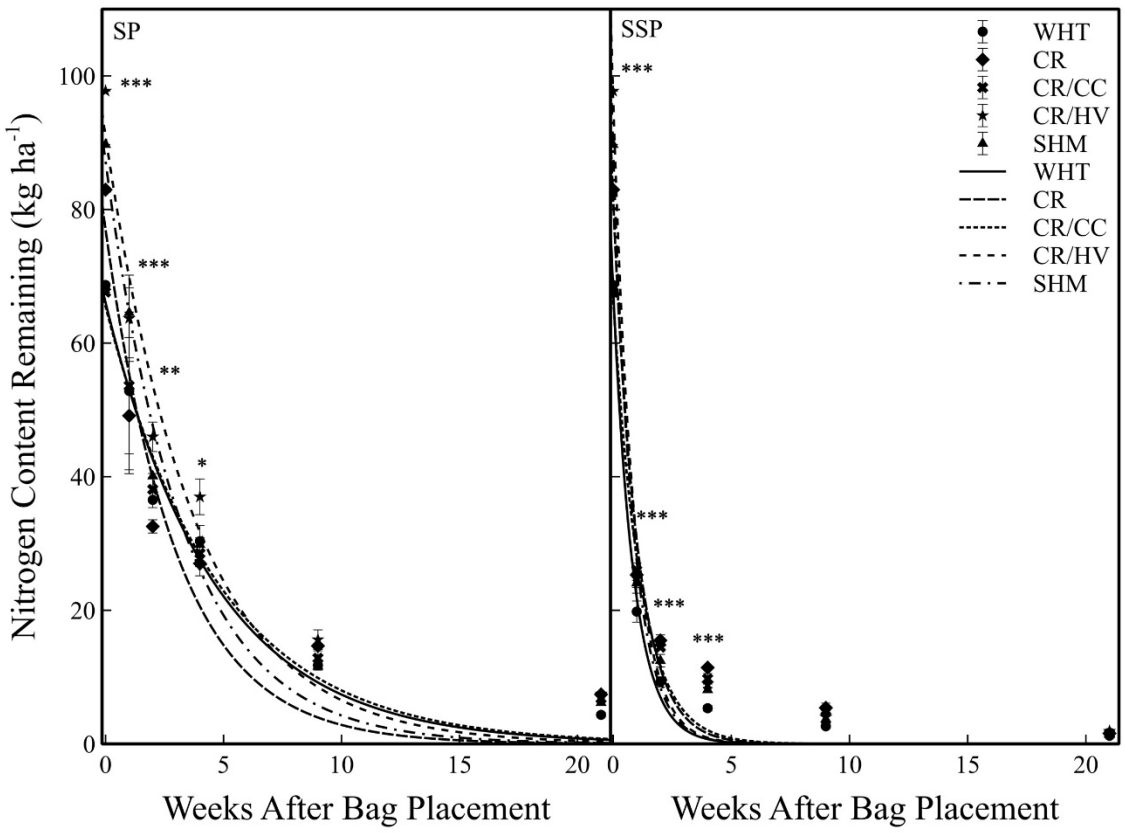


Figure 2.3 Nitrogen (N) content remaining in the cover crop (CC) residues at different times in response to residue placement methods: surface placed (SP) and subsurface placed (SSP). WHT, wheat; CR, cereal rye; CR/CC, cereal rye and crimson clover; CR/HV, cereal rye and hairy vetch; SHM, soil health mix (cereal rye, whole oats, crimson clover, hairy vetch, and daikon radish). Error bars represent standard error of the mean. Asterisks (*) above error bars indicate that CC type had a significant effect on N content remaining in that week. Absence of * indicates that CC treatment had no significant effect on N content remaining. Significance was determined at $P \leq 0.05$ (n=4). The lines derived from the single-parameter exponential decay model used to predict the rate of N release over time (Equation [2]); the model parameters for each CC treatment is given in Table 2.7.

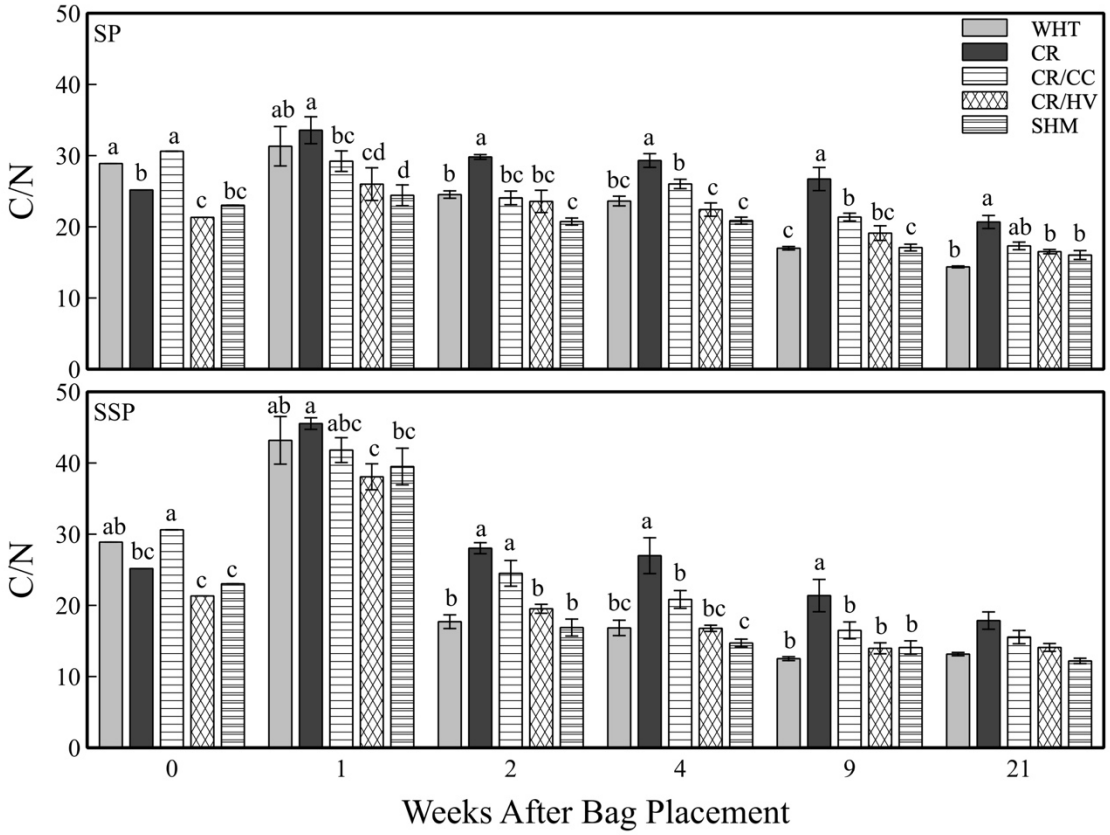


Figure 2.4 Carbon to nitrogen mass ratio (C/N) of the remaining cover crop (CC) residues at different times in response to residue placement method: surface placed (SP) and subsurface placed (SSP).

WHT, wheat; CR, cereal rye; CR/CC, cereal rye and crimson clover; CR/HV, cereal rye and hairy vetch; SHM, soil health mix (cereal rye, whole oats, crimson clover, hairy vetch, and daikon radish). Error bars represent standard error of the mean. Within each sampling time, different lowercase letters above the error bars denote statistically significant means. The absence of letters indicates that CC treatment had no significant effect on C/N in that week. Significance was determined at $P \leq 0.05$ (n=4).

CHAPTER III

TEMPORAL CHANGES IN SOIL NITROGEN POOLS FROM DIVERSE COVER CROP SPECIES IN A NO-TILLAGE SOYBEAN-CORN PRODUCTION SYSTEM IN WEST TENNESSEE

A version of this chapter will be submitted for publication to the Agronomy Journal by Manuel J. Sabbagh, Sindhu Jagadamma, Forbes R. Walker, Jaehoon Lee, Michael E. Essington, Amanda J. Ashworth, and Liesel G. Schneider.

ABSTRACT

Cover crops (CC) can offer an alternative source of nitrogen (N) to row-crops. However, little research has been done on the effects of multi-species CC on soil N dynamics throughout the CC and row-crop growing season, especially in the hot and humid southeastern US. Soil samples were taken 5 times between October 2017 and November 2018 from no-tillage soybean [*Glycine max* (L.) Merr.] plots containing single-species grasses [cereal rye (*Secale cereal* L.) and wheat (*Triticum aestivum* L.)]; double-species grass/legume mixtures [cereal rye and crimson clover (*Trifolium incarnatum* L) and cereal rye and hairy vetch (*Vicia villosa* Roth)]; and a multi-species CC mixture [cereal rye, whole oats (*Avena sativa* L.), crimson clover, hairy vetch, and daikon radish (*Raphanus sativus var. Longipinnatus*)], as well as from a no-CC control. Soil samples were obtained from 3 soil depths (0-5, 5-10, and 10-20 cm). Samples were analyzed for plant available N (PAN), water extractable total soluble N, and potentially mineralizable N (PMN). Significant interactions of CC treatment and soil depth, CC treatment and sampling event, and soil depth and sampling event were observed. Plant available N was greatest in April 2018 (27 mg N kg^{-1}) and May 2018 (24 mg N kg^{-1}) in the 0-5 cm soil layer. Water extractable total soluble N was greatest in May 2018 (34 mg N kg^{-1}) with a 167% increase from April 2018 (13 mg N kg^{-1}). The cereal rye and hairy vetch mixture had the greatest PMN (103 mg N kg^{-1}) compared to the control (88 mg N kg^{-1}). Reducing the time lag between CC termination and row-

crop seeding to the greatest extent possible is suggested to maximize the potential of using CC as an alternative N source.

INTRODUCTION

Cover crops (CC) offer several agronomic services that make them appealing to producers. Cover crops have the ability to control soil erosion and reduce nutrient loss (Osterholz et al., 2019; Siller et al., 2016), control weeds (Cornelius and Bradley, 2017; Crawford et al., 2018), as well as improve soil health based on improvements to soil biological, chemical, and physical properties (Finney et al., 2017; Mitchell et al., 2017). The ecosystem services from growing CC depend on the intensity of tillage practices. In the hot and humid southeastern US, over 20 to 50% of row-crop producers practice no-tillage (Claassen et al., 2018). In a 4-yr tillage and cover cropping study conducted in New York, Nunes et al. (2018) found that cover cropping in combination with no-tillage increased soil organic matter compared to a no-tillage fallow, plow-tillage with CC, and plow-tillage with no CC. When comparing no-tillage to conventional tillage in a Tennessee agro-ecosystem, Jagadamma et al. (2019) reported greater accumulation of soil organic carbon in no-tillage systems compared to conventional tillage systems. In addition, Nouri et al. (2019) reported improved hydro-physical properties under no-tillage cotton (*Gossypium hirsutum* L.) systems in Tennessee, especially when combined with CC. Although cover cropping in combination with no-tillage has been shown to provide improvements in various physical, biological, and chemical soil properties, there has been a special interest in the potential ability of CC to provide nitrogen (N) to subsequent row-crops, potentially reducing synthetic N inputs.

Nitrogen is one of the most important macronutrients for row-crop production. Producers rely on synthetic N fertilizer to meet row-crop demand for optimal yields; however, leaching and volatilization losses can decrease N use efficiency. In a study conducted in Minnesota, Ochsner et al. (2017) estimated nitrate-N (NO_3-N) leaching of 40 to 80 kg ha^{-1} from continuous corn (*Zea mays* L.) and corn-soybean [*Glycine max* (L.) Merr.] production systems, which resulted in 17.9 to 26.7 mg L^{-1} NO_3-N in the groundwater. The NO_3-N leaching from agro-ecosystems has led to deleterious environmental impacts, including the creation of large algal blooms resulting in hypoxic environments in the Gulf of Mexico (Goolsby et al., 2001). As a result, there is a need to reduce the N transport from croplands. One approach is to include cover cropping, which reduces soil erosion, serves as an alternative N source, and scavenges for excess N.

The ability of CC to serve as an alternative N source to row-crops depends on the CC species. Leguminous CC can fix atmospheric N; therefore, providing N credits to subsequent row-crops. Gentry et al. (2013) determined that the N credit from red clover (*Trifolium pratense* L.) can be as high as 50 kg N ha^{-1}. The University of Tennessee Extension has recommended producers decrease their N fertilizer amount by 67 to 90 kg N ha^{-1} if there was a dense establishment of crimson clover (*Trifolium incarnatum* L.) or hairy vetch (*Vicia villosa* Roth) prior to termination (McClure et al., 2018). However, legumes in humid climatic regions can lose approximately 80% of the N in their residues in the first four weeks after termination (Sievers and Cook, 2018), which is the lag time most producers allow before planting row-crops. This lag can cause an asynchrony between row-crop N demand and N turnover from CC residues particularly when growing legume monocultures (Stute and Posner, 1995). Non-leguminous CC, such as cereal rye (*Secale cereale* L.), winter wheat (*Triticum aestivum* L.), and oats (*Avena sativa* L.), decompose

slower than legumes. Sievers and Cook (2018) found 60% of the N in the residues of cereal rye 8 weeks after termination, resulting in reduced N supply to row-crops. In recent years, the United States Department of Agriculture has promoted double-, triple-, and multi-species cover cropping strategies to harness a more diverse array of agronomic services from cover cropping. Subsequently, White et al. (2017) reported that three- and four-species mixtures performed similar to single-species legumes but better than single-species cereal rye at supplying N. Although CC have shown promise to influence soil N dynamics, it is not clear how their influence fluctuates seasonally and at different soil depths.

Plant available N (PAN) in soil can fluctuate during the growing season, as the biotic and abiotic N cycling processes are affected by changes in precipitation, temperature, sunlight exposure, among other variables. Clivot et al. (2017) reported that PAN varies very little in the soil profile during the autumn, winter, and early spring with a markedly greater fluctuation during late spring and summer. In addition to seasonality, soil N pools vary as a function of soil depth. Although cover cropping studies determining PAN at a single soil depth are abundant (Andrews et al., 2018; Coombs et al., 2017; Liebman et al., 2018), studies looking at the depth distribution of PAN are limited. In a cover cropping study conducted on a sandy loam soil in Georgia, Sainju et al. (2015) reported decreased ammonium-N (NH_4-N) concentration with depth. It is important to understand the concentrations of PAN at soil depth increments to gain a better understanding of the availability of N for succeeding row-crops as root growth patterns vary with crop type and crop growth stages. If PAN is concentrated too shallow or too deep compared to root density, the inefficient utilization of N contributed by the CC may be realized.

The temporal effect of PAN in no-tillage systems as a function of soil depth and CC species is not well studied, especially in the hot and humid southeastern US. Therefore, the objective of this study is to determine how different CC species affect soil N pools at different soil depths during the CC and row-crop growing season. We hypothesized that soils with incorporated legumes will contain greater PAN concentrations compared to soils with incorporated single-species grass CC, due to the ability of legumes to fix atmospheric N. However, the increase in PAN concentrations under legumes will only occur for a short period of time after CC termination due to the rapid decomposition of legumes. It is also hypothesized that mixtures of legumes and grasses will have a greater potential to provide PAN throughout the growing season as a result of different rates of decomposition of grasses and legumes in the mixture.

MATERIALS AND METHODS

Study Site and Cover Crop Treatments

This study was conducted from October 2017 to November 2018 at the University of Tennessee Research and Education Center in Milan, TN (34.9421° N, 88.7164° W, elevation of 129 m). The soil at this site is a Lexington silt loam (fine-silty, mixed, active, thermic Ultic Hapludalfs). This region experiences hot and humid summers with mild winters with a mean annual temperature of 14.8°C and total annual precipitation of 1368 mm. A CC field trial was initiated in 2013 on a cropland that was under long-term no-tillage management for more than 30 years. Several winter CC treatments were laid out in a randomized complete block design (RCBD) with four blocks on a no-till corn-soybean production system. Soybean, planted on 14 May 2018, was the row-crop during the study year. Each experimental unit was 4.06 x 9.14 m (5 crop rows). The

CC treatments were: (i) wheat (WHT); (ii) cereal rye (CR); (iii) cereal rye and crimson clover (CR/CC); (iv) cereal rye and hairy vetch (CR/HV); (v) a five-species mixture recommended by NRCS for the State of Tennessee, known as a soil health mix (SHM) which is comprised of cereal rye, whole oats, crimson clover, hairy vetch, and daikon radish (*Raphanus sativus var. Longipinnatus* L.); and (vi) a no-CC control (CTRL). Cover crops were no-till seeded in October 2017 and chemically terminated with glyphosate [*N*-(phosphonomethyl)-glycine] on 30 April 2018. Seeding rates for each CC treatment, which follows recommendations by the NRCS for Tennessee, are included in Table 2.1. Subsequent pre- and post-emergence plant protection chemicals applied on an as-needed basis are listed in Table 2.2. Row-crop and CC management followed production practices recommended by the University of Tennessee for no-tillage systems. Soybeans received fertilizer applications applied through broadcast spraying on 11 April 2018. Diammonium phosphate (18-46-0), muriate of potash (0-0-60), and ammonium sulfate (21-0-0-24) were applied at 20.6 kg P_2O_5 ha^{-1}, 74 kg K_2O ha^{-1}, and 2.4 kg S ha^{-1}. Rates were soil-test based recommendations by the University of Tennessee. Fertilizers reflect commercially available forms available to producers in this region.

Soil Sampling

Soil samples were collected during the CC and soybean growing seasons. Specifically, samples were collected on 30 October 2017 (prior to CC planting), and then four times in 2018: 16 April (before CC termination), 23 May (one month after CC termination), 15 August (during the soybean growing season), and 5 November (after soybean harvest). Samples were collected from three soil depths (0-5, 5-10, and 10-20 cm) using a 2.5 cm diameter soil probe. Ten to 15 soil cores were taken, at random, from each plot for each depth and were then combined and

homogenized. A total of 72 samples were taken at each sampling event (3 depths x 6 treatments x 4 blocks). Soil samples were placed in plastic bags and transported over ice and stored under refrigeration. Soil samples were air-dried and passed through a 2 mm sieve.

Soil Analysis

Air-dried soil samples (<2 mm) were analyzed for PAN (NO_3-N and NH_4-N), water extractable total soluble N (WEN), and potentially mineralizable N (PMN). Nitrate-N and NH_4-N concentrations were analyzed after a 2 mol L^{-1} potassium chloride (2M KCl) extraction procedure adopted from Sikora and Moore (2014). Briefly, a 1:5 (soil to solution) mixture was shaken for 1 hour on a reciprocating shaker at 180 oscillations $minute^{-1}$ and then filtered through Whatman No. 42 filter paper. Filtrates were analyzed using a Continuous Flow Analyzer (CFA; Skalar Analytical B.V., Breda, Netherlands). Plant available N is computed as the sum of mg N kg^{-1} as NO_3 and mg N kg^{-1} as NH_4.

The WEN analysis procedure was adopted from Haney et al. (2012). Briefly, WEN was determined by combining ultra-pure water with soil at a 1:5 soil to water ratio. The suspensions were shaken on a reciprocating shaker for 10 minutes at 200 oscillations $minute^{-1}$, then centrifuged, and filtered through Whatman No. 42 filter paper. Total soluble N concentrations were determined through the principle of oxidative combustion-chemiluminescence with a Total Organic Carbon and Total Nitrogen Analyzer (Shimadzu Corporation, Kyoto, Japan).

Potentially mineralizable N estimates the amount of PAN that potentially become available throughout the growing season of the row-crop (Waring and Bremner, 1964). Many methods

exist to quantify PMN, depending on incubation approaches (anaerobic vs. aerobic) and total time of incubation (Carter, 1993; Ginakes et al., 2018; Osterholz et al., 2017). We followed a short-term (7-day) anaerobic incubation protocol developed by Keeney and Bremner (1966). Briefly, 12.5 mL of water was combined with 5 g of air-dried soil (<2 mm) into 15 mL polypropylene centrifuge tubes, leaving no headspace, and incubated at 40°C for 7 days. After incubation, the soil and water suspension were extracted using 12.5 mL 4M KCl as described for PAN determination, the shaking step performed for 30 minutes at 200 oscillations minute^{-1}. Ammonium-N concentrations from both, non-incubated and incubated, samples were determined using the CFA. Potentially mineralizable N was calculated as the difference between the NH_4-N concentrations of the incubated and non-incubated samples.

Statistical Analysis

Within the RCBD, a two-way analysis of variance was utilized to test the effects of sampling depth and sampling time within each CC treatment on each measured N parameter. The mixed model analysis of variance (ANOVA) was performed using SAS v9.4 software (SAS Institute Inc., Cary, NC, USA). To determine if the assumptions of ANOVA were met, PROC UNIVARIATE and residual plots were assessed. All inferential statistical analyses were conducted using PROC GLIMMIX. The fixed effects comprised of CC treatment, soil depth, and sampling time while the random effects were block, block by CC treatment, and block by soil depth by CC treatment. For models in which the assumptions of normality of residuals could not be met, data were natural log transformed. In these models, inferential statistics were performed on the transformed values and untransformed least squares means are reported. Least square

means were separated using Tukey's significant difference adjustment. Statistical significance level was determined at $P \leq 0.05$.

RESULTS

The ANOVA showed that strong interactions between soil depth and soil sampling events were observed for all the measured parameters (PAN, NO_3-N, NH_4-N, WEN, and PMN; $P<0.0001$; Table 3.1). In addition, CC treatment by soil sampling event interaction was significant for PAN ($P=0.012$), NO_3-N ($P<0.0001$), and PMN ($P=0.008$). There was a significant CC treatment by soil depth interaction for PMN ($P=0.001$).

Plant Available Nitrogen

Plant available N is the sum of exchangeable and soluble NO_3 and NH_4. There was significantly greater PAN concentration in the top soil layer (0-5 cm) compared to the lower layers (5-10 and 10-20 cm) for all soil sampling events except in November 2018, when all soil layers had similar PAN concentration (8-10 mg N kg^{-1}; Fig. 3.1). During the study, the greatest PAN concentrations were observed in the 0-5 cm layer in April and May 2018 (27 and 24 mg N kg^{-1}). In August 2018, the 0-5 cm layer had the second greatest PAN concentration (17 mg N kg^{-1}) which was greater than in the 0-5 cm layer in October 2017 and November 2018 (11 and 10 mg N kg^{-1}). The deeper soil layers from the October 2017, April 2018, and November 2018 samplings had similar PAN concentrations (5-7 mg N kg^{-1}), but there was a significant increase in the 5-10 cm layer in May 2018 (14 mg N kg^{-1}) from the preceding sampling event in April 2018 (6 mg N kg^{-1}). Statistical analysis was also performed to determine if there was an effect of

CC treatment on PAN within each soil depth at each sampling event. Results indicated no significant effect of CC treatments on PAN ($P>0.05$; Table 3.2). Additionally, when the CC treatment by sampling event interaction was analyzed, PAN of all CC treatments in May 2018 (15-16 mg N kg^{-1}) were significantly greater than all other CC treatments from each sampling event (6-12 mg N kg^{-1}) except the CTRL, CR/CC, and WHT in April 2018 (13-14 mg N kg^{-1}) and CR/HV in November 2018 (12 mg N kg^{-1}; Table 3.3). Ultimately, when averaged across all soil depths, CC treatments had no impact on PAN within each soil sampling event.

Nitrate-N followed a similar trend to that of PAN. The greatest concentrations of NO_3-N were observed in the 0-5 cm layer in April and May 2018 (16 and 19 mg N kg^{-1}; Fig. 3.2). In general, the 0-5 cm layer contained greater concentrations of NO_3-N compared to the 5-10 and 10-20 cm layers within each soil sampling event except November 2018, where all soil layers had similar NO_3-N concentrations (4-5 mg N kg^{-1}). In the 5-10 cm layer, there was a significant increase in NO_3-N concentration in May 2018 (10 mg N kg^{-1}) compared to the preceding soil sampling event in April 2018 (2 mg N kg^{-1}). When each soil depth was analyzed separately within each soil sampling event, CC had no effect on NO_3-N concentrations ($P>0.05$; Table 3.2). Furthermore, CC treatments in May 2018 (11-12 mg N kg^{-1}) were significantly greater compared to all other soil sampling events (2-7 mg N kg^{-1}; Table 3.4).

Unlike PAN and NO_3-N, NH_4-N concentrations were greatest in the 0-5 cm layer in April and August 2018 (9 mg N kg^{-1}; Fig. 3.3). In October 2017, NH_4-N concentrations did not differ across soil depths (4-5 mg N kg^{-1}), whereas in all other soil sampling events there was a significant decrease in NH_4-N concentrations with an increase in soil depth. Overall, within each

soil depth at each soil sampling event, CC had no effect on NH_4-N concentrations (P>0.05; Table 3.2).

Water Extractable Total Soluble Nitrogen

Water extractable total soluble N, the sum of water-soluble inorganic and organic N in soil, decreased with soil depth at all the sampling events (Fig. 3.4). At the 0-5 cm layer, WEN concentrations were the greatest in May 2018 (34 mg N kg^{-1}), followed by October 2017 and August 2018 (20 and 22 mg N kg^{-1}) and the least in April and November 2018 (13 and 10 mg N kg^{-1}). The same trend was also observed for the other two soil depths. Cover crops had an effect on WEN only in the 5-10 cm layer in April 2018 (P=0.034; Table 3.2). The CTRL and CR/CC had significantly greater WEN concentrations (4.5 mg N kg^{-1}) compared to all other treatments (3.5-3.8 mg N kg^{-1}) except SHM (4.2 mg N kg^{-1}), which was in between.

Potentially Mineralizable Nitrogen

For the CC treatment and soil depth interaction in the 0-5 cm layer, PMN concentrations under the CR/HV was significantly greater (103 mg N kg^{-1}) than the CTRL and WHT (88 mg N kg^{-1}; Fig. 3.5). There was a consistent decrease in PMN concentrations with depth and the mean values at the 5-10 and 10-20 cm layers were not statistically different among CC treatments. The soil depth and soil sampling event interaction indicates that PMN concentrations were greatest in the 0-5 cm layer in November 2018 (150 mg N kg^{-1}) followed by April 2018 (93 mg N kg^{-1}) which were both greater than the October 2017 sampling event (78 mg N kg^{-1}; Fig. 3.6). In the 5-10 cm layer, PMN concentrations were greatest in November 2018 (41 mg N kg^{-1}) with all other sampling events being statistically similar (23-29 mg N kg^{-1}). In the 10-20 cm layer, PMN

concentrations were statistically similar across soil sampling events (7-17 mg N kg^{-1}). For the CC treatment and soil sampling event interaction, the PMN concentrations of all CC treatments in November 2018 (66-75 mg N kg^{-1}) were significantly greater compared to all other sampling events (32-51 mg N kg^{-1}; Table 3.5). Cover crops, however, did had an effect on PMN in April 2018 in the 0-5 cm layer (P=0.0004; Table 3.2). The CTRL and WHT had significantly lower PMN concentrations (69 and 75 mg N kg^{-1}) compared to all other treatments (95-112 mg N kg^{-1}).

DISCUSSION

The relatively greater PAN (both NO_3-N and NH_4-N) concentrations in April and May 2018 (prior to and soon after CC termination) compared to other soil sampling events (Fig. 3.1, 3.2, and 3.3) is potentially the result of fertilizer N application through diammonium phosphate and ammonium sulfate. Although studies have reported increased mineral N in soils under legumes and grass/legume mixtures compared to non-legumes and a no-CC CTRL (Kramberger et al., 2014; Liebman et al., 2018; Van Eerd, 2018), the overall lack of effect of CC on PAN, NO_3-N, and NH_4-N in April 2018 may be due to the synthetic N input. Relatively greater NH_4-N accumulation in August 2018 could be due to the input of N from the N fixing main crop, soybean. A study reported that 40 to 224 kg N ha^{-1} can be fixed by soybeans, with the N supply being strongly and positively correlated to soybean biomass (Schipanski et al., 2010). Furthermore, PAN and NO_3-N concentrations continued to be greater in May 2018 compared to October 2017 (prior to CC planting), August 2018 (during the soybean growing season), and November 2018 (after soybean harvest) as a result of the decomposition of the CC residues after being terminated. Our results of increased PAN and NO_3-N (Fig. 3.2 and 3.3) within a month of

CC termination are similar to the findings of Radicetti et al. (2017) and Jahanzad et al. (2016). Radicetti et al. (2017) reported greater N mineralization within the first month of CC termination and Jahanzad et al. (2016) reported greater NO_3 levels after CC termination which was attributed to increased soil temperatures. May was warmer and wetter than April (12 vs. 24°C and 161 vs. 210 mm). Increased residue decomposition following CC termination is typical in the southeastern US due to abundant precipitation and relatively high temperatures of this region (Franzluebbers, 2005). The increased PAN concentrations in the intermediary soil layer (5-10 cm) in May 2018 could be attributed to root decomposition. It has been shown that CR and hairy vetch roots can lose 20% and 60% N within the first month of termination (Sievers and Cook, 2018). The spike in PAN concentrations, particularly NO_3-N, in the 5-10 cm layer in May 2018 may also be the result of leaching. In a study conducted on loess-derived soils, Wang and Xing (2016) detected a reduction of NO_3-N content in the surface (0-40 cm) and an accumulation in the subsurface (60-100 cm) soil layers.

Water extractable total soluble N content may be used to estimate the potential N loss from a system through leaching and surface runoff at a given timepoint (Chu et al., 2017). Water extractable total soluble N concentrations were the greatest in May 2018 in the top soil layer (Fig. 3.4), which is due to the decomposition and subsequent release of N from CC residues after termination. This indicates potential N loss out of the soil system following CC termination. It has been shown that soils with high N concentrations and good drainage, when experiencing high precipitation, have a greater risk of N loss (Rosolem et al., 2018). It has also been reported that there is a potential loss of agro-chemicals due to leaching in stable no-tillage loess-derived soils as a result of increased macro-porosity (Essington et al., 1995; Wilson et al., 1998). The soil

series in this study, Lexington, is classified as well-drained with moderate to rapid permeability, further emphasizing the risk of N loss from this soil after CC termination. Ruffatti et al. (2019) reported a CC mixture of 92% CR and 8% daikon radish proved to be an effective strategy in reducing NO_3-N loading. In April 2018, WEN concentrations were significantly lower than the prior sampling event in October 2017. All but one CC treatment contained CR, whether as a single-species or in a mixture, indicating a potential reduction in N loading.

The PMN data revealed that CR/HV had significantly greater PMN than the CTRL and WHT in the top soil layer (Fig. 3.5). Across the soil sampling events, PMN for the top soil layer was significantly greater in November 2018 than all other sampling events (Fig. 3.6). Our results of the CR/HV having greater PMN than the CTRL indicates greater short-term soil organic-N accumulation as a result of the slower decomposition of CR in both the single-species and the CR included mixtures (Table 2.6). The observation that PMN was greatest in November 2018, after soybean harvest, was unexpected. In an incubation experiment determining the rate of decomposition of corn and soybean, Li et al. (2013) reported greater decomposition rates in soybean compared to corn. In another incubation study, Jat et al. (2018) determined greater N mineralization rates for surface placed soybean compared to corn.

Overall, the consistent trend of all soil N parameters (PAN, NO_3-N, NH_4-N, WEN, and PMN) decreasing with soil depth, regardless of CC treatment and soil sampling event, aligned with the expectations of no-tillage production systems with a stratification of nutrients, which is consistent with the findings of several other studies (Murugan et al., 2014; Rorick and Kladivko, 2017; Wang and Weil, 2018).

CONCLUSIONS

From the results, our first hypothesis, greater PAN concentrations from plots with legume CC, was proven false. Cover crops did not have a significant effect on PAN at any sampling time; however, the soil sampling time and soil depth interaction was significant. We found that PAN concentrations were greatest in the top soil layer in April and May 2018 (prior to and one month after CC termination). The greater concentration of PAN prior to CC termination may have been the result of fertilizer application, and that one month after CC termination is the result of the decomposition of CC residues upon termination. Furthermore, due to the high rates of decomposition and N mineralization that have occurred after CC termination, there was a 167% increase in WEN concentrations from April to May 2018. We therefore recommend that producers decrease the lag time between CC termination and row-crop planting in order to utilize most of the N released from the CC to succeeding row-crops and potentially decrease the risk of N loss through leaching. Furthermore, although CC treatment had no direct effect on PAN, the CR/HV showed significantly greater PMN, meaning a greater potential to supply PAN to the entire growing season of the succeeding row-crop. Thus, our second hypothesis, grass/legume mixtures will have a greater potential to supply PAN, was partially proven correct as only one of the mixtures had significantly greater PMN than the no-CC CTRL and single-species grasses. Additional research to correlate yield to row-crop planting dates and different N fertilizer application rates in conjunction with cover cropping is needed to further understand the potential of CC to reduce the use of synthetic N fertilizer in order to improve environmental and water quality while increasing profitability to southeastern US agro-ecosystems.

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APPENDIX III

Table 3.1 Analysis of variance of the fixed effects of CC treatments, soil depths, and soil sampling events on all measured variables.

Fixed Effect	PAN†	NO_3-N	NH_4-N	WEN	PMN
	---------- Pr > F ----------				
Treatment (T)	0.183‡	0.077	0.997	0.851	0.162
Depth (D)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Sampling Event (E)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
T*D	0.759	0.624	0.807	0.446	0.001
T*E	0.012	<0.0001	0.549	0.859	0.008
D*E	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
T*D*E	0.907	0.996	0.978	0.788	0.053

†PAN, plant available nitrogen (NO_3-N and NH_4-N); NO_3-N, nitrate-N; NH_4-N, ammonium-N; WEN, water extractable total soluble nitrogen; PMN, potentially mineralizable nitrogen

‡Significance was determined at $P \leq 0.05$

Table 3.2 Analysis of variance for the effect of cover crop treatments for each soil depth at each soil sampling event on all measured variables.

Fixed Effect	Depth	PAN†	NO_3-N	NH_4-N	WEN	PMN
		---------- Pr > F ----------				
Oct. 17						
CC Treatment	0-5 cm	0.322‡	0.134	0.74	0.092	0.512
CC Treatment	5-10 cm	0.133	0.138	0.103	0.304	0.797
CC Treatment	10-20 cm	0.818	0.076	0.706	0.354	0.812
Apr. 18						
CC Treatment	0-5 cm	0.993	0.923	0.349	0.734	0.0004
CC Treatment	5-10 cm	0.277	0.311	0.272	0.034	0.237
CC Treatment	10-20 cm	0.121	0.201	0.086	0.53	0.445
May 18						
CC Treatment	0-5 cm	0.212	0.685	0.307	0.448	0.367
CC Treatment	5-10 cm	0.473	0.555	0.103	0.318	0.423
CC Treatment	10-20 cm	0.928	0.893	0.133	0.397	0.274
Aug. 18						
CC Treatment	0-5 cm	0.882	0.309	0.755	0.523	0.456
CC Treatment	5-10 cm	0.646	0.888	0.492	0.508	0.908
CC Treatment	10-20 cm	0.887	0.512	0.79	0.475	0.765
Nov. 18						
CC Treatment	0-5 cm	0.629	0.469	0.601	0.503	0.092
CC Treatment	5-10 cm	0.23	0.252	0.79	0.466	0.457
CC Treatment	10-20 cm	0.22	0.815	0.33	0.803	0.574

†PAN, plant available nitrogen (NO_3-N and NH_4-N); NO_3-N, nitrate-N; NH_4-N, ammonium-N; WEN, water extractable total soluble nitrogen; PMN, potentially mineralizable nitrogen

‡Significance was determined at $P \leq 0.05$

Table 3.3 Effect of cover crop treatments at each soil sampling event for plant available nitrogen (PAN). Soil sampling was done once in 2017 (October) and four times in 2018 (April, May, August, and November; n=4).

Treatment†	PAN	SE‡
	--------mg kg^{-1}--------	
Oct. 17		
CTRL	6.08 e§	0.39
WHT	7.3 de	0.72
CR	9.64 cd	1.33
CR/CC	7.88 de	1.16
CR/HV	8.37 cde	0.83
SHM	9.37 cde	1.4
Apr. 18		
CTRL	12.9 bdc	3.55
WHT	14.2 abcd	3.15
CR	11.9 cde	3.39
CR/CC	12.7 bcd	2.94
CR/HV	11.6 cde	2.94
SHM	12.2 cde	3.21
May 18		
CTRL	15 ab	1.9
WHT	16.3 a	2.16
CR	14.6 ab	2.17
CR/CC	15.7 ab	2.63
CR/HV	16.5 a	2.05
SHM	14.5 ab	1.96
Aug. 18		
CTRL	8.95 cde	1.60
WHT	9.5 cde	1.63
CR	9.32 cde	1.7
CR/CC	9.41 cde	1.97
CR/HV	9.34 cde	1.67
SHM	8.75 cde	1.52
Nov. 18		
CTRL	8.19 cde	0.29
WHT	8.45 cde	0.42
CR	8.58 cde	0.31
CR/CC	8.2 cde	0.37
CR/HV	11.6 abc	1.82
SHM	9 cd	0.62

†CTRL, no cover crop; WHT, wheat; CR, cereal rye; CR/CC, cereal rye and crimson clover; CR/HV, cereal rye and hairy vetch; SHM, soil health mix (cereal rye, whole oats, crimson clover, hairy vetch, and daikon radish)

‡Standard error of the mean

§ Means followed by the same lowercase letter are not significantly different at $P \leq 0.05$

Table 3.4 Effects of cover crop treatments by soil sampling event for nitrate-N (NO_3-N). Soil sampling was done once in 2017 (October) and four times in 2018 (April, May, August, and November; n=4).

Treatment†	NO_3-N	SE‡
	--------mg kg^{-1}--------	
Oct. 17		
CTRL	2.3 e§	0.32
WHT	2.94 cde	0.61
CR	4.79 bcd	0.86
CR/CC	3.89 bcde	0.85
CR/HV	4.91 b	0.6
SHM	4.92 bcd	0.98
Apr. 18		
CTRL	7.22 b	2.06
WHT	6.66 bcd	2.18
CR	5.7 de	2.12
CR/CC	6.9 bcd	2.18
CR/HV	6.67 bcde	2.32
SHM	6.63 bcd	2.16
May 18		
CTRL	11.5 a	1.67
WHT	12.2 a	1.74
CR	11 a	1.82
CR/CC	11.6 a	1.7
CR/HV	11.9 a	1.62
SHM	11.6 a	1.53
Aug. 18		
CTRL	4.47 bc	0.42
WHT	5.28 b	0.59
CR	5.5 b	0.74
CR/CC	5.47 b	0.89
CR/HV	5.79 b	1
SHM	4.53 bc	0.48
Nov. 18		
CTRL	4.34 bc	0.14
WHT	4.56 b	0.26
CR	4.87 b	0.23
CR/CC	4.3 bc	0.1
CR/HV	5.18 b	0.33
SHM	4.81 b	0.25

†CTRL, no cover crop; WHT, wheat; CR, cereal rye; CR/CC, cereal rye and crimson clover; CR/HV, cereal rye and hairy vetch; SHM, soil health mix (cereal rye, whole oats, crimson clover, hairy vetch, and daikon radish)

‡Standard error of the mean

§Means followed by the same lowercase letter are not significantly different at $P \leq 0.05$

Table 3.5 Effects of cover crop treatments by soil sampling event for potentially mineralizable nitrogen. Soil sampling was done once in 2017 (October) and four times in 2018 (April, May, August, and November; n=4).

Treatment†	PMN	SE‡
	--------mg kg^{-1}--------	
Oct. 17		
CTRL	32.6 ef	7.64
WHT	39.9 cdef	11.04
CR	37.9 cdef	9.04
CR/CC	34.4 ef	8.82
CR/HV	36.5 cdef	9.97
SHM	36.3 cdef	11.04
Apr. 18		
CTRL	31.8 f	8.27
WHT	42.5 cdef	8.08
CR	44 cdef	10.72
CR/CC	50.7 bc	12.71
CR/HV	49.8 cd	11.86
SHM	47 cde	11.81
May 18		
CTRL	34.2 ef	8.20
WHT	35.3 def	7.76
CR	37.4 cdef	10.35
CR/CC	37.2 cdef	9.57
CR/HV	40 cdef	10.43
SHM	34.4 ef	9.40
Aug. 18		
CTRL	40.3 cdef	8.46
WHT	39.8 cdef	7.68
CR	42 cdef	9.43
CR/CC	38.5 cdef	7.41
CR/HV	38.2 cdef	8.56
SHM	40cdef	8.88
Nov. 18		
CTRL	68.1 a	17.79
WHT	72.4 a	16.97
CR	65.6 ab	16.62
CR/CC	65.6 a	16.39
CR/HV	75 a	18.57
SHM	67.5 a	16.07

†CTRL, no cover crop; WHT, wheat; CR, cereal rye; CR/CC, cereal rye and crimson clover; CR/HV, cereal rye and hairy vetch; SHM, soil health mix (cereal rye, whole oats, crimson clover, hairy vetch, and daikon radish)

‡Standard error of the mean

§Means followed by the same lowercase letter are not significantly different at $P \leq 0.05$

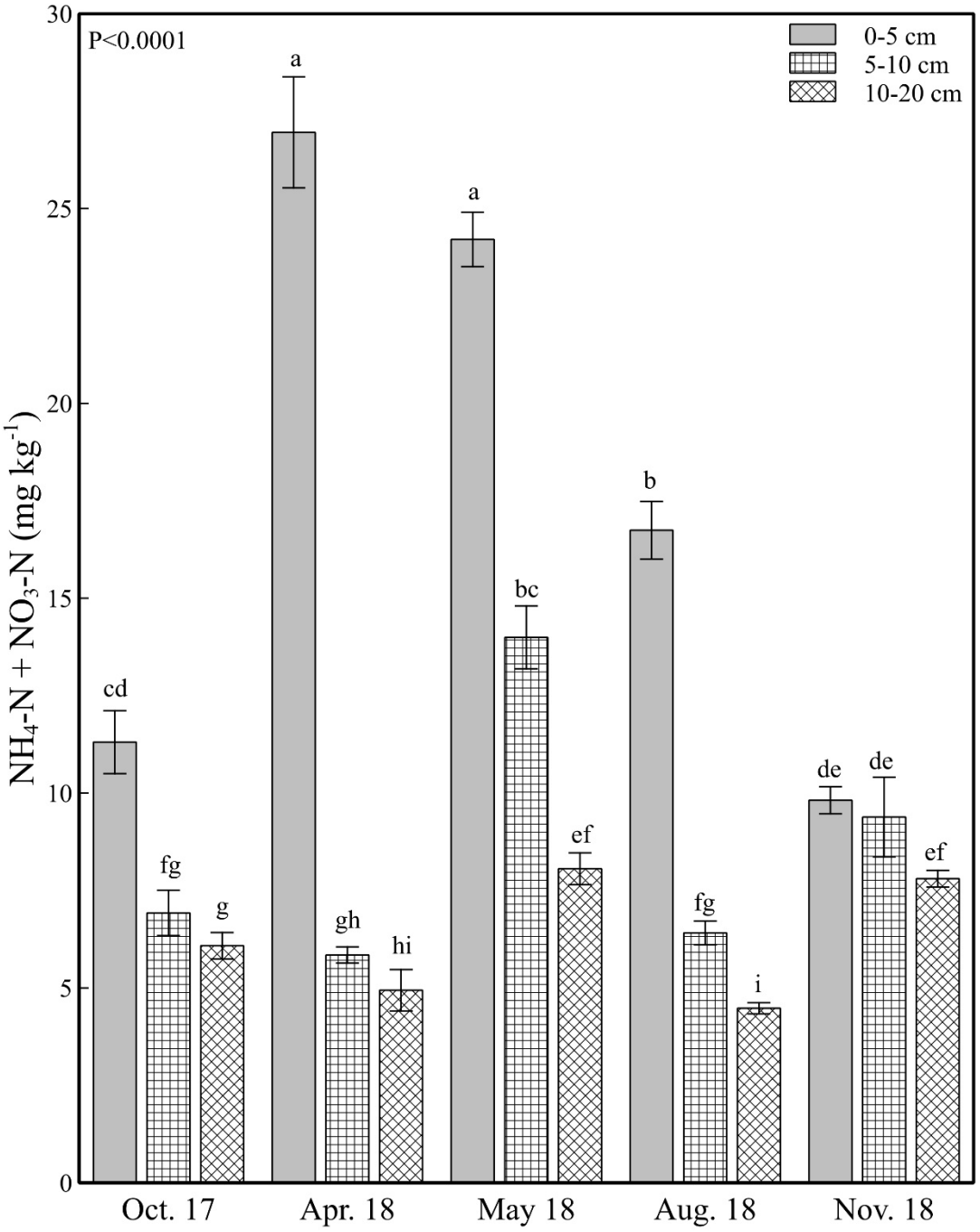


Figure 3.1 Interaction effect of soil sampling event and soil depth on plant available nitrogen (NO_3-N and NH_4-N).

Error bars represent standard error of the mean. Different lowercase letters over the bars denote statistically significant means at $P \leq 0.05$ (n=4).

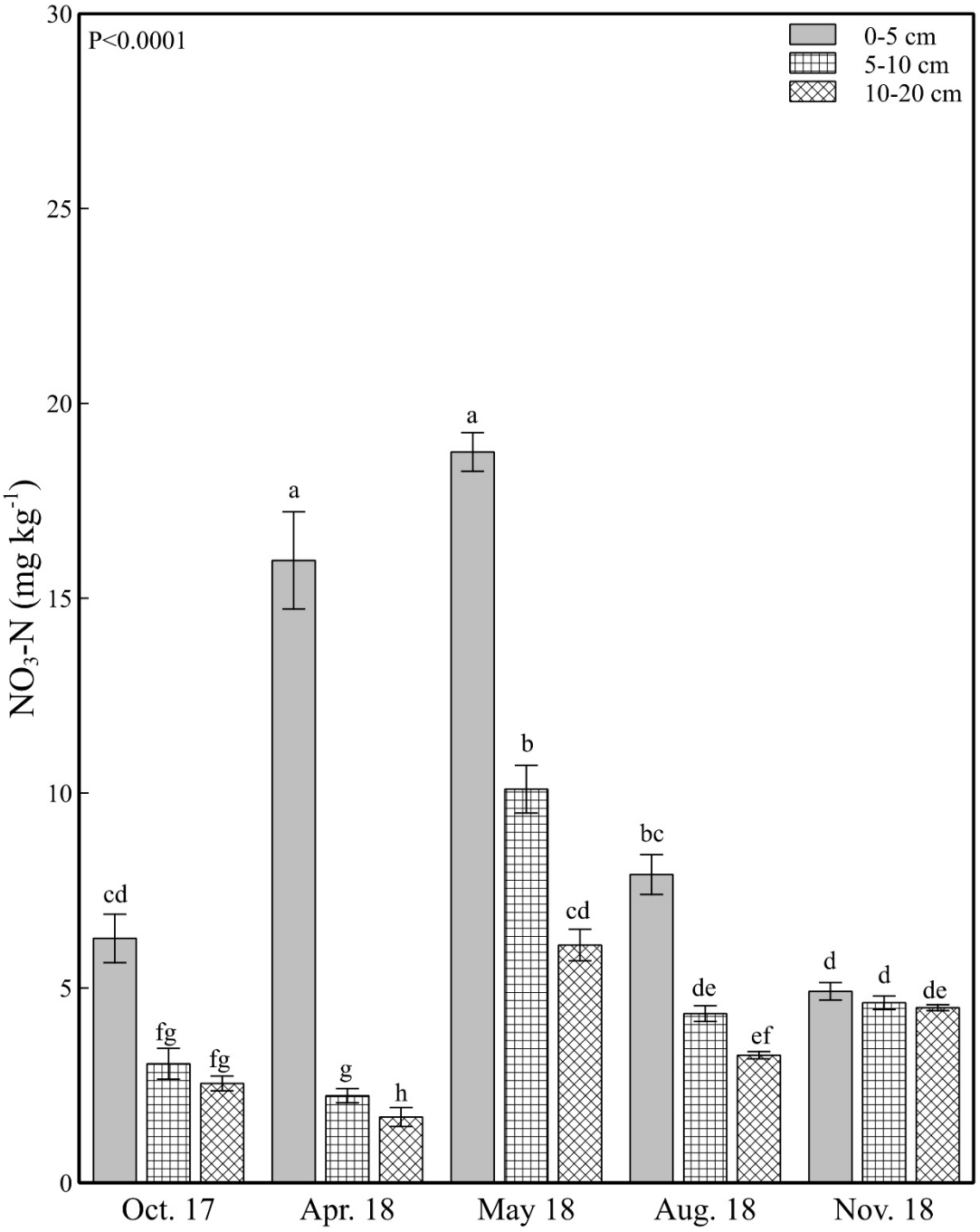


Figure 3.2 Interaction effect of soil sampling event and soil depth on soil nitrate-N (NO_3-N). Error bars represent standard error of the mean. Different lowercase letters over the bars denote statistically significant means at $P \leq 0.05$ (n=4).

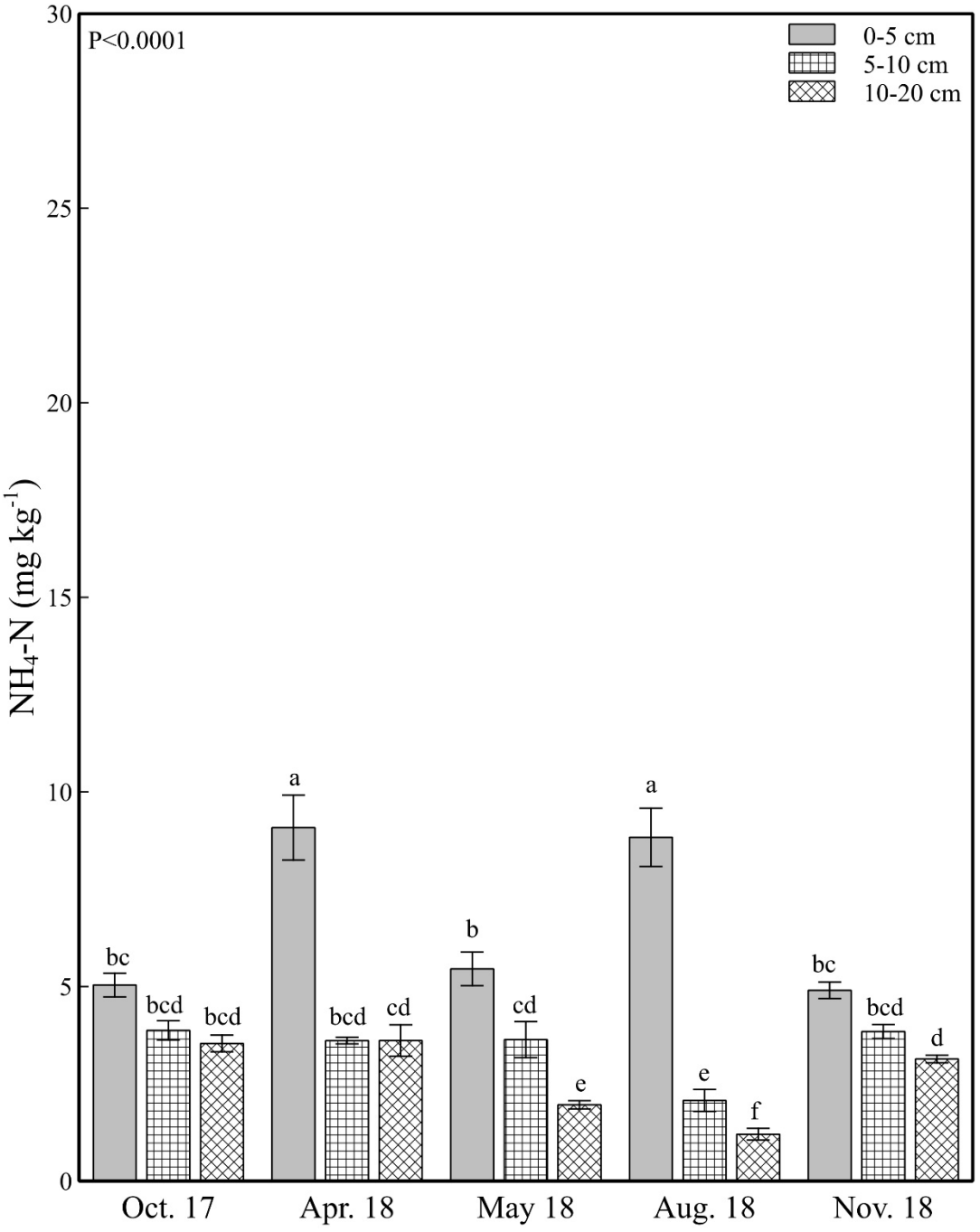


Figure 3.3 Interaction effect of soil sampling event and soil depth on ammonium-N (NH_4-N). Error bars represent standard error of the mean. Different lowercase letters over the bars denote statistically significant means at $P \leq 0.05$ (n=4).

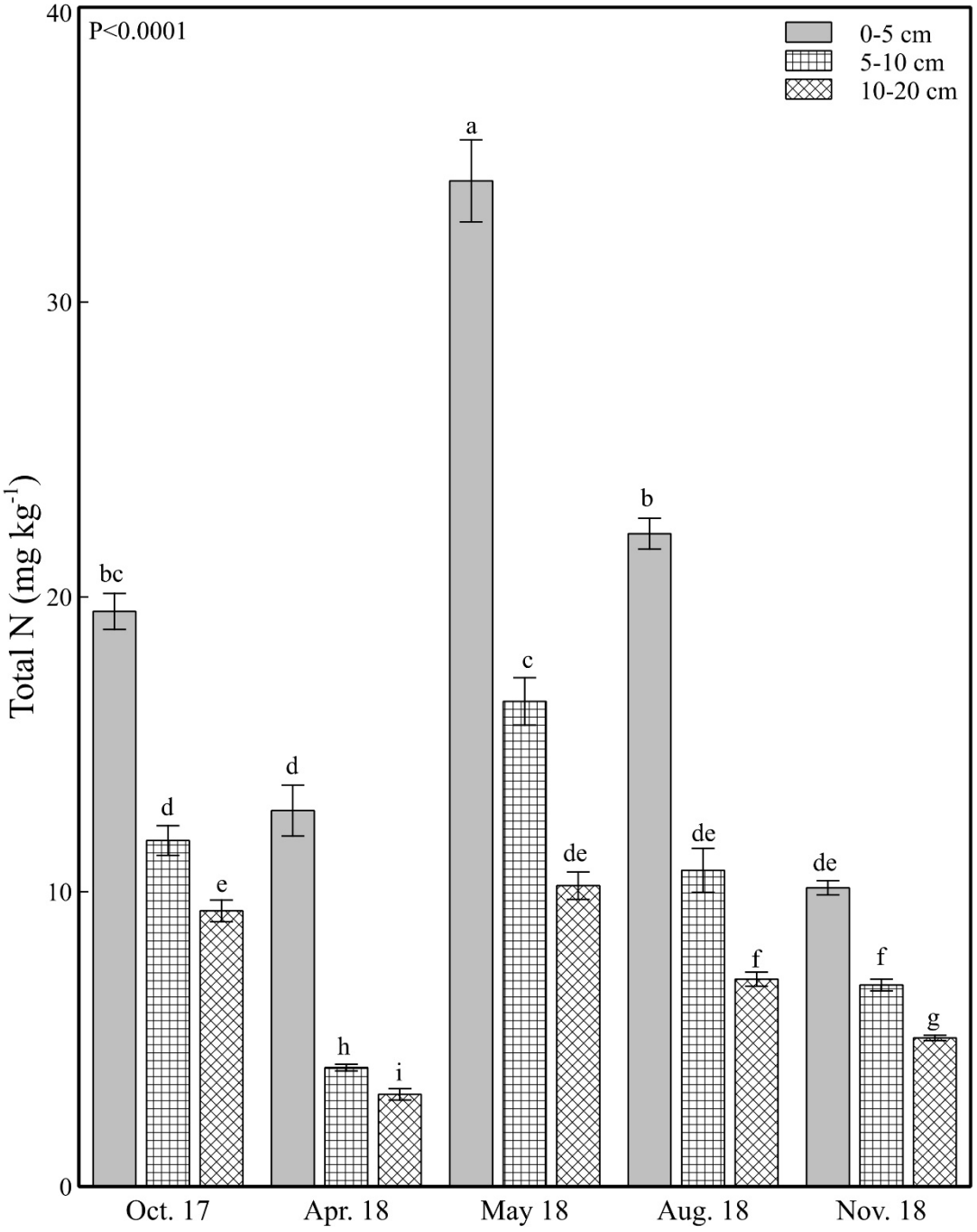


Figure 3.4 Interaction effect of soil sampling event and soil depth on water extractable total soluble nitrogen.

Error bars represent standard error of the mean. Different lowercase letters over the bars denote statistically significant means at $P \leq 0.05$ (n=4).

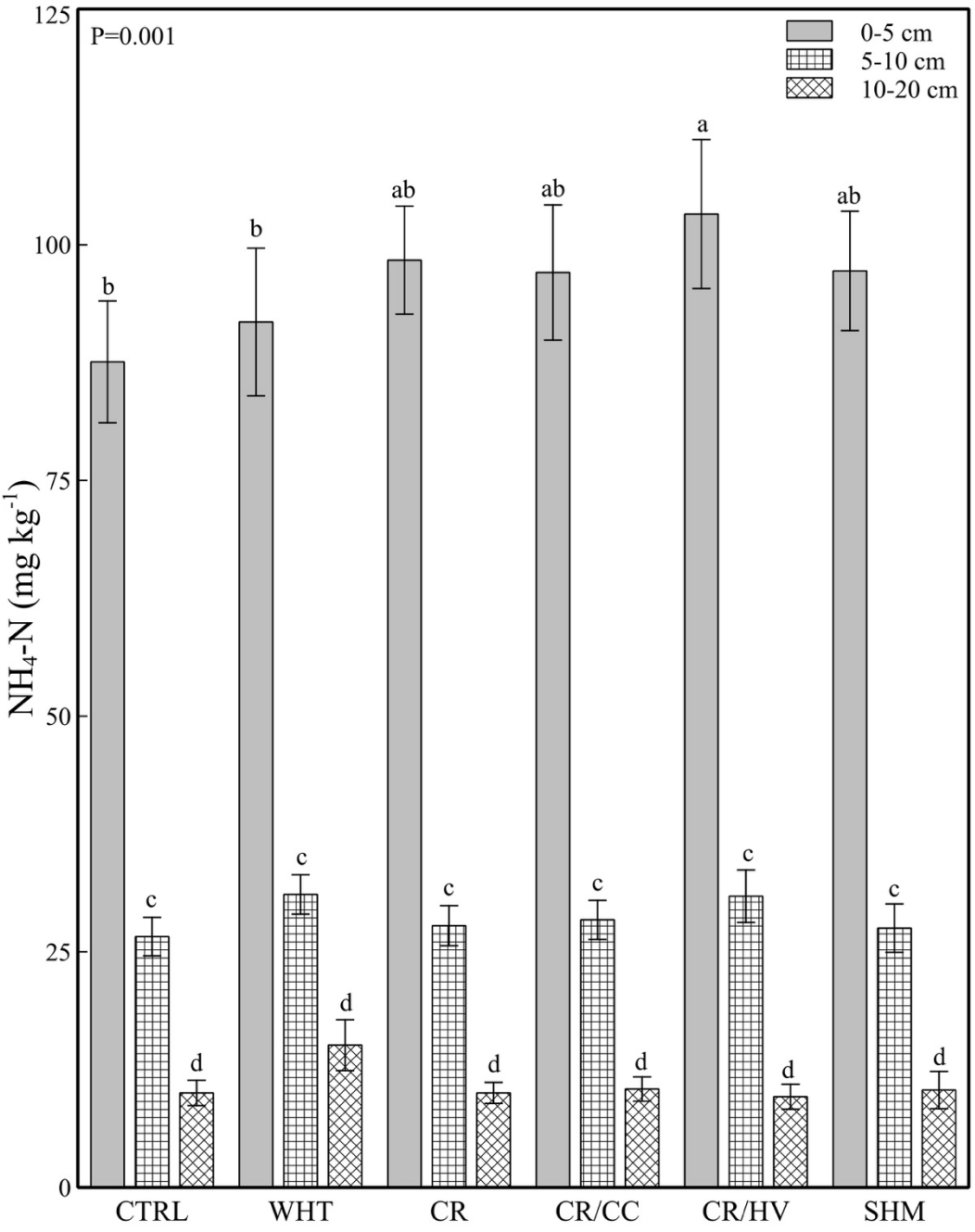


Figure 3.5 Interaction effect of cover crop treatment and soil depth on potentially mineralizable nitrogen.

CTRL, no cover crop; WHT, wheat; CR, cereal rye; CR/CC, cereal rye and crimson clover; CR/HV, cereal rye and hairy vetch; SHM, soil health mix (cereal rye, whole oats, crimson clover, hairy vetch, and daikon radish). Error bars represent standard error of the mean. Different lowercase letters over the bars denote statistically significant means at $P \leq 0.05$ (n=4).

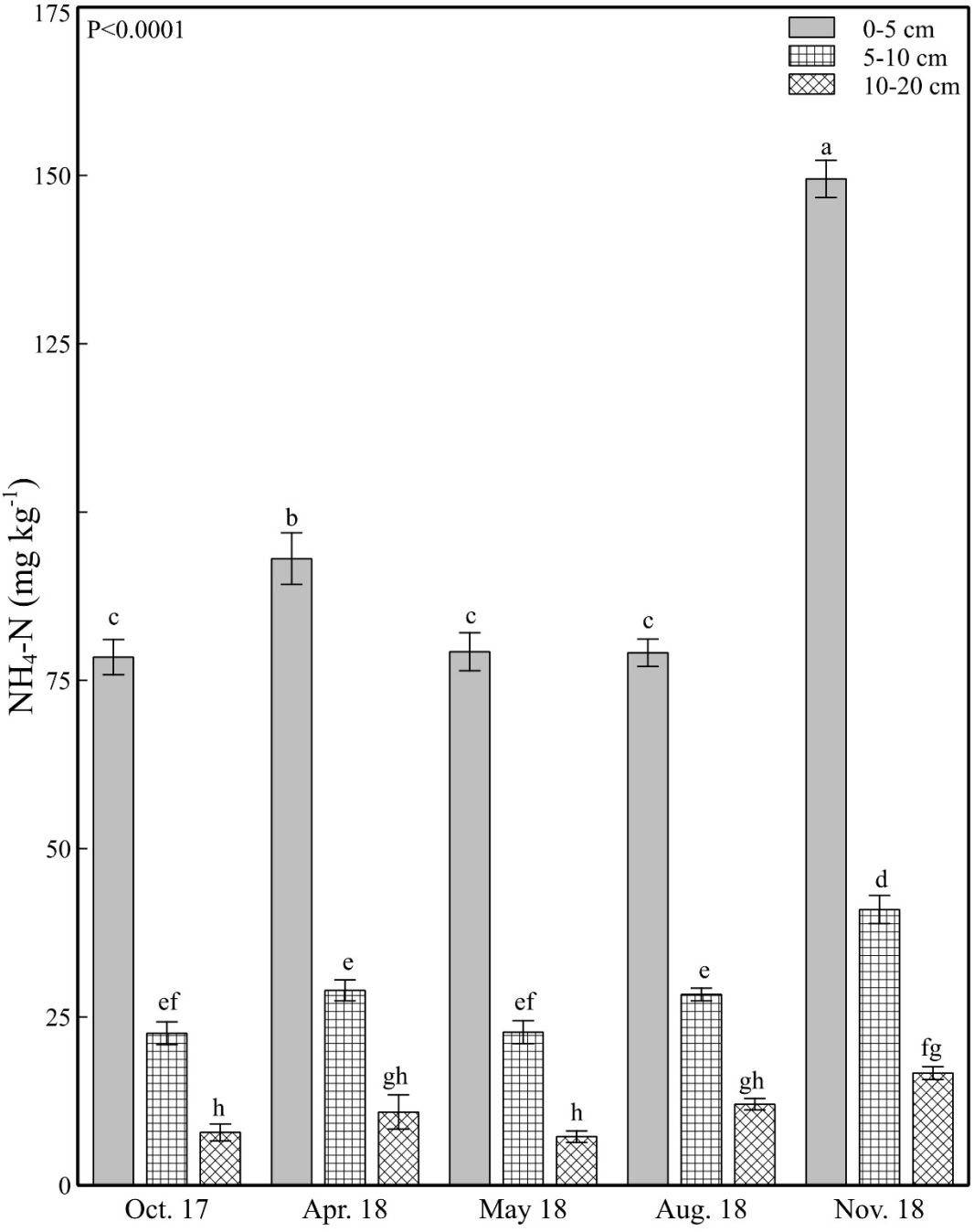


Figure 3.6 Interaction effect of soil sampling event and soil depth on potentially mineralizable nitrogen.

Error bars represent standard error of the mean. Different lowercase letters over the bars denote statistically significant means at $P \leq 0.05$ (n=4).

CHAPTER IV

SUMMARY

The overarching goal of this project is to understand if CC species have any effect on the amount of N available in the soil for the succeeding row-crop. Leveraging a CC field trial started in 2013 at the University of Tennessee's Research and Education Center in Milan, TN, we conducted two studies. The first study was an *in-situ* litterbag study to understand how CC residue decomposition is affected by CC species types and degree of soil disturbance. Results showed that sub-surface placement of residues significantly enhanced the decomposition and N release of CC residues compared to surface placement, validating our hypothesis that CC residue decomposition and N release will increase with an increase in soil disturbance. Within 4 weeks of residue placement in the field, the CC residues in the subsurface placed litterbags had 62 to 79% and 86 to 92% biomass decomposition and N release, across the CC treatments. The single-species wheat (*Triticum aestivum* L.) had the highest rate of biomass decomposition, whereas the 5-species soil health mix treatment had the greatest rate of N mineralization throughout the 21-week study. The residue decomposition from the surface placed litterbags were significantly lower than subsurface placed litterbags with only 40 to 47% and 56 to 67% biomass decomposition and N release, across the CC treatments at week 4. The soil health mix treatment had the greatest decomposition, although the soil health mix and single-species cereal rye (*Secale cereal* L.) both had the greatest N mineralization. Our hypothesis that the soil health mix would have the greatest N release was partially proven correct.

The second study was conducted to understand if the differences in CC biomass decomposition and N release is actually affecting the ability of soil to provide plant available N to the succeeding row-crop. Results showed that CC treatments, soil depth, and soil sampling dates had no significant effect on any of the soil N parameters analyzed such as plant available N, water

extractable total soluble N, and potentially mineralizable N. As a result, our hypothesis that mineral N would be greatest in plots with legume CC, was proven false. However, we observed a strong interaction between soil depth and sampling dates for all soil N parameters. Plant available N concentrations were greatest in the top soil layer in April 2018 and May 2018 (prior to and one month after CC termination, respectively). This resulted in water extractable total soluble N concentrations being greatest in May 2018, with an increase of 167% from April 2018, indicating a greater chance for N loss through leaching. The interaction between CC treatment and soil depth was significant for potentially mineralizable N. Cereal rye, and the cereal rye and hairy vetch (*Vicia villosa* Roth) mixture had the greatest potentially mineralizable N concentration in the top soil layer. As a result, our hypothesis that grass/legume mixtures will have a greater potential to supply plant available N throughout the row-crop growing season was partially correct.

Overall, this study shows that CC have the potential to supply N to succeeding row-crops after termination. Our results show that CC utilization in the winter months instead of leaving fields fallow is beneficial not only to control soil erosion and associated water quality issues, but to also make southeastern US agro-ecosystems more sustainable. However, to fully utilize CC as a supplemental N source for row-crop production, we suggest that producers reduce the lag time between CC termination and row-crop planting as much as possible. To accurately determine the N saving credits from cover cropping, future research is needed to study the combined effect of row-crop planting dates and N fertilizer rates, with and without cover cropping, on row-crop yield.

VITA

Manuel Sabbagh was born in Aleppo, Syria, to Joseph Sabbagh and Lina Tanous. He is the youngest of three siblings; his brother, George, and sister, Darine. He and his family moved to California at the age of 3. Until third grade, Manuel moved around various parts of southern California until finally residing in the small town of San Dimas, CA. He attended Willow Elementary School, Ekstrand Elementary School, Ramona Middle School, and Bonita High School. After earning his diploma in June 2011, Manuel enrolled at the University of California, Berkeley majoring in Molecular Environmental Biology with a concentration in Environment and Human Health. Manuel earned a Bachelor of Science degree in May 2015. In between graduating from the University of California, Berkeley and starting his Master's program at the University of Tennessee, Knoxville, Manuel worked as a Quality Technician at E & J Gallo Winery in Fresno, CA. After that, he worked as a research assistant in the Research & Development department for a plant nutrient company, Actagro, located in Kerman, CA. Soon after enrolling at UT Knoxville, Manuel accepted a Graduate Research Assistantship and later a Graduate Teaching Assistantship. Manuel was a teaching assistant for Dr. Michael Essington, who taught Soil Chemistry, and for Andrew Sherfy, who taught Introduction to Soil Science where he taught one of the lab sections. Manuel graduated with a Master of Science in Environmental and Soil Sciences in August 2019. After obtaining his M.S., Manuel moved on to obtain a Ph.D.